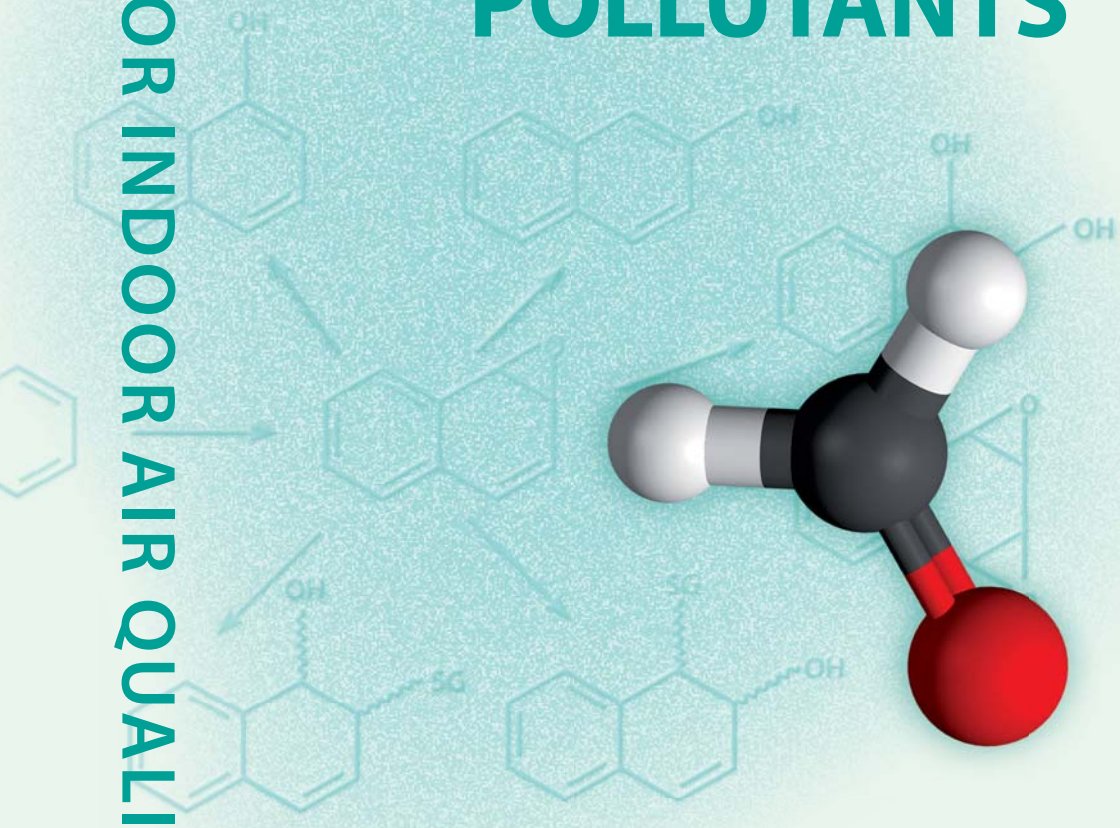
World Health Organization
REGIONAL OFFICE FOR Europe

WHO GUIDELINES FOR INDOOR AIR QUALITY

SELECTED POLLUTANTS



WHO guidelines for indoor air quality: selected pollutants

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Abstract

This book presents WHO guidelines for the protection of public health from risks due to a number of chemicals commonly present in indoor air. The substances considered in this review, i.e. benzene, carbon monoxide, formaldehyde, naphthalene, nitrogen dioxide, polycyclic aromatic hydrocarbons (especially benzo[*a*]pyrene), radon, trichloroethylene and tetrachloroethylene, have indoor sources, are known in respect of their hazardousness to health and are often found indoors in concentrations of health concern. The guidelines are targeted at public health professionals involved in preventing health risks of environmental exposures, as well as specialists and authorities involved in the design and use of buildings, indoor materials and products. They provide a scientific basis for legally enforceable standards.

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Declaration of interests

A standard "Declaration of interests for WHO experts" form was completed by all experts involved in the preparation of the guidelines. Twenty-six experts declared an interest in the subject matter of the meetings/guidelines. All responses were reviewed by the WHO Legal Office and Guidelines Review Committee. The declared interests are listed below.

Potential interests judged by WHO as being insignificant for the guidelines process: experts cleared to participate in the development of the guidelines

Vernon Benignus reported having received remuneration for employment and consultancy from a commercial entity or other organization with an interest related to the subject of air pollution. He also reported having provided an expert opinion or testimony for a commercial entity or other organization as part of a regulatory, legislative or judicial process. He equally stated having held an office or other position, paid or unpaid, where he was expected to represent interests or defend a position related to the subject of air pollution.

Juana M. Delgado Saborit and ***Frank Kelly*** reported them or their research unit having received support for research from an organization that is mainly funded by a commercial entity with a major interest related to air pollution. The organization clarified that its research and reporting are independent, regardless of whether its funding is public or private.

Peter Farmer reported having received remuneration for consultancy from a commercial entity or other organization with an interest related to the subject of air pollution.

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Paul Harrison reported him or his research unit having received support for research from a commercial entity or other organization with an interest related to air pollution. He also reported having provided an expert opinion or testimony for a commercial entity or other organization as part of a regulatory, legislative or judicial process related to the subject of air pollution.

Roy Harrison reported a research contract with and funded travel to annual meetings of a research organization active in the field of health effects of air pollution, which receives funding from the motor vehicle industry and other private organizations.

Rogene Henderson reported having been employed by and having given consultation to a commercial entity or other organization with an interest related to the subject of air pollution. She also reported having provided expert opinion or testimony related to the subject of air pollution for a commercial entity or other organization, as well has having held an office or other position, paid or unpaid, where she may have been expected to represent interests or defend a position related to the subject of air pollution.

Stylianos Kephalopoulos and ***Dimitrios Kotzias*** reported them or their research unit having received support from a commercial entity or other organization with an interest related to the subject of air pollution.

Michael Kleinman reported having received remuneration from a commercial entity or other organization with an interest related to air pollution and having provided an expert opinion or testimony as part of a regulatory, legislative or judicial process related to air pollution.

Gunnar Nielsen reported having provided an expert opinion or testimony as part of a regulatory, legislative or judicial process related to the subject of air pollution for a commercial entity or other organization.

David G. Penney has given numerous testimonies on safe levels of carbon monoxide. The potential interest was judged by WHO as being insignificant for the guidelines process.

Eugene Bruce and ***Regula Rapp*** reported having received research support from a commercial entity or other organization with an interest in the subject of air pollution.

Christophe Rousselle reported having held an office or other position, paid or unpaid, where he may have been expected to represent interests or defend a position related to the subject of air pollution.

Kurt Straif reported having held an office or other position, paid or unpaid, where he may have been expected to represent interests or defend a position related to the subject of air pollution.

Peder Wolkoff reported being a member of a scientific advisory committee for indoor climate.

Hajo Zeeb reported having received remuneration from employment and consultancy for a commercial entity or other organization with an interest related to the subject of air pollution. He also reported having received research support from a commercial entity or other organization with an interest related to the subject of the meeting. He confirmed having held an office or other position, paid or unpaid, where he may have been expected to represent interests or defend a position related to the subject air pollution.

Experts excluded from the development of the guidelines

Alan Buckpitt reported funded travel to and an honorarium from a commercial entity or other organization with an interest related to air pollution. He also reported a research contract with the same entity. His wife has significant stock in a commercial entity with an interest in air quality.

Vincent Cogliano reported holding stocks, bonds, stock options or other securities in a commercial entity with an interest related to air pollution. He also reported having held office or position, paid or unpaid, where he may have been expected to represent interests or defend a position related to the subject of air pollution.

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Foreword

Clean air is a basic requirement of life. The quality of air inside homes, offices, schools, day care centres, public buildings, health care facilities or other private and public buildings where people spend a large part of their life is an essential determinant of healthy life and people's well-being. Hazardous substances emitted from buildings, construction materials and indoor equipment or due to human activities indoors, such as combustion of fuels for cooking or heating, lead to a broad range of health problems and may even be fatal.

Indoor exposure to air pollutants causes very significant damage to health globally – especially in developing countries. The chemicals reviewed in this volume are common indoor air pollutants in all regions of the world. Despite this, public health awareness on indoor air pollution has lagged behind that on outdoor air pollution. The current series of indoor air quality guidelines, focuses specifically on this problem. This volume, the second in the series following that addressing the hazards of dampness and mould, sets guidelines for a range of chemical substances most commonly polluting indoor air. Understanding of the hazards of these substances is a first step in identifying the actions necessary to avoid and reduce the adverse impacts of these pollutants on health. If these guidelines are sensibly applied as part of policy development, indoor exposure to air pollutants should decline and a significant reduction in adverse effects on health should follow.

WHO has a long tradition in synthesizing the evidence on health aspects of air quality and in preparing air quality guidelines defining conditions for healthy air. We are grateful to the outstanding scientists conducting this work. We hope that these new guidelines will be useful globally to people assessing indoor air quality with a view to predicting its effects on health, and also to those with responsibility for introducing measures to reduce health risks from indoor exposure to air pollutants. Prevention of the health effects of poor indoor air quality is needed in all regions of the world, and especially in developing countries. WHO will assist its Member States in implementing the guidelines, synthesizing the evidence on the most effective approaches to indoor air quality management and on the health benefits of these actions. It will continue encouraging the relevant policy developments and intersectoral collaboration necessary for ensuring access to healthy indoor air for everyone.

Zsuzsanna Jakab
WHO Regional Director for Europe

Executive summary

This document presents WHO guidelines for the protection of public health from health risks due to a number of chemicals commonly present in indoor air. The guidelines are based on a comprehensive review and evaluation of the accumulated scientific evidence by a multidisciplinary group of experts studying the toxic properties and health effects of these pollutants.

The substances considered in this review (benzene, carbon monoxide, formaldehyde, naphthalene, nitrogen dioxide, polycyclic aromatic hydrocarbons (especially benzo[*a*]pyrene), radon, trichloroethylene and tetrachloroethylene) have been added to the guidelines considering information on the existence of indoor sources, on the availability of toxicological and epidemiological data and on exposure levels causing health concerns.

Problems of indoor air quality are recognized as important risk factors for human health in both low- and middle- and high-income countries. Indoor air is also important because people spend a substantial proportion of their time in buildings. In residences, day-care centres, retirement homes and other special environments, indoor air pollution affects population groups that are particularly vulnerable owing to their health status or age.

The primary aim of these guidelines is to provide a uniform basis for the protection of public health from adverse effects of indoor exposure to air pollution, and to eliminate or reduce to a minimum exposure to those pollutants that are known or are likely to be hazardous.

The guidelines are targeted at public health professionals involved in preventing health risks of environmental exposures as well as specialists and authorities involved in the design and use of buildings, indoor materials and products. The guidelines are based on the accumulated scientific knowledge available at the time of their development. They have the character of recommendations. Nevertheless, countries may wish to use the guidelines as a scientific basis for legally enforceable standards.

The evidence review supporting the guidelines for each of the selected pollutants includes an evaluation of indoor sources, current indoor concentrations and their relationship with outdoor levels, as well as a summary of the evidence on the kinetics and metabolism and health effects. Based on the accumulated evidence, the experts formulated health risk evaluations and agreed on the guidelines for each of the pollutants as summarized below.

Benzene

Guidelines on exposure levels for indoor air are needed because indoor air is a significant source of benzene exposure and inhalation is the main pathway of

human exposure to benzene. Benzene is present in both outdoor and indoor air. However, indoor concentrations are generally higher than those in outdoor air owing to the infiltration of benzene present in outdoor air and to the existence of many other indoor sources. Typically, indoor concentrations are below the lowest levels showing evidence of adverse health effects. Considering that benzene is present indoors and taking into account personal exposure patterns, which are predominantly indoors, indoor guidelines for exposure are needed.

Benzene is a genotoxic carcinogen in humans and no safe level of exposure can be recommended. The risk of toxicity from inhaled benzene would be the same whether the exposure were indoors or outdoors. Thus there is no reason that the guidelines for indoor air should differ from ambient air guidelines. It is also recommended continuing to use the same unit risk factors. The geometric mean of the range of the estimates of the excess lifetime risk of leukaemia at an air concentration of 1 μg/m^3 is 6×10^{-6}. The concentrations of airborne benzene associated with an excess lifetime risk of 1/10 000, 1/100 000 and 1/1000 000 are 17, 1.7 and 0.17 μg/m^3, respectively.

As noted above, there is no known exposure threshold for the risks of benzene exposure. Therefore, from a practical standpoint, it is expedient to reduce indoor exposure levels to as low as possible. This will require reducing or eliminating human activities that release benzene, such as smoking tobacco, using solvents for hobbies or cleaning, or using building materials that off-gas benzene.

Adequate ventilation methods will depend on the site of the building. In modern buildings located near heavy traffic or other major outdoor sources of benzene, inlets for fresh air should be located at the least polluted side of the building.

Carbon monoxide

Exposure to carbon monoxide reduces maximum exercise ability in healthy young individuals and reduces the time to angina and, in some cases, the time to ST-segment depression in people with cardiovascular disease, albeit at a concentration that is lower than that needed to reduce exercise ability in healthy individuals.

The relationship of carbon monoxide exposure and the carboxyhaemoglobin (COHb) concentration in blood can be modelled using the differential Coburn-Forster-Kane equation, which provides a good approximation to the COHb concentration at a steady level of inhaled, exogenous carbon monoxide. Based on laboratory studies of reduction in exercise capacity in both healthy individuals and volunteers with cardiovascular disease, it was determined that COHb levels should not exceed 2%. The Coburn-Forster-Kane equation is used below to determine the levels of carbon monoxide to which a normal adult under resting conditions for various intervals can be exposed without exceeding a COHb level of 2%.

The previous WHO guidelines were established for 15 minutes to protect against short-term peak exposures that might occur from, for example, an unvented stove; for 1 hour to protect against excess exposure from, for example, faulty appliances; and for 8 hours (which is relevant to occupational exposures and has been used as an averaging time for ambient exposures). We do not recommend changing the existing guidelines.

However, chronic carbon monoxide exposure appears different from acute exposure in several important respects. The latest studies available in 2009, especially those epidemiological studies using very large databases and thus producing extremely high-resolution findings, suggest that the appropriate guideline level for longer-term average concentration of carbon monoxide in order to minimize health effects must be positioned below the 8-hour guideline of 10 mg/m^3. Thus, a separate guideline is recommended to address 24-hour exposures.

Therefore, a series of guidelines relevant to typical indoor exposures is recommended as follows: 100 mg/m^3 for 15 minutes and 35 mg/m^3 for 1 hour (assuming light exercise and that such exposure levels do not occur more often than one per day); 10 mg/m^3 for 8 hours (arithmetic mean concentration, light to moderate exercise); and 7 mg/m^3 for 24 hours (arithmetic mean concentration, assuming that the exposure occurs when the people are awake and alert but not exercising).

Formaldehyde

An indoor air guideline for formaldehyde is appropriate because indoor exposures are the dominant contributor to personal exposures through inhalation and indoor concentrations may be high enough to cause adverse health effects.

The lowest concentration reported to cause sensory irritation of the eyes in humans is 0.36 mg/m^3 for four hours. Increases in eye blink frequency and conjunctival redness appear at 0.6 mg/m^3, which is considered equal to the no observed adverse effect level (NOAEL). There is no indication of accumulation of effects over time with prolonged exposure.

The perception of odour may result in some individuals reporting subjective sensory irritation, and individuals may perceive formaldehyde at concentrations below 0.1 mg/m^3. However, this is not considered to be an adverse health effect. The NOAEL of 0.6 mg/m^3 for the eye blink response is adjusted using an assessment factor of 5 derived from the standard deviation of nasal pungency (sensory irritation) thresholds, leading to a value of 0.12 mg/m^3, which has been rounded down to 0.1 mg/m^3. Neither increased sensitivity nor sensitization is considered plausible at such indoor concentrations in adults and children. This value is thus considered valid for short-term (30-minute) duration, and this threshold should not be exceeded at any 30-minute interval during a day.

Thus, a short-term (30-minute) guideline of 0.1 mg/m^3 is recommended as preventing sensory irritation in the general population.

Evaluations of long-term effects, including cancer, based on a NOAEL and assessment factor approach, as well as estimates from the biologically motivated models, yield similar results, with values of approximately 0.2 mg/m^3. These values are above the guideline for short-term effects of 0.1 mg/m^3. Thus the use of the short-term (30-minute) guideline of 0.1 mg/m^3 will also prevent long-term health effects, including cancer.

The use of low-emitting building materials and products, and preventing exposures to environmental tobacco smoke and other combustion emissions, will minimize exposure-related risk. In addition, ventilation can reduce indoor exposure to formaldehyde.

Naphthalene

The principal health concerns of exposure to naphthalene are respiratory tract lesions, including tumours in the upper respiratory tract demonstrated in animal studies and haemolytic anaemia in humans.

Lesions in the nasal olfactory and, at higher concentrations, also in the respiratory epithelia of rats appear to be the critical non-neoplastic effect. At concentrations about 100-fold higher than the lowest lesion level, severe inflammation and tumours have been reported to occur at these sites.

Increased cell proliferation due to cytotoxicity (cell damage) is considered a key element in the development of airway tumours. The likely involvement of cytotoxic metabolites in the carcinogenic response and the apparent primary non-genotoxicity of naphthalene favour the assumption of the existence of a threshold.

Therefore, the use of a lowest observed adverse effect level (LOAEL)/NOAEL as a threshold, combined with safety factors, is considered to be an appropriate approach for setting indoor air guidelines to minimize the carcinogenic risk to the respiratory tract of naphthalene exposure.

Associated with repeated inhalation exposure of 6 hours per day, 5 days a week for 104 weeks, severe effects in terms of inflammation were observed in almost all rats exposed to the lowest (but still relatively high) naphthalene dose of 53 mg/m^3. In the absence of adequately published data in relation to less severe effects, this can be taken as a LOAEL, even though it is related to severe effects.

Taking this LOAEL as a starting point and adjusting for continuous exposure (dividing by a factor of 24/6 and 7/5), a value of about 10 mg/m^3 is obtained. Further, incorporating a factor of 10 for using a LOAEL rather than a NOAEL, a factor of 10 for inter-species variation and a factor of 10 for inter-individual variation, a guideline value of 0.01 mg/m^3 is established. This guideline value should be applied as an annual average.

Extensive use or misuse of naphthalene mothballs may lead to haemolytic anaemia. Knowledge of the impact of exposure to naphthalene on the risk of haemolytic anaemia in susceptible individuals (glucose 6-phosphate dehydroge-

nase deficiency) cannot be used to define a guideline owing to the lack of adequate exposure data.

In the absence of mothballs or other sources such as combustion of biomass, indoor air concentrations of naphthalene are just above the typical limit of detection of about 0.001 mg/m^3. Since the concentration of naphthalene in the residential environment increases up to 100-fold when mothballs are used, the most efficient way to prevent high exposures would be to abandon (ban) the use of naphthalene-containing mothballs.

Nitrogen dioxide

A 1-hour indoor nitrogen dioxide guideline of 200 μg/m^3, consistent with the existing WHO air quality guideline, is recommended.

At about twice this level, asthmatics exhibit small pulmonary function decrements. Those who are sensitized may have small changes in airway responsiveness to a variety of stimuli already at this level. Studies of the indoor environment provide no evidence for an indoor guideline different to the ambient guideline.

An annual average indoor nitrogen dioxide guideline of 40 μg/m^3, consistent with the existing WHO air quality guideline, is recommended.

The ambient annual average guideline of 40 μg/m^3 was initially based on a meta-analysis of indoor studies. It was assumed that having a gas stove was equivalent to an increased average indoor level of 28 μg/m^3 compared to homes with electric stoves, and the meta-analysis showed that an increase in indoor nitrogen dioxide of 28 μg/m^3 was associated with a 20% increased risk of lower respiratory illness in children.

Homes with no indoor sources were estimated to have an average level of 15 μg/m^3. Several exhaustive reviews to further develop ambient guidelines have not challenged these findings.

Recent well-conducted epidemiological studies that have used measured indoor nitrogen dioxide levels support the occurrence of respiratory health effects at the level of the guideline.

Polycyclic aromatic hydrocarbons

Some polycyclic aromatic hydrocarbons (PAHs) are potent carcinogens and, in air, are typically attached to particles. The primary exposure to carcinogenic PAHs found in air occurs via inhalation of particles. PAHs occur in indoor air as complex mixtures, the composition of which may vary from site to site. Experimental data on metabolism, gene expression and DNA adducts suggest that interactions between PAHs in mixtures may be complex and highly unpredictable for various PAH compositions (inhibitory, additive, synergistic).

In view of the difficulties in developing guidelines for PAH mixtures, benzo[*a*]pyrene (B[*a*]P) was considered to represent the best single indicator compound. Its toxicology is best known, most single PAH concentration data in ambient and

indoor air are for B[*a*]P, and B[*a*]P has widely been used as an indicator compound for exposure in epidemiological studies.

The health evaluation data suggest that lung cancer is the most serious health risk from exposure to PAHs in indoor air. B[*a*]P is one of the most potent carcinogens among the known PAHs.

In its evaluation of PAHs as ambient air pollutants in 2000, WHO expressed a unit cancer risk as a function of the concentration of B[*a*]P taken as a marker of the PAH mixture. Use of the same unit risk factor for indoor air implies that B[*a*]P represents the same proportion of carcinogenic activity of the PAH mixture as in the occupational exposure used to derive the unit risk. This assumption will not always hold, but the associated uncertainties in risk estimates are unlikely to be large.

Reducing exposure to B[*a*]P may also decrease the risk of other adverse health effects associated with PAHs.

Based on epidemiological data from studies on coke-oven workers, a unit risk for lung cancer for PAH mixtures is estimated to be 8.7×10^{-5} per ng/m^3 of B[*a*]P. This is the guideline for PAH in indoor air. The corresponding concentrations for lifetime exposure to B[*a*]P producing excess lifetime cancer risks of 1/10 000, 1/100 000 and 1/1 000 000 are approximately 1.2, 0.12 and 0.012 ng/m^3, respectively.

Radon

Radon is classified by the International Agency for Research on Cancer as a human carcinogen (Group I). There is direct evidence from residential epidemiological studies of the lung cancer risk from radon. The exposure–response relationship is best described as being linear, without a threshold. The excess relative risk, based on long-term (30-year) average radon exposure is about 16% per increase of 100 Bq/m^3, and on this relative scale does not vary appreciably between current smokers, ex-smokers and lifelong non-smokers. Therefore, as the absolute risk of lung cancer at any given radon concentration is much higher in current smokers than in lifelong non-smokers, the absolute risk of lung cancer due to radon is appreciably higher for current and ex-smokers than for lifelong non-smokers. For ex-smokers, the absolute risks will be between those for lifelong non-smokers and current smokers.

The cumulative risk of death from radon-induced lung cancer was calculated for lifelong non-smokers and for current smokers (15–24 cigarettes per day). The derived excess lifetime risks (by the age of 75 years) are 0.6×10^{-5} per Bq/m^3 and 15×10^{-5} per Bq/m^3, respectively. Among ex-smokers, the risk is intermediate, depending on the time since smoking cessation. The radon concentration associated with an excess lifetime risk of 1 per 100 and 1 per 1000 are 67 Bq/m^3 and 6.7 Bq/m^3 for current smokers and 1670 Bq/m^3 and 167 Bq/m^3 for lifelong non-smokers, respectively.

As part of the management of the radon problem, the WHO International Radon Project has recommended that there should be a reference level as an essential tool in this process.[1]

> A national Reference Level does not specify a rigid boundary between safety and danger, but defines a level of risk from indoor radon that a country considers to be too high if it continues unchecked into the future. However, protective measures may also be appropriate below this level to ensure radon concentrations in homes are well below that level. In view of the latest scientific data, WHO proposes a Reference Level of 100 Bq/m^3 to minimize health hazards due to indoor radon exposure. However, if this level cannot be reached under the prevailing country-specific conditions, the chosen Reference Level should not exceed 300 Bq/m^3 which represents approximately 10 mSv per year according to recent calculations by the International Commission on Radiation Protection.

A guide for radon management should include, in addition to the setting of a reference level, building codes, measurement protocols and other relevant components of a national radon programme.

Trichloroethylene

The existence of both positive and negative results has in the past led risk assessors to different interpretations of trichloroethylene (TCE) toxicity and to divergent estimates of human cancer risk. For a health risk evaluation, bearing in mind recent data on a mechanism of action that is not species-specific, the evidence for weak genotoxicity, and the consistency between certain cancers observed in animals and in humans (in particular liver cancer), it is prudent to consider that the carcinogenicity in animals, the positive epidemiological studies and the plausibility of a human cancer risk leads to the recommendation of a non-threshold approach with a risk estimate rather than a safe level.

Therefore, carcinogenicity (with the assumption of genotoxicity) is selected as the end-point for setting the guideline value. The unit risk estimate of 4.3×10^{-7} $(\mu g/m^3)^{-1}$, derived on the basis of increased Leydig cell tumours (testicular tumours) in rats, is proposed as the indoor air quality guideline. This was also the conclusion of WHO in 2000, the European Union in 2004 and the French Agency for Environmental and Occupational Health in 2009.

The concentrations of airborne TCE associated with an excess lifetime cancer risk of 1/10 000, 1/100 000 and 1/1 000 000 are respectively 230, 23 and 2.3 $\mu g/m^3$.

Tetrachloroethylene

Carcinogenicity is not selected as the end-point for setting the guideline value for tetrachloroethylene, for three reasons: the epidemiological evidence is equiv-

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[1] *WHO handbook on indoor radon: a public health perspective*. Geneva, World Health Organization, 2009.

ocal, the animal tumours detected are not considered relevant to humans, and there are no indications that tetrachloroethylene is genotoxic. The derivation of a guideline value is at present based on two non-neoplastic effects as the critical end-point: impaired neurobehavioural performance and early renal changes.

On the basis of a long-term LOAEL for kidney effects of 102 mg/m^3 in dry cleaning workers, a guideline value of 0.25 mg/m^3 has been calculated. In deriving this guideline value, the LOAEL is converted to continuous exposure (dividing by a factor of 4.2 (168/40)) and divided by an uncertainty factor of 100 (10 for use of an LOAEL and 10 for intra-species variation). Recognizing that some uncertainty in the LOAEL exists because the effects observed at this level are not clear-cut and because of fluctuations in exposure levels, an alternative calculation was made based on the LOAEL in mice of 680 mg/m^3 and using an appropriate uncertainty factor of 1000. This calculation yields a guideline value of 0.68 mg/m^3.

A chronic inhalation minimal risk level (MRL) of 0.28 mg/m^3 (0.04 ppm) has been derived by the Agency for Toxic Substances and Disease Registry based on the LOAEL of 15 ppm. The MRL was calculated from this concentration by expanding to continuous exposure (8/24 hours, 5/7 days) and dividing by an uncertainty factor of 100 (10 for use of a LOAEL and 10 for human variability). This reference found significantly prolonged reaction times in workers occupationally exposed to an average of 15 ppm for about 10 years.

The value and appropriateness of establishing a short-term guideline value is questionable because acute effects occur only at very high concentrations of 50 ppm (340 mg/m^3) and higher, compared to generally observed levels in close proximity to dry cleaning facilities. Establishing a long-term value is more protective of human health.

On the basis of the overall health risk evaluation, the recommended guideline for year-long exposure is 0.25 mg/m^3. This is the same as the previous WHO guideline.

Summary table

A synthesis of the guidelines for all pollutants considered in this volume is presented in Table A overleaf.

Table A. Summary of indoor air quality guidelines for selected pollutants

Pollutant	Critical outcome(s) for guideline definition
Benzene	• Acute myeloid leukaemia (sufficient evidence on causality) • Genotoxicity
Carbon monoxide	Acute exposure-related reduction of exercise tolerance and increase in symptoms of ischaemic heart disease (e.g. ST-segment changes)
Formaldehyde	Sensory irritation
Naphthalene	Respiratory tract lesions leading to inflammation and malignancy in animal studies
Nitrogen dioxide	Respiratory symptoms, bronchoconstriction, increased bronchial reactivity, airway inflammation and decreases in immune defence, leading to increased susceptibility to respiratory infection
Polycyclic aromatic hydrocarbons	Lung cancer
Radon	Lung cancer Suggestive evidence of an association with other cancers, in particular leukaemia and cancers of the extrathoracic airways
Trichloroethylene	Carcinogenicity (liver, kidney, bile duct and non-Hodgkin's lymphoma), with the assumption of genotoxicity
Tetrachloroethylene	Effects in the kidney indicative of early renal disease and impaired performance

Guidelines	Comments
• No safe level of exposure can be recommended • Unit risk of leukaemia per 1 µg/m^3 air concentration is 6×10^{-6} • The concentrations of airborne benzene associated with an excess lifetime risk of 1/10 000, 1/100 000 and 1/1 000 000 are 17, 1.7 and 0.17 µg/m^3, respectively	
• 15 minutes – 100 mg/m^3 • 1 hour – 35 mg/m^3 • 8 hours – 10 mg/m^3 • 24 hours – 7 mg/m^3	
0.1 mg/m^3 – 30-minute average	The guideline (valid for any 30-minute period) will also prevent effects on lung function as well as nasopharyngeal cancer and myeloid leukaemia
0.01 mg/m^3 – annual average	The long-term guideline is also assumed to prevent potential malignant effects in the airways
• 200 µg/m^3 – 1 hour average • 40 µg/m^3 – annual average	No evidence for exposure threshold from epidemiological studies
• No threshold can be determined and all indoor exposures are considered relevant to health • Unit risk for lung cancer for PAH mixtures is estimated to be 8.7×10^{-5} per ng/m^3 of B[*a*]P • The corresponding concentrations for lifetime exposure to B[*a*]P producing excess lifetime cancer risks of 1/10 000, 1/100 000 and 1/1 000 000 are approximately 1.2, 0.12 and 0.012 ng/m^3, respectively	B[*a*]P is taken as a marker of the PAH mixture
• The excess lifetime risk of death from radon-induced lung cancer (by the age of 75 years) is estimated to be 0.6×10^{-5} per Bq/m^3 for lifelong non-smokers and 15×10^{-5} per Bq/m^3 for current smokers (15–24 cigarettes per day); among ex-smokers, the risk is intermediate, depending on time since smoking cessation • The radon concentrations associated with an excess lifetime risk of 1/100 and 1/1000 are 67 and 6.7 Bq/m^3 for current smokers and 1670 and 167 Bq/m^3 for lifelong non-smokers, respectively	WHO guidelines provide a comprehensive approach to the management of health risk related to radon
• Unit risk estimate of 4.3×10^{-7} per µg/m^3 • The concentrations of airborne trichloroethylene associated with an excess lifetime cancer risk of 1:10 000, 1:100 000 and 1:1 000 000 are 230, 23 and 2.3 µg/m^3, respectively	
0.25 mg/m^3 – annual average	Carcinogenicity is not used as an end-point as there are no indications that tetrachloroethylene is genotoxic and there is uncertainty about the epidemiological evidence and the relevance to humans of the animal carcinogenicity data

Introduction

Human beings need a regular supply of food and water and an essentially continuous supply of air. The requirements for air and water are relatively constant (10–20 m^3 and 1–2 litres per day, respectively). That all people should have free access to air and water of acceptable quality is a fundamental human right. Recognizing the need of humans for clean air, in 1987 the WHO Regional Office for Europe published the first edition of *Air quality guidelines for Europe (1)*, containing health risk assessments of 28 chemical air contaminants.

In 2000, WHO published a second edition of the guidelines *(2)* and a "global update" was published in 2006 *(3)*. The second edition focused on the pollutants considered in the first edition. The global update focused on a small group of pollutants (particulate matter, ozone, nitrogen dioxide and sulfur dioxide) but also included chapters that addressed some health-related general subjects of importance to the air pollution field, including a chapter on indoor air quality. The WHO air quality guidelines have played an important role in providing information and guidance for regulatory authorities working in the air pollution field. In Europe, the guidelines are now seen as the key source on which the European Commission's directive on air quality is based.

That people are exposed to air pollutants both outdoors and indoors is obvious. Globally, people are spending an increasing amount of time indoors. There they are exposed to pollutants generated outdoors that penetrate to the indoor environment and also to pollutants produced indoors, for example as a result of space heating, cooking and other indoor activities, or emitted from products used indoors.

The first edition of the *Air quality guidelines for Europe* published in 1987 *(1)* included a chapter on radon and an annex on tobacco smoke, indoor air pollutants with significant adverse public health impacts. The second edition published in 2000 *(2)* provided a section on indoor air pollutants and added man-made vitreous fibres to radon and tobacco smoke.

The 2005 global update of the air quality guidelines *(3)* drew attention to the large impact on health of indoor air pollution in developing countries. The high concentration of particulates and gases found indoors in houses using solid fuel, including biomass, were noted and it was estimated that exposure might be responsible for nearly 1.6 million excess deaths annually and about 3% of the global

burden of disease. This is a huge impact on health; indeed, far larger than that imposed by exposure to outdoor air pollutants.

Work on assessing the health effects of indoor air pollution has lagged behind that on outdoor air pollution for a number of reasons, including:

- the fact that policy development in the air pollution field has focused on outdoor air pollution as a result of the correctly perceived need to deal with the high levels of outdoor air pollutants associated with both coal smoke and photochemical smog;
- the ready applicability of standards to outdoor concentrations of air pollutants;
- the feasibility of monitoring concentrations of outdoor air pollutants on a large scale;
- the focus of epidemiologists on defining coefficients linking outdoor concentrations of air pollutants with effects on health; and
- the fact that the science and policy communities have focused on the public health impacts of air pollution in wealthy developed countries, while often disregarding the larger burden of disease due to indoor air pollution from solid fuel burning in the developing world.

Questions such as: "how could air quality standards be enforced indoors?" have delayed work on specific indoor air quality guidelines. However, WHO has not ignored the problem of indoor exposure to air pollutants and has stressed since the publication of the first edition of the guidelines in 1987 *(1)* that they should be applicable to both indoor and outdoor air. This was reinforced in the global update published in 2006 *(3)* and the guidelines were recommended for application in all microenvironments. It should be noted that the workplace has been specifically excluded: WHO air quality guidelines have not been seen as a basis for occupational exposure standards.

Developing indoor air quality guidelines

Acknowledging that indoor air has a special role as a health determinant and that the management of indoor air quality requires approaches different from those used for outdoor air, the working group preparing the global update of the WHO air quality guidelines *(3)* recommended that WHO should also prepare guidelines for indoor air quality. This is in line with the recommendations of an earlier WHO working group formulating a set of statements on "The right to healthy indoor air" and in particular with Principle 6, which states that "Under the principle of accountability, all relevant organizations should establish explicit criteria for evaluating and assessing building air quality and its impact on the health of the population and on the environment." *(4)*.

The WHO working group that subsequently met in Bonn in October 2006 acknowledged the applicability of the existing WHO guidelines for air quality *(2,3)*

to indoor air and identified a number of chemical substances for which specific indoor air guidelines should be recommended *(5)*. The working group also recommended developing guidelines for two additional categories of risk factor of particular importance for health in indoor environments: biological agents and indoor combustion. Following these recommendations, the WHO guidelines on dampness and mould were published in 2009 *(6)*.

The working group defined the following criteria for selecting compounds for which the development of WHO guidelines for indoor air could be recommended:

- existence of indoor sources
- availability of toxicological and epidemiological data
- indoor levels exceeding the levels of health concern (no observed adverse effect level (NOAEL) and/or lowest observed adverse effect level (LOAEL)).

Based on these criteria, pollutants considered were divided into two categories (Table 1). Group 1 included pollutants for which WHO guidelines for indoor air were needed and WHO was requested to plan their development. Group 2 included pollutants of potential interest, but the group concluded that further investigation would be needed before it was clear whether there was sufficient evidence to warrant their inclusion in the guidelines at present.

Table 1. Pollutants considered for inclusion in the WHO indoor air quality guidelines by the WHO working group in October 2006

Group 1. Development of guidelines recommended	Group 2. Current evidence uncertain or not sufficient for guidelines
Benzene	Acetaldehyde
Carbon monoxide	Asbestos
Formaldehyde	Biocides, pesticides
Naphthalene	Flame retardants
Nitrogen dioxide	Glycol ethers
Particulate matter ($PM_{2.5}$ and PM_{10})	Hexane
Polycyclic aromatic hydrocarbons, especially benzo-[*a*]-pyrene	Nitric oxide
Radon	Ozone
Trichloroethylene	Phthalates
Tetrachloroethylene	Styrene
	Toluene
	Xylenes

Source: WHO Regional Office for Europe *(5)*.

The group concluded that the WHO guidelines for environmental tobacco smoke (ETS) published in the second edition of *Air quality guidelines for Europe (2)*, stating that there is no evidence for a safe exposure level, are clear and still valid. Therefore, ETS is not included in the current work. Furthermore, the guidelines for other pollutants should be developed based on the assumption that ETS is eliminated from indoor spaces.

The steering group[1] assisting WHO in designing the indoor air quality guidelines concluded that there is no convincing evidence of a difference in the hazardous nature of particulate matter from indoor sources as compared with those from outdoors and that the indoor levels of PM_{10} and $PM_{2.5}$, in the presence of indoor sources of PM, are usually higher than the outdoor PM levels. Therefore, the air quality guidelines for particulate matter recommended by the 2005 global update *(3)* are also applicable to indoor spaces and a new review of the evidence is not necessary at present. Consequently, the work on developing indoor air quality guidelines for selected pollutants focused on nine out of the ten compounds listed in Group 1 of Table 1, i.e. all except particulate matter. As decided at the working group meeting in 2006, the guidelines are intended to address various levels of economic development, cover all relevant population groups, and allow feasible approaches to reducing health risks from exposure to the selected pollutants in various regions of the world.

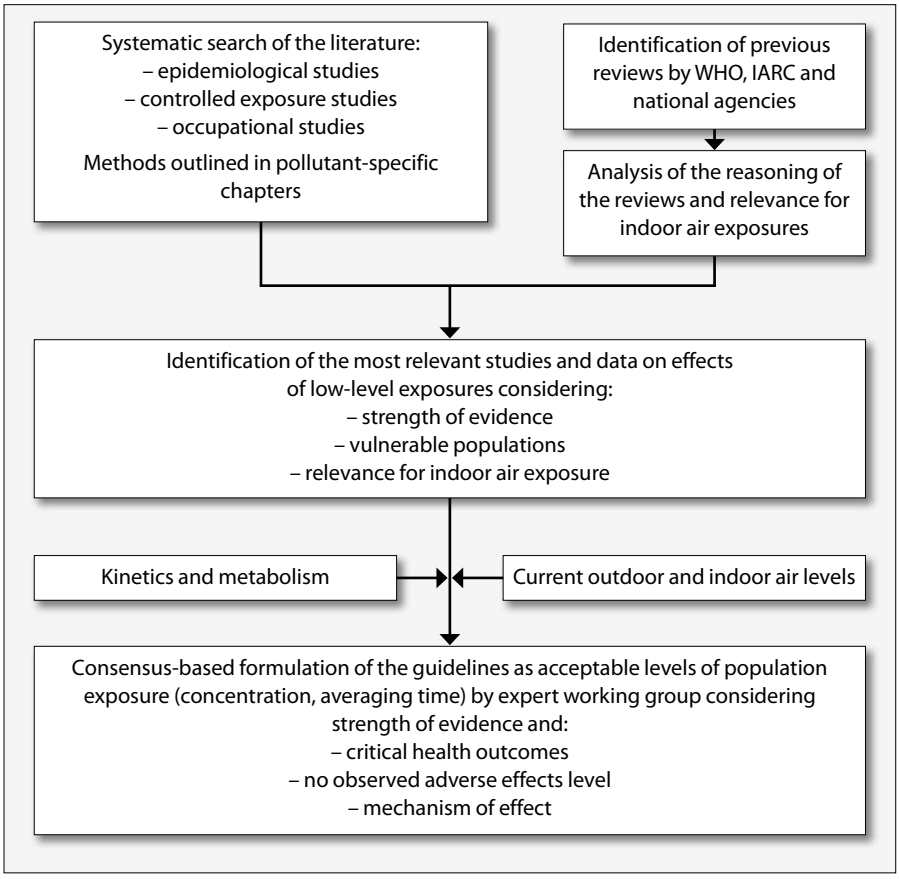
Setting indoor air quality guidelines

The general approach and terminology used in setting air quality guidelines has been presented in a previous WHO publication *(2)*. It is based on a careful review and interpretation of globally accumulated scientific evidence linking exposure to a selected pollutant in the air with the health outcomes of that exposure, using the approaches proposed by the WHO guidelines on assessing human health risks of chemicals *(7)* and on the evaluation of epidemiological evidence for environmental health risk assessment *(8)*. For each of the selected substances, a search of bibliographic databases was conducted to identify relevant studies, according to the search protocols described in each of the pollutant-specific chapters. Major reviews conducted by WHO, the International Agency for Research on Cancer (IARC) or national agencies were also considered an important source of information. The process followed in setting the guidelines is schematically presented in Fig. 1.

In reviewing the available information, a systematic review of the peer-reviewed publications was undertaken. This included specifically studies of the effects of indoor exposure to the compounds considered and also evidence gathered from studies of outdoor exposure. The evidence comes from epidemiological, toxicological and clinical research, examining associations between exposures to the pollutants and health as well as studying physiological mechanisms of the effects. The latter includes experiments based on controlled human exposure or using animals. Much of the available health evidence is indirect, based on exposures to mixtures of pollutants or to single pollutants in concentrations higher than usually encountered indoors. The advantages and disadvantages of

[1] Steering group members: Ross Anderson, Aaron Cohen, Severine Kirchner, Erik Lebret, Lars Mølhave, Aino Nevalainen, Bernd Seifert and Kirk Smith.

Fig. 1. The process followed in guidelines formulation



various types of study used to assess health effects of air pollution are summarized in introductory chapters of the 2005 global update *(3)*.

The review of the evidence focuses on the papers considered to be most relevant for development of the guidelines, and in particular on the studies providing quantitative links between health outcomes and the exposures (as determined by the concentrations of pollutants and the duration of exposure) encountered in indoor environments. The strength of evidence for a link between exposure and health outcome was classified according to the criteria used in the *WHO guidelines for indoor air quality: dampness and mould (6)*, based on the approach developed by the Institute of Medicine *(9)* and presented in Box 1. The evidence was classified according to the professional judgement of the experts of the clarity of the reported findings with consideration of the strength, quality, diversity and number of studies. Understanding of biological mechanisms responsible for associations observed in epidemiological studies, and described in the "kinetics and metabolism" sections of each pollutant-specific chapter, strengthened the conclusions reached.

BOX 1 Classifying the strength of evidence

The categories in this box refer to the association between exposure to an agent and a health outcome and not to the likelihood that any individual's health problem is associated with or caused by the exposure. These categories are used for classifying the evidence in this review, in the *WHO guidelines for indoor air quality: dampness and mould (6)* and in that of the Institute of Medicine *(9)*.

Sufficient evidence of a causal relationship
The evidence is sufficient to conclude that a causal relationship exists between the agent and the outcome. That is, the evidence fulfils the criteria for "sufficient evidence of an association" and, in addition, satisfies the following evaluation criteria: strength of association, biological gradient, consistency of association, biological plausibility and coherence and temporally correct association.

The finding of sufficient evidence of a causal relationship between an exposure and a health outcome does not mean that the exposure inevitably leads to that outcome. Rather, it means that the exposure *can* cause the outcome, at least in some people under some circumstances.

Sufficient evidence of an association
The evidence is sufficient to conclude that there is an association. That is, an association between the agent and the outcome has been observed in studies in which chance, bias and confounding could be ruled out with reasonable confidence. For example, if several small studies that are free from bias and confounding show an association that is consistent in magnitude and direction, there may be sufficient evidence of an association.

Limited or suggestive evidence of an association
The evidence is suggestive of an association between the agent and the outcome but is limited because chance, bias and confounding could not be ruled out with confidence. For example, at least one high-quality study shows a positive association, but the results of other studies are inconsistent.

Inadequate or insufficient evidence to determine whether an association exists
The available studies are of insufficient quality, consistency or statistical power to permit a conclusion regarding the presence or absence of an association. Alternatively, no studies of the association exist.

Limited or suggestive evidence of no association
Several adequate studies are consistent in not showing an association between the agent and the outcome. A conclusion of "no association" is inevitably limited to the conditions, magnitude of exposure and length of observation covered by the studies available.

In estimating the health risks of exposure, it was not possible to apply the techniques of formal meta-analysis to the evidence base; marked differences in study design and, in some cases, the very limited number of studies available made this impossible.

The evaluation of health risks, which follows the presentation of the most important studies, sets out the conclusions of the experts based on the accumulated evidence. It includes risk characterization (i.e. a summary, integration and evaluation of the major scientific evidence) and considers the relevance to health of

indoor exposures encountered in various non-occupational settings as well as the conclusions of other reviews.

Ideally, guideline values should represent concentrations of chemical compounds in air that would not pose any hazard to the human population. Realistic assessment of hazards to human health, however, necessitates a distinction between absolute safety and acceptable risk. To produce a guideline with a high probability of offering absolute safety, one would need a detailed knowledge of dose–response relationships in individuals in relation to all sources of exposure, the types of toxicological effect elicited by specific pollutants or their mixtures, the existence or non-existence of "thresholds" for specified toxicological effects, the significance of interactions, and the variation in sensitivity and exposure levels within the human population. Such comprehensive and conclusive data on indoor contaminants are generally unavailable. Very often, the relevant data are scarce and the quantitative relationships uncertain. The professional judgement of the scientists evaluating the evidence and consensus therefore play an important role in establishing guidance that can be used to indicate acceptable levels of population exposure. Value judgements are needed and the use of subjective terms such as "adverse effects" is unavoidable.

Distinction between adverse and non-adverse effects is difficult. For example, changes in a physiological variable such as an index of lung function that might be regarded as minor and reversible could imply a significant short- or perhaps long-term effect on health. In developing these guidelines, concerns were often expressed about the possible long-term effects of repeated insults that individually produce only small changes in physiological end-points. It was also noted that physiological changes that had previously been seen as indicative of only minor effects (such as a small increase in carboxyhaemoglobin concentration) might not explain all the effects of exposure to a pollutant.

Although it might be accepted that a certain risk can be tolerated, the risks to individuals within a population may not be equally distributed: there may be subpopulations that are at considerably increased risk. Therefore, groups at special risk in the general population must be taken specifically into account in the risk management process. Even if knowledge about groups with specific sensitivity is available, unknown factors may exist that change the risk in an unpredictable manner. During the preparation of these guidelines, attention was paid to defining specific sensitive subgroups in the population.

Preparation of the guidelines

As recommended by the working group that met in 2006 to plan the development of the guidelines, a steering group was established to advise on the scientific issues concerning their development. This group recommended potential authors, who would be invited to review the evidence and develop the first draft of the background material during the summer and autumn of 2008. The steer-

ing group also recommended other experts to act as reviewers of the background material. All invited experts, including the members of the steering group, are internationally recognized scientists conducting research in academic or public health institutions and active in the assessment of health risks related to exposure to chemicals. Their expertise includes exposure assessment, toxicology, epidemiology and risk assessment. All experts were requested to disclose any circumstances that could give rise to a potential conflict of interests, i.e. any interests that might affect or might reasonably be perceived to affect the experts' objectivity and independence. A standard "Declaration of interests for WHO experts" form was used, and any positive responses to a set of questions in the Declaration were evaluated by the WHO Legal Office and the representatives of the WHO Guidelines Review Committee. Only experts with no declared conflicts of interests, or for whom the declared activities were not considered to create such conflicts, participated in the formulation of the guidelines.

The background material on each of the nine pollutants reviewed contained sections on:

- general description of the compound;
- indoor sources and pathways of exposure;
- current indoor levels and relationship with outdoor levels;
- kinetics and metabolism (including experimental evidence on pathogenic mechanisms from animal and in vitro studies); and
- health effects.

The authors submitted the drafts to WHO in November/December 2008. The complete drafts on each of the compounds were distributed to the reviewers with a request that they evaluate the completeness of the scientific evidence used to prepare the manuscript, the scientific reliability of the evidence review and the clarity of the conclusions of the review. The comments received from the reviewers and collated by the WHO secretariat, were used by the authors to prepare the second drafts, including, in addition to the sections listed above, a first draft of the "Health risk evaluation" section. These second drafts were made available to the WHO working group in advance of its meeting in Bonn on 2–6 November 2009.

The working group meeting was convened to agree on the risk evaluation for each of the pollutants and to formulate WHO guidelines for protecting public health from these risks. Existing national and international guidelines, experience in indoor air quality regulation and the results of completed international reviews supported the discussion and its conclusions. The meeting brought together 47 experts from 15 countries, who had reviewed the evidence and prepared the background papers, as well as members of the steering group. It also involved three observers from national agencies potentially interested in using WHO indoor air quality guidelines in shaping policies and actions addressing

health risks of indoor air pollutants, as well as five scientists from WHO headquarters, the WHO Regional Office for Europe and IARC. Robert Maynard and Bernd Seifert chaired the meeting.

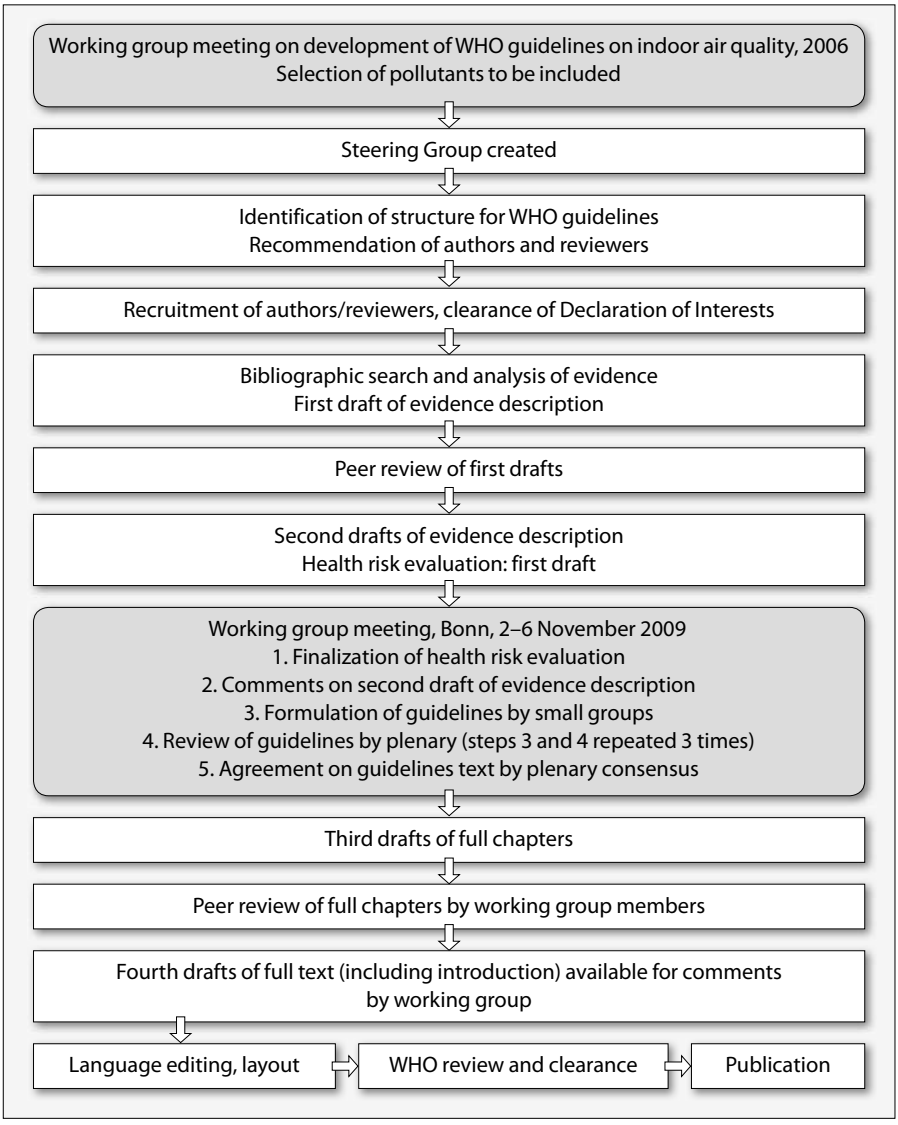
Several consecutive drafts of the guidelines section for each pollutant were prepared by small groups of experts and discussed in plenary. The final text was reviewed and approved by consensus at the plenary session. Besides agreeing on the health risk evaluations of the pollutants and formulating the guidelines sections, the experts provided comments on the final text of the background material. These comments were used by the authors to finalize the background sections summarizing the evidence supporting the guidelines in the two months following the meeting. The complete unedited draft was made available to all the working group members for final comments. Final changes to the background sections (but not in the guidelines), as well as to the boxes summarizing the main decisions leading to setting the guidelines, were made following language editing. The edited text was then reviewed by the WHO Guidelines Review Committee. Fig. 2 illustrates the major steps in the process leading to the publication of the guidelines.

Combined exposures

This volume contains an evaluation of the health effects of specific chemicals. However, exposure to combinations of air pollutants is inevitable. Data dealing with the effects of co-exposure to air pollutants are very limited and, in most cases, it is not possible to recommend guidelines for such combinations. Notable exceptions are guidelines on particulate matter, assessed on the basis of mass concentration of particles of a broad range of physical and chemical properties. Of course, measures taken to control air pollution frequently lead to the reduction in concentrations of more than one pollutant. This is often achieved by controlling sources of pollutants rather than by focusing on individual pollutants. This is especially important in the indoor environment. In developing countries, the use of solid fuel, often including biomass, in poorly ventilated buildings leads to exposure to a mixture of air pollutants. Combinations of pollutants can lead to additive or synergistic effects: the combinations of exposure to tobacco smoke and radon and asbestos fibres provide examples of synergistic effects. These are well-known effects. Less well-known is, for example, the possibility that cataract formation may be linked to exposure to the mixture of pollutants generated by burning biomass indoors. Whether this effect is due to a single pollutant or to co-exposure to a group of pollutants is unknown.

A good example of difficulty in attributing health effects to one of the components of indoor air pollution mixture is provided by particulate matter. The measures to assess or control particle mass concentration are rarely effective in respect to very small particles, often referred to as ultrafine (< 0.1 μm), and most commonly measured as number concentration. Operation of combus-

Fig. 2. Major stages in preparation of the WHO guidelines on indoor air quality: selected pollutants



tion sources always results in the emission of ultrafine particles as well as many other pollutants, and particularly as discussed in this document, benzene, carbon monoxide, nitrogen dioxide and polycyclic aromatic hydrocarbons. Possibly synergistic effects of exposure to these pollutants and ultrafine particles are not known at this point. It is also not known whether some of the pollutants act as surrogates for ultrafine particles (or vice versa).

Some of these problems will be addressed by the guidelines on indoor combustion, to be developed following the recommendations of the WHO work-

ing group from 2006 *(5)*. Nevertheless, it is important to emphasize the need to consider the health risks of all pollutants for which guidelines are available in activities to improve indoor air quality. This, to some extent, is addressing the health hazard of combined exposure. Furthermore, situations where following the guideline for one pollutant adversely affects other aspects of indoor air quality should be avoided.

When strategies to protect public health are under consideration, the air quality guidelines on selected substances need to be placed in the perspective of total chemical exposure. The interaction of humans and the biosphere is complex. Individuals can be exposed briefly or throughout their lifetimes to chemicals in air, water and food; exposures may be environmental or occupational. In addition, individuals vary widely in their response to exposure to chemicals; each person has a pre-existing status (defined by, for example, age, sex, pregnancy, pulmonary disease, cardiovascular disease, genetic make-up) and a lifestyle, in which such factors as exercise and nutrition play key roles. All these different elements may influence a person's susceptibility to chemicals.

Use of the indoor air quality guidelines in protecting public health

The primary aim of these guidelines is to provide a uniform basis for the protection of public health from adverse effects of indoor exposure to air pollution, and to eliminate or reduce to a minimum exposure to those pollutants that are known or are likely to be hazardous. The guidelines are targeted at the public health professionals involved in preventing health risks of environmental exposures, as well as the specialists and authorities involved in the design and use of buildings, indoor materials and products. The guidelines are based on the scientific knowledge available at the time of their development. They have the character of recommendations, and it is not intended or advocated that they be adopted as standards. Nevertheless, countries may wish to transform the recommended guidelines into legally enforceable standards.

In the process of moving from a "guideline" or a "guideline value" to a "standard", a number of factors beyond the exposure–response relationship need to be taken into account. These factors include current concentrations of pollutants and exposure levels of a population, the specific mixture of air pollutants and the specific social, economic and cultural conditions encountered. In addition, the standard-setting procedure may be influenced by the likelihood of implementing the standard. Broader discussion of these considerations has been presented in an earlier edition of the WHO air quality guidelines *(2)*.

Establishing legally binding concentration-based standards of indoor air quality requires, inter alia, determination of the methods for enforcement of the regulation, including compliance testing. This poses difficulties for the use of standards as a means of reducing the effects on health of indoor exposure to air pollutants. Routine monitoring in people's homes is unlikely to be widespread,

and the application of measures to enforce standards is often not feasible or, at least, difficult.

The concentrations of pollutants in both indoor and outdoor air can be reduced by controlling the primary factor that determines their presence in the air: their sources. In the outdoor environment, this is all that can be done because the secondary factors that control concentrations – dispersion and dilution – are not generally under control as they depend largely on meteorological conditions. In the indoor environment, dispersion (to and from the outdoor environment) can be influenced by controlling the ventilation of the indoor space. This provides an additional means of changing indoor concentrations of pollutants. It also creates a difficulty: a source that might be entirely acceptable as regards output of pollutants in a well-ventilated space might be unacceptable in a poorly ventilated space. Of course, significant indoor sources should not be allowed to release pollutants into the indoor space: conducting pollutants to the outside space by flues and chimneys is clearly desirable. It should be noted, however, that indoor sources can make a substantial contribution to outdoor concentrations, especially in places where there are large, widespread sources of indoor pollution. This should be avoided by control of emission from indoor sources.

Thus, acceptable indoor air quality can be achieved through source control and pollutant dispersion, and in particular through:

- application of low-emission materials and products;
- proper selection of the devices and fuels used for combustion indoors;
- the venting of products to the outdoor air; and
- ventilation control.

In many countries, these means of control are encapsulated in product standards and building standards or regulations. In practice, both sets of standards are derived from calculations or experiments and are implemented without routine monitoring of pollutant levels indoors. Surveys of indoor concentrations of pollutants act as a means of checking that the standards are appropriate or, more often, that they are being appropriately applied. Here there is another difference between the approaches taken indoors and outdoors: monitoring outdoor concentrations of air pollutants is standard practice in many countries but routine monitoring of indoor concentrations hardly exists.

The development of product and building standards requires targets for acceptable indoor concentrations of air pollutants: here guidelines can play an important role. This is not very different from the approach adopted outdoors. Even in periods of high outdoor concentrations of air pollution, reduction of emissions is seldom more than an ad hoc solution, although reducing vehicle usage or industrial emissions at such times has been tried in a number of cities. Much more important than this are calculations based on inventories of sources and atmospheric dispersion modelling that form the basis of "product standards" as

applied, for example, to motor vehicle engines and to the fuels they burn, i.e. to the permanent characteristics of the sources. Such calculations are easier when applied to outdoor air; the wide range of types of dwelling and their levels of ventilation makes calculations more difficult for the indoor environment. Nevertheless, adoption of the guidelines presented in this volume as benchmarks for such models and actions is a useful option for reducing the adverse effects of indoor air pollution on health.

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1. Benzene

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General description

Benzene (CAS Registry Number 71-43-2; C_6H_6; molecular weight 78.1 g/mol) is an aromatic compound with a single six-member unsaturated carbon ring. It is a clear, colourless, volatile, highly flammable liquid with a characteristic odour and a density of 874 kg/m^3 at 25 °C *(1)*.

At 1 atmosphere of pressure, benzene has a melting point of 5.5 °C, a relatively low boiling point of 80.1 °C and a high vapour pressure (12.7 kPa at 25 °C), causing it to evaporate rapidly at room temperature. It is slightly soluble in water (1.78 g/l at 25 °C) and is miscible with most organic solvents *(2)*. Benzene is soluble in lipids, has a log K octanol–water partition coefficient of 2.14 *(1)* and a log K soil organic carbon–water partition coefficient of 1.85 at 25 °C. Its Henry's Law constant is 550 Pa.m^3/mol at 25 °C, implying that it will have a tendency to volatilize into the atmosphere from surface water *(3)*.

Benzene in air exists predominantly in the vapour phase, with residence times varying between one day and two weeks, depending on the environment, the climate and the concentration of other pollutants. Reaction with hydroxyl radicals is the most important means of degradation, with a rate constant of 1.2×10^{-12} cm^3.molecule^{-1}.s^{-1} at 298 K *(4)*.

Other oxidants such as ozone and nitrate radicals can also contribute to a lesser extent to the degradation of benzene indoors, with rate constants of 2.7×10^{-17} cm^3.molecule^{-1}.s^{-1} at 298 K for nitrate radicals *(5)* and 1.7×10^{-22} cm^3.molecule^{-1}.s^{-1} at 298 K for ozone *(1–3,6,7)*.

Conversion factors

At 760 mmHg and 20 °C, 1 ppm = 3.248 mg/m^3 and 1 mg/m^3 = 0.308 ppm; at 25 °C, 1 ppm = 3.194 mg/m^3 and 1 mg/m^3 = 0.313 ppm.

Indoor sources

Benzene in indoor air can originate from outdoor air and also from sources indoors such as building materials and furniture, attached garages, heating and cooking systems, stored solvents and various human activities. Indoor concentrations are also affected by climatic conditions and the air exchange rate due to forced or natural ventilation.

Indoor concentrations are affected by outdoor levels owing to the exchange of indoor and outdoor air. Outdoor benzene concentrations are mainly due to traffic sources and are affected by season and meteorology. Other outdoor sources of benzene are petrol stations and certain industries such as those concerned with coal, oil, natural gas, chemicals and steel *(8)*.

Materials used in construction, remodelling and decorating are major contributors to indoor benzene concentrations *(9)*. Certain furnishing materials and polymeric materials such as vinyl, PVC and rubber floorings, as well as nylon carpets and SBR-latex-backed carpets, may contain trace levels of benzene. Benzene is also present in particleboard furniture, plywood, fibreglass, flooring adhesives, paints, wood panelling, caulking and paint remover *(3,10,11)*. Therefore, new buildings or recently redecorated indoor environments have been associated with high concentrations of benzene from materials and furniture. The rate of emission of benzene from materials and furniture will decay and eventually these sources will reach a quasi-steady emission rate in new buildings within weeks or months or up to a year *(12)*.

Attached garages are a potential source of gasoline vapour owing to evaporation and exhaust emissions. In addition to cars, petrol, oil, paint, lacquer and hobby supplies often stored in garages can lead to increased levels of benzene indoors *(13)*. Some 40–60% of benzene indoors may be attributable to the presence of an attached garage *(13–16)*, with indoor benzene concentrations rising to 8 $\mu g/m^3$ when garages are connected to the main living environment *(14)*.

The use of fuels such as coal, wood, gas, kerosene or liquid petroleum gas (LPG) for space heating and cooking leads to higher concentrations of benzene indoors *(17–20)*.

The problem of indoor pollution from the use of domestic cooking stoves attains greater importance in developing countries owing to poor ventilation and the extensive use of low-efficiency stoves and biofuels. Benzene concentrations of 44–167 $\mu g/m^3$ have been found to be associated with the use of kerosene stoves *(21)*.

In the past, benzene was widely used as a solvent, mainly in industrial paints, paint removers, adhesives, degreasing agents, denatured alcohol, rubber cements and arts and crafts supplies. The imposition of lower occupational exposure limits led to a reduction in these uses *(3)* but benzene content may still be an issue in some parts of the world, such as some African countries.

Indoor benzene is also associated with human activities such as cleaning *(18)*, painting *(18,22,23)*, the use of consumer products *(24)* and mosquito repellents *(25)*, photocopying *(26)* and printing *(27)*, the storage and use of solvents, and smoking tobacco.

Environmental tobacco smoke (ETS) is considered one of the main indoor sources of benzene. Benzene emissions from cigarette smoking range from 430 to 590 μg per cigarette *(28)*. An increase in benzene concentration of at least 30–

70% is expected *(3,18,20,29,30)* when ETS is present indoors, with increases in some cases of 300% *(31)* to levels of 16 μg/m^3 *(18)*.

To sum up, outdoor benzene provides the baseline for benzene concentrations indoors, upon which will be superimposed benzene given off from building materials and indoor artefacts. The presence of attached garages and combustion sources (especially smoking) and other human activities will be the main determinant of the concentration of benzene indoors.

Pathways of exposure

Inhalation accounts for more than 95–99% of the benzene exposure of the general population, whereas intake from food and water consumption is minimal *(3,32)*. In the United States, daily benzene intake from ambient and indoor air has been calculated to range between 180 and 1300 μg/day, and that in food and water up to about 1.4 μg/day *(2)*. The average daily intake for an adult in Canada was estimated to be 14 μg/day from ambient air, 140 μg/day from indoor air, 1.4 μg/day from food and drinking-water and 49 μg/day from car-related activities, giving a total of about 200 μg/day *(33)*. Wallace *(30)* estimated the corresponding average intake in the United States to be 320 μg/day.

Cigarette smoking has been found to contribute significantly to the amount of benzene inhaled *(34)*. Exposure to ETS is widespread in most countries *(35)*. A survey conducted in the United States in 2006 found that more than 40% of non-smoking adults and almost 60% of children aged 3–11 years were exposed to ETS *(36)*. Another survey, conducted among young people in 132 countries, found that 44% had been exposed to ETS at home and 56% in public places, while another survey found that the exposure of young people at home ranged between 30–87% and 53–98% in public places *(37)*. Active smoking may add as much as 400–1800 μg/day *(2,34)*, while inhalation due to passive smoking will represent an additional 14–50 μg/day to the average daily intake *(2,38)*. Driving a car during the rush hour may give a significant additional intake of 20 μg/day *(34,39)*. Fromme *(40)* calculated the relative intake from food and uptakes from ambient air, indoor air and air inside cars to be 8%, 9%, 53% and 30%, respectively. In a study carried out in Germany in the 1990s, it was found that indoor exposure to ETS and car-related activities (refuelling and time in transit) could account for 20% and 12%, respectively, of personal exposure to benzene *(2)*.

A study carried out in the United Kingdom estimated a daily dose of benzene of 70–75 μg/day for rural non-smokers and 89–95 μg/day for urban non-smokers. The daily dose rose to 116–122 μg/day for urban passive smokers and to over 500 μg/day for urban smokers. Children's daily exposures were estimated to be 15–20 μg/day and 30–40 μg/day for infants and children, respectively, while exposure to ETS led to a daily exposure of 26 μg/day and 59 μg/day for a urban infants and children, respectively *(34)*. Most of the children's exposures were produced in the home *(41)*.

A European study estimated a daily inhaled benzene dose of 102 μg/day, where 36%, 32%, 2% and 30% of the exposure was attributed to indoor home, indoor work, outdoor and in transit, respectively *(42)*. In some Asian cities, where high levels of benzene were reported in homes and offices *(25,43)*, the daily inhalation dose of benzene from indoor sources can be as high as 480–580 μg/day.

Indoor concentrations

Mean indoor concentrations are typically higher than the respective ambient levels and have been consistently shown to be higher in the colder than the warmer seasons *(16,44,45)*. Indoor levels measured in the United States are in the range 2.6–5.8 μg/m^3 *(13,14,46,47)*, which are levels similar to those measured in established buildings in Australia *(22)* and Europe *(48)*.

In European cities, a trend has been observed of increasing indoor concentrations from north to south. Low indoor concentrations (2 μg/m^3) were measured in Finnish homes *(49)*, while they ranged from 2 to12 μg/m^3 in central European cities *(17,44,50–54)* and from 10 to 13 μg/m^3 in southern cities such as Milan and Athens *(48)*. Indoor levels measured in Turkey were in the range 7–14 μg/m^3 *(55)*.

Studies carried out in Asian cities have found much higher indoor benzene concentrations than those reported from cities in the developed world. Houses in India that used kerosene stoves were reported as having average indoor levels of 103 μg/m^3 *(21)*. Higher concentrations have been reported from some Chinese cities, with levels as high as 57.4 μg/m^3 in Guanzhou *(56)*. On the other hand, indoor levels of benzene in Japan are similar to those found in Australia, Europe and the United States, with arithmetic mean values ranging from 0.7 to 7.2 μg/m^3 *(45,57–59)*.

Indoor concentrations in buildings in Singapore were 18.4–35.4 μg/m^3 *(43)*, and similar levels of 23–35 μg/m^3 were found in the Republic of Korea *(25)*. However, a previous study in the Republic of Korea at the end of the1990s found lower concentrations (8.2 μg/m^3 in homes and 12.6 μg/m^3 in offices) than those reported in 2003 by Son et al. *(60)*. Another study performed in India reported indoor concentrations of 10.7 μg/m^3 *(23)*. The lowest concentrations were reported from the Hong Kong Special Administrative Region of China (Hong Kong SAR), with values of 0.5–4.4 μg/m^3 in different indoor environments such as houses, offices and shopping centres *(61,62)*.

Cigarette smoke is an important source of benzene in indoor air, and benzene concentrations measured indoors increase when ETS is present *(2)*. Indoor benzene levels measured in the United States showed arithmetic values of 5.54–10.5 μg/m^3 in homes exposed to ETS compared to 3.86–7.0 μg/m^3 in ETS-free homes *(20,63)*. A similar situation was reported in Italy, with levels of 32.2 and 18.9 μg/m^3 in ETS and ETS-free homes, respectively *(64)* and in Germany, with levels of 11.0 and 6.5 μg/m^3, respectively *(40)*.

Indoor concentrations measured in offices are generally higher than those measured in residential buildings, owing to the presence of sources such as photocopiers and printers. The mean office level in eight European countries was 14.6 μg/m^3, while 87.1 μg/m^3 was measured inside an office in Singapore *(43)*. A recent study in United Kingdom offices reported lower benzene levels in the range of 0.4–4.0 μg/m^3 (1.3 μg/m^3 arithmetic mean) *(53)*.

Benzene levels measured in restaurants ranged from 1.1 to 22.7 μg/m^3, while higher levels of 5.1–78.8 μg/m^3 were reported in pubs *(18,53,60,61,65,66)*, with discotheques/clubs being the locations with the highest mean concentrations (193 μg/m^3) in a study carried out in Germany *(66)*. Benzene concentrations measured in several public indoor spaces such as shopping centres, libraries and cinemas ranged from 0.7 to 15.5 μg/m^3 *(18,53,62)*.

Benzene concentrations measured in vehicles are generally higher than those outdoors. Levels of benzene measured in vehicles in Europe ranged from 13 to 42 μg/m^3 (65,67), while lower levels of 1.3–3.8 μg/m^3 were measured in a recent United Kingdom study *(53)*. Benzene levels measured in Mexico and the United States ranged from 1.7 to 42 μg/m^3 *(68,69)* and a similar range (0.5–47 μg/m^3) was found in several Asian cities *(61,70)*. The highest in-vehicle benzene levels were measured in Italy in the early 2000s, with geometric means ranging from 17 to 101 μg/m^3 *(64)*.

Relatively high benzene concentrations indoors have been attributed to sources such as incense burning, with benzene concentrations peaking at up to 117 μg/m^3 *(48)*; new buildings (e.g. up to 30 μg/m^3) *(22)*; attached garages (e.g. 16–19 μg/m^3); tobacco smoke (e.g. 16–193 μg/m^3) *(18,23,66)*; cleaning (e.g. 13 μg/m^3) *(18)*; painting (e.g. 9–13 000 μg/m^3) *(18,23)* and using a kerosene stove (e.g. 166 μg/m^3) *(21)*.

Indoor–outdoor relationship

Indoor concentrations of benzene are normally higher than those in outdoor air *(9)* as a consequence of the entry and accumulation of benzene from outdoor sources and the presence of dominant benzene sources indoors. Viewed across published studies, indoor concentrations of benzene ranged from 0.6 to 3.4 (arithmetic mean 1.8) times the outdoor concentrations and are greatly influenced by those outdoors. This occurs in part because there are numerous indoor sources of benzene and because the relatively low rates of ventilation typically used in residences and offices prevent the rapid dispersal of airborne contaminants *(9)*.

Indoor–outdoor ratios close to unity (i.e. 0.96–1.10) have been reported in some Asian countries where outdoor air concentrations were particularly high (25–35 μg/m^3) *(25,55,60,71)*. High indoor–outdoor ratios have been traditionally associated with strong indoor sources such as attached garages (ratio > 3) *(13,14)*, combustion sources such as kerosene stoves (ratio 3.3) *(21)*, gas and charcoal cooking (ratio 2) *(60)* or ETS (ratio 1.6–2) *(23,48,60)*.

Kinetics and metabolism

The toxicity of benzene is dependent on its metabolism, as shown by its lower toxicity (*a*) in the presence of toluene, an inhibitor of benzene metabolism; (*b*) in animals that have had a partial hepatectomy; and (*c*) in mice that lack the enzyme CYP2E1 *(72)*. Many studies have been completed in animals and to some extent in humans to determine the metabolism of benzene and its toxicokinetics.

Toxicokinetics

Absorption

Following inhalation exposure, the fraction absorbed is concentration-dependent, with a higher fraction absorbed at lower concentrations. In rats exposed for six hours to 11 or 130 ppm benzene, approximately 95% of the inhaled benzene was absorbed, while only 52% was absorbed after exposure to 930 ppm benzene *(73)*.

Two studies in humans indicate that 50% of the quantity of inhaled benzene is absorbed *(74,75)*. Cigarette smoke is a source of benzene exposure; the benzene concentration in the blood of 14 smokers was significantly higher (median 493 ng/l) than that in 13 non-smokers (median 190 ng/l) *(76)*. Absorption of benzene is also rapid via the oral and dermal routes. Rats absorb and rapidly metabolize oral doses of benzene up to approximately 50 mg/kg. However, after an oral dose of 150 mg/kg, about 50% of the dose is exhaled as non-metabolized benzene *(73)*.

Distribution

After entry into the human organism, benzene is distributed throughout the body and, owing to its lipophilic nature, accumulates preferentially in fat-rich tissues, especially fat and bone marrow. In humans, benzene crosses the blood–brain barrier and the placenta and can be found in the brain and umbilical cord blood in quantities greater than or equal to those present in maternal blood *(77,78)*.

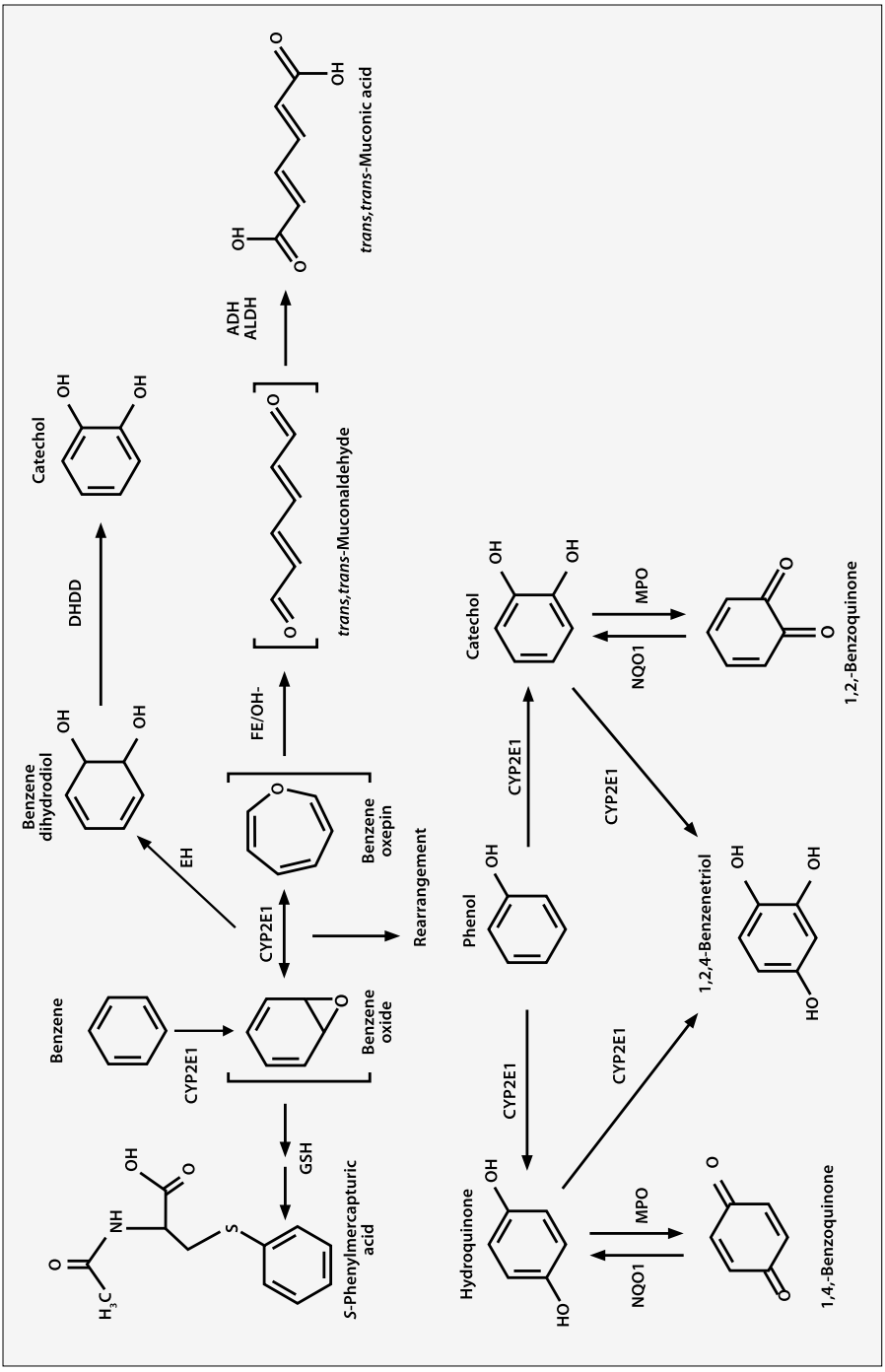
Elimination

Following all routes of exposure in rats and mice, absorbed benzene is rapidly metabolized (mostly within 48 hours), mainly by the liver, and approximately 90% of the metabolites are excreted in the urine *(72)*. Elimination of non-metabolized benzene is by exhalation.

Metabolism

Qualitatively, the metabolism and elimination of benzene appear to be similar in humans and laboratory animals *(79)*. Benzene is metabolized mainly in the liver but also in other tissues, such as the bone marrow. A diagram of benzene metabolism is presented in Fig. 1.1 *(80)*.

Fig. 1.1. Metabolism of benzene



Source: Agency for Toxic Substances and Disease Registry *(80)*.

Note: ADH: alcohol dehydrogenase; ALDH: aldehyde dehydrogenase; CYP 2E1: cytochrome P450 2E1; DHDD: dihydrodiol dehydrogenase; EH: epoxide hydrolase; GSH: glutathione; MPO: myeloperoxidase; NQO1: NAD(P)H:quinone oxidoreductase.

The first step consists in oxidation to benzene oxide and benzene oxepin (formation in equilibrium). This step is mainly catalysed by the enzyme CYP2E1 *(81)*. There are three major pathways by which benzene oxide is further metabolized. It can go through a series of ring-breakage reactions to form t,t-muconaldehyde, which is further oxidized to the acid; it can go through a series of reactions to form a conjugate with glutathione, which is eventually excreted in the urine as phenyl mercapturic acid; or it can rearrange non-enzymatically to form phenol *(82)*.

Phenol can be excreted in the urine directly or it can be further oxidized by CYP2E1 to catechol or hydroquinone. Catechol can be oxidized to trihydroxybenzene, and hydroquinone can be oxidized to the highly reactive bipolar benzoquinone. All of the phenolic compounds can form conjugates (glucuronides or sulfates) and be excreted in the urine *(72,83–86)*. The enzyme myeloperoxidase (MPO), which is present in bone marrow, can also oxidize phenolic compounds into quinones *(79,87–89)*.

The metabolites responsible for benzene toxicity are not yet fully understood. The key toxic metabolites for cytotoxicity and the induction of leukaemia are thought to be benzoquinone, benzene oxide and muconaldehyde *(1,90–95)*. The genotoxic activity of benzene metabolites is thought to be clastogenic (causing chromosomal damage) rather than acting through point mutations (see section on mechanism of action below). Benzoquinone and muconaldehyde are both reactive, bipolar compounds known to be clastogenic and the pathways leading to their formation are favoured at low concentrations in both mice and humans *(72,96,97)*.

Two enzymes are active in the detoxification of benzene metabolites *(98)*. One is NAD(P)H:quinone oxidoreductase (NQO1), which reduces the quinone metabolites to the less toxic diols *(87,99)*; the other is the microsomal epoxide hydrolase, which hydrolyzes the epoxide group on benzene oxide.

There are species differences in the metabolism of benzene. Rats convert most of the benzene to phenol, a marker of a detoxification pathway, while mice form greater amounts of hydroquinone, hydroquinone glucuronide and muconic acid, all markers of toxification pathways. Human metabolism resembles that of mice, the species more sensitive to benzene toxicity *(79,100–102)*.

Biomarkers of exposure

In the past, urinary phenol was commonly used as a biological exposure index in industrial settings to evaluate the exposure of workers to benzene. However, phenol is a good marker only of high-level benzene exposure and, with increased regulation of exposures, urinary phenol is no longer sensitive enough to be useful. More sensitive than phenol are urinary S-phenyl mercapturic acid and t,t-muconic acid, but the most sensitive exposure biomarker studied so far is the parent compound, benzene, in the urine *(103,104)*.

Polymorphisms

Polymorphisms in genes involved in benzene metabolism are thought to influence individual susceptibility to various levels of benzene exposure. Lin et al. *(105)* concluded that, among the GST genotypes investigated, only the GSTT1 genotype was related to the level and dose-related production of S-phenyl mercapturic acid.

NQO1 also exists in polymorphic form. The wild NQO1*1 allele encodes the normal enzyme NQO1, whereas the NQO1*2 allele encodes a mutated NQO2 enzyme presenting negligible activity. Approximately 5% of the Caucasian and Afro-American population, 15% of the American–Mexican population and 20% of the Asian population are homozygotes for the NQO1*2 allele *(106,107)*. Rothman et al. *(108)* demonstrated that workers in which the enzymatic activity of NQO1 was negligible presented a higher risk of benzene poisoning. The same is true for those expressing a rapid cytochrome CYP2E1 activity. Workers who simultaneously had a negligible NQO1 activity and a rapid CYP2E1 had a sevenfold higher risk of benzene poisoning than workers not presenting this dual polymorphism. Deletion of the glutathione S-transferase T1 (GSTT1) gene also showed a consistent quantitative 35–40% rise in DNA single strand break (DNA-SSB) levels.

Mechanism of action

In addressing the mechanism of action of benzene toxicity, one must consider two types of toxicity. At high exposure levels, benzene acts as a narcotic that depresses the central nervous system and causes cardiac sensitization *(109)*. The study of the mechanism for induction of leukaemia and other haematotoxic effects from low-level chronic exposures to benzene has been hampered by the lack of a good animal model for the induction of acute myeloid leukaemia, the major toxic end-point observed in humans. As mentioned above, benzene acts mainly as a clastogenic agent, rather than causing point mutations. The benzoquinones and t,t-muconaldehyde have dual reactive sites that make them capable of clastogenic activity towards DNA. The phenolic metabolites formed in the liver can be transported in the blood to the bone marrow, a major site for toxic effects, and be oxidized to the highly reactive quinones by myeloperoxidases in the marrow. The reactive quinones can cause clastogenic damage to the DNA, such as mitotic recombinations, chromosome translocations and aneuploidies *(110,111)*.

The observed effects of benzene may also be due to the metabolite, benzene oxide. Benzene oxide adducts have been found in the blood (haemoglobin) and bone marrow of mice exposed to benzene *(112)*. Benzene oxide and its adducts have been detected in the blood of workers exposed to benzene *(113–117)*. The studies by Liu et al. *(118)* and Nilsson et al. *(119)* suggested that the metabolites of benzene activate oxygenated radical species, which can lead to DNA changes and the formation of hydroxylated bases such as 8-hydroxy-2-deoxyguanosine.

The toxicity of benzene may also be due to combinations of metabolites *(83–86)*. All the non-conjugated metabolites of benzene, with the exception of phenol and 1,2,4-benzenetriol, are known to induce a reduction in erythropoiesis *(120)*. In mice, a mixture of phenol and hydroquinone induces an increase in loss of cellularity of the bone marrow and an increase in DNA modification *(85,121)*. Phenol–hydroquinone or phenol–catechol mixtures are more toxic for the haematopoietic system than the metabolites alone *(122)*. Catechol stimulates the activation of hydroquinone via peroxidase and triggers a genotoxic effect on lymphocytes, which is amplified in comparison with hydroquinone alone.

Health effects

Identification of studies

The acute non-carcinogenic effects of exposure to high concentrations of benzene and the carcinogenic effects of long-term exposure to lower concentrations are well-established research fields. Therefore, the sections on health effects and toxicokinetics are based on a consultation of summary reports published by various organizations up to December 2006: IARC (*123)*, the Agency for Toxic Substances and Disease Registry (ATSDR) *(80)*, the US Environmental Protection Agency (USEPA) (*124)*, the European Commission (*48)*, INERIS *(125)*, WHO (*2)* and the summary document produced by IARC in 2009 (*126)*.

The sections on mechanisms of action of benzene were supplemented by expert knowledge and by a search in the database PubMed with the following keywords: benzene and health effects, metabolism, kinetics, cancer, leukaemia, genetic polymorphism. This search revealed 37 published papers related to mechanisms of carcinogenicity of benzene up until 2008.

Non-carcinogenic effects

Acute non-carcinogenic effects

There are many reports of human deaths from inhaling high concentrations of benzene *(127,128)*. Death occurred suddenly or a few hours after exposure. The benzene concentrations to which the victims were exposed were often not known. However, it has been estimated that exposure to 20 000 ppm (64 980 mg/m^3) for 5–10 minutes is generally fatal and associated with cerebrovascular ischaemia *(129)*. Death is often attributed to asphyxia, respiratory arrest or central nervous system depression. When autopsies could be performed, cyanosis, haemolysis and ischaemia or haemorrhage of the organs were observed *(127,130,131)*.

In mild forms of poisoning, excitation is reported followed by speech problems, headaches, dizziness, insomnia, nausea, paraesthesia in the hands and feet and fatigue. These symptoms are generally observed for benzene concentrations ranging between 300 and 3000 ppm (975–9750 mg/m^3) *(128,129,132)*. More exactly, inhalation of 50–100 ppm (162–325 mg/m^3) for 30 minutes leads to fatigue

and headaches, while 250–500 ppm (812–1625 mg/m^3) causes dizziness, headaches, faintness and nausea.

INRS (the French National Research and Safety Institute) *(133)* gives the following thresholds for neurological symptoms triggered by acute exposure to benzene: no effect at 25 ppm (81 mg/m^3), headaches and asthenia from 50 to 100 ppm (162–325 mg/m^3), more accentuated symptoms at 500 ppm (1625 mg/m^3), tolerance for only 30–60 minutes at 3000 ppm (9720 mg/m^3) and death in 5–15 minutes at 20 000 ppm (64 980 mg/m^3).

Subchronic and chronic effects

Haematological effects. It is well known from numerous epidemiological studies conducted among workers that subchronic or chronic exposure to benzene leads to adverse haematological effects. Most of these blood effects (aplastic anaemia, pancytopenia, thrombocytopenia, granulopenia, lymphopenia and leukaemia) have been associated with inhalation exposure.

Bone marrow alteration is one of the first signs of chronic benzene toxicity. Aplastic anaemia is one of the most severe effects; the stem cells never reach maturity. Aplastic anaemia can progress to a myelodysplastic syndrome, and then to leukaemia *(134)*. Cytokine changes and chromosomal abnormalities are proposed explanations of the progression of aplastic anaemia to myeloproliferative syndrome and development of leukaemia (see the section on carcinogenic effects below).

Numerous studies conducted by Aksoy have described haematotoxicity. In a population of 217 male workers exposed for between 4 months and 17 years to a concentration of 15–30 ppm (48.8–97.5 mg/m^3), 51 developed leukopenia, thrombocytopenia, eosinophilia and pancytopenia *(135)*. In an additional cohort including 32 people working in the shoe industry, who used benzene for between 4 months and 15 years and were exposed to concentrations of 15–30 ppm (49–98 mg/m^3) outside working hours or 210–640 ppm (683–2080 mg/m^3) during their work, the workers developed pancytopenia with bone marrow changes *(136)*. In another study, conducted 2–17 years following the last exposure to benzene, 44 patients presented with pancytopenia following exposure to concentrations of 150–650 ppm (487.5–2112.5 mg/m^3) for between 4 months and 15 years *(137)*.

The study by Li et al. *(138)*, conducted over the period 1972–1987, examined 74 828 workers exposed to benzene in 672 factories and 35 805 workers not exposed to benzene in 109 factories, the factories studied being located in 12 Chinese cities. A slight increase in the relative risk of developing a lymphoproliferative disorder in both sexes was observed among workers from the chemicals, rubber and paint industries. Rothman et al. *(139,140)* compared 44 men and women exposed to 31 ppm (101 mg/m^3) as median 8-hour time-weighted average with 44 paired control subjects. The numbers of white blood cells, lym-

phocytes, platelets and red blood cells and the haematocrit were lower in exposed subjects. In a subgroup of 11 workers with a mean exposure value of 7.6 ppm (25 mg/m^3) with no exposure over 31 ppm (101 mg/m^3), only the absolute number of lymphocytes was significantly reduced. However, after having conducted a retrospective, longitudinal study on a cohort of 459 rubber workers, Kipen et al. *(141)* observed a negative correlation between benzene concentration and the number of white blood cells. These data were re-analysed by Cody et al. *(142)*, who reported a significant reduction in the number of white and red blood cells in a group of 161 workers compared with data before exposure for the period 1946–1949.

Results reported for exposures below 1 ppm (3.25 mg/m^3) showed a significant reduction in the number of red blood cells, leukocytes and neutrophils. For example, Qu et al. *(143,144)* observed such decreases in 130 workers chronically exposed to benzene at 0.08–54.5 ppm (0.26–177 mg/m^3) compared to a control group of 51 non-exposed workers. Even those in the lowest exposure group (0.82 mg/m^3 and lower) showed reductions in circulating red and white blood cells. Lan et al. *(145)* studied 250 Chinese workers exposed to benzene for a mean duration of 6.1 years (± 2.9 years) and 140 Chinese workers not exposed to benzene. Three groups of workers were studied on the basis of their exposure level: < 1 ppm, from 1 to < 10 ppm and ≥ 10 ppm (< 3.25 mg/m^3, from 3.25 to < 32.5 mg/m^3 and ≥ 32.5 mg/m^3). The control population worked in a factory where benzene concentrations were below the limit of detection (0.04 ppm or 0.13 mg/m^3). For a mean exposure to benzene of one month, a decrease in the number of blood cells of 8–15% was observed for the lowest exposure concentration (< 1 ppm); for the highest concentration (≥ 10 ppm), this decrease was 15–36%. The haemoglobin concentration also decreased, but only for the group exposed to the highest benzene concentration (≥ 10 ppm). A small decrease was observed in a group of workers exposed to benzene concentrations of less than 1 ppm for the previous year.

In contrast, studies on United States petrochemical workers found no association between exposures to low levels of benzene and the development of haematotoxicity *(143–149)*. The studies were based on a review of 200 workers exposed to benzene concentrations of 0.01–1.4 ppm (0.03–4.55 mg/m^3) and 1200 employees working in the petrochemical industry for whom the mean 8-hour time-weighted average of benzene exposure was 0.6 ppm (1.95 mg/m^3) between 1977 and 1988 and 0.14 ppm (0.45 mg/m^3) between 1988 and 2002.

Thus, the haematological effects reported for benzene exposure concentrations of less than 1 ppm (3.25 mg/m^3) are controversial. In a recent review of benzene toxicity *(1)*, it was suggested that the differences in results between the studies in Chinese and United States workers might be due to differences in patterns of exposure or, alternatively, to the fact that the Chinese studies were purposely designed to test the effects of low-level benzene exposure and were thus superior

in their exposure assessment and timing of biological sampling in relation to exposure.

Immunological effects. Exposure to benzene affects the humoral and cellular immune system. These effects were reported for occupational exposures.

Cellular immunity is affected by changes in circulating lymphocytes, leading to a global leukopenia *(135,136,141,142,150–155)*. The benzene levels in workplace air ranged from 1 to 1060 ppm. In one study, routine leukocyte counts conducted every three months on employees of a small-scale industry in China revealed leukopenia in workers exposed to as little as 0.69–140 ppm (mean 6 ppm) for an average of 5–6 years *(156)*.

Another indicator of the alteration of cellular immunity is the change in leukocyte alkaline phosphatase activity. Increased activity is an indicator of myelofibrosis and is associated with both decreased white blood cell counts and with changes in bone marrow activity. Songnian et al. *(157)* showed an increase in the activity of this enzyme in benzene workers chronically exposed to about 31 ppm. This type of effect is confirmed by animal studies *(89,158–161)*.

Carcinogenic effects

Genotoxicity

The genotoxic effect of benzene has been shown to be mainly clastogenic rather than the induction of point mutations. Numerous studies have demonstrated that benzene and its primary metabolites cause chromosomal aberrations (hypodiploidy and hyperdiploidy, deletion and breaking) in humans after chronic exposure *(162–177)*. These chromosomal aberrations were observed in workers exposed to benzene concentrations high enough to induce dyscrasia. They are frequently localized in the peripheral blood lymphocytes and bone marrow. The main limitations of these studies lie in the lack of precise data concerning measurement of exposure, possible co-exposure to other substances and the absence of a suitable control group. Analysis of peripheral lymphocytes in workers exposed to benzene vapour (mean 30 ppm) revealed a significant increase in monosomy for chromosomes 5, 7 and 8, as well as an increase in trisomy or tetrasomy for chromosomes 1, 5, 7 and 8 *(176,177)*.

A significant increase in hyperploidy for chromosomes 8 and 21, along with an increase in translocations between chromosomes 8 and 21, have been observed in workers exposed to benzene at a mean concentration of 31 ppm (100.75 mg/m^3) *(173)*. Kim et al. *(178)* showed around a twofold increase in micronuclei and chromosomal aberrations. In contrast, studies showed a decreased level of t(14;18) chromosome translocation in workers *(179)*. Lichtman *(180)* did not find any chromosomal band damage.

The studies by Liu et al. *(118)* and Nilsson et al. *(119)* suggested that the metabolites of benzene activate oxygenated radical species, which can lead to DNA

changes and the formation of hydroxylated bases such as 8-hydroxy-2-deoxyguanosine. Navasumrit et al. *(181)* showed a significant twofold increase of leukocyte 8-hydroxy-2-deoxyguanosine and DNA strand breaks in temple workers. Buthbumrung et al. *(182)* reported a similar result in schoolchildren exposed to benzene.

The genotoxic capacities of benzene are due to its metabolites. Pandey et al. *(183)* showed with the micronucleus assay that metabolites of benzene, especially p-benzoquinone, produce significant DNA damage. Keretetse et al. *(184)* showed DNA damage with the comet assay. Galván et al. *(185)* showed that the WRN gene protects against DNA damage. For the first time, Shen et al. *(186)* reported an association between benzene exposure and increased mitochondrial DNA copy number.

Carcinogenesis

Animals. Chronic exposure of both rats and mice to benzene leads to an increased incidence of tumours, though mice are more sensitive *(100–102)*. The tumours formed include hepatomas, Zymbals gland tumours, lymphomas and tumours of the lung and ovary. However, there is no animal model for the induction of acute myeloid leukaemia, the major neoplastic lesion in humans. A study by Ross *(88,99)* in mice deficient in some detoxification enzymes showed that the genetically modified mice developed myeloid cell hyperplasia. Animal studies also showed that intermittent lifetime exposures to benzene at 980 mg/m^3 were more tumorigenic than short-term high-level exposures at 3900 mg/m^3 *(187)*.

Humans. Epidemiological studies have clearly demonstrated a causal relationship between exposure to benzene or solvents containing benzene in the workplace and the development of acute myeloid leukaemia *(123,124,188–191)*.

Rinsky et al. *(190)* studied a cohort of 1165 male workers employed in the Pliofilm[1] manufacturing industry between 1940 and 1965 up to 1981. The control data were the mortality data of American individuals of the same age as those studied in the cohort. An increase in mortality due to leukaemia was observed (9 cases observed instead of 2.7 expected), i.e. an SMR (standardized mortality ratio) of 3.37 (95% CI 1.54–6.41), along with an increase in mortality linked to multiple myeloma (4 cases observed, 1 case expected (SMR 4.09; 95% CI 1.10–10.47). The same evaluation repeated 15 years later reported a reduction in SMR for both leukaemia (SMR 2.56; 95% CI 1.43–4.22) and multiple myeloma (SMR 2.12; 95% CI 0.69–4.96) *(189)*. A significant increase in leukaemia, including myeloid leukaemia but not multiple myeloma, was observed with an increase in cu-

[1] Pliofilm is a trade name. It is a plastic, derived from rubber, that is impermeable to water and used to package or store equipment or food, for example.

mulative exposure to benzene (200 ppm-years,[2] i.e. 650 mg/m^3-years) *(190,192)*. An analysis of 4417 workers did not clearly reveal an increased risk of acute non-lymphocytic leukaemia, multiple myeloma or other types of lymphohaematopoietic cancers, with a low cumulative exposure to benzene, i.e. between 1 and 72 ppm-years (3.25 and 234 mg/m^3-years).

Kirkeleit et al. *(193)* performed a historical cohort study of workers employed in Norway's petroleum industry exposed to crude oil and other products containing benzene. Workers in the job category "upstream operator offshore", having the most extensive contact with crude oil, had an excess risk of haematological neoplasms (blood and bone marrow) (rate ratio (RR) 1.90; 95% CI 1.19–3.02). This was ascribed to an increased risk of acute myeloid leukaemia (RR 2.89; 95% CI 1.25–6.67) *(190)*. A peak exposure number of more than 100 ppm (325 mg/m^3) benzene over 40 days or more therefore appears to be a better indicator of the risk of leukaemia and multiple myeloma than long-term exposure to benzene *(194,195)*.

Within the most recently updated Pliofilm cohort, Paxton et al. *(196,197)* conducted an extended regression analysis with exposure description for the 15 leukaemia cases and 650 controls. They used all three exposure matrices, which gave estimates of 0.26–1.3 excess cancer cases among 1000 workers at a benzene exposure of 1 ppm (3.2 mg/m^3) for 40 years.

A study resulting from collaboration between the National Cancer Institute (NCI) and the Chinese Academy of Preventive Medicine (CAPM) analysed different types of haematopoietic disease, malignant or otherwise (development of the disease and mortality rate linked to the disease), in a cohort of 74 828 workers exposed to benzene. A group of 35 805 workers not exposed to benzene were used as a control. All the workers included in the study came from 672 factories in 12 Chinese cities *(189,198–201)*. The workers were employed from 1972 to 1987 for a mean duration of 12 years. A significant increase in the relative risk of haematological malignancies was observed (RR 2.6; 95% CI 1.4–4.7) as well as the risk for all leukaemias (RR 2.5; 95% CI 1.2–5.1), acute non-lymphocytic leukaemia (RR 3.0; 95% CI 1.0–8.9) and the combination of acute non-lymphocytic leukaemia and precursor myelodysplastic syndromes (RR 4.1; 95% CI 1.4–11.6) *(189)*. Analysis of these risks as a function of atmospheric benzene concentrations (< 10 ppm, 10–24 ppm and ≥ 25 ppm) or cumulative exposure to benzene per year (< 40 ppm-years, 40–99 ppm-years and ≥ 100 ppm-years) indicated that the risk for all haematological malignancies was increased significantly at benzene concentrations of less than 10 ppm (32.5 mg/m^3) and at cumulative benzene concentrations of less than 40 ppm-years (less than 130 mg/m^3-years). The risk of acute non-lymphocytic leukaemia and the combination of acute non-lymphocytic leukaemia and precursor myelodysplastic syndromes was significant

[2] Cumulative benzene exposure over a one-year period.

for a benzene concentration of between 10 and 24 ppm (32.5 and 78 mg/m^3) and for cumulative exposures of between 40 and 99 ppm-years (130 and 322 mg/m^3-years). Some criticisms may limit the utility of these data to develop a risk model. Limitations include the possibility of concurrent chemical exposures and a lack of reliable exposure data *(124)*.

Analysis of these results on the basis of exposure duration (< 5 years, 5–9 years and ≥ 10 years) demonstrated that the risk does not increase with exposure duration, irrespective of the disease studied. Analysis on the basis of different factories and sectors demonstrated that the risks are similar irrespective of the factory's activity, suggesting that the risks calculated are indeed attributed to benzene and not to other pollutants that may be found in the factories. A study conducted in workers employed in a shoe-making factory in Italy demonstrated the same results as the Chinese study *(202,203)*. The cohort was monitored from 1950 to 1999 and included 891 men and 796 women exposed to benzene concentrations of 0–92 ppm (0–299 mg/m^3). The cumulative mean exposure was 71.8 ppm-years (233 mg/m^3-years) for men and 43.4 ppm-years (141 mg/m^3-years) for women. A significant increase in the risk of leukaemia was observed in both sexes for the highest benzene concentration among the four concentration categories. This increase was more apparent in men. For cumulative exposures divided into the following four categories: < 40 ppm-years, 40–99 ppm-years, 100–199 ppm-years and > 200 ppm-years (<130, 130–322, 325–647 and > 650 mg/m^3-years), the SMR values for men were, respectively, 1.4 (95% CI 0.2–5), 3.7 (95% CI 0.1–20.6), 3.0 (95% CI 0.4–10.9) and 7.0 (95% CI 1.9–18.0). The type of leukaemia was not indicated.

A meta-analysis was conducted on 19 cohorts of workers in the petrochemical sector in the United Kingdom and the United States *(204)*. The overall cohort included 208 741 workers. Mean exposures and mean cumulative exposures to benzene, for the most exposed posts, were 1 ppm and 45 ppm-years (3.25 mg/m^3 and 233 mg/m^3-years), respectively. No increase in mortality due to acute myeloid leukaemia, chronic myeloid leukaemia, acute lymphocytic leukaemia and chronic lymphocytic leukaemia was observed in this study.

Recently, Richardson *(205)* evaluated data from a cohort of 1845 rubber hydrochloride workers. He reported an association between leukaemia mortality and benzene exposure at greatest magnitude in the 10 years immediately after exposure: RR 1.19 (95% CI 1.1–1.29). The association was smaller in the period 10–20 years after exposure.

Recent data indicate that benzene exposure is haematotoxic at less than 1 ppm. A decrease in circulating lymphocytes has been observed in workers exposed for six months to a mean exposure concentration of less than 1 ppm (3.25 mg/m^3) *(143–145)*. For leukaemia, the studies by Hayes et al. *(189,198,199)* and Yin et al. *(200,201)* in a cohort of approximately 75 000 workers and 36 000 controls indicated that the risk for acute myeloid leukaemia and precursor myelodysplastic

syndromes increased at between 10 and 24 ppm (32.5 and 78 mg/m^3) and, for cumulative exposures, between 40 and 99 ppm-years (130 and 322 mg/m^3-years).

The study by Rinsky et al. *(190)* described above demonstrates an increase in mortality related to the development of multiple myeloma in 1165 male workers followed up for one year. However, this result was not demonstrated in the other cohort studies *(200,201,203,206,207)*. A follow-up analysis by Rinsky et al. *(191)* indicated an increased but non-significant risk of multiple myeloma, with no evidence of an exposure–response relationship. In addition, case-control studies conducted in hospital populations indicate that exposure to benzene was probably not related to an increased risk of developing multiple myeloma *(208–213)*. Kirkeleit et al. *(193)* reported an increase in RR for multiple myeloma (RR 2.49; 95% CI 1.21–5.13) in workers exposed to crude oil and other products containing benzene employed in Norway's upstream petroleum industry.

The results of studies on non-Hodgkin's lymphoma appear to be less clear *(191,194,195)*. In a meta-analysis of 25 occupational cohorts, no association of non-Hodgkin's lymphoma was found *(207,214)*. A possible link between exposure to benzene and the development of non-Hodgkin's lymphoma was suggested by analysis of the results of the Chinese (NCI/CAPM) cohort described above *(189)*. The relative risk of mortality linked to non-Hodgkin's lymphoma in the overall cohort was 3 (95% CI 0.9–10.5). This increase was not statistically significant. However, the risk of non-Hodgkin's lymphoma increased significantly at the highest benzene concentration and for the longest exposure duration. For mean exposure to benzene concentrations < 10 ppm, 10–24 ppm and ≥ 25 ppm, the relative risks for non-Hodgkin's lymphoma were, respectively, 2.7 (95% CI 0.7–10.6), 1.7 (95% CI 0.3–10.2) and 4.7 (95% CI 1.2–18.1) ($P = 0.04$). For cumulative benzene exposures of < 40, 40–99 and ≥ 100 ppm-years, the relative risks for non-Hodgkin's lymphoma were, respectively, 3.3 (95% CI 0.8–13.1), 1.1 (95% CI 0.1–11.1) and 3.5 (95% CI 0.9–13.2) ($P = 0.02$). In addition, the risk of developing non-Hodgkin's lymphoma increases significantly with an increase in benzene exposure duration. The relative risks are, respectively, 0.7 (95% CI 0.1–7.2), 3.3 (95% CI 0.7–14.7) and 4.2 (95% CI 1.1–15.9) for workers exposed for less than 5 years, for between 5 and 9 years and for more than 10 years ($P = 0.01$). The other cohort studies did not reveal any positive relationship between exposure to benzene and an increase in mortality due to non-Hodgkin's lymphoma *(191,194,206)*. Kirkeleit et al. *(193)* reported no statistical differences between the groups in respect to non-Hodgkin's lymphoma.

Recently, Steinmaus et al. *(215)* conducted a meta-analysis of cohort and case-control studies of benzene exposure and non-Hodgkin's lymphoma and a meta-analysis of non-Hodgkin's lymphoma and refinery work. Results for the 22 studies indicated that the summary relative risk for non-Hodgkin's lymphoma was 1.22 (95% CI 1.02–1.47) ($P = 0.01$). When the authors excluded unexposed subjects in the "exposed group" (9 studies), the RR increased to 1.49. When studies based

solely on self-reported work history were excluded (7 studies), the RR rose to 2.12 (95% CI 1.11–4.02). In refinery workers, the summary RR for non-Hodgkin's lymphoma in all 21 studies was 1.21 (95% CI 1.00–1.46) ($P = 0.02$). When adjusted for the healthy worker effect, this RR estimate increased to 1.42 (95% CI 1.19–1.69). These results suggest that effects of benzene on non-Hodgkin's lymphoma might be missed in occupational studies if these biases are not accounted for.

In addition, a recent review by IARC concluded that there is limited evidence of an association between benzene exposure and acute lymphocytic leukaemia or non-Hodgkin's lymphoma *(216)*.

Table 1.1 collates studies on carcinogenic effects linked to human exposure to benzene, along with significant causal relationships between cancer and benzene exposure (subchronic and chronic).

In conclusion, the different studies available (in humans, in animals and in vitro) have demonstrated that benzene metabolites trigger chromosomal aberrations (translocation, monosomy, trisomy). The carcinogenic mechanism of action of benzene is linked to its genotoxic effects and the critical health outcomes are blood dyscrasias and leukaemia, particularly acute myeloid leukaemia.

Health risk evaluation

Critical health outcomes

Inhalation is the dominant route of exposure in humans. Inhaled benzene at concentrations found indoors is rapidly absorbed and distributed throughout the body. Benzene is rapidly metabolized in the liver and bone marrow to bipolar metabolites, which are responsible for its toxicity through clastogenic activity on DNA.

The critical health outcomes are blood dyscrasias and leukaemia, particularly acute myeloid leukaemia. The evidence is sufficient to conclude that a causal relationship exists between benzene exposure and both types of health effect observed. In addition, based on a recent review by IARC, there is limited evidence of an association between exposure to benzene with acute lymphocytic leukaemia and non-Hodgkin's lymphoma. Haematotoxicity is a risk factor for leukaemia *(108)*. This has been observed in many epidemiological studies in many countries. The studies were completed in occupational settings. A decrease in circulating lymphocytes has been observed in workers exposed for six months to a mean exposure concentration of less than 1 ppm (3.25 mg/m^3) *(143–145)*.

The association of benzene exposure with leukaemia was shown in studies of a cohort of male workers employed in the Pliofilm manufacturing industry between 1940 and 1965 *(190,217–219)*. These studies were updated by Paxton et al. *(196,197)* and confirmed the association of benzene exposure with the development of myelogenous leukaemia. Later studies by Hayes et al. *(189,198,199)* and Yin et al. *(200,201)* in a cohort of approximately 75 000 Chinese workers and 36 000 controls indicated that the risk for acute myeloid leukaemia and precur-

sor myelodysplastic syndromes increased at between 10 and 24 ppm (32.5 and 78 mg/m^3) and for cumulative exposures at between 40 and 99 ppm-years (130 and 322 mg/m^3-years).

In considering the exposure–response relationship, while there may be thresholds for these responses (blood dyscrasias and acute myeloid leukaemia) in individuals, there is no evidence of thresholds in population responses. Sensitive subpopulations have been found in which individuals have metabolic polymorphisms consisting of fast CYP2E1 oxidation activity or deficiencies in detoxification enzymes such as NQO1, or both. As regards the shape of the models describing the exposure–response relationship, Crump *(220)* found that multiplicative risk models described the data better than additive risk models and cumulative exposures better than weighted exposures. Crump *(220)* suggested that concentration-dependent non-linear models were more suited than linear models. Nevertheless, although there are biological arguments to support the use of concentration-dependent models, these results are only preliminary and need to be further developed and peer-reviewed.

Health relevance of indoor air exposures

Indoor concentrations of benzene are commonly higher than concentrations in outdoor air *(9)* as a consequence of the entry of benzene from outdoor sources (such as heavy traffic, petrol stations or industrial sites) and the presence of dominant benzene sources indoors. Indoor sources of benzene are mainly due to ETS, solvent use, building materials, attached garages and various human activities. On the other hand, in some regions unvented heating or cooking are the dominant sources indoors.

Also, the relatively low rates of ventilation typically found in houses and offices prevent the rapid dispersal of airborne contaminants. In areas where cooking and heating are provided by open fires in poorly ventilated housing, indoor levels of contaminants, including benzene, may reach high levels.

Indoor levels of benzene in homes and offices without strong indoor sources (e.g. ETS or unvented kerosene cooking/heating stoves) are generally less than 15 μg/m^3 (24-hour average), which are well below any of the lowest levels showing evidence of adverse health effects in either epidemiological or animal studies. In areas with high levels of ETS (e.g. discotheques), peak levels of 200 μg/m^3 have been observed. Incense burning or the use of unvented heating or cooking with kerosene stoves can drive peak indoor levels up in the 100–200 μg/m^3 range, with 24-hour levels in the range of 10–50 μg/m^3.

Conclusions of other reviews

IARC *(123,126)* classifies benzene as a known human carcinogen (Group 1). The USEPA lists benzene as Group A, a known human carcinogen, and lists the cancer risk for lifetime exposure to 1 μg/m^3 of benzene as 2.2–7.8 in a million

Table 1.1. Review of SMR and RR values identified in the literature for chronic human exposure to benzene (occupational and environmental studies) for carcinogenic effects

Reference	Exposure duration	Number and type of individuals exposed
Aksoy et al. *(146,147)* Retrospective study of workers exposed during the production of shoes, handbags and derived products between 1950 and 1965	1–28 years Mean exposure = 9.7 years	28 500 shoe industry workers in Turkey; controls: general population
Infante *(216,217)* Pliofilm cohort	1–10 years	748 workers; controls: general population
Rinsky et al. *(188,215)* Pliofilm cohort	1–14 years	1165 workers; controls: general population
Paci et al. *(201)* Retrospective study of workers in Florence employed from 1939 to 1984		
Yin et al. *(198,199)* Retrospective study of workers exposed in 233 paint, shoe, rubber and leather factories in China	More than 1 year	28 460 workers; 28 257 controls (workers not exposed in 83 factories)

Effect taken into account and measured	Concentration (1 ppm = 3.25 µg/m³)	Statistically significant association between effects measured and exposure
Mortality due to aplastic anaemia and acute leukaemia	150–210 ppm for 1–28 years Peak: 210–640 ppm	Positive association at all concentrations
Mortality due to myeloid leukaemia	Categories of exposure: < 40 ppm-years; 40–199 ppm-years; 200–400 ppm-years; > 400 ppm-years	Increase of standardized mortality rates in all categories with clear exposure–response trend
Mortality due to leukaemia	Total 1–39 ppm-years 40–199 ppm-years 200–399 ppm-years > 400 ppm-years	No significant association No association No significant association Positive association Positive association
Mortality due to lymphatic and haematopoietic tumours	Total	No association
Mortality due to multiple myeloma	Total < 40 ppm-years > 40 ppm-years	Positive association Positive association Positive association
Mortality due to aplastic anaemia	Exposure level not reported, exposure for ≥ 29 years	Positive association
Mortality due to leukaemia		Positive association
Mortality due to leukaemia	From 2 to 345 ppm (samples)	Positive association at all concentrations
Mortality due to lung cancer	From 2 to 345 ppm (samples)	Positive association at all concentrations

Reference	Exposure duration	Number and type of individuals exposed
Hayes et al. *(185)* Follow-up of the retrospective study of workers exposed to benzene in factories in 12 Chinese cities	Mean exposure duration = 12 years	74 828 workers; 25 805 controls
Ireland et al. *(193)* Study of chemical factory workers in the United States with a low degree of exposure to benzene		4127 workers; controls: general population
Kirkeleit et al. *(191)*	22 years	27 919 offshore oil workers ; 366 114 general working population matched by age, gender and place of residence
Steinmaus et al. *(213)*		Meta-analysis of 22 studies of benzene exposure and 21 studies of refinery workers
Richardson *(203)*	Up to 25 years	1845 rubber hydrochloride workers

Effect taken into account and measured	Concentration (1 ppm = 3.25 µg/m³)	Statistically significant association between effects measured and exposure
Development of all haematological cancers	< 40 ppm-years	Positive association
	40–99 ppm-years	Positive association
	≥ 100 ppm-years	Positive association
Development of leukaemia	< 40 ppm-years	No significant association
	40–99 ppm-years	Positive association
	≥ 100 ppm-years	Positive association
Development of non-Hodgkin's lymphoma	40 ppm-years	No significant association
	40–99 ppm-years	No significant association
	≥ 100 ppm-years	No significant association
Development of acute non-lymphocytic leukaemia	< 40 ppm-years	No significant association
	40–99 ppm-years	Positive association
	≥ 100 ppm-years	Positive association
Combination of myeloblastic syndromes and acute non-lymphocytic anaemia (development)	< 40 ppm-years	No significant association
	40–99 ppm-years	Positive association
	≥ 100 ppm-years	Positive association
Mortality due to leukaemia	0.5 ppm-years	No significant association
	3.5 ppm-years	No significant association
	12 ppm-years	Positive association
Acute myeloid leukaemia	Exposure classified by job description	Positive association
Multiple myeloma	Exposure classified by job description	Positive association
Non-Hodgkin's lymphoma	Exposure classified by job description	No significant association
Non-Hodgkin's lymphoma	"High" exposures (see paper for definition)	Positive association in both benzene and refinery workers
Age and temporal association between benzene exposure and leukaemia mortality	10 years since exposure (35–144 ppm-years)	Greatest association; RR 1.19; CI 1.10–1.29
	10–20 years since exposure	Smaller association; RR 1.05; CI 0.97–1.13
	> 20 years since exposure	No significant association

(124,221). The California Environmental Protection Agency lists the unit cancer risk for the same exposure as 29 in a million.

Guidelines

Guidelines on exposure levels are needed for indoor air because indoor air is a significant source of benzene exposure and inhalation is the main pathway of human exposure to benzene. Benzene is present in both outdoor and indoor air. However, indoor concentrations are generally higher than concentrations in outdoor air owing to the infiltration of benzene present in outdoor air and to the existence of many other indoor sources. Typically, indoor concentrations are below the lowest levels showing evidence of adverse health effects. Considering benzene is present indoors and taking into account personal exposure patterns, which are predominantly indoors, indoor guidelines for exposure are needed.

Benzene is a genotoxic carcinogen in humans and no safe level of exposure can be recommended. The risk of toxicity from inhaled benzene would be the same whether the exposure were indoors or outdoors. Thus there is no reason that the guidelines for indoor air should differ from ambient air guidelines.

Previous WHO benzene guidelines for ambient air were calculated using the Pliofilm cohort studies *(220)*. Since these studies, new data have become available, such as those on the large Chinese workers cohort *(189)*. However, the unit risks and risk assessment analysis based on these data are still not available. Hence we recommend continuing to use the same unit risk factors calculated from the Pliofilm cohort studies. The geometric mean of the range of the estimates of the excess lifetime risk of leukaemia at an air concentration of 1 $\mu g/m^3$ is 6×10^{-6}. The concentrations of airborne benzene associated with an excess lifetime risk of 1/10 000, 1/100 000 and 1/1000 000 are 17, 1.7 and 0.17 $\mu g/m^3$, respectively.

As noted above, there is no known exposure threshold for the risks of benzene exposure. Therefore, from a practical standpoint, it is expedient to reduce indoor exposure levels to as low as possible. This will require reducing or eliminating human activities that release benzene, such as smoking tobacco, using solvents for hobbies or cleaning, or using building materials that off-gas benzene. Adequate ventilation methods will depend on the site of the building. In modern buildings located near heavy traffic or other major outdoor sources of benzene, inlets for fresh air should be located at the least polluted side of the building.

The guidelines section was formulated and agreed by the working group meeting in November 2009.

Summary of main evidence and decision-making in guideline formulation

Critical outcome(s) for guideline definition

- Acute myeloid leukaemia (sufficient evidence on causality).
- Genotoxicity *(162–178,181–184,186)*.

Source of exposure–effect evidence

Occupational cohort study of male workers employed in Pliofilm manufacturing industry in China *(190–192,196,197,217–220)*.

Supporting evidence

Occupational cohort studies in China *(189,198–201)*, Italy *(202,203)*, Norway *(193)*, United States *(194,195,205)*.

Results of other reviews

- IARC: Group I (known human carcinogen) *(123,126)*.
- USEPA: Group A (known human carcinogen); the cancer risk for lifetime exposure to 1 μg/m^3 benzene is 2.2–7.8 in a million *(124,221)*.

Guidelines

- No safe level of exposure can be recommended.
- Unit risk of leukaemia per 1 μg/m^3 air concentration is 6×10^{-6}.
- The concentrations of airborne benzene associated with an excess lifetime risk of 1/10 000, 1/100 000 and 1/1000 000 are 17, 1.7 and 0.17 μg/m^3, respectively.

Comments

No change in the guideline as compared to *Air quality guidelines for Europe (2)*.

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2. Carbon monoxide

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General description

Carbon monoxide (CO) is a colourless, non-irritant, odourless and tasteless toxic gas. It is produced by the incomplete combustion of carbonaceous fuels such as wood, petrol, coal, natural gas and kerosene. Its molecular weight is 28.01 g/mol, melting point –205.1 °C, boiling point (at 760 mmHg) –191.5 °C (–312.7 °F), density 1.250 kg/m^3 at 0 °C and 1 atm and 1.145 kg/m^3 at 25 °C and 1 atm, and relative density (air = 1) 0.967 *(1,2)*. Its solubility in water at 1 atm is 3.54 ml/100 ml at 0 °C, 2.14 ml/100 ml at 25 °C and 1.83 ml/100 ml at 37 °C.

The molecular weight of carbon monoxide is similar to that of air (28.01 vs approximately 29). It mixes freely with air in any proportion and moves with air via bulk transport. It is combustible, may serve as a fuel source and can form explosive mixtures with air. It reacts vigorously with oxygen, acetylene, chlorine, fluorine and nitrous oxide. Carbon monoxide is not detectible by humans either by sight, taste or smell. It is only slightly soluble in water, blood serum and plasma; in the human body, it reacts with haemoglobin to form carboxyhaemoglobin (COHb).

The relationship of carbon monoxide exposure and the COHb concentration in blood can be modelled using the differential Coburn-Forster-Kane equation *(3)*, which provides a good approximation to the COHb level at a steady level of inhaled exogenous carbon monoxide.

Conversion factors

At 760 mmHg and 20 °C, 1ppm = 1.165 mg/m^3 and 1 mg/m^3 = 0.858 ppm; at 25 °C, 1 ppm = 1.145 mg/m^3 and 1 mg/m^3 = 0.873 ppm.

Indoor sources

Inhalation is the only exogenous exposure route for carbon monoxide. Anthropogenic emissions are responsible for about two thirds of the carbon monoxide in the atmosphere and natural emissions account for the remaining one third. Small amounts are also produced endogenously in the human body *(4,5)*. Exposure to low levels of carbon monoxide can occur outdoors near roads, as it is also produced by the exhaust of petrol- and diesel-powered motor vehicles. Parking areas can also be a source of carbon monoxide *(6)*.

Carbon monoxide is produced indoors by combustion sources (cooking and heating) and is also introduced through the infiltration of carbon monoxide from outdoor air into the indoor environment *(7)*. In developed countries, the most important source of exposure to carbon monoxide in indoor air is emissions from faulty, incorrectly installed, poorly maintained or poorly ventilated cooking or heating appliances that burn fossil fuels. In homes in developing countries, the burning of biomass fuels and tobacco smoke are the most important sources of exposure to carbon monoxide. Clogged chimneys, wood-burning fireplaces, decorative fireplaces, gas burners and supplementary heaters without properly working safety features could vent carbon monoxide into indoor spaces. Incomplete oxidation during combustion may cause high concentrations of carbon monoxide in indoor air. Tobacco smoke can be a major source of indoor exposure, as can exhaust from motor vehicles operating in attached garages *(6)*.

Combustion of low-grade solid fuel and biofuels in a small stove or fireplace can generate high carbon monoxide emissions, which may become lethal to occupants unless the flue gases are vented outdoors via a chimney throughout the entire combustion process. At the beginning of combustion, the pollutants released are dominated by particulate matter (elemental and organic carbon) but carbon monoxide dominates towards the end. Combustion of high-grade fuels such as natural gas, butane or propane usually produces much less carbon monoxide, provided that sufficient air is supplied to ensure complete combustion. Nevertheless, even devices using such fuels can cause lethal carbon monoxide intoxication if they are not properly maintained or vented or if air : fuel ratios are not properly adjusted.

Table 2.1. Indoor concentrations of carbon monoxide and indoor : outdoor (I : O) ratios

Study	Location	AM (SD) (mg/m^3)	GM (SD) (mg/m^3)	Median (mg/m^3)
Europe				
Maroni et al. *(9)*	Athens	1.3		
Georgoulis et al. *(10)*	Athens		4	
Chaloulakou et al. *(11)*	Athens	3.7		
Maroni et al. *(9)*	Basel	2.0		
Alm et al. *(12)*	Helsinki	2.1	1.6 (2.3)	1.8
Maroni et al. *(9)*	Helsinki	1.2		
Georgoulis et al. *(10)*	Helsinki	1.2		
Scotto di Marco et al. *(13)*	Helsinki	9.0	5.7	
		7.1	5.3	
		5.7	3.7	
		4.3	3.3	
		2.6	2.1	
		2.0	1.8	

Note: AM = arithmetic mean; GM = geometric mean; SD = standard deviation.

Incense burning in homes and public buildings such as stores and shopping malls can be a source of exposure to carbon monoxide. Jetter et al. *(8)* reported emission rates of 23 different types of incense, such as rope, cones, sticks, rocks and powder, that are typically used indoors. The measured emission rates of carbon monoxide ranged from 144 to 531 mg/hour. The authors estimated a peak concentration of 9.6 mg/m^3 caused by incense burning and therefore concluded that carbon monoxide concentrations could exceed the USEPA's National Ambient Air Quality Standard of 10 mg/m^3 for an 8-hour average, depending on the room volume, ventilation rate and the amount of incense burned. Incense burning might be a significant contributor to carbon monoxide exposure in cultures where incense is burned frequently, for example in religious rituals.

Indoor levels and relationship with outdoor levels

Results of recent studies on carbon monoxide concentrations in indoor air are summarized in Table 2.1. The studies are listed by continent. Studies concerning accidental or peak exposures are presented separately in Table 2.2. Representativeness and data quality, as well as the form in which the data are presented, vary greatly between the studies and make detailed comparisons meaningless except when comparing data within the same study. The general levels of carbon monoxide, however, vary so much between the locations and studies that patterns are easily discernible.

In the absence of indoor sources, current concentrations of carbon monoxide in indoor air in European and North American cities are well below the levels of existing air quality guidelines and standards. In the 1950s and 1960s, carbon

Range (mg/m^3)	I : O ratio (range)	Sources	Other information	Averaging time
		Smoking	Homes	
			Offices	1-hour
		Ambient air, gas stoves and ETS	Personal 24-hour exposures of children	
			Homes	
		Non-ETS	Exposure	
		ETS		Max. 15-minute
		Non-ETS		
		ETS		Max. 1-hour
		Non-ETS		
		ETS		Max. 8-hour
		Non-ETS		

Study	Location	AM (SD) (mg/m³)	GM (SD) (mg/m³)	Median (mg/m³)
Maroni et al. *(9)*	Prague	0.6		
Pan et al. *(14)*	Anhui			
Maroni et al. *(9,15)*	Milan	2.4		
Bruinen de Bruin et al. *(16)*		1.8 (1.3)	1.4 (2.2)	
		2.4 (1.5)	1.9 (1.9)	
		2.9 (1.6)	2.4 (1.8)	
		3.4 (2.2)	2.8 (1.9)	
		1.9 (1.7)	1.4 (2.2)	
		1.6 (1.2)	1.2 (2.0)	
		2.5 (2.2)	1.8 (2.4)	
		6.5 (2.5)	6.2 (1.4)	
		3.5 (2.9)	2.8 (1.9)	
Valerio et al. *(17)*				
Maroni et al. *(18)*	Italy	12–23		
Ross *(19)*	United Kingdom			
Raw et al. *(20,21)*	United Kingdom		0.4	
			0.3	
			0.8	
			0.9	
			0.7	
			0.4	
			0.3	
			0.4	
			0.5	
			0.7	
	(spring)		0.3	
	(summer)		0.2	
	(autumn)		0.5	
	(winter)		0.5	
Ditmitroulopoulou et al. *(22)*	London	1.9		
		2.3		
		2.0		
Milner et al. *(23)*	London			
Lai et al. *(24)*	Oxford	1.1	0.5 (3.9)	
			1.0 (2.3)	
Akland et al. *(25)*		0.5 (1.6)		
		15.4 (18.1)		
		10.5 (9.3)		
		6.5 (7.7)		
		5.6 (6.5)		
		5.0 (7.1)		
		4.3 (4.4)		
		4.1 (4.2)		
		3.9 (4.8)		
		3.7 (5.6)		

Note: AM = arithmetic mean; GM = geometric mean; SD = standard deviation.

Range (mg/m³)	I : O ratio (range)	Sources	Other information	Averaging time
1.6 – 3				
2.1–3.9		Gas cooking	Homes	
	0.85	None	Homes	
	0.89	Gas cooking	Homes	
	1.45	ETS	Homes	
	1.10	Gas cooking & ETS	Homes	
	1.0	None	Offices	
	1.0	ETS	Offices [a]	
	1.19	None	Other indoor	
	2.95	Gas cooking	Other indoor	
	2.19	ETS	Other indoor	
15 (peak)			Shops	8-hour
18 (peak)			Bars	8-hour
35 (peak)			Bars, restaurants	
0.2–2.7			Homes	
		All-electric homes	Home kitchen	
		Gas oven/cooking	Home kitchen	
		Unflued heater	Home kitchen	
		ETS	Bedroom	
		Non-ETS	Bedroom	
			Rural	
			Suburban	
			Urban	
			City centre	
			All homes	
			All homes	
			All homes	
			All homes	
– 2.7	1.1	Marylebone Road	Lounge	
– 7.6	1.4	Gas cooking	Kitchen	
– 3.6	1.2	Smoking	Kitchen	
0.05–0.6	0.2–4.1	Busy street	Office building, 15-minute averages	
		No smoking	Personal exposure	
		Smoking		
			Public garage	
			Service station / car repair shop	
			Repair shop	
			Shopping mall	
			Residential garage	
			Restaurant	
			Office	
			Sports arena, concert hall	
			Store	
			Health care facilities	

[a] Problem in the self-reported exposures in the offices analysed in Ref. 16.

Study	Location	AM (SD) (mg/m³)	GM (SD) (mg/m³)	Median (mg/m³)
		2.5 (4.3)		
		2.5 (3.3)		
		2.3 (4.1)		
		1.9 (2.8)		
		1.8 (3.4)		
Junker et al. *(26)*	Switzerland	3.5		
Pennanen et al. *(27)*	Finland	20–33		
North America				
Kim et al. *(28)*	Toronto	1.4 (0.5)		1.3
Levesque et al. *(29)*	Quebec	2.5		
Central America				
Lee & Park *(30)*	Costa Rica	1.6		
Naeher et al. *(31)*	Guatemala	28.6		
Clark et al. *(32)*	Guatemala	11		10
		0.5		0.4
Australia				
Brown et al. *(33)*				
		<1.2–4.6		
		20		
		34		
Asia				
Fischer & Koshland *(34)*	China, rural village	4.2 (0.7)	2.6 (2.7)	2.9
Jin et al. *(35)*	China, Gansu	11.3		
	China, Guizhou	1.8		
	China, Inner Mongolia	7.3		
	China, Shaanxi	10.8		
Hui et al. *(36)*	China, Hong Kong SAR	1.0 (0.3)	0.4 (1.4)	
Jo et al. *(37)*	Korea, Daegu			
	(summer)	0.5 (0.4)		0.5
	(winter)	0.9 (0.5)		0.8
	(summer)	0.4 (0.5)		0.3
	(winter)	0.8 (0.6)		0.8
Kim et al. *(38)*	Seoul	2.09		
Lawrence et al. *(39)*	India	1.2 (0.4)		
		1.2 (0.3)		
		2.1 (0.4)		
Gupta et al. *(40)*	India, New Delhi			

Note: AM = arithmetic mean; GM = geometric mean; SD = standard deviation.

Range (mg/m^3)	I : O ratio (range)	Sources	Other information	Averaging time
			Other public buildings Residence School Church	
5.2 (peak)			Concert hall	Event (5½ hours)
			Five indoor ice rinks	Max 1-hour
0.1–3.8	0.4–1.0		Personal 24-hour	
		Open fire	Kitchen	
9–13		Open fire	Kitchen	
0.2–5.5		*Plancha*	Kitchen	
		Unflued gas heaters Normal conditions Gas supply restricted Misaligned burner	Laboratory room chamber test	1-minute
5.0–26.8 (1-hour peak)			Kitchen	24-hour
6.9–15.7		LPG for cooking & heating	33 bed/living rooms	24- hour
1.6–2.0		Coal for cooking & heating	32 cooking/living rooms	24- hour
6.6–7.9		Biomass for cooking & heating	65 cooking/living/ bedrooms	24- hour
6.3–15.3		Coal & biomass for cooking; coal for heating	24 bedrooms	24- hour
0.2–2.1			Offices	
– 2.3	0.8		Low-floor residences	
– 1.6	1.0			
– 1.1	1.0		High-floor residences	
– 1.3	1.6			
	3.6		Rural homes	
	1.7		Urban homes	
	1.7		Roadside homes	
1.2–3.5			Airport authority building control tower – ground floor	

Table 2.2. Accidental or peak exposure studies

Study	Location	Mean (mg/m³)	Range (mg/m³)
WHO *(41,42)*			60–115 (peak)
IEH *(43)*	United Kingdom		10–182 (peak)
Lebret et al. *(44)*	United Kingdom		5–108 (peak) 3–56 (peak)
El Fadel et al. *(45)*	Beirut		26–140 (peak)
Ross *(19)*	United Kingdom		121 (peak) 6–49 (peak) 3.5–4 (peak) 60 (peak)
Hampson & Zmaeff *(46)*	United States, Virginia		COHb % 6.6–50
Salonen et al. *(47)*	Finland, ice rink	> 140	COHb % 8–24
Guo et al. *(48)*	China, Hong Kong SAR		8–16
Lee & Wang *(49)*	China, Hong Kong SAR		44 (peak) 5.7
Thomassen et al. *(50)*	Camping tent	21.5% COHb (2.4)	200–550
Weaver & Deru *(51)*	Hotels, motels and resorts		

monoxide levels in urban air often approached or even exceeded these reference values, but drastic reductions in emissions from space heating and traffic have substantially reduced anthropogenic emissions in spite of the growing size of cities and increasing traffic *(9,29)*.

The highest reported non-accidental carbon monoxide levels are observed in public or residential garages and in primitive kitchens when cooking with open fires (Guatemala). Aside from open-fire cooking with solid fuels, the most common sources for elevated carbon monoxide concentrations in indoor air are unvented gas appliances, tobacco smoking and proximity to busy traffic. The lowest concentrations are found in homes, churches and schools at some distance (> 500 metres) from busy traffic and with no indoor sources. Carbon monoxide intoxication can be caused by single or repetitively generated high short-term peaks, and carbon monoxide poisoning is the leading cause of death from poisoning (accidental and intentional).

Carbon monoxide is a relatively unreactive gas under ambient air conditions and is not absorbed by building materials or ventilation system filters. Therefore, in the absence of indoor carbon monoxide sources, the indoor air concentration is the same as the concentration of ventilated or infiltrating outdoor air. Under these conditions, the indoor : outdoor (I : O) carbon monoxide concentration ratio should be 1.0; in practice, however, measured I : O ratios vary for two reasons.

- The outdoor air carbon monoxide concentration at the point of measurement may be significantly higher or lower than the concentration at the point

Sources	Other information	Averaging time
	Underground car parks, enclosed ice rinks, etc; homes with gas appliances	Several hours
Gas stove with pilot light	Homes	Peak when using a grill
Gas appliances	Kitchen Kitchen	Max. 1-minute Max .1-hour
	Underground parking	30-minute
Faulty boiler Gas cooking All-electric homes	Homes Homes Homes Homes	1-minute
Portable electric generators	Case studies on carbon monoxide poisoning	
Ice resurfacing machine	Case study of a carbon monoxide poisoning (epidemiological study)	
Petrol-fuelled	Indoor ice skating rink	15-minute
Incense burning Propane-fuelled	Chamber tests	 15-minute
Kerosene cooking stove	Experiment	120-minute
Boilers, water heaters, generators	68 cases of carbon monoxide poisoning, 27 deaths 1989–2004	

of ventilation air intake. Consequently, even in the absence of any indoor sources, the 15-minute I : O for carbon monoxide varies from 0.2 to 4.1 and the daily I : O from 0.4 to 1.2.

- Normal indoor sources, gas appliances and tobacco smoking increase the I : O ratios.

Kinetics and metabolism

Carbon monoxide hypoxia

Since the time of Haldane *(52)*, it has been assumed that the effect of carbon monoxide exposure is due to hypoxic effects *(53)*. Carbon monoxide enters the body via inhalation and is diffused across the alveolar membrane with nearly the same ease as oxygen (O_2). Carbon monoxide is first dissolved in blood, but is quickly bound to haemoglobin (Hb) to form COHb, which is measured as the percentage of haemoglobin so bound. The binding of carbon monoxide to haemoglobin occurs with nearly the same speed and ease as with which oxygen binds to haemoglobin, although the bond for carbon monoxide is about 245 times as strong as that for oxygen *(54–56)*. Thus carbon monoxide competes equivocally with oxygen for haemoglobin binding sites but, unlike oxygen, which is quickly and easily dissociated from its haemoglobin bond, carbon monoxide remains bound for a much longer time. In this way, COHb continues to increase with continued exposure, leaving progressively less haemoglobin available for carrying oxygen. The result is arterial hypoxaemia. Another effect of COHb is to

increase the binding strength of oxygen to haemoglobin, thus making release of oxygen into tissue more difficult *(57)*. The latter effect is quantitatively described as a leftward shift in the oxyhaemoglobin dissociation curve, proportional to the COHb level *(58)*.

The endogenous formation of COHb has been described by Coburn, Forster & Kane *(3)*. The model has also been tested under a wide variety of carbon monoxide exposure conditions and found to predict COHb more accurately than empirical methods *(54,59–66)*.

The most important variables in the formation of COHb are the concentration and duration of carbon monoxide in inhaled air and the rate of alveolar ventilation *(67)*. Alveolar ventilation, largely determined by body energy expenditure (exercise), can vary over a wide range and is thus the major physiological determinant of the rate of COHb formation and elimination.

Carbon monoxide will also reduce the diffusion of oxygen into tissue via myoglobin by formation of carboxymyoglobin. The formation of carboxymyoglobin also acts as another sink for carbon monoxide. This process has been described by a multicompartmental physiological model *(68,69)*. The models estimate the effects of carboxymyoglobin formation on carbon monoxide uptake, but the effect of carboxymyoglobin on tissue function is not clear. It is probable that such effects become important only for high levels of carbon monoxide exposure *(70)*. Binding of carbon monoxide to other proteins (cytochrome P-450 and cytochrome oxidase) have also been demonstrated, but the dosimetry is unclear and the functional significance appears to be limited to high levels of carbon monoxide exposure *(70)*.

Dosimetric compensations for COHb

Carbon monoxide, in addition to being an environmental contaminant, is produced endogenously. Thus, it is not surprising that physiological mechanisms have evolved to compensate for its presence in mammalian blood and tissues. These compensatory mechanisms must be considered when calculating the tissue dosimetry. For acute exposures, as COHb increases, arterial blood flow to the brain increases proportionally. Thus, even though the blood oxygen contents are decreased, in normal people the increased volume of blood tends to keep the amount of oxygen delivered to the brain constant, preventing hypoxia *(71–74)*. These investigators have demonstrated that brain tissue metabolism remains constant as the COHb increases until it approaches 20%, implying that brain tissue hypoxia does not occur with lower COHb levels. Thus it is apparent that the increased compensatory flow is sufficient to account for the shift in the oxyhaemoglobin dissociation curve. This compensatory activity also occurs in neonates and fetuses *(73,74)*. For chronic exposures to carbon monoxide, red cell volume increases or plasma volume decreases *(70)*, thus increasing the amount of oxygen that can be delivered.

Non-hypoxic mechanisms

An accumulating body of evidence indicates that direct carbon monoxide exposure (not COHb) can produce a number of brain cellular events that could potentially lead to serious functional consequences (see the section on health effects below). The direct effect of carbon monoxide on tissue has not been demonstrated in vivo, although such effects have been inferred by the observation of tissue effects in exposures in vivo that are very similar to such effects found with in vitro preparations. It would appear that the presence of carbon monoxide in tissues from in vivo exposure would depend on carbon monoxide dissolved in blood, because it had not yet bound with haemoglobin or because there could be some level of dissociation due to chemical equilibrium reactions. The amount of such dissolved carbon monoxide and the diffusion into various tissues has not been described or modelled. Thus, the dosimetry for putative non-hypoxic effects of carbon monoxide exposure is not known. The amount of dissolved carbon monoxide in blood would seem to be highest for high-level carbon monoxide exposure.

Comprehensive dosimetry

The final dose for carbon-monoxide-induced hypoxic effects is thus seen to be some measure of tissue oxygenation. This is an inverse measure in the sense that, as tissue oxygen increases towards the normal, function improves. As shown above, tissue oxygenation is determined by (*a*) the blood oxygen content (inversely proportional to COHb level), (*b*) the ease of dissociation from blood to tissue (the oxyhaemoglobin dissociation curve), (*c*) the volume of blood delivered to tissue and (*d*) the ability of tissue to utilize the oxygen (tissue respiration). To these we must add the rate of oxygen utilization by the tissue. The final criterion of tissue function is the energy metabolism rate in the tissue.

The issue of dosimetry is complex, but there exist physiologically based mathematical models to estimate many of the above variables and thus to predict tissue function. They are not mathematically trivial, but with modern computation tools the necessary calculations are readily performed *(3,75)*. Many of these models have been combined into "whole-body" models, which hold much promise for estimating physiological function (http://physiology.umc.edu/themodelingworkshop/).

Exposure–response relationship

The information required for regulatory guidance setting is some measure of the biologically critical concentration and duration of carbon monoxide exposure in inhaled air. To estimate environmental guidelines that provide reasonable protection against adverse health effects, information is required about what tissue dose produces what health effects. Given this critical tissue dose, one can estimate the various environmental concentrations, subject characteristics and

subject activities that will produce the critical tissue dose. Thus for a specific environmental case of interest, mathematical simulations can be done to estimate protective regulatory decisions. Therefore, for each health effect of interest, critical tissue oxygenation must be known.

It might be argued that the critical tissue dose is obtained from experimental evidence in which environmental exposure is given in the first place. Experiments, however, are not usually good simulations of actual scenarios of interest. The purpose of the simulations is to be able to simulate any environment of interest without having direct experimental evidence. Unfortunately, in the absence of adequate dosimetric information, and therefore dosimetric models, simulation by models is not possible. Thus for non-hypoxic effects, it is frequently necessary to use less general evidence from empirical environmental data to make estimates of critical exposures. To preserve exposure data from experiments and literature reviews, it would seem to be important to report both COHb and exposure concentration and duration. This would potentially permit calculation of tissue dose for non-hypoxic tissue effects when the dosimetry models are developed. It should be kept in mind that the tissue dose and the eventual health effect are not necessarily contemporaneous. Delayed sequelae may occur and cumulative exposure may be needed to become effective. These are really questions of physiological mechanisms.

Health effects

Identification of studies

For the acute health effects, the literature search was conducted in the PubMed and Web of Science databases, searching the keywords carbon monoxide and health. A special search for behavioural and neurological effects used PubMed with the following keyword statement: ("carbon monoxide" OR CO) AND ("human behaviour" OR "nervous system" OR CNS OR sensory OR "human performance" OR vision OR hearing OR auditory) NOT co- NOT smoking. Similar search statements were used for physiological and mechanistic articles. From these searches, 952 articles were found and, from these, 52 were deemed relevant and used in the review. The references in each of the relevant articles were searched to find any other articles that might have been missed by the automated searches.

A similar strategy was followed for a review of the health effects of chronic exposure. From these articles, 101 were deemed relevant and were used.

Chronic exposure

Definition of the health outcome

This review will discuss concisely and briefly human exposure to carbon monoxide in enclosed (i.e. closed) breathing spaces. Since outdoor air inevitably becomes indoor air, some consideration of carbon monoxide levels in outdoor air

and their effects on humans are required. To that end, there will be some discussion of epidemiological studies involving ultra-low-level carbon monoxide found in outside air. Exposure to high, potentially lethal levels are not considered here at any length and "delayed effects" are not examined because neither would be seen in indoor carbon monoxide exposure situations under normal circumstances. Because animal studies cannot at present provide much useful data about many aspects of the carbon monoxide poisoning syndrome *(76)*, they have been considered only in order to understand basic mechanisms by which carbon monoxide may impair human health.

This review extends the discussion of those issues involving carbon monoxide exposure in humans summarized in the 1999 WHO and 2005 European Union reports *(77,78)*. There has been no major attempt to recapitulate the review of most studies before roughly 1999. Other recent reviews on carbon monoxide exposure are available in monographs by Penney *(79–81)* and Kleinman *(6)*. Recourse to these works is strongly encouraged.

Tikuisis *(82)* reviewed human carbon monoxide uptake and elimination in 1996. Chen & Wang *(83)* reviewed the health effects of carbon monoxide in air pollution in major Chinese cities in 2000. Flachsbart *(84)* reviewed ambient and very low concentrations of carbon monoxide on humans more recently. Penney *(81)* recently reviewed pitfalls in making diagnoses of carbon monoxide poisoning, especially chronic poisoning. "Chronic" is defined as any exposure lasting more than 24 hours; "acute" is an exposure of 24 hours or less *(76)*.

Penney *(85)* reviewed the effects of carbon monoxide exposure on developing animals and humans in 1996. White *(86)* reviewed carbon monoxide poisoning in children in 2000. Public perceptions about carbon monoxide in the northern and southern regions of the United States, some relevant to indoor air, were investigated by Penney and published in 2008 *(87)*.

Penney reviewed the general characteristics of chronic carbon monoxide poisoning in humans in 2000 *(80)* and 2008 *(88)*, as did Hay et al. in 2000 *(89)* and Hay in 2008 *(90)*. In 2000,Greiner & Schwab *(91)* reviewed engineering aspects of carbon monoxide as it occurs in the living space.

Helfaer & Traystman *(71)* reviewed the cerebrovascular effects of carbon monoxide in 1996. In 2000, Hazucha *(92)* reviewed the effects of carbon monoxide on work and exercise capacity in humans. McGrath *(93)* reviewed the interacting effects on humans of altitude and carbon monoxide.

In 1996, Hiramatsu et al. *(94)* reviewed the impairment of learning and memory and neuronal dysfunction resulting from carbon monoxide exposure. In 2008, Hopkins *(95)* and Armstrong & Cunningham *(96)* reviewed the neurocognitive and affective outcomes of carbon monoxide poisoning in adults and children. Helffenstein *(97)* recently reported on a study investigating the neurocognitive and neurobehavioural sequelae of chronic carbon monoxide poisoning.

Early studies of chronic carbon monoxide poisoning

The early studies of Beck *(98,99)*, Lindgren *(100)*, Barrowcliff *(101)*, Wilson & Schaeffer *(102)*, Davies & Smith *(103)*, Trese et al. *(104)*, Kowalska *(105)*, Kirkpatrick *(106)*, Jensen et al. *(107)*, Ryan *(108)*, Tvedt & Kjuus *(109)*, Myers et al. *(110)* and Bayer et al. *(111)* on chronic carbon monoxide poisoning have been reviewed by Penney *(76)*. Other older studies, many coming out of the Second World War, have not been included in published reviews by this author. For example, Helminen *(112)* describes changes in the visual field caused by chronic coal gas (i.e. carbon monoxide) poisoning in 180 patients. The investigation was part of an extensive, systematic examination carried out at the First Medical Clinic of the University in Helsinki, Finland.

Sumari *(113)* describes the method used in Finland in examining victims of coal gas poisoning and the observations made in connection with it. The subject material comprises the results of the examination of 135 patients of which 71 are certain, "pure" chronic carbon monoxide cases. Of the cohort of 71, objective neurological symptoms were found in 60 cases. Out of 69 cases ophthalmologically examined, 66 gave positive results. Out of 65 cases oto-neurologically examined, the reaction of 52 was positive. In some cases the disease seemed to progress, although the patients being examined were then in surroundings free from coal gas.

Lumio, in an extensive 1948 study *(114)*, found fatigue, headache, vertigo, irritation, memory impairment, tinnitus and nausea to be the most frequent symptoms resulting from chronic carbon monoxide poisoning. Hearing disturbances were noted in 78.3% of the patients suffering from chronic carbon monoxide poisoning. A smaller number of hearing disturbances (26.7%) were found in patients exposed to carbon monoxide at work but in whom chronic carbon monoxide poisoning could not be confirmed. Thus, hearing disturbances were present in approximately three times as many patients suffering chronic carbon monoxide poisoning as in patients not affected. The majority of patients had a similar pattern of hearing deficiencies. The threshold of hearing was about normal at frequencies up to 1000 Hz. Hearing loss occurred above that frequency. This pattern of hearing deficiency was noted in 67.7% of patients who had suffered chronic carbon monoxide poisoning, but in only 14% of patients not so affected. Often, patients themselves were not aware of the presence of a hearing deficiency. Of those suffering from chronic carbon monoxide poisoning, 47.9% complained of hearing impairment during the time they were exposed to the carbon monoxide. The audiogram, however, showed changes in 78.3% of the patients with carbon monoxide poisoning. Follow-up examinations revealed that typical hearing losses improved only slightly or not at all. An improvement in hearing was found in only 26.7% of the cases, and it was always slight. The data suggest that typical hearing deficiency may appear during the initial stage of chronic carbon monoxide poisoning, when vestibular symptoms are not yet

present. For additional details see the Carbon Monoxide (CO) Headquarters web site (http://www.coheadquarters.com/ChronicCO/indexchronic2.htm).

Von Zenk *(115)* reported on rhino-cochlear-vestibular symptoms in 80 suspected cases of chronic carbon monoxide poisoning. The cochlear findings showed a perceptive disturbance with a high tone loss and largely retroganglionic damage. Subjective symptoms included vertigo that was accompanied by nystagmus more commonly in the confirmed group. There was also a diminution of the sense of smell.

Komatsu et al. *(116)* examined 733 workers at a steel-making facility. Mean ages of four groups broken out of the cohort was approximately 32 years (no significant difference). Group A1 was exposed to 58–291 mg/m^3, Group A2 to 70–1595 mg/m^3, Group B to < 23 mg/m^3 and Group C to < 12 mg/m^3 carbon monoxide in the course of their normal work. Median COHb saturation was 10–15% in Group A1, 20–25% in Group A2, 1–5% in Group B and 1–5% in Group C. The average frequency of health complaints was much higher for members of Groups A1 and A2 than for those of Groups B and C. A large variety of subjective health complaints were made by Group A1 and especially Group A2 members. For example, the highest frequency of complaints in reports included headache, poor hearing, chest pain, lassitude, fatigue and forgetfulness. A variety of objective health complaints were made by Group A1 and especially Group A2 members. The highest incidences, for example, included pallor, cardiac enlargement (cardiomegaly), coldness of the extremities and hyperactive patellar reflex. Average vital capacity was significantly less for members of Group A at any age than for members of Groups B or C. Average back strength was significantly less for members of Group A at age 30–40 years than for same-age members of Group C. The difference from members of Group B was very large and significant over the entire age range of the two groups.

Smith & Landaw *(117)* reported that smokers develop polycythaemia. Furthermore, smoking at increased elevation dramatically increases the extent of the polycythaemia. This, along with cardiomegaly, has been demonstrated numerous times following chronic carbon monoxide exposure in animals *(118,119)*.

Stern et al. *(120)* studied the effects of carbon monoxide exposure on deaths of New York City bridge and tunnel employees over the period 1952–1981. It was found that the tunnel workers experienced a 35% excess risk compared with the New York City general population; among the less exposed bridge workers the risk was not elevated. The elevated risk among the tunnel workers declined significantly within five years after ending occupational exposure, and there was also a significant decline after 1970, when a new ventilation system lowered carbon monoxide levels inside the tunnels and tunnel booths. The 24-hour average tunnel carbon monoxide concentrations were approximately 58 mg/m^3 in 1961 and 47 mg/m^3 in 1968. During periods of rush hour traffic in 1968, carbon monoxide concentrations in tunnel toll booths were as high as 76–192 mg/m^3.

Retrospective and case studies

Two questionnaire studies (A and B) of chronic carbon monoxide poisoning in North America have been reported by Penney *(76)*. A third questionnaire study (C) of 61 individuals sustaining chronic carbon monoxide poisoning was recently reported by Penney *(121)*. The large questionnaire study conducted in the United Kingdom in 1997 under the title "Carbon monoxide support" has been reviewed by Hay et al. *(89)*.

Two cases of chronic carbon monoxide poisoning in children *(122,123)* have been discussed by White *(86)* and another *(124)* by Hay *(90)*. Armstrong & Cunningham *(96)* report on three cases of chronic carbon monoxide poisoning in young children and the functional and developmental effects that resulted. A review of the effect of chronic or intermittent hypoxia on cognition in childhood *(125)* included carbon monoxide poisoning; it concluded that adverse effects have been noted at even mild levels of oxygen desaturation and that "studies of high-altitude and carbon monoxide poisoning provide evidence for causality".

Other studies looking at neuropsychological aspects of chronic carbon monoxide exposure such as those of Ryan *(108)*, Myers et al. *(110)*, Pinkston et al. *(126)*, Hartman *(127)* and Devine et al. *(128)* have recently been thoroughly reviewed by Helffenstein *(97)*. Helffenstein's findings from his own study of 21 people chronically exposed to carbon monoxide are detailed in that same 2008 source.

Ely et al. *(129)* describe 30 people who developed "warehouse workers' headache". COHb levels in the workers most exposed to exhaust gases were 21.1%. It is understood that this condition in the warehouse had continued for some time, making the exposure "chronic" rather than "acute". A majority of the people experienced acute difficulty with headache, dizziness, weakness, nausea and chest pain. Some complained of shortness of breath, vomiting, muscle cramps, difficulty in concentrating, visual changes and confusion. Follow-up symptoms present two years after the carbon monoxide exposure included numbness in the extremities, restlessness, persistent headaches, irritability, confusion, difficulty in walking or moving the extremities, and memory loss.

Walker *(130)* states that the incidence of chronic carbon monoxide exposure in Great Britain is officially 200 per year, while at the same time "250 000 gas appliances are condemned annually". He speculates that if only 10% of these appliances give off significant amounts of carbon monoxide that reach the breathing space of residents, as many as 25 000 people every year may be exposed to carbon monoxide in their homes. The carbon monoxide support study *(89)* found that "only one case out of 77 was correctly identified (i.e. diagnosed) on the basis of symptoms alone" and that medical professionals were the least likely group to "discover" the fact of the carbon monoxide poisoning.

Thyagarajan et al. *(131)* report on a 37-year-old woman chronically exposed to carbon monoxide for seven years. Her symptoms included seizure, persistent tiredness, problems with balance, headache associated with cognitive symptoms,

personality changes and depression. Magnetic resonance imaging of her brain five years after the end of carbon monoxide exposure showed a well-defined lesion in the globus pallidus, on the left. Hippocampal atrophy was also suggested. This case indicates that unilateral lesioning resulting from carbon monoxide poisoning can occur.

Prochop *(132)* reports on the case of four people chronically exposed to carbon monoxide in an apartment building in Florida as the result of a faulty gas heater. All four suffered transient loss of consciousness immediately prior to discovery of the problem. All four incurred cognitive impairments, while two also experienced residual coordinative deficits. Magnetic resonance imaging of the four people was said to be normal. One victim had an abnormal magnetic resonance spectroscopy scan.

Sari et al. *(133)* investigated an association between chronic carbon monoxide exposure and P-wave and QT interval characteristics of the electrocardiogram in 48 healthy male indoor barbecue workers and 51 age-matched healthy male controls. COHb in the two groups was 6.48% and 2.19%, respectively. Using Pearson analysis, there were significant correlations between COHb level and P-wave duration, maximum QT height, QT duration and corrected QT duration.

In a clinical review, Weaver *(134)* states that "lower level CO exposures can cause headache, malaise, and fatigue and can result in cognitive difficulties and personality changes". This assertion is borne out by Chambers et al. *(135)* (see Hopkins *(95)*), who prospectively followed 256 patients, 55 with "less severe" and 201 with "more severe" carbon monoxide poisoning. Less severe poisoning was defined as no loss of consciousness and a COHb level of ≤ 15%, while more severe poisoning was defined as loss of consciousness or a COHb of >15%. Of the less severely poisoned patients, 39% had cognitive deficits at six weeks. Of those more severely poisoned, 35% had cognitive deficits. In the less vs more severe groups, the incidence of depression was 21% and 16%, respectively, and that of anxiety was 30% and 11%, respectively. There was no difference in cognitive outcomes between the two groups. Interestingly, the prevalence of depression was higher in patients with the less compared with the more severe poisoning at six months. Likewise, the prevalence of anxiety was higher in patients with the less compared with the more severe poisoning at six weeks. These results suggest that loss of consciousness is not a requirement for carbon-monoxide-induced brain damage, and that carbon-monoxide-related cognitive (and other) outcomes may be independent of poisoning severity when that severity is based on COHb saturation.

In a recent clinical study, Keles et al. *(136)* characterized their patients as having acute carbon monoxide poisoning, when in actual fact most had chronic poisoning since the authors cite coal stoves and water heaters as carbon monoxide sources. These devices do not deteriorate overnight. Many studies do not characterize the exposure condition at all, or will characterize it as acute when in fact

Table 2.3. Summary data from five studies on chronic carbon monoxide poisoning

Study	Carbon monoxide (mg/m^3) AM (SD)	N1	COHb (%) AM (SD)	N2
Bayer et al. *(111)*	0–50 (estimated)	56	0.4–5.8	
Penney (A) *(76)*	497 (519)	15	9.65 (8.16)	11
Penney (B) *(76)*	257 (164)	25	9.0 (8.62)	29
Penney (C) *(121)*	174 (131)	23	9.2 (4.50)	12
Helffenstein *(97)*	143 (144)	14	14.5	2

Note: AM = arithmetic mean; SD = standard deviation.

it is chronic. The study found that COHb could not be used to rule out carbon monoxide poisoning. This has been known for some time, i.e. the poor relationship between COHb, symptoms and outcome. The most common symptoms they recorded were headache, nausea, dizziness and syncope.

Table 2.3 provides summary data from five studies on chronic carbon monoxide poisoning: Bayer et al. *(111)*, Penney *(76,121)* and Helffenstein *(97)*. N1 is the number of cases for which air carbon monoxide concentration data are available. N2 is the number of cases for which COHb data are available. It should be noted that, for all five studies, average COHb levels fall within the "less severe" carbon monoxide poisoning group as defined by Chambers et al. *(135)*.

Epidemiological studies

Epidemiological studies reported prior to 2000 dealing with carbon monoxide effects relative to mortality, birth weight, asthma, congestive heart failure, coronary artery disease, psychiatric admissions, etc. in humans have been reviewed by Penney *(76)*. The topic of congestive heart failure and environmental carbon monoxide levels was also reviewed by Morris *(137)*.

Mar et al. *(138)* evaluated the association between mortality in the elderly and air pollutants over a three-year period in Phoenix, Arizona. Total mortality was found to be significantly correlated with changes in ambient carbon monoxide and nitrogen dioxide, whereas cardiovascular mortality was significantly associated with carbon monoxide, nitrogen dioxide, sulfur dioxide, etc.

Moolgavkar *(139)* investigated non-accidental cardiovascular, cerebrovascular and chronic obstructive pulmonary disease deaths over eight years in three American metropolitan areas: two in California and one in Illinois. Carbon monoxide level was particularly found to have a stronger association with mortality than level of particulate matter. This association was noted to be stronger in Los Angeles County. This study is similar to an earlier epidemiological investigation by Hexter & Goldsmith *(140)*, reviewed by Penney *(76)*.

Hajat et al. *(141)* found a relationship between ambient carbon monoxide and asthma consultations for children in London. Sheppard et al. *(142)* examined the relationship between asthma and air carbon monoxide levels in Seattle for data

during the period 1987–1994. They found a 6% increase in the rate of hospital admissions for asthma related to carbon monoxide, with a three-day lag.

Yu et al. *(143)*, in another study in Seattle, found a 30% increase in asthma in children for a 1.2-mg/m^3 increment in carbon monoxide that lagged one day. They estimated 25% increases in the odds of increases in carbon monoxide, conditional on the previous day's asthma symptoms. It was concluded that there is an association between change in short-term air pollution levels and the occurrence of asthma symptoms among children in Seattle.

Karr et al. *(144)* analysed nearly 12 000 diagnoses of infant bronchiolitis between 1999 and 2002 in south-west British Columbia. They looked at infants' exposure within 10 km of home, and were able to account for confounding variables including sex, gestational age, maternal smoking and breastfeeding. An interquartile increase in exposure to nitric oxide, nitrogen dioxide, sulfur dioxide and carbon monoxide increased bronchiolitis risk by 8%, 12%, 4% and 13%, respectively. Infants living within 50 metres of a highway had an increased risk of 6%; those living in an area with higher exposure to wood smoke had an increase of 8% in their risk of bronchiolitis. Carbon monoxide posed the largest risk for bronchiolitis among the pollutants examined.

In studies by Hong et al. *(145,146)*, the occurrence of acute stroke mortality in Seoul is reported to be related to air pollution. Data covering 4- and 7-year periods were analysed. In the first study, stroke mortality increased 4.1% with a two-day lag. In the second study, a significantly increased risk of 1.06 (95% CI 1.02–1.09) was found for carbon monoxide, with a one-day lag. Nitrogen dioxide and ozone also appeared to play a role. This suggests, according to the authors, "an acute pathogenetic process in the cerebrovascular system induced by air pollution".

Yang et al. *(147)*, in a "case cross-over study" carried out on data for Kaohsiung (Taiwan, China), found that carbon monoxide and other air pollutants were significantly associated with increased numbers of admissions for cardiovascular diseases (CVD) on both warm and cool days. This study provides evidence that exposure to "higher levels of ambient contaminants, particularly carbon monoxide, increase the risk of hospital admissions for CVD".

Barnett et al. *(148)*, looking at data from Australia and New Zealand, found an association between outdoor air quality and cardiovascular hospital admissions. They found that for a 1-mg/m^3 increase in carbon monoxide, there were significant increases in hospital admissions of elderly people for total cardiovascular disease (2.2%), all cardiac disease (2.8%), cardiac failure (6.0%), ischemic heart disease (2.3%) and myocardial infarction (2.9%). In matched analyses, carbon monoxide had the most consistent association.

Bell et al. *(149)* studied hospital admissions for cardiovascular disease in 126 urban counties in the United States during 1999–2005. They found a positive and statistically significant association between same-day carbon monoxide exposure and increased risk of hospitalization for multiple cardiovascular outcomes

(ischemic heart disease, heart rhythm disturbances, heart failure, cerebrovascular disease and total cardiovascular disease). A 1.2-mg/m^3 increase in same-day daily 1-hour maximum carbon monoxide was associated with a 0.96% (95% CI 0.79–1.12) increase in risk of cardiovascular admissions.

In 1995, Morris et al. *(150)* reported an association between ambient carbon monoxide levels in seven United States cities and hospital admissions for congestive heart failure among elderly people, which showed a consistent association with daily variations in ambient carbon monoxide. This association was independent of season, temperature and other major gaseous pollutants. In 1997, Burnett et al. *(151)* found a similar association in ten Canadian cities. The logarithm of the daily high-hour ambient carbon monoxide concentration recorded on the day of admission displayed the strongest and most consistent association with hospital admission rates among the pollutants, after stratifying the time series by month of the year and simultaneously adjusting for temperature, dew point and the other ambient air pollutants. The relative risk for a change from 1.2 mg/m^3 to 3.5 mg/m^3, the 25th and 75th percentiles of the exposure distribution, was 1.065.

Yang *(152)* re-examined the reported association between air pollutant levels and hospital admissions for congestive heart failure in Taipei in 2008. The data examined covered the period 1996–2004. The number of admissions for congestive heart failure was significantly associated with the environmental presence of carbon monoxide and several other pollutants. Statistically significant positive effects on increased congestive heart failure admissions on cool days were observed only for the carbon monoxide levels.

Stieb et al. *(153)* conducted a study of nearly 400 000 emergency department visits to 14 hospitals in Canada between the early 1990s and the early 2000s. Twenty-four-hour averages of carbon monoxide and nitrogen dioxide exhibited the most consistent associations with cardiac conditions: 2.1% (95% CI 0.0–4.2) and 2.6% (95% CI 0.2–5.0) increase in visits, respectively, for myocardial infarction and angina per 0.8 mg/m^3 carbon monoxide. Thus, daily average concentrations of carbon monoxide and nitrogen dioxide exhibited the most consistent associations with emergency department visits for cardiac conditions.

Dales et al. *(154)* examined an association between air pollution and daily numbers of hospital admissions for headache in seven Chilean urban centres during the period 2001–2005. Relative risks for migraine associated with interquartile-range increases for carbon monoxide was 1.11 (95% CI 1.06–1.17) for a 1.3-mg/m^3 increase in carbon monoxide concentration. The authors concluded that air pollution increases the risk of headache in Santiago Province. There was no significant effect of modification by age, sex or season.

In a massive epidemiological study, Ritz & Yu *(155)* studied a cohort of 125 573 singleton children born in Los Angeles. Excluded were infants born before 37 or after 44 weeks of gestation, those weighing below 1000 or above 5500 grams at birth, those for whom fewer than 10 days of carbon monoxide measurements

were available during the last trimester, and those whose mothers suffered from hypertension, diabetes or uterine bleeding during pregnancy. Within the cohort, 2813 (2.2%) were low in birth weight (between 1000 and 2499 grams). Exposure to higher levels of ambient carbon monoxide (> 6.4 mg/m^3, 3-month average) during the last trimester was associated with a significantly increased risk for low birth weight (odds ratio (OR) 1.22; 95% CI 1.03–1.44) after adjustment for potential confounders, including commuting habits in the monitoring area, sex of the child, level of prenatal care, and the age, ethnicity and level of education of the mother. Levels of environmental carbon monoxide previously thought to be extremely low were shown to reduce birth weight in women exposed to carbon monoxide during the last trimester of pregnancy.

Maisonet et al. *(156)* followed the Los Angeles study with an investigation on birth weight in Boston, MA, Hartford, CT, Philadelphia, PA, Pittsburgh, PA, Springfield, IL and Washington, DC. Their results suggest that exposure to ambient carbon monoxide (and sulfur dioxide) increases the risk of low birth weight at term. This risk is increased by a unit rise in the average concentration of carbon monoxide in the third trimester.

Chen et al. *(157)* assessed the association between ambient air pollution and daily elementary school absenteeism in Washoe County, Nevada in the period 1996–1998. A total of 27 793 students were enrolled. The daily average absence rate was 5.09% (SD = 1.54%). The daily average carbon monoxide concentration was 3.2 mg/m^3. After adjustment for the effects of weather, day of the week, month, holidays and time trend, they found that carbon monoxide and oxygen were statistically significant predictors of daily absenteeism. For every 1.2-mg/m^3 increase in carbon monoxide concentration, absence increased by 3.79% (95% CI 1.04–6.55).

Two studies examining cardiovascular events and long-term exposure to carbon monoxide at ultra-low levels (i.e. 1.2–1.8 mg/m^3) found no significant association with changes in the carbon monoxide concentration in ambient air *(158,159)*.

Experimental studies

Past reviews of air quality mainly discuss acute studies of carbon monoxide exposure at lower concentrations. Even though hypoxic stress may have been the only underlying mechanism at work, some nonetheless reported positive effects. It can be argued that when considering exposure to air pollution in human residential and work environments, these studies have limited significance and model rather poorly human responses to long-term carbon monoxide exposure.

Symptomatology

Recognizing the onset of carbon monoxide poisoning is crucial, as it can be fatal in just a few minutes. The symptoms are usually non-specific and appear to

involve many of the body systems. Common symptoms include headache, lethargy/fatigue, nausea, dizziness and confusion. A victim may also suffer from shortness of breath, cardiac palpitations, convulsion, paralysis, loss of consciousness, coma and eventually death. Many reviews list the step-wise onset of various symptoms in acute carbon monoxide poisoning as they relate to blood COHb levels. However, the relationship in reality between blood carbon monoxide levels and symptomatology is extremely poor. There is no hyperventilation induced by carbon monoxide poisoning or increased salivation, taste/odour changes, eye watering or coughing, as are produced by carbon monoxide's toxic twin, hydrogen cyanide. Age, anaemia, increased elevation, cardiopulmonary disease and prior exposure to carbon monoxide can increase susceptibility to carbon monoxide toxicity. The median level of COHb in people dying of uncomplicated carbon monoxide poisoning is 53–55%.

An important key to identifying carbon monoxide poisoning is the victim's environment and immediate past living or work situation. Was the victim exposed to sources of carbon monoxide such as uncontrolled fires, motor vehicles, fuel-burning heaters or other internal combustion engines in a poorly ventilated enclosed space? Are others in that environment (e.g. family members or pets living in the same house) displaying similar symptoms? These facts are critical in accurately identifying carbon monoxide poisoning.

First and foremost, the victim must be moved out of the contaminated area into fresh air. Eventually, the carbon monoxide will be eliminated from the blood through normal ventilation, although often serious health damage may be done before this can occur, so emergency measures should be started immediately.

In 1895, John Scott Haldane demonstrated that rats survive carbon monoxide poisoning when placed in oxygen at two atmospheres pressure. In 1942, End & Long treated carbon monoxide poisoning in experimental animals with hyperbaric oxygen. The first human clinical use of hyperbaric oxygen therapy in carbon monoxide poisoning was by Smith & Sharp in 1960 *(80)*. This type of therapy is now recommended for most seriously, acutely poisoned victims, but there have been some studies that fail to show its efficacy *(81)*. If hyperbaric oxygen therapy is to be used, it must be initiated immediately (within 12 hours) on reaching a health care facility.

Pathophysiological mechanisms

Since the time of Haldane *(52)*, it has been presumed that the attachment of carbon monoxide to haemoglobin, thus preventing the carriage of adequate oxygen and the impaired release of oxygen from the remaining oxyhaemoglobin (i.e. hypoxic stress) was the major mechanism by which carbon monoxide exerts its health-damaging effects. At low COHb levels and in the presence of normal vasomotion and hyperaemia, it has been difficult to understand how carbon monoxide can cause immediate or long-term cellular, tissue and organ damage. Evi-

dence for various cellular mechanisms not requiring hypoxic stress has recently appeared. See also http://www.coheadquarters.com/coacute.mech1.htm.

Ischiropoulos et al. *(160)* found in rat studies that the potent oxidant species, peroxynitrite, was generated in the brain from nitric oxide and that a cascade of events could lead to "oxidative stress" in carbon monoxide poisoning. Thom & Ischiropoulos *(161)* reported that platelets released nitric oxide when incubated with carbon monoxide and that carbon monoxide concentrations as low as 12 mg/m^3 were capable of doing this in vitro. They concluded that carbon monoxide levels produced in vivo when humans are exposed to carbon monoxide "can cause endothelial cells to liberate nitric oxide and derived oxidants, and that these products can adversely affect cell physiology". Using microelectrodes in rats, it was seen that carbon monoxide exposure caused nitric oxide concentration to nearly double to 280 nM through the modulation of nitric oxide synthase *(162)*.

It was found that platelet activating factor was involved in the adherence of neutrophils to brain endothelium after carbon monoxide poisoning and that the process required nitric-oxide-derived oxidants *(163)*. Thom et al. *(164)* postulated that carbon monoxide poisoning causes "adduct formation between myelin basic protein (MBP) and malonylaldehyde, a reactive product of lipid peroxidation, resulting in an immunological cascade". It was found that carbon-monoxide-poisoned rats displayed impaired maze-learning that did not occur in similar rats made immunologically tolerant to MBP. They suggest that this mechanism may explain brain damage occurring days after treatment for carbon monoxide poisoning, and be the reason for the observed lack of a simple dose–response relationship between COHb level at presentation and outcome. The use of hyperbaric oxygen following carbon monoxide poisoning in rats prevented deficits in maze-learning performance and MBP immune-mediated neurological dysfunction *(165)*.

In blood obtained from 50 patients who had sustained carbon monoxide poisoning, platelet–neutrophil aggregates were detected and plasma myeloperoxidase concentration was elevated, suggesting that the processes seen in animals also operate in humans *(166)*.

Thus, recent studies suggest that the intracellular uptake of carbon monoxide could be a major cause of neurological damage (i.e. brain damage). When carbon monoxide binds to cytochrome oxidase, it causes mitochondrial dysfunction. The release of nitric oxide from platelets and endothelial cells inside blood vessels, forming the free radical peroxynitrite, further inactivates mitochondrial enzymes and damages the vascular endothelium of the brain. The end result is lipid peroxidation of the brain, which starts during recovery from carbon monoxide poisoning. With reperfusion of the brain, leukocyte adhesion and the subsequent release of destructive enzymes and excitatory amino acids amplify the initial oxidative injury. Such endovascular inflammation may be a major mechanism leading to organ dysfunction.

Other recent studies indicate that carbon monoxide poisoning can cause immune system dysfunction *(164)* that causes decrements in learning not observed in immunologically tolerant animals. This may be based on adduct formation between MBP and malonylaldehyde, a reactive product of lipid peroxidation, resulting in an immunological cascade. Thus, carbon monoxide poisoning appears to trigger immunological reactions, just as a number of other disease states do. Therefore, a third damaging mechanism of carbon monoxide exposure appears to be through its action on the immune system.

The information summarized above suggests that the damaging effects of carbon monoxide are not only due to its action in binding to haemoglobin and interfering with oxygen delivery, i.e. hypoxic stress. Although this process certainly takes place and is undoubtedly important in higher-level and acute carbon monoxide poisoning, other processes not previously known result in endothelial inflammation and immune activation, causing interference with blood flow and the destruction of cellular machinery. The operation of these pathways and their products explain the effects of carbon monoxide at very low air–carbon monoxide and COHb levels, and what occurs during extended exposure, and finally the seeming lack of a dose–response relationship between air–carbon monoxide concentration, COHb, immediate symptoms and the long-term health effects.

Acute exposure

Effects on exercise duration

There have been no reliable demonstrations of health effects due to acute carbon monoxide exposure in normal, healthy people where exposures resulted in COHb levels below 6%, except for limitation of maximal exercise duration. In laboratory experiments, people exposed to carbon monoxide before maximum exercise tests had reduced exercise duration *(167–172)*. The duration was reduced as an inverse function of COHb level. A linear equation was fitted to the data *(167)* but the equation should have been curvilinear. This is clear from inspection of the data because the zero COHb point, had it been included in the fitting, would have been plotted well below the intercept of the fitted curve. At higher COHb, however, the curve is nearly linear. An increase in COHb of 4.5% produced a drop in exercise time of about 30 seconds. In the Ekblom & Huot study *(167)*, the baseline mean exercise duration was about 5.2 minutes. Another metric of the effect magnitude was calculated by estimating the maximum total calories expended from the amount of work performed. Here, a 4.5% increase in COHb level reduced the maximum exercise from a total expenditure of about 112 kcal to some 90 kcal.

The exercise effect of carbon monoxide exposure in healthy subjects was produced by reduced oxygen delivery to the exercising muscle. At 20%, COHb reduced the arterial oxygen content from about 19.8% to about 15.8% by volume.

Normally, one would expect reduced oxygen dissociation from arterial blood into muscle tissue because of the shift in the dissociation curve, but in the case of exercising muscle the oxygen partial pressure of the tissue is likely to have been so low that the dissociation shift did not matter *(167)*.

Also, at maximum exercise, no further increase in blood flow to the muscle was possible. Thus, in this experiment, the only appreciable determinant of tissue oxygenation was the COHb. No account of the possible role of carboxymyoglobin was possible.

When laboratory maximal exercise testing was done with patients who exhibited stable angina pectoris due to coronary artery disease, the results were quite different from normal subjects *(173–178)*. Here the subjects were also given maximal exercise tests, but the criterion for stopping was not exhaustion but the onset of angina. Subjects were also exposed to lower levels of carbon monoxide, producing a maximum of nearly 6% COHb. In the baseline (no carbon monoxide) condition, the mean maximum exercise time was around 8.2 minutes. Allred et al. *(175)* showed that an increase in COHb of 4.5% reduced exercise time by 36 seconds and reduced total maximum energy expenditure from about 64 kcal to about 30 kcal. Thus it is seen that the magnitude of effect produced by an increase in COHb of 4.5% in not dramatically greater than for normal subjects. The difference is that the cardiac impairment has simply reduced the baseline exercise ability.

The angina patient's baseline exercise ability was reduced from a maximum energy expenditure of 112 kcal to 64 kcal by the inability of the heart to supply sufficient blood flow to provide oxygen to the exercising muscles. The further decrease in exercise time was due to the same mechanism as for normal subjects (reduced arterial content of the same magnitude), which produced nearly the same magnitude of effect. To be sure, the percentage exercise reduction is greater for the angina patients than for the normal subjects, but this is simply due to the reduction in baseline exercise ability.

It is not clear whether the slightly greater observed effect of COHb in the patients compared to the normal subjects would be considered statistically significant or physiologically meaningful. Another consideration in the angina data is the fact that COHb was not extended to higher levels as it was for normal subjects. Clearly, this was done for ethical reasons, but the possibility exists that higher exposures would have led to greater magnitudes of effect than for normal subjects.

It might be argued that the data on the effect of carbon monoxide exposure in angina patients contributes little additional information needed for regulatory decisions. However, heart disease is a leading cause of sickness and death worldwide, and it is plausible that coronary artery disease would make patients more susceptible to cardiac failure from increased hypoxic cardiac stress *(179)*, but there are no data to evaluate this hypothesis. On the other hand, individuals

with heart disease represent a large fraction of the population and therefore the angina studies do address an issue of public health concern.

Brain function effects

Clinical reports of symptoms of low-level acute carbon monoxide poisoning (headache and nausea) are commonly cited *(180)* for COHb levels of 10–20% but were not observed in a double-blind study for COHb levels below 20% *(181)*. Headache and nausea were reported in a double-blind study at COHb levels of 25–30% *(182)*.

A large number of behavioural studies were critically reviewed by Benignus *(183,184)* involving sensory, psychomotor, vigilance, cognitive and schedule-controlled behaviour in both humans and rats. Human studies were largely unreliable in the sense that they were not replicable, sometimes even by their original authors. Rat studies were highly consistent but demonstrated statistically significant effects only when COHb exceeded about 20%.

Benignus *(183)* meta-analysed the carbon monoxide literature, fitting dose–effect curves and attempting to relate the rat and human carbon monoxide data and the human hypoxia data. The rat carbon monoxide data were meta-analysed and the internal dose (oxygen delivery by arterial blood) was estimated. The extra behavioural effect of hypothermia (which results from COHb increase) was also estimated and subtracted. The internal dose for humans exposed to carbon monoxide was also calculated, but hypothermia (which does not occur in humans for the duration of acute exposures) was not considered. The internal dose for hypoxic hypoxia in humans was calculated, in addition to the hypocapnia (which occurs due to hyperventilation in hypoxic hypoxia but not carbon monoxide exposure). The carbon monoxide effects were corrected by subtracting the effects of hypocapnia. When all of the internal doses and the behaviourally corrected dose–effect curves were compared, they nearly overlay each other. The conclusion was that, when arterial oxygen content was used as the internal dose and extraneous effects were subtracted, the behavioural effects of carbon monoxide hypoxia and hypoxic hypoxia were of equal magnitude for humans and were equal in rate to the magnitude of carbon monoxide hypoxia. The results were expressed in equivalent of estimated COHb.

The above-mentioned dose–effect curves reached the 10% effective dose (ED-10) at mean COHb ~ 20%, with upper and lower 95% confidence limits of about 22.2% and 18.8% *(184)*. The ED-10 was selected as a point of interest because in the behavioural literature, and with the typical number of subjects, the ED-10 is about the magnitude of effect that becomes statistically significant or behaviourally important. A continuous non-linear function was fitted to the data and thus there is a continuum of magnitude of effect estimates, which may be used to estimate severity of effects between zero and about 30% COHb and higher by extrapolation from rats. It may not be inferred from these results that effects be-

low a COHb of 20% are absent; they gradually diminish towards zero at a COHb of zero.

These results provide an example of compensatory physiological action, i.e. the increased arterial blood flow to the brain sufficient to keep tissue oxygen supply nearly constant *(73,185)*. It was observed by these workers that brain energy metabolism remained statistically unchanged until COHb exceeded 20%, because up to that point blood flow could increase sufficiently to offset the carbon-monoxide-induced hypoxia. At COHb levels of around 30%, the brain metabolism fell precipitously. These physiological results agree almost exactly with the behavioural data.

It is interesting that small decreases in mean brain energy metabolism as well as in mean behaviour are estimated to occur below 20% COHb. This could be attributed to an actual small effect or to some small fraction of susceptible subjects having larger effects or to an inappropriate statistical model for the dose–effect curves. This is an area requiring additional study, since at the present stage of knowledge the question cannot be resolved.

An implication of the above analysis is that if, owing to some pre-existing cardiovascular or pulmonary disease, the compensatory increase in blood flow were impaired, small increases in COHb could produce larger decreases in tissue oxygen and thus larger behavioural effects. No data have been reported to test this hypothesis.

Compromised brain function, in addition to being an adverse effect in itself, can contribute to sensory impairment that could result in failure to detect signs of danger or could impair decision-making capabilities, leading to an inability to respond appropriately to danger. The ability to avoid or flee danger could also be impaired by carbon-monoxide-induced limitations on exercise. Such effects of acute exposure can potentially lead to consequences ranging from minor injuries to serious injuries and death. Behaviourally or physically impaired people exposed to carbon monoxide could also endanger others in their vicinity.

Quality of the exposure and effects measures

It has been customary to specify the "dose" of carbon monoxide as either the amount in blood as COHb or as the concentration in the inhaled air. The effects of carbon monoxide are, however, not strictly determined by either of these metrics. The health effects are a product of tissue functioning and these, in turn, are functions of some tissue dose metric. An effort is made below to specify tissue dosimetry where knowledge permits and to point to gaps in knowledge when appropriate.

Susceptible populations and effect modifiers

Any person with some form of impaired oxygen uptake and delivery would be more sensitive to the acute hypoxic effects of carbon monoxide exposure. Thus,

hypothetically, any cardiac, vascular or pulmonary disease would have such an effect, as would other factors that limit the blood's ability to transport oxygen, such as anaemia. Also, presumably, multiple diseases in a particular person could increase that individual's risk of greater effects; the potential interaction need not necessarily be simply additive. The severity of a given disease state would influence the maximum COHb, possibly before adverse effects became noticeable, and could determine the maximum amount of effort that could be expended. The magnitude of a carbon monoxide effect would depend on the amount of oxygen available for metabolism in the tissue under consideration. Because multiple cardiac, vascular and pulmonary diseases in one person are not uncommon, it would not be surprising if some impaired people were adversely affected by even small increases in COHb. No data are available to evaluate this conjecture, but quantitative physiological analyses to further delimit the range of effects would be possible.

Other possible sensitive groups are pregnant women, whose endogenous COHb is greater, and fetuses, whose haemoglobin has somewhat greater affinity to carbon monoxide than that of adults.

There are numerous situations in which carbon monoxide is not the only source of hypoxia. Until a person is adapted to high altitude, the resulting arterial hypoxia is directly additive (in terms of arterial oxygen content) to carbon monoxide hypoxia *(178)*, and the increased pulmonary ventilatory response also increases carbon monoxide uptake. Increased inhaled carbon dioxide increases pulmonary ventilation and thus carbon monoxide uptake. Hydrogen cyanide inhibits tissue respiration and thus adds to hypoxic effects, in addition to strongly stimulating increased pulmonary ventilation. These effects are of interest because all of the above pollutants are combustion products. These effects are enumerated in detail by Benignus *(184)* and physiological effects and interactions have also been quantitatively estimated in interesting cases by Benignus *(186)* using computerized mathematical models of physiological function. Thus, the presence of any or all of the above combustion gases would exacerbate the effects of carbon monoxide exposure.

The concomitant behaviour of people exposed to carbon monoxide can also make them more sensitive to its effects. Higher rates of physical exercise increase pulmonary ventilation, thereby increasing the COHb formation rate, and increase oxygen metabolism, exacerbating the hypoxia. Increased body temperature from external heat or inappropriate clothing would increase pulmonary ventilation. Those who are anxious owing to emotional or psychological conditions have increased pulmonary ventilation.

Clearly, impaired persons could be exposed to multiple hypoxic toxicants while engaged in situations in which pulmonary ventilation would be elevated. Even though the carbon monoxide in these environments might be insufficient to produce effects in controlled laboratory experiments, the real world is much

more complicated and the possibility of such complex multiple effects cannot be dismissed.

Health risk evaluation

There are several health concerns associated with exposure to carbon monoxide. The best understood health effects appear to be produced by hypoxia due to the binding of carbon monoxide to haemoglobin, which reduces the oxygen-carrying capacity of the blood as well as decreasing the dissociation of oxygen into extravascular tissue. COHb is widely used as a biomarker for carbon monoxide exposure. Carbon monoxide also binds with myoglobin and cytochrome oxidase and P-450, but the magnitude and the effects of such binding are less well explored.

High-level exposures (over several hundred mg/m^3) can cause unconsciousness and death. There can be severe and permanent CNS damage, even in cases where individuals do not experience loss of consciousness. Evidence is also mounting that carbon monoxide can produce a cascade of cellular events leading to adverse effects that are not necessarily ascribable to hypoxia (i.e. COHb may be a less reliable biomonitor for these effects).

Acute exposure

Acute laboratory exposure to carbon monoxide in healthy young people has been shown to decrease duration of maximum exercise tests in a COHb (dose)-related manner. The same phenomena were demonstrated in patients with stable angina, but only at a lower range of COHb. The latter effect is presumably due to limitation of heart oxygen supply because of an inability to increase blood flow in the presence of, for example, stenoses in the coronary arteries.

In early acute laboratory exposures of healthy young people, brain function (as measured by reduced behavioural performance) was reported to be impaired in a COHb-related manner when COHb ranged from 2.5% to around 10%. These studies were, however, not replicable in any case where such replication was attempted. It has been suggested, based on physiological analysis and extrapolation, that brain function should not be reduced by more than 10% until COHb approaches around 18%. With laboratory carbon monoxide exposures of a few hours' duration, no symptoms were reported, even for COHb approaching 20%. Such high effect thresholds were attributed to the compensatory effect of the increased brain blood flow that accompanies increased COHb.

As COHb due to acute exposure increases above 25–30%, people begin to lose consciousness and eventually, as COHb reaches 60% and above, death ensues. Exact COHb values depend on individual susceptibilities, the underlying state of health and, to some extent, the activity level of the individuals concerned.

The above data have been considered as evidence that carbon monoxide hypoxia produced the effects. It may not be assumed, however, that non-hypoxic

physiological events do not contribute to the effects, because such non-hypoxic effects might be correlated in time and magnitude with COHb. Evidence exists that non-hypoxic events are responsible for impairments that sometimes develop several days after reduction of COHb due to high-level acute carbon monoxide exposure.

Chronic, low-level exposure

There is a growing consensus that for carbon monoxide, as with ionizing radiation, a NOAEL exists. Effectively, a so-called "safe level" is arbitrarily set at a point at which a level of health effects is deemed acceptable. Thus, the setting of a guideline for indoor carbon monoxide involves other considerations than simply scientific considerations of carbon monoxide's toxicity.

Long-term exposures to lower levels of carbon monoxide have far wider-ranging implications for human health than do acute carbon monoxide exposures. There are many hundreds of millions, indeed billions of people around the world who are currently chronically exposed to carbon monoxide indoors. Such exposure has been reported to alter health in a number of ways, including physical symptoms, sensory–motor changes, cognitive memory deficits, emotional–psychiatric alterations, cardiac events and low birth weight. The evidence for this is derived from clinical toxicological, medical and neuropsychological case reports, case series and other retrospective studies. It is established that many cases of carbon monoxide toxicity are misdiagnosed because the symptoms mimic other health problems.

Epidemiological studies involving large population groups, where exposures are generally at relatively low carbon monoxide levels, have demonstrated increased incidences of low birth weight, congenital defects, infant and adult mortality, cardiovascular admissions, congestive heart failure, stroke, asthma, tuberculosis, pneumonia, etc. In both accidental exposure and epidemiological studies, toxic substances other than carbon monoxide were often present in the exposed person's inhaled air. Dose–effect relationships are suggested in some epidemiological studies. The body of literature from both kinds of study is large and growing, and is consistent with subtle but often profound health effects at low carbon monoxide levels.

Health damage resulting from chronic, lower-level exposure has been difficult to fully explain on the basis of hypoxia, hypoxaemia and measured COHb, since various physiological mechanisms should quickly compensate. This leads to the conjecture that non-hypoxic mechanisms may be responsible for some of the effects. The lack of good dose–effect relationships in the accidental exposure case study reports also suggests alternative mechanisms of causation. The cellular mechanisms described above from recent experimental studies may well be the avenues by which this health damage occurs.

If COHb and hypoxia are not important factors in chronically generated health effects, then an alternative means of referencing severity of exposure must be used. Since COHb level only recognizes initial carbon monoxide uptake, a better measure is arguably to use the product, carbon monoxide concentration × time (i.e. duration of exposure). This parameter more accurately represents the total dose of carbon monoxide received in long-term carbon monoxide exposure, since duration of exposure is explicitly present.

Specially sensitive people

Groups at highest risk from carbon monoxide exposure include the unborn and those adults, elderly or not, with coronary artery disease, congestive heart failure or potential stroke, those at risk of sudden death, etc. There is almost certainly also a group of individuals who are extraordinarily sensitive to carbon monoxide but who have no obvious health or unusual physiological conditions and thus cannot be readily identified. They represent that fraction of individuals who lie at the left end of the standard curve when health effects are determined in any population with known exposure history. All of these higher risk groups must be considered when setting carbon monoxide guidelines for indoor air or, for that matter, outdoor air, i.e. the guideline must be low enough to protect all those at highest risk.

Quality and weight of evidence

Compelling evidence of carbon-monoxide-induced adverse effects on the cardiovascular system is derived from a series of controlled human exposure studies of individuals with cardiovascular disease at COHb levels relevant to ambient conditions. Carbon monoxide exposure caused decreases the time to angina and ST-segment changes with COHb levels on the range of 2 to 6%.

Recent epidemiological studies of chronic environmental exposures are coherent with the results of the controlled human exposure studies. Positive associations between ambient carbon monoxide exposure and ED visits and hospital admissions for ischemic heart disease, congestive heart failure and cardiovascular disease are seen in multiple locations where ambient carbon monoxide concentrations ranged from 0.6 to 10.9 mg/m^3. These carbon monoxide associations generally remained robust in multiple pollutant models. In addition, newer data on pathophysiological mechanisms offer an eventual possible explanation of the chronic effects. These two lines of data support a direct effect of carbon monoxide exposure on cardiovascular morbidity and are considered to have a high weight of evidence.

The toxicological studies of carbon monoxide effects on human birth outcomes and fetal development have been critically reviewed. There is evidence that carbon monoxide exposure during pregnancy is associated with reduced fe-

tal growth and low birth weight. Because of inconsistencies in data reporting, exposure assessment and possible confounding of effects by co-pollutants the weight of this evidence is considered limited but suggestive of important health effects.

At the present time, the strength of the evidence for important health outcomes is as summarized in Table 2.4.

Table 2.4. Strength of evidence

Sufficient evidence of a causal relationship	Acute exposure-related reduction of exercise tolerance and increase in symptoms of ischaemic heart disease (e.g. ST-segment changes)
Sufficient evidence of a relationship	Chronic epidemiological studies of cardiovascular morbidity (heart attack, congestive heart failure , ischaemic heart disease)
	Associations between short-term exposure to carbon monoxide and hospital admissions or emergency department visits for respiratory complaints derived from chronic time-series studies
Limited or suggestive evidence of a relationship	Low birth weight, congenital defects and infant mortality
	Total mortality
	Increased risk of cardiovascular mortality and stroke
	Asthma, bronchiolitis, sinusitis, tuberculosis, pneumonia, etc.
	Neurological, neuropsychological and psychiatric deficits (human and animal studies)
	Effects on the developing auditory system
	Immunological impairment (animal studies)

Guidelines

The 24-hour guideline

Chronic carbon monoxide exposure is different from acute exposure in several important respects, as noted above. Thus, a separate guideline is needed to address minimal exposure over 24 hours, rather than the 8-hour period used in the acute guidelines. The latest studies available to us in 2009, especially those epidemiological studies using very large databases and thus producing extremely high-resolution findings, suggest that the appropriate level for carbon monoxide in order to minimize health effects must be positioned below the 8-hour guideline of 10.5 mg/m^3, possibly as low as 4.6–5.8 mg/m^3. This is also essential since the minimal exposure time for this guideline is three times longer.

Derivation of a concentration–response factor

Exposure to carbon monoxide reduced maximum exercise ability in healthy, young individuals and reduced the time to angina and, in some cases, the time

to ST-segment depression in subjects with cardiovascular disease, albeit at a concentration that was lower than that needed to reduce exercise ability in healthy individuals.

The relationship of carbon monoxide exposure and the COHb concentration in blood can be modelled using the differential Coburn-Forster-Kane equation *(3)*, which provides a good approximation to the COHb concentration at a steady level of inhaled, exogenous carbon monoxide. Based on the laboratory studies of reduction in exercise capacity in both healthy individuals and volunteers with cardiovascular disease, it was determined that COHb levels should not exceed 2%. The CFK equation is used below to determine the levels of carbon monoxide to which a normal adult under resting conditions for various intervals can be exposed without exceeding a COHb level of 2%.

The previous WHO guidelines were established for 15 minutes to protect against short-term peak exposures that might occur from, for example, an unvented stove; for 1 hour to protect against excess exposure from, for example, faulty appliances; and for 8 hours (which is relevant to occupational exposures and has been used as an averaging time for ambient exposures). We do not recommend changing the existing guidelines. However, chronic carbon monoxide exposure appears different from acute exposure in several important respects. Thus, a separate guideline is recommended to address 24-hour exposures. This is also relevant because the epidemiological studies (based on 24-hour exposures) using very large databases and thus producing extremely high-resolution findings are now available and indicate important population-level effects at levels that might be lower than the current 8-hour limit. We recommend a series of guidelines relevant to typical indoor exposures, as shown in Table 2.5.

Table 2.5. Indoor carbon monoxide guidelines

Averaging time	Concentration (mg/m^3)	Comments
15 minutes	100	Excursions to this level should not occur more than once per day Light exercise
1 hour	35	Excursions to this level should not occur more than once per day Light exercise
8 hours	10	Arithmetic mean concentration Light to moderate exercise
24 hours	7	Arithmetic mean concentration Awake and alert but not exercising

The guidelines section was formulated and agreed by the working group meeting in November 2009.

Summary of main evidence and decision-making in guideline formulation

Critical outcome for guideline definition

Acute exposure-related reduction of exercise tolerance and increase in symptoms of ischaemic heart disease (e.g. ST-segment changes).

Source of exposure–effect evidence

Laboratory dose–effect experiments with human subjects with stable angina exposed to carbon monoxide *(173–178)*. COHb elevated above 2% caused ST-segment changes and decreased time to angina. The CFK equation *(3)* was used to calculate exposure levels to which a normal adult under resting conditions can be exposed for various intervals without exceeding 2% COHb to calculate guideline levels.

Supporting evidence

- Laboratory dose–effect exercise experiments in non-angina (normal) subjects *(167–172)*.
- Numerous epidemiological studies on effects of acute and chronic exposure to carbon monoxide, including studies on health effects when daily mean levels were in the range 0.6–10.9 mg/m^3, provide sufficient evidence of a relationship between long-term exposure and cardiovascular morbidity *(145–157)*.

Results of other reviews

- *Air quality guidelines for Europe*, 2nd ed. *Chapter 5.5, carbon monoxide*. The guidelines were established for 15 minutes (100 mg/m^3), for 1 hour (35 mg/ m^3) and for 8 hours (10 mg/ m^3) *(41,42)*.
- European Commission's INDEX project proposed guidelines: for 1 hour, 30 mg/m^3; for 8 hours, 10 mg/m^3 *(78)*.

Guidelines

15 minutes – 100 mg/m^3.
1 hour – 35 mg/ m^3.
8 hours – 10 mg/ m^3.
24 hours – 7 mg/m^3.

Comments

The addition of a guideline for 24 hours (7 mg/m^3) to the WHO 2000 guidelines *(41)* to address the risk of long-term exposure.

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3. Formaldehyde

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General description

Formaldehyde (molecular formula H_2-C=O; CAS number 50-00-0) is a colourless gas, flammable and highly reactive at room temperature. Formaldehyde can also be obtained commercially as a 30–50% (by weight) aqueous solution, known as formalin.

In ambient air, formaldehyde is quickly photo-oxidized in carbon dioxide. It also reacts very quickly with the hydroxyl radicals to give formic acid. The half-life estimated for these reactions is about one hour depending on the environmental conditions.

The main chemical and physical properties (of the pure substance) are as follows *(1,2)*: molecular mass 30.03 g/mol; relative vapour density 1.03–1.07 (air = 1); melting point –92 °C; and boiling point –19.1 °C. Formaldehyde is soluble in water (around 400 g/l at 20 °C), ethanol and chloroform and miscible with acetone, benzene and diethylether. The octanol/water partition coefficient (log K_{ow}) is 0.35, the vapour pressure is 5.19×10^5 Pa at 25 °C and the Henry's Law constant is 3.41×10^{-2} Pa.m^3/mol at 25 °C.

Formaldehyde is ubiquitously found in the environment, because it is formed primarily by numerous natural sources and anthropogenic activities. In the environment, it is released through biomass combustion (forest and bush fires) or decomposition and through volcanoes, for example. Anthropogenic sources include direct ones such as on-site industrial emissions and fuel combustion from traffic. Other combustion processes (power plants, incineration, etc.) also represent sources of formaldehyde emissions in the atmosphere. However, formaldehyde is also extensively produced industrially worldwide for use in the manufacture of resins, as a disinfectant and fixative, or as a preservative in consumer products.

All these man-made products and uses are the major indirect sources of formaldehyde, in particular indoors. Finally, it should be noted that secondary formation of formaldehyde occurs in air through the oxidation of volatile organic compounds (VOCs) and reactions between ozone (mainly from outdoors) and alkenes (especially terpenes) have been widely described. The contribution of these secondary chemical processes to the ambient and indoor concentrations is still not fully quantified.

Common techniques to measure formaldehyde concentrations include both integrated active and passive methods. Formaldehyde is generally trapped on a sorbent impregnated with 2,4-dinitrophenylhydrazine (2,4-DNPH). Analysis is then conducted in the laboratory by high-performance liquid chromatography and ultraviolet detection at 350 nm. Detection and quantification limits around 1 μg/m^3 can be achieved. The use of an ozone scrubber is recommended to remove the latter from the sample stream to prevent interference during the analysis. Recent comparisons of formaldehyde measurement techniques have shown that, in the presence of low relative humidity, 2,4-DNPH-based methods could underestimate concentrations *(3,4)*.

Conversion factors

At 760 mmHg and 20 °C, 1 ppm = 1.249 mg/m^3 and 1 mg/m^3 = 0.801 ppm; at 25 °C, 1 ppm = 1.228 mg/m^3 and 1 mg/m^3 = 0.814 ppm.

Sources and pathways of exposure

Indoor sources may be combustion processes such as smoking, heating, cooking, or candle or incense burning *(1,5)*. However, major sources in non-smoking environments appear to be building materials and consumer products that emit formaldehyde *(5,6)*. This applies to new materials and products *(7)* but can last several months, particularly in conditions with high relative humidity and high indoor temperatures *(8)*.

Formaldehyde sources in indoor environments include: furniture and wooden products containing formaldehyde-based resins such as particleboard, plywood and medium-density fibreboard; insulating materials (in the early 1980s, urea formaldehyde foam insulation was a major source of indoor pollution); textiles; do-it-yourself products such as paints, wallpapers, glues, adhesives, varnishes and lacquers; household cleaning products such as detergents, disinfectants, softeners, carpet cleaners and shoe products; cosmetics such as liquid soaps, shampoos, nail varnishes and nail hardeners; electronic equipment, including computers and photocopiers; and other consumer items such as insecticides and paper products.

As mentioned above, secondary formation of formaldehyde occurs indoors through chemical reactions between, for example, ozone and terpenes *(9,10)*.

Taking all the indoor sources of formaldehyde into account, it is difficult to identify the major ones that contribute to indoor levels. During a large-scale indoor survey carried out between 1997 and 1999 in 876 homes in the United Kingdom, Raw et al. *(11)* found that, depending on the age of the building, the presence of particleboard flooring in the home was the second most important determinant of indoor concentration. Clarisse et al. *(12)* measured formaldehyde in the bedroom, the kitchen and the living room of 61 Parisian flats with no previous history of complaint for olfactory nuisance. They found that indoor levels

depended on the age of wall or floor coverings (renovations less than one year old), smoking and ambient parameters (carbon dioxide levels and temperature). Using emission factors from the literature, the German Federal Institute for Risk Assessment found that pressed wood products were the major sources contributing to exposure through inhalation at home *(13)*. Marchand et al. *(14)* carried out aldehyde measurements in 162 homes in the Strasbourg area in 2004–2005. Variance analyses showed that formaldehyde concentration was a function of the age of the ceiling coverings for both bedrooms and living rooms. Formaldehyde concentrations tended to decrease with increasing furniture age for both living rooms and bedrooms, but the analyses were not significant. In Canada, Gilbert et al. *(15)* measured formaldehyde levels in 96 homes in Quebec City in 2005. Formaldehyde concentrations were negatively correlated with air exchange rates. They were significantly elevated in homes heated by electricity, in those with new wooden or melamine furniture purchased in the previous 12 months, and in those where painting or varnishing had been done in the sampled room in the previous 12 months. Similarly, relative high levels that can be measured in schools are usually considered to be linked to the high density of furniture in the classrooms (and to poor ventilation).

The possible routes of exposure to formaldehyde are inhalation, ingestion and dermal absorption. Almost no data are available in the literature on dermal exposure *(16)*. Concerning the oral pathway, exposure through food may not be negligible. Estimates of daily formaldehyde intake by six age groups of the general population in Canada were carried out to determine the relative contributions from different media *(17)*. These calculations indicate that daily formaldehyde intake via inhalation is much lower than for intake from food. However, since critical effects associated with exposure to formaldehyde are directly linked to the site of contact, inhalation and ingestion are usually considered separately. Considering exclusively inhalation, indoor exposure contributes up to 98% to the integrated exposure (considering time–activity patterns and daily inhalation volume) *(16)*.

Indoor concentrations and relationship with outdoor levels

A large review of formaldehyde concentrations worldwide in all types of indoor environment, including mobile homes, has been summarized by Salthammer et al. *(5)*. A second large review compiles information on indoor, outdoor and personal exposures to formaldehyde *(18)*.

During a large indoor air survey carried out in homes by the Building Research Establishment (BRE) in the United Kingdom in 1997–1999, the geometric mean, 95th percentile and maximum value of three-day samples of formaldehyde in bedrooms (n = 833) were, respectively, 22.2, 61.2 and 171 $\mu g/m^3$ *(11)*.

During Phase IV of the German longitudinal environmental survey 2003–2006 (GerES IV), formaldehyde was measured through passive samplers for one

week in bedrooms of a randomly selected population of children and teenagers. The geometric mean, 95th percentile and maximum concentration (n = 586) were, respectively, 23.3, 47.7 and 68.9 μg/m³ *(19)*. These levels were lower than the concentrations measured previously in the framework of the GerES.

In the EXPOLIS study in Helsinki, the average air concentration of formaldehyde in homes was 41.4 μg/m³ (range 8.1–77.8 μg/m³) and at the workplace 15 μg/m³, whereas average personal exposure was 26.8 μg/m³ *(20)*.

Hutter et al. measured formaldehyde concentrations in 160 Austrian homes and found a median concentration of 25 μg/m³ and a maximum value of 115 μg/m³ *(21)*.

The French Observatory on Indoor Air Quality carried out a large monitoring campaign in 567 randomly selected dwellings between 2003 and 2005. The median concentration, 95th percentile and maximum value of formaldehyde following seven days of passive sampling in bedrooms (n = 554) were, respectively, 19.6, 46.7 and 86.3 μg/m³ *(22)*.

In Canada, Gilbert et al. *(15)* measured formaldehyde levels in 96 homes in Quebec City between January and April 2005. The indoor concentrations ranged from 9.6 to 90 μg/m³, with a geometric mean of 29.5 mg/m³.

In the National Human Exposure Assessment Survey (NHEXAS) in Arizona, the median and 90th percentile indoor concentrations were, respectively, 21 and 46 μg/m³, about the same levels as those measured in Europe *(21)*.

Dingle & Franklin *(23)* observed, in a study carried out in 185 homes in Perth, Australia, indoor formaldehyde concentrations of between 2.5 and 133.7 μg/m³, i.e. the same range of concentrations as measured in other countries.

Formaldehyde concentrations in Japanese dwellings have been regularly measured within large-scale monitoring campaigns since the 1090s *(24,25)*. The National Institute of Health Sciences conducted a first national field survey in 230 houses in 1996 and found an arithmetic mean concentration of 78 μg/m³ (range 5–600 μg/m³). During the last survey conducted in 2005 (n = 1181 homes), the arithmetic mean decreased to 31 μg/m³ (maximum concentration 300 μg/m³). In between, the Japanese authorities amended the national building codes and instituted restrictions on the use of formaldehyde-emitting materials for interior finishing.

In China, a large number of monitoring results are available for new homes, since it is mandatory to check whether the maximum allowable formaldehyde concentration in residential buildings (100 μg/m³) has been exceeded *(26)*. The mean concentration in approximately 6000 recently refurbished dwellings in urban areas was 238 μg/m³ (remodelled after one year or less; measurements conducted between 1999 and 2006; mean outdoor level around 12 μg/m³).

Formaldehyde concentrations in dwellings vary according to:

- the age of the building, since the release of formaldehyde decreases with time *(11)*;

- temperature and relative humidity *(8)*;
- the air exchange rate *(11,15)*; and
- the season *(11)*.

Moreover, indoor concentrations can reach more than 200 μg/m^3 close to somebody who is smoking in a room *(27)*. There are many fewer data on offices compared to the residential environment.

A large monitoring campaign carried out in Germany between 2001 and 2004 in 419 rooms found a median indoor formaldehyde concentration of 28 μg/m^3 *(28)*.

Over the period 2004–2007, the EU's Joint Research Centre in Ispra, Italy monitored priority pollutants, including formaldehyde, in European public buildings and environments where children frequently stay, such as schools and kindergartens *(29)*. Formaldehyde concentrations in offices in public buildings (n = 94) varied from 3 to 33 μg/m^3.

Formaldehyde concentrations were measured between 2001 and 2006 in office buildings in southern Finland *(30)*. The occupants had complained of symptoms, but inspection by indoor air experts had not revealed any sources of pollutants. The mean formaldehyde concentration and maximum value were found to be 11 and 44 μg/m^3, respectively.

In the United States, within the framework of the Building Assessment Survey and Evaluation (BASE) study *(31)*, 100 office buildings were investigated between 1994 and 1998. Formaldehyde was detected in all the buildings. The 50th and 95th percentiles were 15 and 32 μg/m^3, respectively.

In China, the mean formaldehyde concentration in 351 offices located all over the country (data from 1996–2005) was of the same order of magnitude as in recently refurbished dwellings, i.e. 256 μg/m^3 *(26)*. In Hong Kong SAR, formaldehyde was measured in 422 air-conditioned offices; the geometric mean was found to be equal to 32 μg/m^3 (± 2.7 μg/m^3) *(32)*.

In France, in the frame of the International Study on Asthma and Allergies in Childhood (ISAAC), formaldehyde was measured in 1999 in 401 classrooms in 108 schools located in 6 cities (Strasbourg, Créteil, Reims, Marseille, Bordeaux and Clermont-Ferrand) *(33)*. Concentrations varied from 4 to 100 μg/m^3 with a mean value of 27 μg/m^3. In 50 Parisian kindergartens studied between 1999 and 2001, both in winter and in summer (n = 222), indoor formaldehyde concentrations ranged from 1.5 to 56 μg/m^3 with a median value of 14 μg/m^3 *(34)*.

In Germany, the indoor air quality was evaluated in 92 classrooms in the winter of 2004/2005 and in 75 classrooms in the summer of 2005 in southern Bavaria. Indoor formaldehyde concentrations ranged from 3.1 to 46.1 μg/m^3 *(35)*.

Formaldehyde concentrations measured in European kindergartens by the EU's Joint Research Centre between 2004 and 2007 (n = 57) varied from 1.5 to 50 μg/m^3, with an arithmetic mean of 17.4 μg/m^3 *(29)*.

In Japan, formaldehyde concentrations measured in 50 schools in 2000 were around 14 μg/m^3 in winter and 30 μg/m^3 in summer *(36)*.

Outdoor air does not contribute to indoor pollution (or the contribution is minor) since ambient levels are generally rather low. Mean ambient air background concentrations remain low compared to those indoors, typically around 1–4 μg/m^3. Data from the HEXPOC report *(16)*, collected from Brazil, Canada, Germany, Italy, Mexico, the Netherlands and the United States, provide ambient concentrations of 1.5–16.4 μg/m^3 with a mean value of 7.2 μg/m^3 (SD = 5.1 μg/m^3). Consequently, the indoor : outdoor ratio is always far above 1. Formaldehyde can be qualified as a very specific indoor pollutant.

Kinetics and metabolism

Absorption

Owing to its solubility in water, formaldehyde is rapidly absorbed in the respiratory and gastrointestinal tracts and metabolized. More than 90% of inhaled formaldehyde gas is absorbed and rapidly metabolized to formate in the upper respiratory tract *(37)*. In rats, it is absorbed in the nasal passages *(38,39)*; in primates, some absorption takes place in the nasal cavity as well as in the nasopharynx, trachea and bronchi *(40,41)*. The mucociliary apparatus is an important defence system in the respiratory tract and may provide protection of the underlying epithelium from gases and vapours *(42)*. Given the solubility of formaldehyde in mucus (water) and estimates of total mucus flow, as much as 22–42% of inhaled formaldehyde may be removed by mucus flow *(37,43)*. It has been shown that when formaldehyde is mixed with particles, more of it is retained by the respiratory tract than when it is inhaled alone. This suggests that some particles can bind with gases and increase the retained dose of a gas *(44)*. However, some estimates show that the deposited dose of formaldehyde in the particle phase is substantially smaller than the dose from the vapour phase *(45)*.

Formaldehyde is absorbed rapidly and almost completely from the rodent intestinal tract *(39,46)*. Although formaldehyde or its metabolites can penetrate human skin – it induces allergic contact dermatitis in humans – dermal absorption appears to be very slight *(47,48)*.

Endogenous sources of formaldehyde

In humans, as in other animals, formaldehyde is an essential metabolic intermediate in all cells. It is produced endogenously from serine, glycine, methionine and choline, and it is generated in the demethylation of N-, O- and S-methyl compounds. It is an essential intermediate in the biosynthesis of purines, thymidine and certain amino acids *(49)*.

Owing to its high reactivity at the site of contact and rapid metabolism, exposure of humans, monkeys or rats to formaldehyde by inhalation does not alter the concentration of formaldehyde in the blood from that endogenously present,

which is about 2–3 mg/l for each of the three species. This concentration represents the total concentration of both free and reversibly bound endogenous formaldehyde in the blood. The absence of an increase is explained by the fact that formaldehyde reacts rapidly at the site of contact and is swiftly metabolized by human erythrocytes, as described below. From a mathematical model describing the absorption and removal of inhaled formaldehyde in the human nose, it was predicted that exposures in the range of 0.125–12.5 mg/m^3 only cause extremely small increases in formaldehyde concentrations compared to the pre-exposure concentrations *(50)*. Intravenous administration of formaldehyde to dogs, cats and monkeys also does not result in accumulation of formaldehyde in the blood, largely owing to its rapid metabolism *(1,39,46)*.

Distribution

Following a 6-hour inhalation exposure of rats to formaldehyde, about 40% of the inhaled compound was eliminated as expired carbon dioxide over a 70-hour period; 17% was excreted in the urine, 5% was eliminated in the faeces and 35–39% remained in the tissues and carcass, indicating that absorbed formaldehyde and its metabolites are rapidly removed by the mucosal blood supply and distributed throughout the body *(39)*. In dogs, orally administered formaldehyde results in a rapid increase in formate levels in the blood. In rats, oral exposure results in about 40% being eliminated as carbon dioxide within 12 hours, 10% being excreted in the urine and 1% being excreted in the faeces *(51)*.

Rodents excreted about 6.6% of the dermally applied dose in the urine over 72 hours, while 21–28% was collected in air traps, likely due to the evaporation of formaldehyde from the skin *(52)*. Approximately 22–28% of the compound or its metabolites remained in the body, including the blood and skin at the site of application. In monkeys, less than 1% of dermally applied dose was excreted or exhaled, in contrast to rodents in which nearly 10% was eliminated by these routes. Coupled with the observation of lower blood levels in monkeys than in rodents, the results suggest that the skin of monkeys may be less permeable to aqueous formaldehyde than that of rodents.

Metabolism and elimination

Formaldehyde reacts rapidly at the site of contact and is swiftly metabolized in humans by erythrocytes, which contain the enzymes formaldehyde dehydrogenase and aldehyde dehydrogenase *(53–56)*. Formaldehyde reacts virtually instantaneously with primary and secondary amines, thiols, hydroxyls and amides to form methylol derivatives. Formaldehyde acts as an electrophile and can react with macromolecules such as DNA, RNA and protein to form reversible adducts or irreversible cross-links *(1)*.

Formate, the metabolic product of formaldehyde, is incorporated in normal metabolic pathways or further oxidized to carbon dioxide. This becomes

important when performing fate and transport studies with radio-labelled formaldehyde, as the label appears in all tissues due to the one-carbon pool. Formaldehyde disappears from the plasma with a half-time of about 1–1½ minutes, most of it being converted to carbon dioxide and exhaled via the lungs. Smaller amounts are excreted in the urine as formate salts and several other metabolites *(47)*.

The primary metabolism system for formaldehyde involves an initial spontaneous reaction with glutathione to form S-hydroxymethylglutathione, followed by reaction facilitated by alcohol dehydrogenase to convert the intermediate to S-formylglutathione *(57,58)*. This intermediate is then further metabolized by S-formylglutathione hydrolase to yield formate and reduced glutathione.

Biomarkers of exposure

To determine whether formate is a useful biomarker for human exposure to formaldehyde, urine was examined in veterinary medical students exposed to low concentrations of formaldehyde *(59)*. Exposed students (formaldehyde air concentration < 0.61 mg/m^3 over a 3-week period) were compared to control subjects. The average baseline level of formate in the urine of 35 unexposed subjects was 12.5 mg/l, but this varied considerably both within and among subjects (range 2.4–28.4 mg/l). No significant changes in concentration were detected. Thus formate in urine does not appear to be a useful biomarker for human exposure, especially at low exposure concentrations.

Inhalation of formaldehyde leads to the formation of DNA–protein cross-links in cells at the site of contact, particularly in the nasal respiratory mucosa of rats and monkeys. The formation of these cross-links is a sublinear function of the formaldehyde concentration in inhaled air from 0.86 to 18.4 mg/m^3, and the yield of DNA–protein cross-links at a given inhaled concentration is approximately an order of magnitude lower in monkeys than in rats. There is no detectable accumulation of DNA–protein cross-links during repeated exposure. Application of a pharmacokinetic model to the data obtained in rats and monkeys indicates that the concentration of DNA–protein cross-links in the human nasal mucosa would be lower than those in rats and monkeys *(1,41,60,61)*. No data are available on DNA–protein cross-links in humans *(1)*. Carraro et al. *(62)* have suggested that an immunological assay that measures the humoral immune response of adducts of formaldehyde and human serum albumin could be used as a biomarker of environmental exposure to formaldehyde, but such a marker has not been developed.

Health effects

Identification of studies

The literature for the cancer part was indentified in PubMed with search terms that included "formaldehyde AND DNA-protein crosslink/crosslinks", "formal-

dehyde AND genotoxic/genotoxicity AND blood AND lymphocyte", "lymphatic AND tissue AND nose AND review", "micronucleus AND test AND review", "formaldehyde AND cancer AND meta-analysis", "formaldehyde AND cancer AND humans", "unit risk AND formaldehyde", "Epstein-Barr AND nasopharyngeal cancer AND review", "Hauptmann M AND nasopharyngeal carcinoma", "Hauptmann M AND silver smithing", "silver smithing AND nasopharyngeal carcinoma", "silver smithing AND cancer", "acid AND nasopharyngeal carcinoma AND review", "nickel AND nasopharyngeal carcinoma", "unit risk AND cancer AND review" and "Zhang L AND formaldehyde". References were also obtained from IARC (*1*), Bosetti et al. (*68*) and the European Commission (*64*). Approximately 200 articles were deemed relevant and read. Of these, more than 120 were evaluated in detail; the relevance of these articles is discussed by Nielsen & Wolkoff *(65)*. However, articles were only included here if they were used directly in the derivation of the WHO indoor air guidelines.

For non-cancer effects, publications from the period 1997–2009 were searched with special emphasis on human effects. Except in special cases, animal and in vitro studies were excluded owing to the huge amount of human data. Formaldehyde was searched in combination with the following terms: allergy, asthma(tics), airway (irritation), bronchoconstriction, children, eye (irritation), inflammation, homes, IgE, (nasal) irritation, kindergartens, lung effects, lung function, offices, odour, schools, sensory irritation, sick-building syndrome, sensitization and trigeminal stimulation.

Eczema was not included except if retrieved in the above-mentioned searches. In addition to databases such as PubMed and Google Scholar, recent comprehensive reviews were considered *(66–68)*, including Gilbert *(69)* and international reports *(1,17,64)*.

Of the 170 papers identified (and listed by Wolkoff & Nielsen *(70)*), 90 were included in the discussion presented below addressing human exposure and epidemiological issues (65 studies), children (11 studies), animal studies (8 studies), cell studies (3 studies) and dust (4 studies).

Respiratory effects of formaldehyde

Nasal retention of formaldehyde in the moist layers covering the nasal mucosa exceeds 90–95%. For example, a maximum of 5% formaldehyde reaches the lower airways in dogs *(71)*. The high retention is also deduced from a mouse bioassay, because only sensory irritation of the upper airways has been observed below 5 mg/m^3 formaldehyde *(72)*. Recent computational fluid dynamic calculations at boundary conditions of fast formaldehyde uptake indicate similar total nasal extraction in adults and children (on average 90%), and thus a limited amount of formaldehyde may traverse the nasal cavity *(73)*.

Human exhaled air contains formaldehyde in concentrations in the order of 0.001–0.01 mg/m^3, with an average value of about 0.005 mg/m^3 *(74–76)*.

Table 3.1. Effects on the airways in humans after acute and short-term exposure to formaldehyde

Study	Formaldehyde concentration (mg/m^3)	Subjects
Falk et al. *(78)*	0.13	8 with nasal congestion [a] 8 without nasal congestion
Lang et al. *(79)*	0.3–0.5 0.6 0.6 0.6	21 healthy [a]
Casset et al. *(80)*	0.1	19 dust mite asthmatics [a]
Wantke et al. *(81)*	0.13–0.41 0.27 (mean)	27 medical students [b]
Ezratty et al. *(82)*	0.5	12 grass pollen asthmatics [a]
Krakowiak et al. *(83)*	0.5	10 healthy 10 asthmatics
Harving et al. *(84)*	0.85	15 asthmatics
Kriebel et al. *(85)*	1.3 (mean)	38 physical therapy students [b]
Airaksinen et al. *(86)*	0.08–1.4	95 patients
Chia et al. *(87)*	0.9/0.6 (personal/mean)	150 medical students [b,c] 189 medical students [d]
Akbar-Khanzadeh & Mlynek *(88)*	1.6–3.1 (breathing zone)	50 medical students [b] 36 physical therapy students
Kim et al. *(89)*	0.2–11.2 3.7 (mean)	167 medical students [b] 67 premedical

[a] Double-blind exposure study.
[b] Field epidemiological study.
[c] First-year students.
[d] Control group, third- and fourth-year students.

Effects after acute and short-term exposure to formaldehyde at indoor levels (non-cancer effects)

The effects include odour (which may cause discomfort), sensory irritation to the eyes and upper airways, lung effects (asthma and allergy) and finally eczema. These effects have been discussed in comprehensive reviews during the last decade *(64,66–69,77)*, including international reports *(1,17)*. Selected key studies from the last decade about exposure–response relationships are listed in Table 3.1. They represent controlled, usually double-blind exposure studies, including both sexes, of which some were tested with both questionnaires and objective methods. In addition, Table 3.1 lists a number of epidemiological studies with lung function testing.

Exposure time (minutes)	Health effects
120	Swelling of nasal mucosa among those suffering from nasal congestion
240	Subjective sensory irritation in eyes Increased eye blink frequency No effects on nasal flow and resistance, peak flow or eye redness No effects on lung function (PEF, FEV_1, MMEF)
30	No effects on lung function (PEF, FEV_1) after mouth pre-exposure of formaldehyde Possible decrease of lung function (PEF, FEV_1) after Der p 1 post-exposure
70 days	No significant decrease in PEF 4 students (one smoker) possibly IgE sensitized No specific formaldehyde IgG antibodies of significance No correlation between IgE and symptoms
60	No effects on lung function (FVC and FEV_1) No effects post-exposure to grass pollen
120	No change in inflammatory mediators after 4 and 24 hours No effects on lung function (FEV_1) No specific formaldehyde IgE antibodies No differences between healthy and asthmatics
90	No effects on airway resistance, lung function (FEV_1) and bronchial activity No delayed reactions
150/week	1–1.5 % decrease of lung function (FEV_1) Effect diminished after 4 weeks
30	No or few effects on lung function Few cases of rhinitis
	No differences in $FEV_1$and FVC among 22 randomly selected male and female subjects after first day and after end of dissection period
60–180	No dose–response relationship between increase in lung function (FVC, FEV_1, FEV_3, FEF) and formaldehyde exposure IgE not associated with formaldehyde exposure

Odour. A large number of odour thresholds have been reported for formaldehyde, varying from 0.05 to 0.5 mg/m^3 *(90)*, some of which are listed in Table 3.2. Two recent studies, carried out under controlled olfactometric conditions, indi-

Table 3.2. Selected odour thresholds for formaldehyde

Study	Odour detection threshold (mg/m^3)	Subjects
Berglund & Nordin *(93)*	0.068	22 non-smoking (women, age-matched)
	0.116	22 smoking (women, age-matched)
Lang et al. *(79)*	0.19–0.36	21 healthy (men and women) [a]
Nagata *(91)*	0.2–0.3	6 adults (expert panellists)

[a] Olfactory perception differs significantly from background.

cate that the odour threshold lies between 0.2 and 0.4 mg/m^3 *(79,91)*; this also agrees with the fact that 33 subjects (mean age 30 years) perceived formaldehyde at about 0.3 mg/m^3 (0.25 ppm) *(92)*. Lower values down to about 0.1 mg/m^3, obtained under conditions of careful generation and monitoring of formaldehyde, have been reported for women *(93)*. Olfactometric determination of odour thresholds depends on a number of experimental factors, such as air purity of the background, and possibly also personal factors such as smoking status and previous olfactory experience; generally, however, it is considered that lower values have higher validity than higher values *(94)*. In addition, recent olfactometric studies indicate less intra- and inter-variability of sensitivity among subjects than in previous studies *(94)*. In view of the above-mentioned studies, it is considered that a significant fraction of the population may perceive formaldehyde at or below 0.1 mg/m^3.

Both eye and upper airway sensory symptoms may be over-reported by odour cues, which cause perceptual uncertainty because of the difficulty of separating the simultaneous and integrated input from odours and sensory irritants *(95,96)*. The perceived odour intensity will depend on a number of psychological factors, such as information about the risk of the chemical *(97)*.

Sensory irritation. Generally, sensory irritation (nasal pungency) is perceived as an unpleasant sensation from the eyes and airways caused by stimulation of the trigeminal nerve endings by airborne sensory irritants *(95)*. A number of reviews have assessed the threshold for self-reported sensory irritation. In general, the eyes are considered to be more sensitive to such irritants than the upper airways *(95)*. Values have been suggested of from 0.15 up to 1.25 mg/m^3 *(66,67,77)*. Raw data on exposure–response relationships obtained from reported human exposure studies about irritating effects were used in a regression model. A value below 0.94 mg/m^3 formaldehyde was considered safe against sensory irritation of the eyes for all workers; about 6% of workers may experience moderate irritation between 0.94 and 1.25 mg/m^3, while none would experience severe irritation *(98)*.

One of the key experimental studies involved 21 healthy subjects exposed double-blind and randomly to formaldehyde for 4 hours *(79)*. Questionnaires and objective methods were used to evaluate eye and airway irritation and lung function. Eye irritation was found to be the most significant effect. Subjective sensory irritation was perceived at as low as 0.38 mg/m^3 for the eyes and 0.63 mg/m^3, with peaks up to 1.25 mg/m^3, for the nose. Adjustment for the personal trait of negative affectivity (e.g. anxiety), however, led to a value of 0.63 mg/m^3 for the eyes at constant exposure and 0.38 mg/m^3 plus four brief peak exposures at 0.63 mg/m^3. An increase in eye blink frequency, which reflects sensory stimulation of the trigeminal nerve but not necessarily in perception thereof, was observed at 0.63 mg/m^3 formaldehyde baseline exposure plus four brief peak exposures at 1.25 mg/m^3, but not without the peak exposures. The nasal flow

resistance and lung function remained unaffected. Eye and nasal irritation did not occur in parallel in the low dose range because eyes are more sensitive; it also to some extent depended on personal factors (e.g. trait and odour). The authors concluded that a corrected lowest observed effect level (LOEL) is 0.63 mg/m^3 without peak exposure, which agrees with the observations of Kulle et al. *(99)*. Lang et al. *(79)* also concluded that the NOAEL for both subjective and objective eye irritation would be close to the LOEL, i.e. 0.63 mg/m^3 at constant exposure; the effects were considered weak, because "less" and "somewhat" were ranked nearly equal. In addition, a slightly lower NOAEL was considered to be 0.38 mg/m^3 with peaks of 0.75 mg/m^3 formaldehyde. Sensory irritation in humans can be predicted from airway responses in mice *(100)*. Further support for the Lang et al. *(79)* estimate is obtained from the mouse bioassay *(72)*, because the NOAEL was found experimentally to be 0.38 mg/m^3.

As a first approximation, the sensory effect of formaldehyde together with other sensory airway irritants is additive *(101)*. However, in a study of 130 women (mean age 27 years) exposed to 0.04 mg/m^3 formaldehyde in a mixture of 23 typical indoor VOCs at a total of 25 mg/m^3 plus ozone (0.08 mg/m^3) for about 140 minutes, neither significant reported sensory irritation nor indication of nasal inflammation was observed *(102,103)*.

No epidemiological study has been identified that unequivocally shows a direct association between formaldehyde and sensory irritation. In general, mixed exposures have encumbered definite conclusions about the effects of formaldehyde *(104–107)* and other explanations have been proposed for the reported symptoms, including psychosocial factors *(108)*. Further, two studies reported no correlation between sensory irritation and formaldehyde concentrations in 23 offices *(30)* and in 59 kitchens *(109)*. Mixed exposures also occur in the wood industry and hamper the interpretation of the effect of formaldehyde. Nasal irritation dominated relative to that of the eyes and throat and was highest among those working with medium-density fibreboard and other wood products. It was concluded that 0.17 mg/m^3 formaldehyde was of minor importance for the reporting of symptoms *(110)*.

The threshold for objective sensory irritation appears to be about 1 mg/m^3 for workers. For the indoor environment (24 hours), a value of 0.125 mg/m^3 was considered safe for the entire population against sensory irritation, including chronic sensory irritation *(66,77)*. This value agrees with results obtained from a recent controlled human exposure study, where no subjective sensory irritation occurred in the eyes and upper airways below 0.38 mg/m^3 formaldehyde *(79)*. Two approaches have been used to protect the potentially more sensitive part of the population. An assessment factor of 4 has been suggested for extrapolation from the NOAEL to a level below the threshold for sensory irritation *(101)*. An assessment factor of 5 has been derived from the standard deviation of nasal pungency thresholds *(111)*. Thus, applying an assessment factor of 5 on the sug-

gested NOAEL of 0.63 mg/m^3 from the studies by Lang et al. *(79)* and Kulle et al. *(99)*, a value of 0.125 mg/m^3 is obtained. This value is also considered valid for children, because there is no indication that children are more susceptible to formaldehyde exposure than adults.

There is no indication that extending exposure beyond four hours would increase the formaldehyde irritative response or the sensitivity. This is based on the fact that the chemical reaction of formaldehyde on the TRPA1 receptor site is reversible *(112,113)*. Inflammation may increase the receptor sensitivity, but neither eye nor airway inflammation has been reported at indoor concentrations of formaldehyde. Further, neither nasal damage nor inflammation was observed in rats during life-long exposure to 1.2 mg/m^3 (1 ppm) formaldehyde *(114)*.

Nasal histopatological changes. A Swedish study *(115)* investigated 70 workers in a chemical plant in which formaldehyde and products based on formaldehyde were produced as resins and, for example, used for impregnation of paper. Additionally, 100 workers employed in the furniture industry were investigated. The 36 controls were mainly clerks. The mean formaldehyde concentration was 0.3 mg/m^3 (range 0.05–0.5 mg/m^3) with frequent formaldehyde peaks above 1 mg/m^3 in the chemical plant. The mean duration of exposure was 10.4 years. The furniture workers were exposed to 0.2–0.3 mg/m^3 formaldehyde that seldom exceeded 0.5 mg/m^3. The mean wood dust concentration was 1–2 mg/m^3 and the mean duration of exposure was 9 years. Controls were exposed to a mean formaldehyde concentration of 0.09 mg/m^3. Nasal biopsies were performed and evaluated by means of a nine-point scale (score 0–8), where category 1 was "stratified cuboid epithelium with loss of ciliated epithelium" and category 2 "mixed stratified cuboid/stratified squamous epithelium".

The mean nasal biopsy score was 1.56 (range 0–4) in the controls, 2.07 (range 0–6) in the furniture workers (not statistically significant) and 2.16 (range 0–4) in the chemical plant (statistically significant). Within the formaldehyde exposure groups themselves, the histopathology scores were not exposure-dependent; exposure metrics were current formaldehyde concentrations (both formaldehyde groups were divided into exposure groups 0.1–0.24, 0.25–0.49 and ≥ 0.5 mg/m^3 formaldehyde), current wood dust concentrations (furniture workers were divided into exposure groups 0.1–1, 1.1–2 and 2.1–4.9 mg/m^3), cumulative formaldehyde exposures (both formaldehyde groups were divided into < 1.5, 1.5–4.99 and ≥ 5 mg/m^3.years) and duration of exposure (< 5, 5–14, 15–24 and ≥ 25 years). Overall, this study cannot be used for risk assessment owing to the lack of an exposure-dependent effect.

Lung effects (non-cancer)

Formaldehyde alone does not cause IgE sensitization *(116,117)*. Recent epidemiological studies of the occupational environment have not indicated an increase

in sensitization to formaldehyde exposure (see below). Nevertheless, formaldehyde-induced sensitization has been hypothesized. Two causes have been suggested, inflammation and formaldehyde acting as an adjuvant for allergens, but they are not supported at normal indoor air concentrations. Inflammatory mediator response was absent on the exposure of human lung epithelial cells at 0.25 mg/m^3 formaldehyde compared to clean air *(118)* and inflammation was not observed in life-long exposure of rats to 1.25 mg/m^3 formaldehyde *(119)*. However, increased lung inflammation, reduced lung function and higher allergen-specific IgE antibody levels have been reported in rodents immunized by intraperitoneal administration to the allergen ovalbumin and followed by different airborne formaldehyde exposures *(116,120,121)*. The interpretation of these studies is not clear in terms of the risk assessment of combined human indoor exposure to formaldehyde and allergens due to intraperitoneal administration in the animals. Thus, evidence of lung effects must depend on human data.

Experimental studies. A number of human exposure studies have been carried out with lung function testing during the last decade (see Table 3.1).

The human exposure studies generally show that lung function is unaffected in both healthy and asthmatic people exposed for 1–4 hours to formaldehyde below 1 mg/m^3 *(79,83,84,88)*. The limited effect on lung function and rhinitis is in agreement with a study in which formaldehyde inhalation had no effect on 95 patients with both upper and lower airway symptoms, when adjusted for placebo effects *(86)*; further, the authors concluded that IgE-mediated formaldehyde allergy was nearly non-existent.

Two studies with asthmatics sensitive to grass pollen and dust mites (Der p 1), respectively, were investigated, in which formaldehyde exposure was combined with post-exposure to the allergens (in a season without grass pollen). In one study, increasing doses of inhaled grass pollen after exposure to 0.5 mg/m^3 formaldehyde for one hour did not affect the lung function over an 8-hour period; a non-significant protective effect of formaldehyde was observed *(82)*. The particle size distribution of the grass pollen was 20–40 μm (V. Ezratty, personal communication, 2010). In the other study, oral breathing of 0.09 mg/m^3 formaldehyde for 30 minutes followed by exposure to dust mites (mean particle size 11 μm) resulted in a bronchial response at a lower dust mite allergen concentration relative to background air with 0.03 mg/m^3 formaldehyde; the geometric mean PD_{20} for Der p 1 was 34 ng after formaldehyde and 45 ng after placebo ($P = 0.05$) *(80)*. An alternative statistical test (Wilcoxon signed-rank test) of the published data showed no significance, thus illustrating how sensitively the statistical outcome depends on the test applied. Further, the effect is considered to have no clinical relevance, because an estimated inhaled allergen dose for 8 hours while resting would be less than 1 ng, based on standard respiratory rates for males, sampled dust mites, e.g. in mattresses *(122)* and assuming a room particle concentration

of 100 μg/m^3. This agrees with measured airborne concentrations of dust mites (Der f 1) in bedrooms *(123)*.

Epidemiological studies (children and adults). Gilbert *(69)* reviewed epidemiological studies on formaldehyde and lung effects in the indoor environment. Studies from the occupational environment have also been evaluated *(1,68)*. Key studies with objective lung function testing are summarized in Table 3.1.

Children. Some case-control and cross-sectional studies have indicated a possible association between low formaldehyde exposure and asthma or sensitization to certain allergens *(106,124–128)*. Briefly, these studies have complex co-exposures, which encumber the establishment of direct cause–effect and dose–response relationships for formaldehyde and the evaluation of confounding effects *(69)*.

Formaldehyde measured in the bedrooms of 224 healthy children aged 6–13 years was not found to be associated with effects on lung function (FEV), but an increase in exhaled nitric oxide was associated with formaldehyde levels greater than 0.06 mg/m^3 *(124)*. Another study was carried out in 80 homes with 148 children aged 7–14 years, of which 53 were asthmatics. An association (OR = 1.40) between formaldehyde exposure and atopy was found with a 0.01-mg/m^3 increase in formaldehyde in the bedrooms. However, no association was identified between formaldehyde in the bedrooms and asthma incidents and lung effects *(125)*. The result is difficult to interpret, because about one third of the children were also exposed to environmental tobacco smoke and possibly pollutants from nearby coal mines and power stations *(129)*. In a third case-control study, formaldehyde was measured twice in homes (bedroom and living room) of 88 asthmatic children under three years of age and a non-asthmatic control group of 104 children *(126)*. A formaldehyde concentration > 0.06 mg/m^3 in the bedroom was found to be associated with an increased risk of asthma. Potential bias could be created by gas heating and new materials in the homes, and the general difficulty of diagnosis in children. Further confounding factors are discussed by Gilbert *(69)*. The most important confounding factor, however, is the presence of combustion products as indicated by reported high concentrations of traffic pollutants such as benzene, toluene, xylenes, nitrogen dioxide and sulfur dioxide in the homes of the children *(130)*. Such pollutants are known to be associated with asthma in children *(131)*, that is, the reported cases may be different apart from asthma and formaldehyde exposure *(132)*. For further information about this particular study and the impact of combustion products and lung effects, see Nielsen et al. *(133)*.

Measured formaldehyde in living rooms and bedrooms did not differ in a univariate analysis between 90 matched pairs of homes of young asthmatics and non-asthmatics aged 4–17 years *(134)*.

The formaldehyde-specific IgE level decreased in 8-year-old children when they moved to a new school with a lower formaldehyde level *(128)*, although no association was identified between formaldehyde and reported symptoms, which encumbers the interpretation. One school study indicated an association between low formaldehyde values and airway effects *(106)*, while another study failed to do so *(135)*. However, the multiple co-exposure of animal allergens, moisture damage (fungi), traffic pollution and socioeconomic factors encumbers interpretation. In addition, chance significance is possible because of the high number of comparisons.

Formaldehyde-specific IgE was measured in 155 Japanese children randomly recruited from outpatient clinics, 122 asthmatics (mean age 9.5 years) and 33 without allergy (mean age 8.8 years) *(136)*. No correlation was found between severity of asthma and IgE levels and formaldehyde, which agrees with the findings of Kim et al. *(137)*. Formaldehyde-specific IgE was detected in only two asthmatic children and only at low levels. One child suffered from severe asthma, while the other had mild asthma.

In a cross-sectional case-control study, comparison of formaldehyde, total volatile organic compounds and dampness in the homes of 193 children (aged 9–11 years) with persistent wheezing and 223 controls showed that formaldehyde may increase wheezing. However, this may be interfered with or dominated by the effects of dampness *(138)*.

In a similar cross-sectional case-control study of children aged 9–11 years, 245 with asthma symptoms within the last year and 329 controls, no association was found between formaldehyde exposure (median concentration 0.037 mg/m^3) in the home and reported asthma, allergy, adverse lung function, bronchial hyperreactivity or sensitization *(139)*.

Adults. Mean exposures of 1.4 ± 0.7 mg/m^3 caused a minor decrease in lung function among students dissecting cadavers *(85)*, an effect that diminished over weeks. Three other studies with exposed students and controls failed to find dose–response relationships *(87–89)*. A limited effect on lung function, and rhinitis, is in accord with a study of 95 patients with both upper and lower airway symptoms related to work that were challenged with inhaled formaldehyde *(86)*. Formaldehyde had no effect when adjusted for placebo effects, and the authors concluded that IgE-mediated formaldehyde allergy was nonexistent.

In a prospective study of 998 pregnant Japanese women, a possible association was identified between formaldehyde levels (median 0.030 mg/m^3, maximum 0.164 mg/m^3) and atopic eczema, but not with asthma, allergy or rhinitis *(140)*. Another prospective study involved 143 Japanese medical students exposed to 3.0 mg/m^3 formaldehyde that responded to a questionnaire before and after a course in anatomy. Two students, one of whom was atopic, showed skin reaction to 1% formaldehyde solution *(141)*. No association was found between reported

asthma in 182 inhabitants from 59 homes and measured formaldehyde levels in their kitchens *(109)*. Eczema, but not allergic respiratory effects, was reported in a study among Finish metal workers exposed to, inter alia, formaldehyde and metalworking fluids *(142)*.

In a cross-sectional study, VOCs and formaldehyde emitted from newly painted surfaces were found to be associated with exacerbated asthma in a study of 252 asthmatics that were compared with 310 non-asthmatics *(127)*. The low number of affected people, multiple exposures (e.g. wood smoke and pets), socioeconomic status and the possibility of chance significance have been suggested as potential sources of bias *(133)*.

In summary, consistent cause–effect and dose–response relationships between formaldehyde and measurable lung effects have not been found in controlled human exposure studies and epidemiological studies below 1 mg/m^3. In general, associations between formaldehyde and lung effects or sensitization in children in homes and schools have not been convincing owing to confounding factors and chance effects *(17,77,132)*.

Release of formaldehyde from wood particles. It has been proposed that particles, such as allergens, may carry formaldehyde down to the lower airways *(132,143)*. Indeed, combined effects between formaldehyde and particles have been reported.

In the only human exposure study, subjects reported more coughing and effects on the lungs when exposed to 0.5 mg/m^3 active charcoal particles (1.4 μm diameter) and 3.5 mg/m^3 formaldehyde *(144)*. These effects are supported by studies on mice and guinea-pigs *(145–147)*, although the results are difficult to interpret for risk assessment because the concentrations are in general orders of magnitude higher than normally found indoors. Further, the amount of releasable formaldehyde from wood particles (> 6 μm) into the respiratory tract has been estimated to be negligible under the conditions in which the particles (5 mg/m^3) were exposed to 0.4 mg/m^3 formaldehyde *(148)*.

The release of formaldehyde from medium-density fibreboard has been measured to lie between 100 and 1000 μg/g dust during 6 hours in water at 35–37 °C *(110,149)*. This shows that the maximum amount of releasable formaldehyde from inhaled dust particles is 2 μg/day for a respirable particle concentration of 100 μg/m^3 and a respiratory rate of 20 m^3/day. Thus, estimated formaldehyde release is insignificant compared to the inhaled amount of gaseous formaldehyde per day (1 mg) at a concentration of 0.05 mg/m^3 *(45,150)* and in agreement with formaldehyde on ambient particles *(151)*.

In summary, the reported studies on formaldehyde in the wood industry indicate that release of formaldehyde into the airways from inhaled particles in indoor environments is negligible compared to the inhalable formaldehyde.

Susceptible groups (non-cancer)

Formaldehyde exposure alone

Paustenbach et al. *(77)*, in their comprehensive review, concluded that hypersensitive groups (elderly people, asthmatics and children) could not be identified, nor could they identify any indication of sensitization by exposure to formaldehyde. This has been supported by comprehensive reviews during the last decade *(66,67)*. Increased sensitivity is not considered biologically plausible. No studies on formaldehyde have been reported that show elderly people to be more susceptible; on the contrary, the elderly are generally less sensitive to sensory irritation *(95)*, possibly decreasing after the age of 60 years *(152,153)*.

Children may breathe more oronasally than adults, in addition to having higher respiration. DNA–protein cross-linking (DPX) has been shown in a computational fluid dynamic nasal model to be about 1.5 higher in adults than in children *(154)*. This suggests that children are not more susceptible than adults, which agrees with predicted formaldehyde adsorption rates per unit surface area of the nasal cavity being equal in children and adults *(73)*.

Combined exposure

One study showed that asthmatics sensitive to grass pollen are insensitive to formaldehyde prior to inhalation of grass pollen *(82)*. Another study indicated that dust mite asthmatics may be more sensitive to a dust mite dose after formaldehyde exposure by mouth *(80)*. The effect is not considered to have clinical relevance. Healthy people that suffer from nasal distress in their homes have been shown to exhibit swelling of the mucosa following exposure to 0.13 mg/m^3 formaldehyde for two hours when compared with a control group *(78)*.

In summary, the experimental and epidemiological literature on formaldehyde does not indicate an increase in susceptibility among children, elderly people and asthmatics. Nevertheless, people with a personal trait of negative affectivity may report more symptoms.

Long-term (carcinogenic) effects of exposure to formaldehyde at indoor levels

Formaldehyde is classified by IARC as carcinogenic to humans (Group 1) *(1)*. In addition to sufficient evidence in experimental animals for upper airway carcinogenicity, IARC concluded that there is sufficient epidemiological evidence that formaldehyde causes nasopharyngeal cancer in humans. This was based on results from the U.S. National Cancer Institute (NCI) cohort and supported by the primarily positive findings in other studies. IARC *(1)* found only limited epidemiological evidence that formaldehyde causes sinonasal cancer in humans and the overall balance of epidemiological evidence did not support a causal role for formaldehyde-induced cancer at other sites, including the oral cavity, oro- and hypopharynx, pancreas, larynx, lung and brain. IARC recently accepted that

there is sufficient evidence that formaldehyde may cause myeloid leukaemia in humans *(155)*. The change in classification of myeloid leukaemia was supported by two new studies *(156,157)*.

Formaldehyde is genotoxic in multiple in vitro models and in exposed humans and laboratory animals *(1,64)*. Studies in humans showed increased DPX in workers exposed to formaldehyde, and genotoxicity and cytotoxicity are considered to play important roles in the carcinogenesis of formaldehyde in nasal tissues *(1)*, where cell proliferation due to cytotoxicity is considered a key element in the development of airway cancer *(64,158)*. For this type of carcinogenic effect, the NOAEL and the use of assessment factors are considered appropriate for setting standards or guidelines for airborne exposures *(159)*. On the contrary, the early risk assessments used linear low-dose extrapolations, which do not account for the sub-linearities in the observed concentration–response relationship *(1)*. The NOAEL approach has been used for setting health-based occupational exposure limits for formaldehyde, for example in Europe *(64)*, Germany *(160)*, Japan *(161)* and the United States *(162)*, and for setting outdoor air standards in Germany *(66)*.

Biological mechanisms

Formaldehyde is a normal component of the blood. In humans, exposure to about 2.5 mg/m^3 airborne formaldehyde did not increase the blood level and exposure to less than 0.6 mg/m^3 did not increase urinary formate excretion owing to rapid metabolism *(1)*. From a mathematical model describing the absorption and removal of inhaled formaldehyde in the human nose, it was predicted that exposures in the range of 0.125–12.5 mg/m^3 cause only extremely small increases in blood formaldehyde levels compared to pre-exposure levels *(50)*. In monkeys, 7.5 mg/m^3 formaldehyde for 6 hours a day, 5 days a week for 4 weeks produced no increase in blood formaldehyde level. In rats, the half-time of formaldehyde is about one minute in the plasma after intravenous administration *(1)*. This indicates that normal indoor air levels of formaldehyde are not expected to increase internal organ exposures.

The mucosal effect in Wistar rats was studied at exposures to 0, 0.125, 1.25 or 12.5 mg/m^3 formaldehyde for 6 hours a day, 5 days a week for 1 year *(163)* and 28 months *(119)*. No histological effect was apparent at 1.25 mg/m^3. In another study, nasal epithelial effects were observed at 2.5 mg/m^3 in Fischer 344 rats exposed for 6 hours a day, 5 days a week for 6–24 months *(164)*. This indicates a NOAEL of 1.25 mg/m^3 for histopathological changes.

In the nasal tissue, formaldehyde reacts with glutathione to form S-(hydroxymethyl)glutathione, which is oxidized by the formaldehyde-dependent alcohol dehydrogenase to produce formate *(1)*. The half saturation of the enzyme is estimated to occur at 3.25 mg/m^3 formaldehyde in the air *(60)* and, thus, higher exposure levels are expected to cause a disproportionate increase in cellular lev-

els of formaldehyde. Formaldehyde causes DPX formation, which is non-linearly related to formaldehyde concentration. A conspicuous increase in DPX formation occurs above 2–4 mg/m^3 *(1)*. In the nasal tissue of animals, DPX is removed rapidly and not accumulated over the exposure period *(1)*.

Nasal cancer in inhalation studies in rats

Chronic exposure to about 7.5 mg/m^3 formaldehyde and above caused squamous cell carcinoma of the nasal cavity of rats *(67,158,165)* with a non-linear concentration–response relationship *(66,158,165)*. Exposure-dependent squamous cell carcinoma has not been observed at 2.5 mg/m^3 (2 ppm) and lower formaldehyde concentrations *(1)*. Further, animal data mostly suggest that organs that are not in direct contact with formaldehyde do not develop neoplasms, presumably due to the fact that formaldehyde is highly reactive and rapidly metabolized locally *(158)*.

The development of squamous cell carcinoma is considered to be related to a genotoxic effect that may be due to DPX *(63,156,164)* in addition to cytolethality-regenerative cellular proliferation *(156,165)*; increased cell proliferation in the rat nose is considered to occur at about 2.5 mg/m^3 formaldehyde and above *(67,158)*.

Lymphohaematopoietic malignancies in animals

Drinking-water studies. Formaldehyde was administered in the drinking-water in a 2-year study in Wistar rats *(168)*. Males were dosed with 0, 1.2, 15 or 82 mg/kg per day and females with 0, 1.8, 21 or 109 mg/kg per day. Each group comprised 50 rats of each sex. Treatment-related pathological effects were limited to changes in the stomach and the kidney in both sexes in the high-dose group; the kidney effect was considered secondary to the reduced intake of liquid. The incidence of tumours did not vary markedly between the groups. Thus, the number of tumour-bearing rats and the total number of tumours were lower in the high-dose males than in the control males.

Haematological tumours were limited to generalized histiocytic sarcoma in one male and myeloid leukaemia in another male, both in the high-dose group. No lymphoma appeared in the high-dose group and no exposure-dependent lymphoma appeared from the study of the auxiliary lymph nodes and the small intestine.

In another study *(169)*, formaldehyde was administered to Wistar rats for up to 24 months at 0, 10, 50 or 300 mg/kg per day. Each group comprised 20 males and 20 females. None of the animals survived 24 months of exposure in the 300-mg/kg group and severe lesions were observed in the stomach. Additionally, serum urea nitrogen increased significantly in both sexes, suggesting an effect on the kidneys. There was no significant difference in the incidence of any kind of tumour among the groups.

In a 104-week study *(170)*, Sprague-Dawley rats were exposed to 10, 50, 100, 500, 1000 or 1500 mg formaldehyde per litre drinking-water. Another group was treated with 15 mg methanol per litre. The treated groups each consisted of 50 males and 50 females, while a control group given tap water consisted of 100 males and 100 females. The animals were observed until they died. There was no difference in survival among the groups, but the number of tumour-bearing animals was significantly higher among males in the highest exposure group. In the female control, methanol and 10, 50, 100, 500, 1000 and 1500 mg/l formaldehyde groups, the percentages of animals with haemolymphoreticular neoplasia were 7, 10, 10, 14, 16, 14, 22[1] and 20[1], respectively. In the males, the percentage was 8, 20, 8, 20, 26[2], 24[1], 22[1] and 46[2], respectively. The study has a number of limitations *(1)*. This applies to the "pooling" of lymphomas and leukaemias ("haemolymphoreticular neoplasia"), the lack of reporting of non-neoplastic lesions, and the absence of information on the incidence of haemolymphoreticular tumours in the historical controls. Further, the incidence in comparison with the methanol-treated group was significantly increased only in the high-dose males, but the dose–response relationship was statistically significant.

Overall, the drinking-water studies showed no consistent increase in lymphohaematopoietic malignancies. Where significant, the effects were at the high formaldehyde levels and exposure–response relationships were apparently non-linear.

Inhalation studies. Groups of approximately 120 male and 120 female Fischer 344 rats and C57BL/6 × $C3HF_1$ mice were exposed to 0, 2.5, 7 or 18 mg/m^3 formaldehyde for 6 hours a day, 5 days a week for 24 months. The exposure period was followed by up to 6 months of non-exposure. Gross pathological examinations were performed on all animals that died or were sacrificed; histopathology was performed on 50 tissue samples per animal in the control and highly exposed groups.

Significantly increased mortality was observed both in male and female rats in the high-dose group and in males in the intermediate group. Survival in female mice was not affected by formaldehyde exposures. Exposed male mice had a slightly poorer survival, but this was not statistically significant. The significant formaldehyde-induced lesions were restricted to the nasal cavity and proximal trachea in both species *(164)*.

The slides from the Kerns et al. *(164)* study were re-evaluated by Woutersen *(114)* as well as by a recent IARC working group (see Baan et al. *(155)* for a list of the working group members) to investigate the occurrence of lymphohaematopoietic malignancies. A mortality-adjusted trend test (the Peto mortality-

[1] $P < 0.05$.
[2] $P < 0.01$.

prevalence test) was used to take into account early deaths due to nasal cancer that might have limited the detection of lymphohaematopoietic malignancies *(114)*. No associations between formaldehyde exposure and leukaemia were seen in male or female rats at the end of the 24-month exposure period or in the 6-month recovery period. In male mice, rare lymphomas were seen at the end of the 24-month exposure period (1%, 1%, 1% and 0%, respectively, in the 0-, 2.5-, 7- and 18-mg/m^3 exposure groups), whereas the trend was highly significant in female mice (17%, 16%, 9% and 29%, respectively). It was concluded that formaldehyde may induce lymphoma in female mice, which is clearly driven by the incidence in the top exposure group.

The IARC working group noted that 12, 17, 16 and 7 out of 120 female rats developed undifferentiated leukaemia in the 0-, 2.5-, 7- and 18-mg/m^3 exposure groups, respectively, and that there was a markedly decreased survival in the 18-mg/m^3 group. Based on a survival-adjusted analysis, the incidence of leukaemia in females exposed to 18 mg/m^3 was increased compared to controls ($P = 0.0056$; Tarone extension of the Cox test, $P < 0.0167$). The working group noted that this is a very common, spontaneously occurring neoplasm in the F344 rat strain.

These re-evaluations permit two conclusions. First, leukaemia may or many not be induced in Fischer 344 rats at 24 months of exposure to 18 mg/m^3, at which a high incidence of nasal tumours occurred. Second, if lymphoma is induced by formaldehyde in female mice, the occurrence is at the very high exposure level at which there was high incidence of nasal tumours in rats. Thus, in rats the occurrence of nasal tumours is a more sensitive end-point than lymphohaematopoietic malignancies.

In another study, 100 Sprague-Dawley rats were exposed to 18 mg/m^3 formaldehyde for 6 hours a day, 5 days a week for life. Complete necropsy was performed on each animal. Histological sections were performed from each lobe of the lung, trachea, larynx, liver, kidney, testes and other organs where gross pathology was present. There was an increased mortality in the formaldehyde group compared with the control group. In the formaldehyde group, three malignant lymphomas were observed. In the similar control group of 99 rats, two malignant lymphomas were observed, while three were observed in 99 colony controls *(171)*.

In a 28-month study, male F-344 rats in groups of 32 were exposed to formaldehyde for 6 hours a day, 5 days a week at 0, 0.38, 2.5 or 18.8 mg/m^3, plus a room control group. The number of rats alive at 18 months or later and thus available for histopathology was 19, 22, 17, 7 and 16, respectively. Haematological, biochemical and pathological examinations were performed. Tissues for histopathology were pituitary, thyroid, nasal region, trachea, oesophagus, stomach, small and large intestine, prostate gland, urinary bladder, muscle, femur, sciatic nerve, spinal cord, mesenteric lymph nodes and any other gross lesion. Increased

mortality was observed at the highest exposure concentration. No microscopic lesions were attributed to formaldehyde exposure except those in the nasal cavity. Also, there was no exposure-related abnormal haematological finding *(172)*.

Overall, the occurrence of lymphohaematopoietic malignancies in inhalation studies in rats and mice is not convincing. In general, there is lack of consistency across species *(165,173)*. Nevertheless, if it is assumed that there is a causal association, the association was seen at high exposure levels, which caused a high incidence of nasal cancer in rats. Also, the exposure–response relationship seems to be non-linear.

Assessment of cancer hazards in meta-analyses

Oral cavity and pharynx, sinus and nasal cavity, and lung. Bosetti et al. *(63)* conducted a meta-analysis based on six cohorts of industrial workers and professionals (pathologists, anatomists, embalmers and undertakers). No significant excess cancer risk was found in industrial workers and professionals for all cancers or for oral and pharyngeal cancer. The lung cancer risk was not affected in industrial workers (RR 1.06; 95% CI 0.92–1.23), whereas the risk was reduced in the professionals (RR 0.63; 95% CI 0.47–0.84). The study concluded that there was no appreciable risk for cancer of the oral cavity and pharynx, sinus and nasal cavity and lung. IARC *(1)* also concluded that the overall balance of epidemiological evidence did not support a causal role for formaldehyde in cancer in the oral cavity, oro- and hypopharynx and lungs.

In the meta-analysis by Bosetti et al. *(63)*, the nasopharyngeal cancer risk was increased in industrial workers, but this was not statistically significant (RR 1.33; 95% CI 0.69–2.56). This was based on eight cancers in one study where six cancers were in one of ten plants and one cancer was from another cohort. No excess brain cancer risk was apparent in the industrial workers (RR 0.92; 95% CI 0.75–1.13) but the risk was significantly increased in the professionals (RR 1.56; 95% CI 1.24–1.96). The brain cancer risk is not consistent across the two types of study and it is not biologically plausible that formaldehyde causes brain cancer. This is in agreement with the evaluation of IARC *(1)*.

Pancreatic cancer was addressed in a meta-analysis that comprised 14 epidemiological studies. No exposure-dependent effect was apparent *(174)*. This is in agreement with the IARC evaluation *(1)*.

Leukaemia was studied in a meta-analysis comprising 18 epidemiological studies *(175)*. Heterogeneity was observed across studies and differences appeared between the RR of formaldehyde exposures in American (RR 1.2; 95% CI 1.0–1.4) and European workers (RR 0.9; 95% CI 0.7–1.1). Furthermore, the RR was different for various types of job: industrial workers (RR 0.9; 95% CI 0.8–1.0), embalmers (RR 1.6; 95% CI 1.2–2.0) and pathologists and anatomists (RR 1.4; 95% CI 1.0–1.9). Only three of the studies *(176–178)* evaluated leukaemia rates by exposure level. This meta-analysis concluded that the data do not pro-

vide consistent support for a relationship between formaldehyde exposure and leukaemia.

In the meta-analysis by Bosetti et al. *(63)*, significantly reduced risks of lymphatic and haematopoietic cancer were observed in the industrial workers (RR 0.85; 95% CI 0.74–0.96). In contrast, the risk was significantly increased in the professionals (RR 1.31; 95% CI 1.16–1.48), comprising pathologists, anatomists and embalmers. No excess in leukaemia risk appeared in industrial workers (RR 0.90; 95% CI 0.75–1.07) but the risk was significantly increased in the professionals (RR 1.39; 95% CI 1.15–1.68).

The most recent meta-analysis that includes all relevant cohort and case-control studies published through May 2009 found no increase in leukaemia. The meta-analysis summary RR was 1.05 (95% CI 0.93–1.20) for cohort studies and the summary OR was 0.99 (95% CI 0.71–1.37) for case-control studies *(179)*.

While the three meta-analyses discussed above reported on the contrast between ever- vs never-exposed subjects and various combinations of lymphohaematopoietic cancers, a recent meta-analysis evaluated especially myeloid leukaemia from the highest exposure group of each study *(180)*. Where several RRs were reported in a study, one RR was selected from each study in the order: peak exposure, average exposure intensity, cumulative exposure, exposure duration. For example, the accepted study groups were exposed to more than 2 ppm on average, with peak exposures above 4 ppm, or were exposed for more than 10 years. In the analysis by Zhang et al. *(180)*, the fixed effects model and the random effect model showed similar results, and therefore the results are from the fixed effects model. Thus, an increased risk was observed for all types of cancer combined (RR 1.25; 95% CI 1.12–1.39; N = 19), for all leukaemia (RR 1.54; 95% CI 1.24–1.91; N = 15), for myeloid leukaemia (RR 1.90; 95% CI 1.41–2.55; N = 6) and for multiple myeloma (RR 1.31; 95% CI 1.02–1.67; N = 9) but not for Hodgkin's lymphoma (RR 1.23; 95% CI 0.67–2.29; N = 8) or non-Hodgkin's lymphoma (RR 1.08; 95% CI 0.86–1.35; N = 11).

The increases in leukaemia, myeloid leukaemia and multiple myeloma in the Zhang et al. *(180)* study were not consistently observed in the other studies *(63,175)*. This may be explained by the fact that, if these types of cancer are caused by formaldehyde, they appear at high levels of formaldehyde.

Cancer hazard studies in occupational cohorts

To obtain concentration–response relationships for formaldehyde exposures based on human experiences, the cancer risk due to formaldehyde exposure is reviewed from the three largest and recently updated occupational cohorts, which were identified from IARC *(1)*, from the formaldehyde documentation for setting a health-based occupational exposure limit by the Scientific Committee on Occupational Exposure Limits *(64)* and a recent review *(63)*. The NCI cohort comprised 25 619 workers employed in 10 facilities producing or using for-

Table 3.3. Cancer risks from formaldehyde exposure[a]

Cancer	Study[b]											
	NCI cohort *(175,179)*[c]			NCI cohort *(180)*			British cohort *(174)*[d]			USA garment worker cohort *(176)*[e]		
	ICD-8[f]	O/E	SMR	ICD-8[f]	O/E	SMR	ICD-9[f]	O/E	SMR	ICD-9[f]	O/E	SMR
All cancers	140–209	1916[g]/–[h]	0.90*	–	–	1.07*	140–208	1511/1375.2	1.10*	140–208	608/–	0.89*
Nose and nasal sinuses	160	3/–	1.19	–	–	–	160	2/2.3	0.87	160	0/0.16	–
Pharynx				–	–	–	146–149.1	15/9.7	1.55	146–149	3/–	0.64
Nasopharynx	147	8[i]/–	2.10*				147	1/2	–	147	0/0.96	–
Larynx	161	23/–	0.95	–	–	–	161	14/13.1	1.07	161	3/–	0.88
Lung	162	641/–	0.97	–	–	–	162	594/486.8	1.22*	162	147/–	0.98
Bone	170	7/–	1.57				170	6/3.5	1.73	–		
Prostate	185	131/–	0.90	–	–	–	185	80/99.4	0.80	185	11/–	1.58
Hodgkin's lymphoma	201	20/–	1.26	201	25/–	1.42	201	6/8.5	0.70	201	2/–	0.55
Non-Hodgkin's lymphoma	200	44/–	0.61*	200	94/–	0.85	200	31/31.7	0.98	200	5/–	0.85
	202			202			202.0					
							202.1					
							202.8					
Multiple myelomas	203	28/–	0.88	203	48/–	0.94	203.0	15/17.5	0.86	–		
Leukaemia	204–207	65/–	0.85	204–	116/–	1.02	204–208	31/34.1	0.91	204–208	24/–	1.09
Lymphatic leukaemia				207	36/–	1.15						
Myeloid leukaemia	205	–/–	–	204	44/–	0.90			0.89[j]	205	15/–	1.44
Stomach	–			205	–	–	151	150/114.4	1.31*	151	13/–	0.80
All digestive	150–159	420/–	0.89*	–			–		–	150–159	16/–	0.77*

[a] Standardized mortality ratio (SMR), observed cases (O), expected cases (E) and the ratio (O/E). When the 95% CI does not include 1.00, it is marked with an asterisk (*).

[b] Comparison with national death rates.

[c] Median average intensity 0.45 and range 0.01–4.25. Exposure to ≥ 2 occurred in 4.7% and 22.6% had peak exposures at ≥ 4.

[d] Range 0.1 to > 2.

[e] Geometric mean 0.15 and geometric standard deviation 1.90. Range 0.09–0.2. Past exposures may have been substantially higher.

[f] International Classification of Diseases, 8th revision (ICD-8) and 9th revision (ICD-9).

[g] In the Hauptmann et al. 2003 study *(177)*, the number of formaldehyde workers who had died was 1916 (2-year lag interval), while in the Hauptmann et al. 2004 study *(181)*, the number was 1723 (15-year lag interval). The lag interval was 2 years in the Freeman et al. study *(182)*.

[h] – = not indicated.

[i] Hauptmann et al. *(181)* (Table 2) report eight nasopharyngeal cancers among formaldehyde-exposed workers that were used for the SMR calculation. Although one subject was misclassified on the death certificate, this subject was retained in the SMR calculation since population reference rates are based on death certificates. Also, the exact 95% CI was reported to be 0.91–4.14 and thus the SMR value of 2.10 is not statistically significant. The seven cases in the text and in Tables 3–6 of Hauptmann et al. *(181)* were used for calculation of relative risks.

[j] Estimated by Cole & Axten *(183)* for the highly exposed group (> 2 ppm).

maldehyde. Workers were employed prior to 1 January 1966 and were followed through to 31 December 1994 *(177,181)* and recently through to 31 December 2004 for lymphohaematopoietic malignancies *(182)*. A cohort from six British factories, comprising 14 014 men employed after 1937, was followed through to December 2000 *(176)*. The U.S. National Institute for Occupational Safety and Health (NIOSH) established a cohort of 11 039 employees in three garment facilities; the study was updated through to 31 December 1998 *(178)*.

The cancer risks obtained from the three studies are shown in Table 3.3. The table is limited to anatomical sites that are directly exposed to airborne formaldehyde and to other sites where excess risks have been reported.

Nasopharyngeal cancer

The relative risk of nasopharyngeal cancer was further evaluated by four metrics: average exposure intensity (mg/m^3), highest peak exposure (mg/m^3), cumulative exposure (mg/m^3-years) and duration of exposure (years). In the average exposure intensity metric and the highest peak exposure metric, RRs were obtained with the unexposed group as the reference group. In the three average intensity exposure groups, > 0 to < 0.63 mg/m^3, 0.63 to < 1.25 mg/m^3 and ≥ 1.25 mg/m^3, the respective RRs were: not obtainable (0/3640 deaths), 0.38 (1/1405 deaths) and 1.67 (6/1450 deaths). Apparently, the increased risk was due to exposures ≥ 1.25 mg/m^3, although the trend was not statistically significant. With the peak exposure metric, all exposed deaths were in the highest peak exposure group (≥ 5 mg/m^3) and the trend was statistically significant. An exposure-dependent trend was found for the cumulative exposure metric *(181)*, which was apparently driven by the highest exposure level.

Later, it was shown that the excess occurrence of nasopharyngeal cancer in the NCI study was driven by one of the 10 plants studied, where 6 of the 10 cases occurred. In this plant, the cases might or might not have been caused by formaldehyde exposure but by other risk factors such as "silver smithing" and "silver smithing or other metal work" *(184)*. The only established occupational risk factor, wood dust, was considered a priori, but dropped because of very small numbers *(184)*. Additionally, a low number of nasopharyngeal cancers in the reference group can cause unstable RR estimates *(185)*. However, a recent IARC working group noted that it was unlikely that confounding or bias could explain the observed association *(186)*.

It can be considered, however, for the purposes of indoor air guideline setting, that no excess nasopharyngeal cancer was reported at a mean formaldehyde exposure level at or below 1.25 mg/m^3 and with peak exposures below 5 mg/m^3.

Lymphohaematopoietic malignancies

The NCI study also evaluated the effect of average intensity and peak exposures on the occurrence of lymphohaematopoietic malignancies leading to 178 deaths

(177). The lowest exposure groups were used as reference for evaluation of RRs. For the average exposure intensity, the reference group comprised exposures of 0.125–0.5 mg/m^3.

The two higher exposure groups comprised exposures of 0.6–1.1 and ≥ 1.25 mg/m^3. Lymphohaematopoietic malignancies were significantly increased in both groups, with a borderline significant trend. Hodgkin's lymphoma was significantly increased in the 0.6–1.1-mg/m^3 group, with a significant exposure-dependent trend. Myeloid leukaemia was significantly increased at the highest exposure level, but the trend was not significant. For the peak exposure, the exposure in the reference group was 0.125–2.4 mg/m^3 and the exposure in the two higher exposure groups was 2.5–4.9 and 5 mg/m^3, respectively. Significantly increased RRs were observed for lymphohaematopoietic malignancies and leukaemia in the two highest exposure groups.

In the highest exposure group, the RR for myeloid leukaemia was also increased. For these three diseases, the trend in exposure-dependent effect was statistically significant. Additionally, the exposure-dependent trend was statistically significant for Hodgkin's lymphoma. The RR for leukaemia was not significantly associated with cumulative exposure.

When the study by Hauptmann et al. *(177)* was reanalysed by Marsh & Youk *(187)*, it was shown that excess leukaemia and myeloid leukaemia were strongly influenced by a lower death rate in the reference groups compared to the national and local county SMRs. Using the national and local ratios, the SMRs for all leukaemia and myeloid leukaemia were very close to unity and were not significantly increased in the highest peak exposure category (≥ 5 mg/m^3). To evaluate the robustness of the categorizations, new average exposure intensity categories were constructed whereby the highest exposure category was ≥ 0.93 mg/m^3. Again, using the national and local county rates showed that the SMRs for all leukaemia and myeloid leukaemia were very close to unity and not significantly increased. Also, in this case, cumulative formaldehyde exposures were not associated with the development of leukaemia or myeloid leukaemia. Although this reanalysis does not support a causal association between formaldehyde exposure and leukaemia and myeloid leukaemia, for indoor air guideline setting one can take into account the fact that no excess lymphohaematopoietic malignancies occurred at a mean exposure level of formaldehyde below 0.93 mg/m^3 and where peak exposures were below 5 mg/m^3.

Recently, the NCI study updated lymphohaematopoietic risks through to 31 December 2004 *(182)*. SMRs were estimated from the United States mortality rate (Table 3.3). For lymphohaematopoietic malignancies, the 319 deaths resulted in similar SMRs in exposed and unexposed people: 0.94 (95% CI 0.84–1.06) and 0.86 (95% CI 0.61–1.21), respectively. Exposure-dependent trends were evaluated from exposure categories similar to the previous follow-up. For lymphohaematopoietic malignancies in the average formaldehyde intensity metric, neither

of the two highest exposure groups showed an increased RR, nor was the exposure trend statistically significant. In the new follow-up, the RR for Hodgkin's lymphoma was significantly increased in the 0.63- to < 1.25-mg/m^3 group but not in the highest exposure group (≥ 1.25 mg/m^3). The trend was statistically significant. Similar results appeared in the previous follow-up. Multiple myeloma was significantly increased among the non-exposed but not in the exposed groups. In the previous follow-up, the increase was not significant. In the peak exposure metric, lymphohaematopoietic malignancies were increased significantly in the highest exposure group (≥ 5 mg/m^3) and the trend was significant. Apparently, it is driven by the highest exposure group. Thus, the RR in the next highest exposure group was not remarkably increased (1.17 (95% CI 0.86–1.59)) and close to the RR among the unexposed, which was 1.07 (95% CI 0.7–1.62). In the previous follow-up, the RRs in the two highest exposure groups were similar (1.71 and 1.87, respectively) and significantly increased in both groups. The trend was also significant. In the new follow-up, the RR of Hodgkin's lymphoma was increased significantly in the two highest exposure groups: 3.30 (95% CI 1.04–10.50) in the 2.5- to < 5.0-mg/m^3 group and 3.96 (95% CI 1.31–12.02) in the ≥ 5-mg/m^3 group) with an exposure-dependent trend. In the previous follow-up, the trend was increased significantly but the RRs were approximately of the same size as in the recent follow-up. Except for a statistically increased RR of multiple myeloma in the non-exposed, no other remarkable RR appeared in the peak exposure group in the new follow-up study. For example, the RRs for multiple myeloma were 2.74 (95% CI 1.18–6.37) among the non-exposed, 1.0 in the reference group (≥ 0.13 to < 2.5 mg/m^3), 1.65 (95% CI 0.79–3.61) in the 2.5- to < 5.0-mg/m^3 group and 2.04 (95% CI 1.01–4.12) in the highest peak exposure group (≥ 5 mg/m^3) with no exposure-dependent trend. In this case, the RRs in the exposed groups were lower than in the non-exposed group, which does not support a formaldehyde-dependent effect. In the similar peak exposure groups, the RRs of myeloid leukaemia were 0.82 (95% CI 0.25–2.67), 1.0, 1.30 (95% CI 0.58–2.92) and 1.78 (95% CI 0.87–3.64) with a non-significant trend. In the earlier follow-up, myeloid leukaemia was significantly increased in the highest exposure group (3.46 (95% CI 1.27–9.43)) with a highly significant trend ($P \leq 0.009$).

Summarizing the NCI study, it is of note that the RRs for Hodgkin's lymphoma increase abruptly from that in the reference group (peak exposure > 0 to < 2.5 mg/m^3 and average intensity > 0 to < 0.63 mg/m^3). Overall, as the RRs in the reference group and the non-exposed group were not significantly different, an exposure guideline for formaldehyde should consider that peak exposures should be below 2.5 mg/m^3 and average exposures below 0.63 mg/m^3 to protect against lymphohaematopoietic malignancies in general.

The United Kingdom cohort from six British factories comprised 14 014 men employed after 1937 and followed through to December 2000 *(176)*. By the end of the follow-up, 5185 of the men had died. The overall mortality from all can-

cers was slightly higher than expected from national death rates (SMR 1.10; 95% CI 1.04–1.16), as was that from lung cancer (SMR 1.22; 95% CI 1.12–1.32) and from stomach cancer (SMR 1.31; 95% CI 1.11–1.54) (see Table 3.3). Lung and stomach cancers were further analysed using the local geographical variations in mortality. Lung cancer increased significantly (SMR 1.28; 95% CI 1.13–1.44) only in the highest exposed group where the formaldehyde level was greater than 2.5 mg/m^3. No trend was seen at lower levels and, for example, the SMR in the range 0.75–2.5 mg/m^3 was 0.99 (95% CI 0.74–1.30). However, there was a statistically non-significant decrease in the risk of death from lung cancer with duration of high exposure. The risk showed no increasing trend with time since first exposure. The authors interpreted lung cancer in the highest exposed group to be "rather large to be explained simply by a confounding effect of smoking" (which was not taken into account). Using the local mortality rate, stomach cancer was not exposure-dependent and was considered by the authors to be a less plausible outcome. For setting an indoor air guideline, the key information from this study is that no increase in lung cancer was apparent at formaldehyde levels of 5 mg/m^3 or lower. No results on peak exposures and risk for myeloid leukaemia were provided.

NIOSH established a cohort of 11 039 employees in three garment facilities (The USA garment worker cohort). The study was updated through to 31 December 1998, by which time 2206 of the employees had died. The mortality from all malignant neoplasms was significantly less than expected (SMR 0.89; 95% CI 0.82–0.97), as was that for all digestive neoplasms (SMR 0.77; 95% CI 0.63–0.92). Myeloid leukaemia (ICD-9: 205) was significantly increased (13 deaths; SMR 1.91) after 20 or more years since first exposure, but the trend was not significant. Among workers with both 10 or more years of exposure and 20 years or more since first exposure, multiple-cause mortality from leukaemia was significantly increased almost two-fold (15 deaths; SMR 1.92; 95% CI 1.08–3.17). In addition to underlying cause of death, all causes listed on the death certificate were analysed using multiple cause mortality. Multiple cause mortality from myeloid leukaemia was significantly increased among this group (8 deaths; SMR 2.55; 95% CI 1.10–5.03) *(178)*.

Recent studies on lymphohaematopoietic effects

Haematopoietic tissue damage was studied in 43 formaldehyde-exposed workers and 51 controls. The 8-hour time-weighted average was 1.6 and 0.03 mg/m^3 and the 90 percentile 3.14 and 0.03 mg/m^3, respectively. Peak exposure concentrations were not reported. Formaldehyde exposures were associated with reduced blood lymphocyte, granulocyte, platelet, red blood cell and total white blood cell counts; the total white blood cell count was reduced by 13.5% in the formaldehyde-exposed workers. Urinary benzene concentrations were low in both groups, thus excluding benzene exposure as a confounder. The findings

were considered consistent with a bone-marrow-toxic effect due to formaldehyde. Peripheral blood cells from formaldehyde-exposed and control workers were cultivated to derive blood myeloid progenitor cells. The colony formation fell non-significantly by 20% in the formaldehyde-exposed workers and this was considered a toxic effect on the myeloid progenitor cells. Blood mononuclear cells from volunteers were cultivated in vitro to derive different lines of progenitor cells.

The addition of different dilutions of formalin to the cultures showed that formaldehyde reduced the number of generated colonies from all progenitor cell lines. This showed that formaldehyde can inhibit the proliferation of all progenitor cells if the endogenous formaldehyde level is increased due to formaldehyde exposure. Blood progenitor cells of the myeloid line were derived from 10 highly exposed workers (8-hour time-weighted median concentration 2.67 mg/m^3 and 90th percentile 5.18 mg/m^3) and 12 controls (8-hour time-weighted median concentration 0.03 mg/m^3 and 90th percentile 0.03 mg/m^3). Formaldehyde-exposed workers showed increased monosomy (loss) of chromosome 7 and an increase in trisomy of chromosome 8; these cytogenic changes are observed in myeloid leukaemia and myelodysplastic syndromes *(154)*.

It should be noted that the study has limitations in relation to risk assessment of formaldehyde exposure at indoor air concentrations. First, the exposures are extremely high and thus the unreported peak exposure concentrations may have been at extremes. Second, no exposure–response relationship is established. Third, the very high exposure concentrations may be expected to cause mucosal damage that may influence both the nasal metabolism and absorption into the blood compartment; no information is available on the mucosal tissue. Fourth, the in vitro cell culture study is relevant for mechanistic considerations only, because no increase in formaldehyde has been observed in the blood compartment of humans due to formaldehyde exposure. This is supported by model calculations at about 2.5 mg/m^3 *(50)*. Similar results were reached for extrapolations up to 12.5 mg/m^3, but such extrapolations may be invalidated by the toxic effects on the mucosal membrane above 2.5 mg/m^3. Overall, the interpretation of this study in relation to risk assessment is unclear. For the sake of transparency, it would have been desirable that all measured exposures other than to formaldehyde had been reported.

In a case-control study in the United States *(157)*, 168 professionals employed in the funeral industry who died from lymphohaematopoietic malignancies were compared with 265 deceased matched controls from the same industry. The 8-hour time-weighted average formaldehyde intensity was about 0.125–2.5 mg/m^3, the average intensity while embalming was about 1.9–2.25 mg/m^3 and peak exposure was about 10–13 mg/m^3. Four people died from nasopharyngeal cancer, but only two had been involved in embalming (OR 0.1 (95% CI 0.01–1.2)). No increase was observed in lymphoid malignancies (ICD-8 200–204), in-

cluding Hodgkin's lymphoma (OR 0.5 (95% CI 0.1–2.6)), which was consistently elevated in the previous industrial cohort studies *(177,182)*. The study observed a specific association between embalming and myeloid leukaemia (ICD-8 205). Thus, using a reference group of newer exposed with one case subject, the OR was 11.2 (95% CI 1.3–95.6).

The first analysis of myeloid leukaemia used a reference group of subjects that had not performed embalming. The duration of working in jobs that involved embalming showed a significant trend ($P = 0.02$): in the categories > 0–20, > 20–34 and > 34 years, the OR was 5.0 (95% CI 0.5–51.6), 12.9 (95% CI 1.4–117.1) and 13.6 (95% CI 1.6–119.7), respectively. No significant trend was observed with the number of embalmings. However, several significant ORs were observed. Thus, the number of performed embalmings were divided into > 0–1422, > 1422–3068 and > 3068, where the OR was 7.6 (95% CI 0.8–73.5), 12.7 (95% CI 1.4–116.7) and 12.7 (95% CI 1.4–112.8), respectively. Exposure–response relationships for the different formaldehyde metrics were established. The peak exposure metric was the only metric that showed a significant trend ($P = 0.036$). Peak formaldehyde exposures were divided into > 0–8.75 mg/m^3, > 8.75–11.6 mg/m^3 and > 11.6 mg/m^3, where the OR was 15.2 (95% CI 1.6–141.6), 8.0 (95% CI 0.9–74.0) and 13.0 (95% CI 1.4–116.9), respectively. The cumulative formaldehyde exposures (mg/m^3-hours) were divided into > 0–5073, > 5073–11 566 and > 11 566, where the OR was 10.2 (95% CI 1.1–95.6), 9.4 (95% CI 1.0–85.7) and 13.2 (95% CI 1.5–115.4), respectively. The average formaldehyde intensity while embalming was > 0–1.75 mg/m^3, > 1.75–2.38 mg/m^3 and > 2.38 mg/m^3, where the OR was 11.1 (95% CI 1.2–106.3), 14.8 (95% CI 1.6–136.9) and 9.5 (95% CI 1.1–86.0), respectively. The 8-hour time-weighted formaldehyde intensity was divided into > 0–0.125 mg/m^3, > 0.125–0.225 mg/m^3 and > 0.225 mg/m^3, where the OR was 8.4 (95% CI 0.8–79.3), 13.6 (95% CI 1.5–125.8) and 12.0 (95% CI 1.3–107.4), respectively. The cumulative formaldehyde exposure, the average formaldehyde intensity while embalming and the 8-hour time-weighted average intensity showed no statistically significant formaldehyde exposure-dependent trend. It is noted that, within each of the formaldehyde exposure metrics, the ORs showed little difference and had highly overlapping confidence intervals. This suggests that the statistical significances are driven mainly by exposure vs non-exposure and less by differences in exposure levels. Also, in each of the formaldehyde metrics, none of the trend tests within the formaldehyde groups themselves was statistically significant.

Because of the small number of exposed cases and related instability of the reference group, the authors performed additional exposure–response analyses with a larger reference group, including subjects with low exposure. The second analysis of myeloid leukaemia used a reference group whose members had performed fewer than 500 lifetime embalmings, allowing 5 case subjects in the reference group.

The duration of working in jobs with embalming showed a significant trend ($P = 0.02$). In the categories < 20, > 20–34 and > 34 years, the OR was 0.5 (95% CI 0.1–2.9), 3.2 (95% CI 1.0–10.1) and 3.9 (95% CI 1.2–12.5), respectively. No significant trend was observed with the number of embalmings, but significant ORs were observed at the highest exposure level. Thus, the numbers of performed embalmings were divided into ≥ 500–1422, > 1422–3068 and > 3068, where the OR was 1.2 (95% CI 0.3–5.5), 2.9 (95% CI 0.9–9.1) and 3.0 (95% CI 1.0–9.2), respectively.

The peak exposure metric was the only formaldehyde metric that showed a significant trend ($P = 0.036$). Peak formaldehyde exposures were divided into ≤ 8.75 mg/m^3, > 8.75–11.6 mg/m^3 and ≥ 11.6 mg/m^3, where the OR was 2.9 (95% CI 0.9–9.8), 2.0 (95% CI 0.6–6.6) and 2.9 (95% CI 0.9–9.5), respectively. The trend was not statistically significant in the cumulative formaldehyde exposure, the average formaldehyde intensity while embalming and the 8-hour time-weighted intensity group. Only the highest cumulative formaldehyde exposure group (> 11 566 mg/m^3-hours) had a statistically elevated OR of 3.1 (95% CI 1.0–9.6). Except for this, the ORs were elevated (2.0–2.9) and very similar within each of the metrics, but none was significantly increased.

Also, in each of the metrics, none of the trend tests within the formaldehyde groups themselves was statistically significant. It is noted that the overall picture was similar to that in the first analysis except for the fact that the ORs fell by one third in this analysis, where a higher number of case subjects were available in the control group. Only one significant OR appeared in the formaldehyde exposure metrics, which was in strong contrast to the 10 significantly elevated ORs in the first analysis.

It is noted that there is a lack of exposure-dependent differences in OR within the different formaldehyde exposure levels in the different metrics. A lack of exposure-dependent effect could be due either to an inappropriate exposure assessment or to a lack of causality between formaldehyde exposure and myeloid leukaemia; the reference groups contained a low number of case subjects. The method of formaldehyde exposure has limitations, as the estimates were predicted by means of interviews and mathematical modelling rather than being based on measured exposures. Also, it is mentioned by the authors that the peak model was not validated. On the whole, this study cannot be used for risk assessment as it does not provide a convincing exposure–response relationship.

Comparison of the Zhang et al. *(156)* and the Hauptmann et al. *(157)* studies reveals some differences. The Zhang et al. study suggests an effect on all progenitor cells that results in decreased production of lymphocytes, granulocytes, platelets and red blood cells. Similar results were obtained from the in vitro cell cultures with different progenitor cell lines. In the Hauptmann et al. study, the effect was selective at the myeloid progenitor line. Overall, these studies have very high exposure intensities and thus do not contradict the fact that lymphohaemat-

opoietic malignancies are not observed at lower levels, as derived from the 2003 study by Hauptmann et al. *(177)* and its re-analysis by Marsh & Youk *(187)*.

The meta-analysis based on the highest exposure levels reported that formaldehyde caused leukaemia and especially myeloid leukaemia *(180)*. Three hypotheses were proposed. First, formaldehyde could be transported by the blood to the bone marrow, where it could cause initiation in a stem or progenitor cell. Second, as a portion of the bone marrow stem and progenitor cells circulate in the peripheral blood, they may be initiated by formaldehyde absorbed into the blood. Third, initiation of the primitive pluripotent stem cells presented within the nasal mucosa could occur, followed by transport to the bone marrow. Similar arguments were analysed by Pyatt et al. *(173)* and the two first hypotheses were not considered likely owing to the negligible amount of formaldehyde reaching the blood. However, nasal (portal-of-entry) effects caused by high formaldehyde exposure levels could be a plausible mechanism for Hodgkin's lymphoma. However, this was not consistent with the Zhang et al. meta-analysis *(180)*. In summary, potentially offending levels can be considered to be in the range where formaldehyde has shown nasal effects in rats, as no lymphohaematopoietic malignancies were observed with mean exposures below 0.63 mg/m^3 and peak exposures below 2.5 mg/m^3, if caused by formaldehyde at all.

Prediction of nasal cancer

Formaldehyde can induce squamous cell carcinoma of the nasal cavity in rats. As a nasal effect would be consistent across species, it is considered the key to setting an indoor air guideline for carcinogenic effects of formaldehyde. The NOAEL approach for setting a guideline value is based mainly on the strongly non-linear relationship between formaldehyde exposure and development of squamous cell carcinoma in rats, largely corroborated by epidemiological studies. This approach accepts that the fall-off of the carcinogenic effect is so rapid that the observed NOAEL resembles a true NOAEL. Accepting these arguments, an indoor air guideline value can be set by dividing the appropriate NOAEL by one or more assessment factors *(159)*. This approach considers the NOAEL for squamous cell carcinoma in rats (2.5 mg/m^3), the NOAEL for nasal cytotoxicity in rats (1.25 mg/m^3) and the potential development of malignancies in humans, which have not been encountered at mean exposures below 0.63 mg/m^3 and peak exposures below 2.5 mg/m^3 formaldehyde.

To obtain a deeper knowledge and thus a better risk assessment, a biologically motivated model has been developed that models exposures by computational fluid dynamics and the development of cancer from a two-stage clonal growth model *(17,167,188)*. Formaldehyde was assumed to act as a direct mutagen with the effect considered proportional to the concentration of the pro-mutagenic DPX lesion. The DPX formation is considered linearly related to the formaldehyde concentration; the linear relation between formaldehyde and DPX concen-

trations can be considered a worst-case scenario in the low-dose range. At high concentrations, the model includes that cytolethality is followed by cell proliferation. Mutations are considered to occur during cell division, and a tumour cell arises when an initiated cell (modelled by DPX levels) acquires a second mutation *(17,167)*. The relationship between formaldehyde exposure and the average cell division rate was J-shaped in rats. The rapid increase in cell proliferation occurred at a level that was not significantly different from a threshold model with a NOAEL set above 2.5 mg/m^3 *(167)*. The two-stage clonal growth model was shown to predict nasal tumours in rats using a lifetime cumulative probability of squamous cell carcinoma with 13 animals with squamous cell carcinoma among 7684 control rats from the U.S. National Toxicology Program historical control database, and where several of the parameters were estimated from the best fit of the model to the experimental data.

The biologically motivated model was extended to humans and took into account that humans are oronasal breathers *(17,188)*. For the general population, the predicted additional risk of upper respiratory tract cancer for non-smokers, associated with an 80-year continuous exposure to 0.125 mg/m^3 formaldehyde, was about 2.7×10^{-8} *(17)*. The additional risk was estimated to be 10^{-6} or less for non-smokers exposed continuously to 0.25 mg/m^3 formaldehyde *(188)*.

The robustness of the model has been challenged by sensitivity analyses *(189–191)*. Thus, the estimate is sensitive to the DPX half-life in the nose *(190)*; the DPX half-life was accepted as 1.78 hours in the 2003 study by Conolly et al. *(167)* that was based on in vivo rat studies, but it was assumed to be 12.3 hours in the sensitivity analysis *(190)* on the basis of the half-life in immortalized cell lines *(192)*. The estimated risks were sensitive to the incidence of squamous cell carcinoma in the rat control group data *(189,190)* and to the data used for rates of nasal cell replication and death *(189,191)*. For example, the instability of the estimates was seen when the current control group, comprising no squamous cell carcinoma among 341 controls, was used *(189)*, although this frequency is in overall agreement with what would have been expected from the cumulative group by proportional scaling (0.57/341). Overall, the sensitivity analyses highlight the limited possibility of predicting risks from the rare events in the unexposed control group.

The importance of the replication rate of the initiated cell was addressed *(189,191)*. Introducing a minor arbitrarily selected increase in cell division rate by formaldehyde exposure and using the entire historical control group of rats, the predicted human risk of respiratory cancer by the age of 80 years from lifetime exposure varied from about 0.02 to about 1 at 0.1 ppm formaldehyde. Thus, the sensitivity analysis showed that the assumed cell division rate of the initiated cell has a tremendous effect on the predicted risk. This led to the conclusion that the Conolly et al. model *(175)* is not reliable for estimating human risk, irrespective of whether the predictions by Crump et al. *(193)* are at odds with human

epidemiology, which was the main point of critique of the sensitivity analyses *(194)*.

The 2004 estimate of Conolly et al. *(188)* has to be taken cautiously. It is not an upper boundary ("worst-case consideration"), which can reach values – depending on the assumptions in the sensitivity analysis – that are incompatible with epidemiological findings. Beside the key event of cell proliferation in formaldehyde-induced nasal cancer identified in animal studies, the estimates by the International Programme on Chemical Safety *(17)* and Conolly et al. *(188)* qualify the discussion about the size of the formaldehyde-induced risk as well as providing input to the selection of the assessment factor in the NOAEL approach.

A recent model study showed that formaldehyde exposure of children would result in less DPX formation than it would in adults exposed at the same level *(154)*. Consequently, children are not expected to be more sensitive to any carcinogenic effect of formaldehyde than adults and are thus not considered separately in the further evaluation.

Health risk evaluation

Exposure evaluation

The major exposure route for formaldehyde is inhalation. Although concentrations above 0.2 mg/m^3 may be encountered in new or renovated buildings, in new furnishings and at hot and humid times of the year, levels on the average are less than 0.05 mg/m^3 in homes and about half that in public buildings (Table 3.4). The most important way to control the formaldehyde concentration is the air exchange rate and the use of low-emitting materials and products. Environmental tobacco smoke and ozone-initiated reactions of alkene compounds may

Table 3.4. Mean exposure concentrations of formaldehyde in various environments, sampled over several days

Source	Concentration (mg/m^3)
Outdoor air	
General	< 0.01
Highly urbanized or industrial areas	0.02
Indoor air	
General	0.01–0.1
Homes	
General	< 0.05
Range	0.005–0.25
Schools/Kindergartens	
General	< 0.05
Range	0.002–0.05
Public buildings	
General	< 0.025
Range	0.005–0.15

contribute to temporary peak levels. Outdoor concentrations are considerably lower, except in some major cities.

Formaldehyde is a normal component of blood. Exposure to 2.4 mg/m^3 did not increase the blood level and exposure to 0.5 mg/m^3 did not result in an increase in urinary formate excretion due to rapid local metabolism *(1,37,43)*.

Critical health outcomes

Effects of formaldehyde in indoor air are generally expected to be limited to effects at the site of contact, specifically the eyes and nasal and upper airways. Effects are due to direct reactions with formaldehyde itself and do not appear to require metabolism.

Non-cancer

The acute symptom of formaldehyde at indoor exposure concentrations is sensory irritation of the eyes and upper airways. Human exposure studies indicate that 0.63 mg/m^3 is the threshold for trigeminal stimulation of the eyes (e.g. increased blink frequency) and 0.38 mg/m^3 is the threshold for subjective sensory irritation.

In general, the concentration perceived by the olfactory system is lower than that triggering sensory irritation of the eyes and airways, and people may therefore report symptoms at levels below its sensory irritation threshold.

Irritation effects of formaldehyde are not cumulative, based on the reversibility of the chemical reactions of formaldehyde-induced irritation and the lack of detectable accumulation of DNA protein cross-links during repeated exposures.

There is no evidence indicating an increased sensitivity to sensory irritation to formaldehyde among people often regarded as susceptible (asthmatics, children and older people).

Although some studies suggest that formaldehyde plays a role in airway sensitization, an association between formaldehyde and lung effects or sensitization in children have not been convincing owing to confounding factors in the studies, including exposure to traffic-related co-pollutants.

Lung function remains unaltered in adults at exposures below 1 mg/m^3 formaldehyde.

Cancer

Formaldehyde can induce squamous cell carcinoma of the nasal cavity in rats and nasopharyngeal cancer in humans. Long-term exposure to 7.5 mg/m^3 formaldehyde and above caused squamous cell carcinoma of the nasal cavity of rats with a non-linear, biphasic concentration–response relationship having the break point at or above 2.5 mg/m^3. In humans, no excess nasopharyngeal cancer has been observed at mean exposure levels at or below 1.25 mg/m^3 and with peak exposures below 5 mg/m^3.

Exposure to formaldehyde has been suspected of leading to lymphohaematopoetic malignancies. However, most long-term inhalation carcinogenicity studies in rats, mice and hamsters do not suggest induction of lymphohaematopoetic malignancies by formaldehyde at levels associated with nasal cancer. In humans, the overall conclusions from three meta-analyses, as well as a recent study in embalmers, suggest that formaldehyde may be causally associated with lymphohaematopoetic malignancies. The recent study in embalmers found evidence of myeloid leukaemia but not other haematopoietic malignancies; the 8-hour time-weighted average formaldehyde intensity was 0.125–0.25 mg/m^3, the average formaldehyde intensity while embalming was about 1.9–2.25 mg/m^3, and peak exposure was about 10–13 mg/m^3. This suggests that an effect on bone marrow or blood progenitor cells is possible at high exposure concentrations. However, since exposure to formaldehyde concentrations up to 2.5 mg/m^3 has negligible influence on the endogenous formaldehyde blood level, protection against nasal cancer should also protect against leukaemia.

Relevance for health of indoor air exposure

The major exposure route of formaldehyde is inhalation from indoor sources. Formaldehyde is a normal component of blood. Exposure of humans to 2.5 mg/m^3 formaldehyde did not increase the blood levels and exposure to 0.5 mg/m^3 did not result in an increase in urinary formate excretion due to rapid metabolism. This suggests that formaldehyde levels normally encountered in indoor air, not exceeding 0.2 mg/m^3, are not expected to increase internal organ exposure.

Conclusions of other reviews

Regulatory agencies in many countries have established guideline values for concentrations of formaldehyde in indoor air. IARC has classified formaldehyde as a human carcinogen (Group 1) based on sufficient epidemiological evidence of nasopharyngeal cancer, and a recent IARC working group also found sufficient evidence for myeloid leukaemia.

Guidelines

An indoor air guideline for formaldehyde is appropriate because indoor exposures are the dominant contributor to personal exposures through inhalation and indoor concentrations may be high enough to cause adverse health effects.

The lowest concentration reported to cause sensory irritation of the eyes in humans is 0.38 mg/m^3 for four hours. Increases in eye blink frequency and conjunctival redness appear at 0.6 mg/m^3, which is considered equal to the NOAEL. There is no indication of accumulation of effects over time with prolonged exposure.

The perception of odour may result in some individuals reporting subjective sensory irritation, and individuals may perceive formaldehyde at concentrations

below 0.1 mg/m^3. However, this is not considered to be an adverse health effect. The NOAEL of 0.6 mg/m^3 for the eye blink response is adjusted using an assessment factor of 5 derived from the standard deviation of nasal pungency (sensory irritation) thresholds, leading to a value of 0.12 mg/m^3, which has been rounded down to 0.1 mg/m^3. Neither increased sensitivity nor sensitization is considered plausible at such indoor concentrations in adults and children. This value is thus considered valid for short-term (30-minute) duration, and this threshold should not be exceeded at any 30-minute interval during a day.

Thus, a short-term (30-minute) guideline of 0.1 mg/m^3 is recommended as preventing sensory irritation in the general population.

There is sufficient evidence that formaldehyde causes nasal cancer in animals and nasopharyngeal cancer in humans with a non-linear, biphasic concentration–response relationship. Carcinogenicity studies in rats, mice and hamsters do not show a consistent association between formaldehyde and lymphohaematopoetic malignancies. Associations between exposure to formaldehyde and nasopharyngeal malignancies and leukaemia in humans are limited to high exposure concentrations.

Increased cell proliferation due to cell damage is considered a key mechanism for the development of nasal malignancies following exposure to formaldehyde. Overall, indoor air effects of formaldehyde are expected to be limited to the site of contact, generally the nasal and upper airways. Increasing cell proliferation in the nasal mucosa of rats occurs at concentrations at and above 2.5 mg/m^3 formaldehyde. The NOAEL for cell proliferation is 1.25 mg/m^3 for long-term exposures.

Thus a threshold approach to setting a guideline for cancer effects is appropriate. Starting with the NOAEL of 1.25 mg/m^3, assessment factors are applied. An interspecies assessment factor of 3 is proposed because the effect is local (non-systemic) and directly due to formaldehyde itself; for inter-individual variation, an assessment factor as low as 2 is proposed because sensitivity differences are not seen among different populations (asthmatics, children and older people). This would lead to a proposed guideline of 0.21 mg/m^3 for the protection of health for long-term effects, including cancer.

An alternative approach was taken by several other groups, using a biologically motivated model. Their assessments led to a predicted additional risk of 2.7×10^{-8} for continuous lifetime exposure to 0.125 mg/m^3 and a predicted additional risk of 10^{-6} or less for non-smokers continuously exposed to 0.25 mg/m^3.

These two assessments (using a NOAEL/assessment factor approach and estimates from the biologically motivated models) yield similar results, with values of approximately 0.2 mg/m^3. These values are above the guideline for short-term effects of 0.1 mg/m^3. Thus use of the short-term (30-minute) guideline of 0.1 mg/m^3 will also prevent long-term health effects, including cancer.

The use of low-emitting building materials and products, and preventing exposures to environmental tobacco smoke and other combustion emissions, will

minimize exposure-related risk. In addition, ventilation can reduce indoor exposure to formaldehyde.

The guidelines section was formulated and agreed by the working group meeting in November 2009.

Summary of main evidence and decision-making in guideline formulation

Critical outcome for guideline definition

Sensory irritation.

Source of exposure–effect evidence

Experimental study reporting conjunctival redness and increases in eye blink frequency at a four-hour exposure of 0.63 mg/m^3 considered as the NOAEL *(79)*. This was adjusted using an assessment factor of 5 derived from the standard deviation of nasal pungency (sensory irritation) thresholds, leading to a value of 0.12 mg/m^3, which has been rounded down to 0.1 mg/m^3.

Supporting evidence

- Several reviews on sensorial irritation at exposure levels between 0.15 and 1.25 mg/m^3 *(66,67,77)*.
- 12 controlled, mostly double-blind studies on respiratory effects at exposures of 0.08–11.2 mg/m^3 *(78–89)*.

Results of other reviews

IARC: Group I (known human carcinogen) *(1,155,186)*.

Guidelines

0.1 mg/m^3 (30-minute average concentration).

Comments

- The short-term guideline will also prevent effects on lung function as well as long-term health effects, including nasopharyngeal cancer and myeloid leukaemia.
- No change in the guideline as compared to *Air quality guidelines for Europe*, 2nd ed.

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4. Naphthalene

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General description

Naphthalene (CAS Registry Number 91-20-3; molecular formula $C_{10}H_8$) is a white crystalline powder with a characteristic odour (of mothballs). It is a two-ring aromatic hydrocarbon isolated from coal tar. Synonyms used are antimite, naphthalin, naphthaline, naphthene and tar camphor. Naphthalene is the most volatile polycyclic aromatic hydrocarbon (PAH) with a gas-phase part of 90–100%, and has a relatively short half-life of 3–8 hours in the atmosphere. Its physicochemical properties are as follows *(1–7)*: molecular weight 128.17 g/mol; melting point 80.2 °C; boiling point 218 °C; relative vapour density 4.42g/cm^3 at 20 °C and 1 atm; vapour pressure 10 Pa at 25 °C; and diffusion coefficient 7.20×10^{-2} cm^2/s at 298 K. It is soluble in alcohol and acetate but not in water.

Conversion factors

At 760 mmHg and 20 °C, 1 ppm = 5.331 mg/m^3 and 1 mg/m^3 = 0.188 ppm; at 25 °C, 1 ppm = 5.241 mg/m^3 and 1 mg/m^3 = 0.191 ppm.

Sources and pathways of exposure

Naphthalene is produced from coal tar fractions by distillation and crystallization. It is used as feedstock in the manufacture of phthalic anhydride for the synthesis of phthalate plasticizers and synthetic resins. It is also used as feedstock for naphthalene sulfonic acids often used in the production of plasticizers for concrete, as ingredients for plasterboards, as dispersants in synthetic and natural rubbers and as tanning agents in the leather industry. Naphthalene is also used in paints and in the production of the insecticide carbaryl, used in home yards and gardens. Still predominant in the exposure of consumers worldwide is the production and use of crystalline (pure) naphthalene as a moth repellent and disinfectant. Its use as a solid block deodorizer for toilets is also reported. Wood smoke, fuel oil and gasoline also contain naphthalene. The major constituent of creosote, used for timber impregnation, is naphthalene and its alkyl homologues.

Outdoor naphthalene sources mainly originate from fugitive emissions and motor vehicle exhaust. Spills to land and water during the storage, transport and disposal of fuel oil and coal tar are released to the atmosphere by volatilization,

photolysis, adsorption and biodegradation. Usual indoor sources of naphthalene are unvented kerosene heaters and tobacco smoke *(8)*.

Outdoor sources can contribute to low levels of indoor naphthalene. The highest indoor concentrations, however, usually orders of magnitude above the outdoor air levels, come from consumer products such as multipurpose solvents, lubricants, herbicides, charcoal lighters and hair sprays, unvented kerosene heaters, tobacco smoke, rubber materials and – most importantly – naphthalene insect repellents (mothballs) used to protect textiles stored indoors in closets (although this use has decreased, mainly in western Europe).

It is assessed that the primary route of exposure is inhalation, especially in the vicinity of heavy traffic, petrol stations and oil refineries. Although inhalation is the major route of the total human exposure to naphthalene, dermal exposure is not to be neglected. Preuss et al. *(9)* assessed the total daily naphthalene intake from air, food and house dust (including soil) at 1.127, 0.237 and 0.235 μg/kg per day, respectively, for a 70-kg adult. Since people spend most of their time indoors, inhalation of indoor air plays the major role in human total exposure to naphthalene.

Indoor concentrations and exposures

There is limited information available in the literature on indoor air concentrations and personal exposure levels of naphthalene. In Europe, two large-scale population-based studies, EXPOLIS *(10)* and the German Environmental Survey (GerES) *(11)*, provide useful data on indoor air exposure and outdoor air concentrations of naphthalene. Some other studies have been reviewed in the course of the INDEX project *(12,13)*. Results from some studies carried out in and outside Europe are summarized in Table 4.1 and are discussed below.

In Europe, indoor concentrations and personal exposures are usually low, typically below 1–2 μg/m^3 *(14)*. In a large-scale study representative of the Federal Republic of Germany before reunification (n = 479), a mean naphthalene concentration of 2.0 μg/m^3 in residential indoor air within a range of individual samples from 0.7 to 14 μg/m^3 was reported *(9)*. In a follow-up study, 555 dwellings in 150 cities were monitored between May 2003 and May 2006 (child's bedroom; passive sampling for one week) *(15)*. The indoor concentration of naphthalene was below the detection limit (1 μg/m^3) in 93% of the houses. The median concentration and 90th percentile were below the detection limit, whereas the 95th percentile and maximum value were respectively 1.2 and 4.9 μg/m^3.

In contrast to this, naphthalene exposures in Athens were found to be remarkably higher. Here, the USEPA's 2006 inhalation reference concentration of 3 μg/m^3 *(16)* and the INDEX project's long-term guideline value of 10 μg/m^3 *(13)* were exceeded in every personal exposure, and the mean and median concentrations were 54.0 and 22.6 μg/m^3, respectively. In Athens, there were five

participants whose personal exposures were considerably higher than the rest of the population and ranged from 74 to 469 μg/m^3. Indoor concentrations were even higher, ranging from 114 to 989 μg/m^3, respectively *(14)*.

Few data could be found on naphthalene concentrations in public spaces, transport and schools, and these are summarized in Table 4.1. Only two European studies carried out in Germany deal with schools and hospitality venues, respectively. In addition, some non-European studies were reviewed.

In Schleswig-Holstein, 285 classrooms from 105 schools and day-care centres were investigated for VOCs (active sampling) between July 2005 and February 2007 *(17)*. In 216 classrooms (76%), the naphthalene concentration was below the detection limit of 1 μg/m^3. The median concentration, 90th and 95th percentiles and maximum value were respectively <1.0, 1.0, 3.7 and 22 μg/m^3. Naphthalene was not measured in a previous campaign carried out in Schleswig-Holstein in 1990–1993, so no comparison can be provided.

Active sampling of indoor air was conducted for 4 hours during the main opening hours in 28 hospitality venues in the cities of Augsburg and Munich, from April 2005 to May 2006 at a time when smoking was allowed *(18)*. Median levels of naphthalene were 80.0 μg/m^3 in restaurants and cafés (n = 11), 59.0 μg/m^3 in pubs and bars (n = 7) and 98.5 μg/m^3 in discotheques (n = 10).

In Table 4.1, the naphthalene concentrations vary widely between 0.036 and 143.9 μg/m^3. Although it would be more appropriate to differentiate between the data measured in different ways, such a differentiation is not reflected in Table 4.1.

In the studies reviewed in the European INDEX project, residential indoor concentrations were elsewhere low, typically averaging below 2 μg/m^3, whereas in Athens clearly higher indoor levels were measured, being on average 90 μg/m^3 *(10)*. Personal exposures to naphthalene elsewhere ranged from 1 to 3 μg/m^3 *(10,11)*, whereas in Athens the average exposure was 46 μg/m^3. In general, we can conclude that exposures to naphthalene are usually low in Europe, but in Athens (and presumably also other countries in eastern and southern Europe) remarkably higher indoor levels of naphthalene were present.

Maroni et al. *(19)* reported typical median and 90th percentile naphthalene concentrations in indoor air in Italy of 2 μg/m^3 and 5 μg/m^3, respectively. Kostiainen et al. *(20)* detected slightly lower indoor concentrations in Helsinki, 0.44 μg/m^3 and 1.63 μg/m^3 being the mean and maximum concentrations. Bituminous materials commonly used in the United Kingdom for damp-proofing floors emit naphthalene *(21)*. Naphthalene concentrations up to 970 μg/m^3 were found in homes having an objectionable smell, where a damp-proof membrane had been applied, compared with less than 300 μg/m^3 for control homes *(22)*. Rubber flooring may also emit naphthalene in odorous amounts. In an Italian study, the average indoor naphthalene concentration was 11 μg/m^3 and the maximum level 70 μg/m^3 *(23)*.

In tropical areas, indoor naphthalene concentrations seem to be generally higher. Mean values in Burundi and Taiwan, China were about 30 μg/m^3 *(9)*. Zuraimi et al. *(24)* compared the characteristics of VOCs and the associated factors affecting them in office buildings in Europe (EU) and in Singapore. They found that concentrations of naphthalene were significantly higher (mean and maximum 144 μg/m^3 and 745 μg/m^3, respectively) in Singapore buildings compared to the EU buildings (mean and maximum 6.5 μg/m^3 and 68.5 μg/m^3, respectively, see Table 4.1).

Area-specific emission rates of naphthalene were also significantly higher and ventilation rates significantly lower in Singapore buildings. Higher levels of naphthalene in ETS-free Singapore buildings were associated with human activity.

Jia et al. *(25)* measured VOCs in indoor and outdoor environments in Michigan, United States to assess their health risk drivers. Monitoring was conducted during two seasons inside and outside 159 residences in industrial, urban and suburban cities. Outdoor concentrations were elevated in winter in the suburban community and were highest in the industrial community. Indoor concentrations were higher in the summer. Seasonal changes were small or inconsistent. Indoor levels of naphthalene exceeded the inhalation reference concentration of 3 mg/m^3 in 12% of residences. The highest level measured was 91.7 μg/m^3.

Yu *(26)* pointed out that indoor naphthalene pollution may also be an issue in Chinese archives. The Chinese Government banned the production and sale of mothballs in 1993, but the use of mothballs in archives and libraries is still permitted for the protection of documents and specimens. It was estimated that up to 10–12 mothballs per m^2 were used in a typical Chinese archive, but unfortunately no measurements have been reported for such an environment.

Lu et al. *(27)* modelled the regional distributions and human exposures to naphthalene in southern California. Petrol and diesel engine exhaust, with related vaporization from fuels, were found to contribute roughly half of the daily total naphthalene burden in southern California. Based on their analysis, the mean hourly naphthalene exposure of the population was 0.27 μg/m^3 in the summer and 0.43 μg/m^3 in the winter. Higher exposures are experienced by a fraction of the population. More than one million people were exposed to naphthalene levels greater than 1 μg/m^3 during wintertime and nearly 100 000 were exposed to average concentration exceeding 2 μg/m^3.

Lu et al. *(28)* reported the results of a PAH pollution survey in the air in public places in Hangzhou, China. The most serious PAH pollution was found in indoor air in shopping centres and the least in railway stations. The highest naphthalene concentration (23.5 μg/m^3) was measured in a shopping centre (see Table 4.1). The authors concluded that emissions of 2–4-ring PAHs occurred from indoor sources in shopping centres and supermarkets, whereas 5–6-ring PAHs originated predominantly from outdoor air. In temples, PAHs in indoor air

mainly originated from incense burning. Naphthalene was the largest contributor (62.4%) to the total health risk when risks associated with the inhalation of PAHs were assessed.

To understand PAH generation in kitchens, Zhu & Wang *(29)* surveyed six representative homes and four commercial kitchens in Hangzhou, China. The highest naphthalene concentrations in a commercial kitchen, in a domestic kitchen of a non-smoking family and in a kitchen of a smoking family were 3.0, 2.7 and 9.9 μg/m^3, respectively. Naphthalene was identified as the most predominant PAH, mostly resulting from the evaporation of mothballs used to protect clothes.

Liu et al. *(30)* measured PAHs simultaneously in the indoor and outdoor air of eight homes in Hangzhou, China. Of the 12 PAHs, naphthalene was the most abundant in both indoor (0.122–26.9 μg/m^3) and outdoor air (0.072–25.1 μg/m^3). Both in summer and in autumn, it contributed more than 60% to the sum of PAHs.

Using standard methods, Lin et al. *(31)* studied the role of incense burning on human exposure to 21 PAHs and total suspended particulates (TSP) in and around a temple in Taiwan, China. Indoor mean total PAH, particle-bound PAH and TSP concentrations were 6.26 μg/m^3, 490 μg/g and 1.32 μg/m^3, respectively. Values for outdoor readings were 0.23 μg/m^3, 245 μg/g and 73 μg/m^3, respectively. With respect to concentrations of individual PAHs (particulate + gas phase), the naphthalene concentration was the second highest at 1.26 μg/m^3. The median indoor : outdoor ratio for naphthalene was 8.6. Median values for indoor : outdoor ratios of individual PAHs ranged from 5.7 to 388, which implied that the temple was a significant PAH source. Moreover, the PAH content of the tested stick incense and ash was low. PAH levels inside the temple were much higher than those measured in the vicinity and inside residential houses, and were in fact close to levels measured at a nearby traffic intersection.

Li & Ro *(32)* measured 15 PAHs simultaneously in the indoor and outdoor air of 14 homes in the Taipei urban area during the summer and winter seasons. They reported that indoor PAH concentrations generally exceeded the corresponding outdoor PAH concentrations. In homes using incense, PAHs could be attributed mainly to incense burning. The most abundant PAH found indoors was naphthalene.

In Australia, several studies have been conducted to detect naphthalene but so far no direct indoor naphthalene concentration data have been forthcoming. The only two indoor studies on indoor naphthalene are summarized below.

Zou et al. *(33)* investigated PAH profiles from the combustion of different Australian firewood species in a domestic wood heater in a laboratory. The 16 PAH emission rates obtained varied between 5965 and 11 508 μg/kg for four firewood species and they were mainly emitted in the gaseous phase (91–98.8%). Overall, gaseous naphthalene accounted for up to 69% of total PAHs in the air.

Table 4.1. Naphthalene concentrations in air reported in the reviewed scientific literature

Reference	Country/city	Period	Environment
Residential settings, European studies			
Jantunen et al. *(10)*	Athens	1996–1997	Residences, indoors
	Basel	1996–1997	Residences, indoors
	Helsinki	1996–1997	Residences, indoors
	Milan	1996–1997	Residences, indoors
	Oxford	1998–2000	Residences, indoors
	Prague	1996–1997	Residences, indoors
Jantunen et al. *(10)*	Athens	1996–1997	Personal exposure
	Basel	1996–1997	Personal exposure
	Helsinki	1996–1997	Personal exposure
	Oxford	1998–2000	Personal exposure
	Prague	1996–1997	Personal exposure
Hoffman et al. *(11)*	German survey	1990–1992	Personal exposure
KUS *(15)*	German survey	2003–2006	Residences. indoors
Non-European studies			
Jia et al. *(25)*	Michigan, USA	2004–2005	Residences, indoors Residences, outdoors
Zhu & Wang *(29)*	Hangzhou, China	1999–2000	Domestic kitchen, non-smoking Domestic kitchen, smoking Commercial kitchen
Ohura et al. *(35)*	Shimizu, Japan	2000 2001	Residences, indoors, summer Residences, indoors, winter
Public spaces			
Lu et al. *(28)*	Hangzhou, China	2006	Railway station, indoors Shopping centre, indoors Supermarket, indoors Supermarket, indoors/outdoors Hotel, indoors Temple, indoors Temple, indoors/outdoors
Zuraimi et al. *(24)*	Singapore Europe	2006 2006	Office buildings Office buildings
Heinzow & Ostendorp *(17)*	Germany	2005–2007	Schools
Bolte et al. *(18)*	Germany	2005–2006	Hospitality venues
Lin et al. *(31)*	Taiwan, China	1996	Temple, indoors Temple, outdoors

[a] AM = arithmetic mean, SD = standard deviation, GM = geometric mean, max = maximum value.

Averaging time	No. of samples	Concentration (µg/m³) [a]			
		AM	SD	GM	Max
30 hours	42	83.5	197		
30 hours	47	0.7	0.3		
30 hours	188	0.6	0.5		
30 hours	41	21.0	81.6		
30 hours	40	1.3	1.5		
30 hours	46	2.0	1.9		
48 hours	46	47.1	78.0		
48 hours	50	0.8	0.6		
48 hours	193	0.7	0.2		
48 hours	42	0.8	0.5		
48 hours	49	2.4	2.8		
1 week	113	2.3		2.1	
1 week		< 1			4.9
3–4 days	226 samples	3.5			91.8
3–4 days	252 samples	0.3			4.7
12 hours	3 kitchens	1.8			2.7
12 hours	3 kitchens	5.3			9.9
12 hours	4 kitchens	2.3			3.0
24 hours	25 houses	1.1			
24 hours	22 houses	1.0			
12 hours	2 samples	2.7			
12 hours	2 samples	23.5			
12 hours	2 samples	19.7			
12 hours	20 samples	2.38	0.59		3.5
12 hours	2 samples	16.3			
9 hours	2 samples	16.1			
9 hours	16 samples	4.14	1.98		7.1
–	8 buildings	143.9	93.0		745
–	50 buildings	6.5	4.3		68.5
	105 schools	< 1			22
4 hours	28 venues				
	Restaurants & cafés (11)	80.0			
	Pubs and bars (7)	59.0			
	Discotheques (10)	98.5			
8 hours	6 samples			1.22	
24 hours	6 samples			0.16	

Duigu et al. *(34)* examined PAH composition on the surface films from the glass windows of 18 residential buildings. The results indicated an average naphthalene concentration on the films of 33.7 ± 44.2 ng/m^2.

Comparison of indoor with outdoor concentrations

Average outdoor naphthalene concentrations are low in Europe, ranging typically from 1 to 4 μg/m^3 *(10)*. Even lower outdoor levels, below 1 μg/m^3, have been reported in Taiwan, China and the United States (see Table 4.1). The outdoor concentration of naphthalene in air is generally lower in rural than in urban areas.

The indoor mean concentration of naphthalene is reported to range up to a maximum of 143.9 μg/m^3, although the majority of studies report average naphthalene indoor levels below 10 μg/m^3.

Table 4.1 shows the naphthalene air concentrations reported in a number of scientific publications. However, several different sampling techniques were used in these studies. For example, Ohura et al. *(35)* employed glass fibre filters and XAD-2 resin for particulate and gaseous naphthalene sampling, respectively, while the EXPOLIS project utilized only a Tenax TA tube to collect both phases of naphthalene. It was also reported by Lin et al. *(31)* that polyurethane foam had been used to sample gas-phase naphthalene with other vapour PAHs.

Biomarkers of human exposure to naphthalene

Urinary 1- and 2-naphthol are well-established human biological exposure indices to evaluate the exposure to naphthalene of workers as well as the general population. Median 1-naphthol concentrations found in non-smokers without known occupational exposure range from 1 to 5 μg/l urine and median 2-naphthol concentrations from 1 to 3.6 μg/l *(36)*. Smokers show significantly higher naphthol concentrations *(9,36)*.

Both 1- and 2-naphthol were used to check the impact of genetic polymorphisms on naphthalene metabolism *(37–39)*. Urinary 2-naphthol concentrations were higher in smokers with the CYP2E1 genotypes c1/c2 or c2/c2 than in smokers with the more common c1/c1 genotype. Higher concentrations of 1- and 2-naphthol were found in the urine of smokers deficient in glutathione S-transferase M1. In recent studies, 2-naphthol was used as a biomarker to evaluate polymorphisms in patients with lung cancer or oral squamous cell carcinoma *(40,41)*.

A glucose-6-phosphate dehydrogenase deficiency has been suggested to lead to an increased susceptibility to haemolytic anaemia in children and newborn infants exposed to naphthalene, but exposure levels of naphthalene were not estimated in most reports. Haemolytic anaemia observed in neonates could also be explained by a lower ability to metabolize naphthalene and eliminate naphthalene metabolites. In a Nigerian study, five neonates presenting with jaundice or

tetanus showed very high urinary 1-naphthol concentrations *(42)*. Three of them were deficient in glucose-6-phosphate dehydrogenase. In this group, the 1-naphthol concentrations ranged from 1140 to 11 690 μg/l urine, similar to those of the non-deficient newborn infants (750–9550 μg/l). Such high naphthol concentrations have been reported in occupational settings but not in humans *(9)*.

It is recommended that 1- and 2-naphthol be measured simultaneously, since both metabolites correlate. An elevated level of 1-naphthol alone may be an indicator of an additional exposure, such as to the biocide 1-naphthyl methylcarbamate (carbaryl) or to some hair dyes *(43)*. A study in the Republic of Korea on non-smoking municipal middle-school students showed significant correlations between urinary 2-naphthol concentrations and the daily mean total suspended particulate level estimated for 1–2 days before and for the day of the survey *(44)*.

In a recent study, a method was developed for measuring urinary 1,2- and 1,4-dihydroxynaphthalene *(45)*. Strong correlations were observed among these naphthadiols and both naphthols in urine. Further, the urinary concentrations of 1,2-dihydroxynaphthalene were significantly correlated with the serum concentrations of 1,2-naphthoquinone albumin adducts.

Kinetics and metabolism

Kinetics

There are no published studies that document the precise bioavailability of naphthalene after oral, dermal or inhalation exposure. It is clear from human poisoning cases *(46)*, the exposure of air force personnel to jet fuel containing naphthalene *(47,48)* and numerous animal studies *(49)* that naphthalene can be absorbed by all three routes. In exposed human volunteers, dermal administration of naphthalene resulted in relatively rapid uptake of the parent compound, with peak levels observed in approximately 60 minutes. Calculated partition coefficients demonstrate high partitioning of naphthalene in the fat, while toxicokinetic studies in mice after inhalation exposure and in rats after both inhalation and intravenous administration *(49)* demonstrate rapid clearance from the blood. Very little naphthalene is eliminated unchanged in expired breath, a finding consistent with the results of the physiologically based toxicokinetic analysis suggesting that 88–98% of inhaled naphthalene is eliminated as metabolic by-products.

Very recently, work has been taken up to better understand gender and species differences in upper respiratory tract uptake and in situ metabolism of naphthalene *(50)*. At a flow of 150 ml/minute, upper respiratory tract uptake in female F344 rats exposed to naphthalene concentrations of 5, 21, 53 or 181 mg/m^3 was concentration-dependent, with rates of 56%, 40%, 35% and 28%, respectively. These rates were similar to the uptake observed in male rats (57%, 49%, 37% and 36%, respectively). The concentration dependence of naphthalene uptake in the upper respiratory tract is probably due to nasal metabolism of

naphthalene. A significant reduction of naphthalene uptake was observed after pre-treatment with the inhibitor 5-phenyl-1-pentyne.

Metabolism

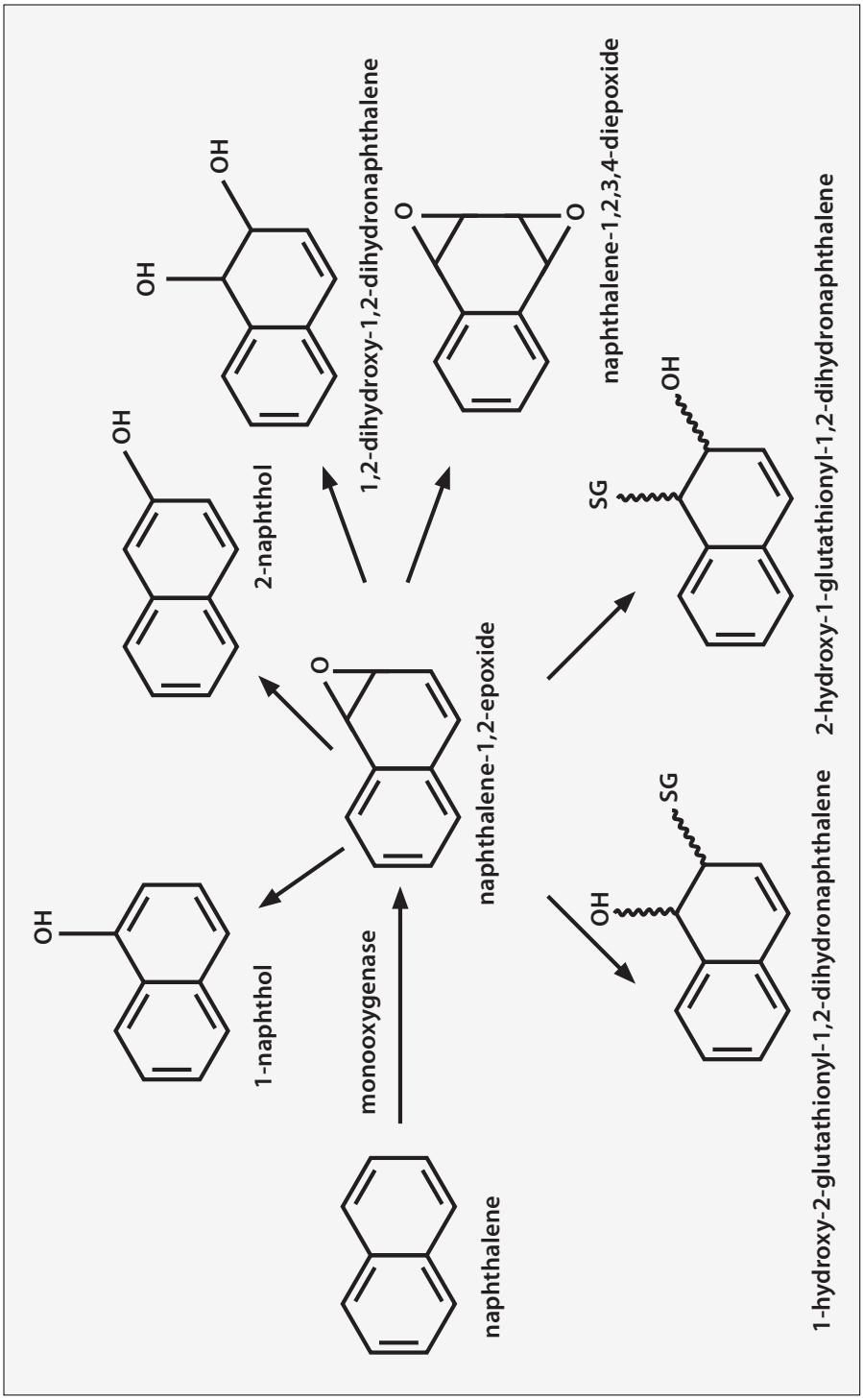
The metabolism of naphthalene to metabolites that can be excreted by mammals occurs as a multi-step process involving both initial oxygenation reactions and subsequent conjugation. The first step in metabolism involves the formation of an unstable 1,2-epoxide (Fig. 4.1) that can be catalysed by several cytochrome P450 monooxygenases. Several (e.g. 2A13, 2E1, 2F1 and 2F2) can oxidize naphthalene to naphthalene-1,2-epoxide and further to 1,2,3,4-diepoxide. Naphthalene-1,2-epoxide can also be rearranged to 1- or 2-naphthol or be transformed by epoxide hydrolases to dihydroxy-dihydro-naphthalene or by glutathione transferases to glutathionyl derivates.

These monooxygenases are discussed in detail below, since the initial formation of naphthalene oxide is a key step in the downstream toxicological activities associated with naphthalene exposure. A number of further metabolites can be generated directly from the epoxide by both enzymatic and non-enzymatic processes. The cytochrome P450 monooxygenases can biotransform naphthalene to a putative diepoxide or diolepoxide *(51,52)*, microsomal epoxide hydrolases generate a trans-dihydrodiol *(53)* and the glutathione transferases form diastereomeric glutathione conjugates *(54)*, which are eliminated primarily as mercapturic acids *(51,52)*. In water, 1- (major) and 2-naphthol (minor) arise from non-enzymatic rearrangement of the epoxide *(55)*. In human liver microsomal incubations, the calculated V_{max} for the formation of 1-naphthol, 2-naphthol and 1,2-dihydroxy-1,2-dihydronaphthalene were 268, 22 and 2860 pmoles/min per mg protein, respectively *(56)*. Each of these secondary metabolites can undergo further biotransformation and with two of these metabolites (1-naphthol and 1,2-dihydroxy-1,2-dihydronaphthalene), more reactive chemical entities can result. The suspected reactive, toxicologically active metabolites include naphthalene epoxide, naphthalene diepoxide (or diol epoxide), 1,2-naphthoquinone and 1,4-naphthoquinone; the formation and disposition of these will be discussed individually. The primary urinary metabolite eliminated in exposed human populations is 1-naphthol glucuronide *(37,57–59)*. In recent surveys in the United States, this metabolite could be detected in the urine of all 2748 individuals sampled, thus establishing widespread exposure of human populations *(60)*.

Naphthalene-1,2-epoxide

The stability of various aromatic and aliphatic hydrocarbon epoxides varies considerably, which in turn affects the interactions with key cellular macromolecules and overall downstream impact *(61)*. In contrast to aflatoxin epoxide, which has an estimated half life of 1 second in water, naphthalene epoxide has a half-life of 2–3 minutes in water and 11 minutes in solutions of albumin *(62)*. Thus, naph-

Fig. 4.1. The first steps in the metabolism of naphthalene



thalene oxide is sufficiently stable to circulate from organs able to rapidly generate this metabolite to those with lower metabolic rates. While there is some evidence that circulating naphthalene oxide can produce injury in the lung *(62)*, there is a strong possibility that such circulating metabolites may enhance the susceptibility of tissues such as the lung to injury by depleting protective thiols such as glutathione *(63)*.

The importance of regiochemistry and stereochemistry in the biological effects of epoxides and diol epoxides of larger PAHs is well-established. Many PAH-specific P450s show remarkable regioselectivity and stereoselectivity in the metabolites they produce. Similarly, several of the P450s show a high degree of stereoselectivity in naphthalene metabolism. By using N-acetylcysteine to trap reactive naphthalene epoxides, van Bladeren et al. *(64,65)* were able to show that cytochrome P450 2B shows a slight preference for the formation of the (1*S*,2*R*)-naphthalene epoxide (74%) whereas cytochrome P450 1A1 preferentially generates (1*R*,2*S*)-epoxide (73–95%). Studies showing marked differences in the ratio of glutathione conjugates formed in microsomal incubations from mouse lung vs liver demonstrated substantial differences in the stereoselectivity of naphthalene epoxide in target (lung) compared to non-target (liver) tissues *(54)*. Approximately equal rates of formation of the (1*R*,2*S*)- and (1*S*,2*R*)-epoxide were observed in liver microsomes, whereas 10 : 1 ratios of the (1*R*,2*S*)- to (1*S*,2*R*)-epoxide were made in the lung. Similarly, subsequent work using dissected airways from susceptible mice and non-susceptible rats showed the same pattern of stereoselectivity. Metabolism in target regions of the respiratory tract of the mouse resulted in highly selective formation of the (1*R*,2*S*)-epoxide whereas approximately equal proportions of the epoxide enantiomers were made by rat lung airways *(66)*.

This high degree of selectivity in the formation of a single stereoisomer of naphthalene oxide was consistent with 60 : 1 ratios in isomer generation catalysed by cDNA-directed expression of cytochrome P450 2F2 in baculovirus-infected SF-21 cells and with immunolocalization experiments showing that that airway epithelial cells were highly stained. Thus, while it appears that cytochrome P450 2F2 is responsible for the rapid and stereoselective formation of (1*R*,2*S*)-naphthalene oxide, it is not at all clear that the stereoselectivity of this process is relevant to the cytotoxicity associated with naphthalene in the respiratory tract. Although it is possible that the toxicological potency of the naphthalene epoxide enantiomers differ, it seems far more likely that the differential susceptibility of rat and mouse airways and mouse lung and liver are due to the rates of initial substrate turnover. Published data in isolated mouse hepatocytes have shown that the intracellular residence time of the epoxide isomers may differ because of different rates of glutathione conjugation or hydrolysis by epoxide hydrolase, and that this does indeed translate into differential toxicity of these two epoxides *(67)*. However, definitive analysis of the importance of the stereochemistry of

epoxidation of naphthalene in the lung is problematic because of the instability of the epoxide. Short incubations of racemic naphthalene epoxide with dissected airways of both the rat and mouse and in proximal vs distal airways showed very little difference in the rates of formation of glutathione conjugates or in the diastereomers produced. Likewise, there were no discernable differences in the rates of dihydrodiol production between rat and mouse airways that appeared to relate to the species differences in response to naphthalene *(66)*.

1-Naphthol

One of the primary metabolites generated from naphthalene oxide in aqueous solutions is 1-naphthol. The ratios of this rearrangement product to the 1,2-dihydroxy-1,2-dihydronaphthalene (dihydrodiol) are dependent on the rates of formation of the epoxide and the activities of microsomal epoxide hydrolase which are, in turn, species-dependent. 1-Naphthol can be metabolized to protein-reactive metabolites both in vitro *(68,69)* and in vivo *(70)*. Conjugation with sulfate and UDP glucuronic acid results in derivatives that, in many species, constitute major urinary metabolites (see above). 1-Naphthol is a precursor to the formation of 1,4-naphthoquinone, a potential cytotoxic metabolite *(56,71)*. The 1,4-naphthoquinone can stimulate the redox cycle *(72)* and binds covalently to proteins in vitro *(73,74)* and in vivo *(75–77)*.

1,2-Dihydroxy-1,2-dihydronaphthalene

The dihydrodiol, generated through metabolism of the epoxide by epoxide hydrolase, is converted by a dihydrodiol dehydrogenase (aldose reductase) *(78–80)* to the 1,2-dihydroxynaphthalene, which auto-oxidizes to a 1,2-quinone. The 1,2-quinone can bind covalently to protein both in vitro and in vivo *(75–77,81)* and forms depurinating adducts on DNA in vitro *(82)*.

1,2-Naphthalene diepoxide (diolepoxide)

Indirect evidence for the formation of a diepoxide/diolepoxide comes from the isolation of the 1,2,3,4-tetrahydroxytetrahydronaphthalene from urine of naphthalene-treated rats *(83)*.

Glutathione is depleted in murine tissues capable of metabolizing naphthalene in a dose/concentration-dependent fashion after either intraperitoneal administration *(84)* or inhalation *(85)*. Glutathione adducts are generated at both the allylic and benzylic carbons of naphthalene *(54)*. Although the initial studies resolved only three diasteromers, with improved techniques a fourth, minor conjugate has been identified. These glutathione conjugates are eliminated primarily as mercapturic acids and account for 25–35% of a dose of naphthalene administered intraperitoneally to either mice or rats. No species differences were noted in the percentage of dose eliminated as mercapturate *(86)*. In mice, exposure to 319 mg/m^3 resulted in levels of mercapturate in the urine that were similar to

those observed after intraperitoneal administration of 50 mg/kg. It is interesting to note that there appears to be a significant species difference in the amounts of naphthalene eliminated as mercapturates in rodents and non-human primates. In both the chimpanzee *(87)* and the Rhesus monkey *(88)*, an increase in urinary thioether elimination, measured after conjugate hydrolysis with the Ellman assay, was not observed in response to orally administered naphthalene. In comparison, diethylmaleate administration resulted in dose-dependent increases in thioether elimination in both species.

The primary products eliminated in the urine of mice following the intravenous administration of naphthalene glutathione conjugates were mercapturic acids, and accounted for 40–85% of the administered dose *(89)*. Small amounts of cysteine conjugate were measured in the urine. There was a significant difference noted in the metabolic disposition of the benzylic compared to the allylic adducts. Some 15–20% of the administered dose of the 1R-glutathionyl-2R-hydroxydihydronaphthalene was excreted as a thiopyruvic acid derivative.

Enzymes involved in naphthalene metabolism

There is considerable experimental evidence showing that metabolism of naphthalene is required for any of the downstream toxicities associated with this compound in animal models. Thus, a substantial amount of effort has been focused on species comparisons in the rates of formation of naphthalene oxide, as well as on understanding the importance of specific pulmonary cytochrome P450 monooxygenases in the metabolic activation of this agent. The contribution of each of these P450 proteins to the conversion of naphthalene to more biologically active derivatives is dependent not only on the amounts of protein present but also on the catalytic activities of each of the proteins. Unfortunately, quantifying the amounts of each of the cytochrome P450 isoforms present in various subcompartments of the lung is difficult and in only a few cases has purified protein been available as standard *(90)*. More information is available on the catalytic properties of some of the P450 monooxygenases through the use of recombinant proteins. Since the environmental levels of naphthalene are quite low, data on the catalytic efficiencies (K_m) of the individual P450 monooxygenases is also a key to assessments designed to determine whether low-level, long-term exposures are a potential risk to human health. Accordingly, the following sections discuss what is known about the overall rates of metabolism of naphthalene in target and non-target tissues of rodents and primates, along with a discussion of P450s known to metabolically activate this substrate.

Comparative metabolism studies in rodents and primates

There are 50–100-fold differences in the rates of naphthalene metabolism to water-soluble metabolites in microsomal incubations prepared from target and non-target rodent tissues and corresponding tissues of the Rhesus monkey and

human *(91,92)*. In general, the rates of metabolism correlate well with the tissue susceptibility to toxicity. At saturating substrate concentrations, mouse lung (target tissue) microsomal naphthalene metabolism occurs at rates of 15 nmoles/mg microsomal protein per minute, compared to less than 2 nmoles/mg per minute in rat lung (non-susceptible). Likewise, the rates of microsomal naphthalene metabolism in rat olfactory epithelial tissues (highly susceptible to naphthalene) are approximately 16 nmoles/mg per minute *(93)*. In comparison, Rhesus monkey lung microsomes metabolize naphthalene at a rate of 0.15 nmoles/mg per minute. Similar rodent-to-primate differences were observed using more specific approaches, where metabolism was measured in target subcompartments *(66,94)*.

Enzymology of naphthalene epoxide formation

CYP2F. Nagata and co-workers *(95)* purified a cytochrome P450 monooxygenase from mouse liver that metabolized naphthalene rapidly and with high stereoselectivity. The gene was cloned and sequenced *(96)* and had 82% sequence homology to a cDNA that had been cloned earlier from human lung *(97)*.

CYP2F2 (mouse). Naphthalene is metabolized with a high degree of stereoselectivity by recombinant mouse CYP2F2 expressed in either yeast *(96)* or in SF-21 insect cells *(98)*. A very high V_{max} (107 nmoles product/nmole P450 per minute) and low K_m (3 μM) for the metabolism of naphthalene by recombinant CYP2F2 are consistent with the importance of this protein in the metabolic activation and toxicity of naphthalene in mouse lung. The low K_m observed is well below the range of expected tissue concentrations in the lung after inhalation exposure at the 53-mg/m^3 level. N-terminally truncated recombinant human keratinocyte growth factor (DeltaN23_KGF) lowers the expression of CYP2F2 in mice, thus reducing the airway injury of naphthalene *(99)*.

CYP2F4 (rat). Immunocytochemistry with antibodies generated to the mouse 2F *(66)* and northern blot analysis initially failed to demonstrate the presence of a P4502F orthologue in the rat. More detailed investigations uncovered a transcript that had 94% similarity in the deduced amino acid sequence to CYP2F2 *(100)*. cDNA-directed expression of CYP2F in SF-21 insect cells yielded a protein with nearly identical catalytic activities to the mouse orthologue. Thus, the substantial differences in susceptibility of mouse compared to rat lung was not likely to be due to differences in the catalytic differences in metabolism by CYP2F, but rather appears to be related to the amounts of protein present as assessed by immunoblot analysis *(101)*.

CYP2F1 (human)/CYP2F5 (monkey). CYP2F1 has been expressed in a number of different recombinant protein expression systems. Although substantial pro-

tein is produced in the baculovirus-infected SF-21 cells, a P450 spectrum could not be obtained. Similiarly, cDNA-directed expression of CYP2F5 from the Rhesus monkey resulted in protein but no haem incorporation. Both proteins were catalytically inactive *(100)*. Expression of CYP2F1 in lymphoblastoid cells *(102)* resulted in the production of a protein with very low rates of naphthalene turnover (~0.035 nmoles conjugate/min per nmole P450). This rate is less than 0.1% the rate of metabolism observed with the mouse orthologue. The recombinant human CYP2F1 showed slight stereopreference in the generation of (1*S*,2*R*)-naphthalene epoxide.

Other cytochrome P450 monooxygenases. While it is likely that CYP2F is primarily responsible for the metabolic activation of naphthalene in mice, other P450 monooxygenases may play an important role in catalysing the turnover of this substrate in humans. Cho et al. *(56)* have published a very thorough investigation of the catalytic activity of various commercially available cytochrome P450 monooxygenases with naphthalene. Cytochrome P450 2E1 has the lowest K_m of any of the proteins tested (10 μM) with a V_{max} that is 8 pmoles/min per pmole P450 for the formation of 1-naphthol. This is 10-fold lower than the V_{max} for CYP2F2. Cytochrome P450 2E1 has been reported in human lungs based on both immunoblotting and activity assays *(103,104)*. Recent work showing high catalytic activities of CYP2A13 *(105)*, a protein reported in human respiratory tissue *(106)*, suggests that this protein may be important in human metabolism of naphthalene. The K_m and K_{cat} for the formation of 1-naphthol were 36 μM and 143 min^{-1}, respectively. Aryl hydrocarbon receptor-mediated enzymes do not contribute significantly to naphthalene bioactivation in mice *(107)*.

Formation and possible importance of protein-bound metabolites

The concept that reactive metabolite formation can, but does not always, lead to cellular necrosis has been well-established with a number of hepatic, renal and pulmonary toxicants. Early studies with naphthalene showed that reactive metabolites become bound covalently to cellular proteins both in vivo and in vitro in a dose/concentration-dependent manner *(84)*. The irreversible binding of reactive metabolites occurs prior to any signs of cellular degradation, and prior treatment with inhibitors of cytochrome P450 or with glutathione depletors alters the extent and severity of cytotoxicity in concert with the amounts of reactive metabolite bound *(108)*. The binding levels generally correlate with target tissue susceptibility. Although considerable progress has been made in identifying proteins that are adducted by a variety of reactive metabolites, including naphthalene *(109–111)*, it has not been demonstrated that a particular protein adduct (or adducts) results in toxicity. What is clear is that there are commonalities in proteins that are adducted by reactive naphthalene metabolites across species, and the 50–100-fold differences in rates of water-soluble metabolite formation

between rodents and primates are not observed when total reactive metabolite binding is compared. Incubations of dissected airways of Rhesus monkeys with naphthalene resulted in levels of covalent adduct varying from 0.3 nmoles/mg protein in the trachea to 1.2 and 1.4 nmoles/mg protein in the distal airway and parenchyma, respectively *(94)*. Under similar conditions, the rates of formation of reactive metabolites that become bound covalently in dissected airways of mouse lung varied from 0.8 to 3.8 nmoles adduct per mg protein from trachea to distal airway *(112)*. Recent comparisons between rat nasal olfactory epithelium, which is highly susceptible to naphthalene *(93)*, and ethmoid tissues from the Rhesus monkey show nearly identical levels of reactive metabolite formation in in vitro incubations *(111)*.

It is important to note that several naphthalene metabolites are protein-reactive, including the epoxide *(67)* and both the 1,2- and the 1,4-naphthoquinones *(113,114)*. Which (if any) of these metabolites are essential to the steps leading to cytotoxic injury is not clear, nor are the relative contributions of each metabolite to the overall levels of adducts measured. At least in rats and mice, Waidyanatha & Rappaport *(77)* have shown that naphthalene oxide is the primary metabolite that adducts albumin and haemoglobin in both species.

Health effects

Most of the data available on the toxic effects of naphthalene have been derived from animal studies conducted either in vivo or with in vitro preparations *(22,46,115)*. There are reports of acute poisoning through unintentional or suicidal naphthalene exposures in humans but, as described below, the epidemiological data are very scarce regarding dose–response relationships for human health effects with acute, subchronic or chronic exposure by any route. The effects in humans are now discussed, followed by a description of animal data. In some cases, reference will be made to literature in humans, which, based on mechanistic data derived from animals, would be consistent with adverse health effects of naphthalene. It is important to note that the associations and consistency with mechanisms does not constitute proof of health effects and the data may be explained in many other ways. This is especially true when exposure occurred not solely to naphthalene but to mixtures containing naphthalene such as PAH.

Identification of studies

Published studies on health effects of exposure to naphthalene were identified by hand searching references in former reviews by IARC *(115)*, ECB *(22)* and ATSDR *(46)* and completed by electronic search in February and September 2009 in PubMed, using the descriptors "naphthalene" and "health effects", "toxicity", "lung", "epidemiology", "susceptibility", "cancer", "mothballs" or "poisoning". Following the last review, only a few new epidemiological studies and about two

dozen toxicity studies in mammals or in vitro studies were found. We excluded studies that referred to PAHs but were lacking a sufficient description of exposure to naphthalene.

Effects on humans

Acute effects

Many of the case reports of human exposure to naphthalene involve ingestion of mothballs. The most serious effects are reported in individuals with glucose 6-phosphate dehydrogenase deficiency, where haemolytic anaemia is the primary adverse effect. Many of these involve poisoning in paediatric patients *(116,117)*. In a recent survey of 24 paediatric patients admitted to hospital with acute haemolysis, nearly 60% were found to have been associated with naphthalene-containing mothballs *(118)*. In one case report, involving accidental prenatal exposure to mothballs, both the mother and, following birth, her preterm infant presented with haemolytic anaemia and methaemoglobinaemia *(119)*. Follow-up of both mother and child a year later revealed nothing remarkable. The effortless availability and widespread domestic use of naphthalene-containing mothballs may further lead to acute naphthalene poisoning, including the non-accidental ingestion of mothballs *(120)*.

Chronic effects

Very few cases have been documented of chronic naphthalene exposure in humans. Two of the reports purportedly showing a link between laryngeal *(121)* or colon cancer *(122)* with naphthalene exposure have been judged by both the US National Toxicology Program (NTP) *(123)* and IARC *(115)* as being sufficiently poorly controlled to be unreliable. In a population-based case-control study among women in New York State, the increase in risk of non-Hodgkin's lymphoma diagnosed between October 1995 and September 1998 was significantly associated with the household use of mothballs *(124)*. The lack of a dose–response among users, the unknown chemical constituent(s) of the mothballs used (naphthalene or para-dichlorobenzene), and selection and recall bias limit the drawing of firm conclusions.

There is an early report of human cataractogenesis induced by naphthalene in a dye manufacturing facility, which is consistent with subsequent work in animal models (discussed below) *(125)*. Some studies suggest an association between exposure to biomass fuel smoke and cataracts or lens opacity *(126,127)*, but exposure levels of naphthalene associated with these effects have not been estimated. The final case is of a middle-aged woman who had been sniffing mothballs containing naphthalene for more than 30 years *(128)*. The patient presented with signs of peripheral neuropathy and renal failure. Naphthalene was thought to be a possible contributing factor, but these symptoms were also likely to be related to diabetes, hypertension and obesity.

Odour perception

Naphthalene has a mothball-like odour. Published odour thresholds of naphthalene range from 0.0075 to 0.42 mg/m^3 *(129,130)*.

In vitro studies

There are a number of studies indicating that human cells are susceptible to naphthalene metabolites in vitro. Tingle et al. *(69)* used human liver microsomes to generate reactive metabolites from naphthalene, which were subsequently tested for cytotoxicity using peripheral blood mononuclear leukocytes. Cell death was dependent on the presence of NADPH. Inhibition of epoxide hydrolase with trichloropropylene oxide enhanced toxicity at all three concentrations of naphthalene studied (1, 10 and 100 μM). Interestingly, no effects were noted in sister chromatid exchange (SCE) frequency in cells incubated with human liver microsomes, with or without NADPH. An increase in SCE frequency was observed with the positive control, aflatoxin B_1. Later studies that tested the toxicity of naphthalene, 1-naphthol, 1,2- and 1,4-naphthoquinone and naphthalene oxide on human mononuclear leucocytes and lymphocytes showed that both quinones resulted in concentration-dependent cytotoxicity and that 1-naphthol required the presence of an activating system to generate metabolites that were cytotoxic *(131)*. The dihydrodiol was not cytotoxic at concentrations up to 100 μM. Similarly, both quinones resulted in increased numbers of SCEs. More recent work with cord blood showed that naphthalene at high concentrations (500 μM) increased the expression of several antiapoptotic proteins, including BCl-2 *(132)*. Similarly, three naphthalene metabolites (1- and 2-naphthol and 1,4-naphthoquinone) produce concentration-dependent decreases in the clonogenicity of colony-forming units, granulocyte-macrophage (CFU-GM) in cord blood from both male and female donors. Ranked IC_{50} (concentrations required to decrease clonogenicity by 50%) values for these metabolites were 2-naphthol > 1-naphthol > 1,4-naphthoquinone *(133)*. The reported IC_{50} for the quinone was 0.5–1.9 μM. Naphthalene was inactive at concentrations as high as 5 mM.

Overall, these studies indicate that human liver microsomes are capable of metabolically activating naphthalene to derivatives that are cytotoxic to human cells, and that the known metabolites of naphthalene are capable of producing cytotoxicity when added to cells. With some of these metabolites, cytotoxicity is observed at relatively low levels.

Effects on experimental animals and in vitro test systems

Animal studies in vivo

Acute/subacute studies. Toxicity to the respiratory tract is the most notable lesion associated with naphthalene exposure in animals but the subcompartments of the respiratory tract targeted by this compound depend highly on the species, the age and sex of the animals and the route of administration (Table 4.2). Ocular

injury has also been observed in a number of species and the mechanisms for this appear to be well-established.

Work examining the acute toxicity of naphthalene administered by inhalation has recently been completed *(134)*. Four-hour exposures to concentrations as low as 11 mg/m^3 resulted in detectable Clara cell injury in the proximal airways of adult male mice. Injury was concentration-dependent and proceeded from the proximal, most sensitive airways to distal and less sensitive airways. As the concentration increased, injury became more severe in the proximal airways and extended down into more distal portions of the lung. At 53 mg/m^3, significant cell disruption was noted at all airway levels in mice. In contrast, airway epithelial injury was not observed at any exposure concentration up to the highest concentration tested (585 mg/m^3). Substantial injury of nasal olfactory epithelium in Sprague-Dawley rats was observed following naphthalene inhalation at low exposure concentrations (18 mg/m^3 for four hours) *(93)*. More recently, olfactory epithelium necrosis occurred in SD and F344 rats after a single six-hour whole-body exposure to 5 mg/m^3 naphthalene *(135)*. Lesions of the respiratory and olfactory epithelium were observed at the 53- and 160-mg/m^3 exposure concentrations in male and female F344 and SD rats. The preliminary report indicates that SD rats appear to be more sensitive and that the threshold for injury may be much lower – in the 0.5–1.6-mg/m^3 range. In a subacute study that was not published but reviewed by the European Chemicals Bureau *(22)* and retained as valuable information in the INDEX project *(13)*, male and female Sprague-Dawley rats were exposed nose-only to 0, 5, 17, 55, 153 or 372 mg/m^3 vaporized naphthalene (D. W. Coombs, unpublished data, 1993). In the nasal olfactory epithelium, local lesions with signs of proliferative repair were observed at all doses down to 5 mg/m^3. The findings were similar to those from a subchronic study (see below).

The olfactory region of the nose is also sensitive to naphthalene after intraperitoneal administration in both the mouse and the rat *(136)*. The rat nasal olfactory epithelium is more sensitive than the mouse epithelium: significant necrosis was observed in the rat at intraperitoneal doses of 200 mg/kg, whereas injury in the mouse was not observed until 400 mg/kg. Finally, more recent studies investigating the sex and strain differences in susceptibility to naphthalene toxicity indicate that female Swiss Webster mice are more susceptible to the cytotoxicity of naphthalene than males *(137)*. These differences were detected primarily by differences in uptake of vital dye and consisted of earlier and more extensive injury following a 200-mg/kg dose. Few differences were noted in the extent of initial injury in different mouse strains *(138)*. As discussed below, the chronic bioassay investigating the possible neoplastic effects of naphthalene showed a sex difference in susceptibility: female mice showed a slight increase in bronchioloalveolar neoplasms over the control, whereas in males there was no effect.

In addition to the lesions observed in the nose, the pulmonary toxicity of naphthalene has been studied extensively by a number of laboratories

(136,137,139,140). More recently, naphthalene has been used as a selective Clara cell toxicant to evaluate progenitor cells involved in the repair of the airway epithelium *(141–143)* and to determine whether co-exposures to pulmonary toxicants alter either the initial response or the later repair of the injury *(144,145)*. Pulmonary regenerative response to naphthalene-induced lung injury in mice depends on gender, showing a significantly greater cell proliferation in female compared to male mice *(146)*. Clara cells lining the airway epithelium of the mouse are the primary target cells for naphthalene toxicity, irrespective of the route of administration. After parenteral administration of low doses of the compound, the only tissue affected is the respiratory tract (Table 4.2). Hepatic necrosis is not observed at any dose of naphthalene tested, while proximal tubular cells of the kidney are injured only in some mouse strains and only at very high doses (400 and 600 mg/kg) *(147)*. Swelling of Clara cells in terminal airways is detected in mice at intraperitoneal doses as low as 50 mg/kg. In contrast, in rats even at LD_{50} intraperitoneal doses (1600 mg/kg) airway Clara cells appear normal. Slight swelling of Clara cells in the hamster is observed at the LD_{50} intraperitoneal dose (800 mg/kg) *(136,140)*. In all of the species tested, no injury to the alveolar type I or II cells has been observed. Recently, naphthoquinone was shown to enhance an antigen-related airway inflammation with goblet cell hyperplasia in mice *(148)*. Following an intratracheal application of naphthoquinone to ICR mice for six weeks, airway hyperresponsiveness was enhanced by naphthoquinone in the presence or absence of an antigen *(149)*.

In contrast to the Clara cell toxicity observed after single doses of naphthalene, multiple daily treatments with naphthalene by either the intraperitoneal or inhalation routes result in tolerance to high challenge doses of the compound *(150–152)*. Although acute 200-mg/kg doses intraperitoneally result in substantial injury to Clara cells of mice, treatment for seven days at this same dose caused slight hyperplasia of the epithelium but no frank necrosis or vacuolation. Seven daily treatments with 200 mg/kg naphthalene markedly attenuated the toxicity observed following a 300-mg/kg challenge dose given 24 hours after the last 200-mg/kg dose in comparison to corn-oil-treated controls challenged with 300 mg/kg naphthalene (Table 4.2). As the time between the last 200-mg/kg dose and the challenge dose was extended from 24 to 96 hours, the lungs regained a portion of their sensitivity to the 300-mg/kg challenge dose. Later studies using inhalation exposures at 80 mg/m^3 showed similar effects *(152)*. Tolerance to repeated naphthalene exposures does not appear to be related to changes in the metabolic activation of naphthalene but rather to faster turnover of glutathione associated with upregulation of γ-glutamylcysteine synthase *(153,154)*. These data, showing that the lung becomes tolerant to multiple doses of naphthalene at dose levels that produced substantial toxicity in airway epithelial cells after single administration, are consistent with the 14- and 90-day oral gavage studies. This work demonstrated no significant alterations in serum enzyme levels, body weight,

Table 4.2. Species, tissue and regional differences in naphthalene toxicity

Species	Dose	Lung	
		Trachea/lobar bronchus	Terminal bronchiole
Mouse, adult, LD_{50} = 380 mg/kg	50 mg/kg	0	+
	100 mg/kg	0	++
	200 mg/kg	+/0	+++
	300 mg/kg	++	++++
	400 mg/kg	+++	++++
	11–27 mg/m^3	+/0	0
	45–61 mg/m^3	+	+/0
	133–165 mg/m^3	++	+
	383–410 mg/m^3	+++	+++
	511–591 mg/m^3	+++	+++
Mouse, adult, tolerance	200 mg/kg x 7	ND	0
	200 mg/kg x 7 + 300 (24 hours)	ND	0
	200 mg/kg x 7 + 300 (48 hours)	ND	+
	200 mg/kg x 7 + 300 (96 hours)	ND	+++
	200 mg/kg x 7 + 300 (144 hours)	ND	++++
Rat, adult, LD_{50} = 1600 mg/kg	100 mg/kg	ND	ND
	200 mg/kg	0	0
	400 mg/kg	0	0
	800 mg/kg	0	0
	1600 mg/kg	0	0
	585 mg/m^3	0	0
	0.5–1.6 mg/m^3 x 6 hours	ND	ND
	18 mg/m^3	ND	ND
	127 mg/m^3	ND	ND
Hamster, adult, LD_{50} = 800 mg/kg	200 mg/kg	0	0
	400 mg/kg	0	0
	800 mg/kg	+	0

[a] ND = not determined.

organ weight or various indices of immune function in CD-1 mice treated daily with doses up to 267 mg/kg (14 days) or 133 mg/kg (90 days) *(155)*.

Long-term exposure and carcinogenesis studies. In a subchronic study that was not published but reviewed by the European Chemicals Bureau *(22)* and evaluated in the INDEX project *(13)*, groups of 10 male and 10 female Sprague-Dawley rats were exposed snout-only to 0, 11, 51 or 306 mg/m^3 vaporized naphthalene (D. W. Coombs et al., unpublished data, 1993). Gross pathological examinations on a wide range of tissues revealed no significant changes. There were also no toxicologically relevant haematological or clinical chemistry findings. Microscopic pathology revealed treatment-related effects in the nasal passages at all

Nasal epithelium			Comments
Parenchyma	Olfactory	Respiratory	
0	0	0	No toxicity noted in liver or kidney
0	0	0	of male SW mice; ICR mice showed
0	0	0	lesions of proximal tubule at
0	ND[a]	ND	highest doses (400 and 600 mg/kg)
0	+++	0	*(136,140,147)*
0	ND	ND	West et al. *(134)*
0	ND	ND	
0	ND	ND	
0	ND	ND	
0	ND	ND	
ND	ND	ND	Areas of bronchiolar epithelial cell
ND	ND	ND	hyperplasia observed after 7 days
	ND	ND	*(150,151)*
	ND	ND	
	ND	ND	
ND	+	0	Plopper et al. *(136,140)*
0	+++	0	
0	+++	0	
0	+++	0	
0	+++	0	
0	ND	ND	
ND	+/0	0	Dodd et al. *(135)*
ND	++	0	Lee et al. *(93)*
ND	+++	0	
0	0	0	
0	+++	0	
0	+++	0	

dose levels. Degenerative changes seen in the olfactory epithelium included slight disorganization, atrophy and erosion, loss of subepithelial Bowman´s glands and signs of proliferative lesions of the olfactory epithelium. Changes were generally dose-related in that the more severe lesions and the more severe grades of all lesions occurred in the intermediate- and high-dose groups. At the lowest dose, no relevant treatment-related changes were observed in the nasal respiratory epithelium or in the lung.

Chronic exposure of B6C3F1 mice to naphthalene (53- or 160-mg/m^3) resulted in inflammation in the nose, metaplasia of the olfactory epithelium and hyperplasia of the respiratory epithelium *(156,157)*. The incidence of these lesions was 100% at both the 53- and 160-mg/m^3 exposure levels in both males and

females. The target sites for hyperplasia and metaplasia were identical to those susceptible to necrosis following acute exposures (see above). Alveolar/bronchiolar adenomas occurred in exposed male mice but the incidence did not achieve a level of statistical significance. Likewise, a small incidence of alveolar/bronchiolar carcinomas occurred in males but exposed animals did not differ statistically from unexposed. In contrast, a statistically significant though small increase in alveolar/bronchiolar adenomas was noted in high-dose (160 mg/m^3) females. Inflammation was observed in the lung of both males and females that was dose-dependent and occurred in approximately 40% of the animals in the high-dose group. There were no male/female differences in the incidence of chronic inflammation.

In similar chronic exposure studies in F 344/N rats, animals were exposed to vapour concentrations of 0, 53, 160 and 319 mg/m^3 for 105 weeks. The nasal epithelium was found to be a primary target for these exposures *(157–159)*. A dose-dependent increase in adenoma of the respiratory epithelium of the nose was noted in males, affecting 31% of the exposed population at the highest exposure levels. A much lower incidence of this lesion was observed in female rats and the incidence in exposed groups was not statistically different from that in controls. In females but not in males, there was a statistically significant increase in olfactory epithelial neuroblastomas. In several of the animals, nasal masses were observed, some of which had begun to invade the central nervous system *(159)*. A high incidence of non-neoplastic effects was observed in the nasal epithelium of both male and female rats. In the olfactory epithelium, the incidence of hyperplasia and chronic inflammation was nearly 100%, even at the lowest concentration tested (53 mg/m^3). In contrast, the respiratory epithelium was less sensitive, with 40–60% incidence for hyperplasia and inflammation in exposed animals. No differences were noted between males and females. These targets correlate well with the susceptibility of the nasal olfactory region to acute naphthalene-induced cytotoxicity and with the ability of those regions of the nasal epithelium to activate the parent substrate *(93)*.

Cataract formation. Sensitive animal models for studying naphthalene cataractogenesis have been established in rabbits *(160)*, rats *(161)* and mice *(162)*, and several in vitro methods have been used to more clearly define the mechanisms associated with the biological effects of naphthalene on the eye *(161,163)*. Doses required to produce the lesions are high: 1 g/kg per day in rabbits (number of days not specified), 1 g/kg per day for 14 days in rats and 750 mg/kg (single dose) in mice to produce a high incidence of cataracts. Van Heyningen & Pirie *(160)* presented evidence for the formation of 1,2-naphthoquinone and its involvement in cataract formation. The 1,2-quinone was thought to arise from metabolism of the parent hydrocarbon in the liver, with further processing of metabolites in the eye. Later work in mice *(162)* appears to implicate either the 1,2- or the 1,4-naph-

thoquinone. This conclusion is supported by the finding that (*a*) trichloropropylene oxide, an epoxide hydrolase inhibitor, does not alter the incidence of cataract formation and (*b*) 1-naphthol is intermediate in potency between naphthalene and the naphthoquinones, which are equipotent. Studies in rat lens cultures showed that 1,2-dihydroxy-1,2-dihydronaphthalene produced lesions similar to those observed when naphthalene was given to rats in vivo. This observation, along with the finding that an aldose reductase inhibitor blocked the lens opacity induced by the dihydrodiol, supports the importance of 1,2-naphthoquinone in mediating cataractogenesis in rats.

As indicated above, the doses used to produce cataracts in animal models are high. Lower doses, such as those reported in the subchronic oral naphthalene studies in mice (as high as 267 mg/kg per day for 14 days or 133 mg/kg per day for 90 days) apparently did not result in untoward effects in the eye *(155)*. Likewise, the chronic inhalation cancer bioassays in mice or rats did not report lesions in the eye *(123,164)*. Overall, whether these finding are relevant to humans is uncertain, since there are no reliable data on cataract formation in humans following naphthalene exposure.

Haemolytic anaemia. This principal toxicological effect of naphthalene observed in humans has not been seen in experimental animal studies with rats, mice or rabbits. The reason for this is not known. Therefore, for this end-point, there are no relevant data for extrapolation from experimental animal studies to human exposure.

Animal cells/explants/perfused tissues in vitro

As discussed in the kinetics and metabolism module, naphthalene is metabolized to several reactive metabolites that have the potential to produce the toxicities associated with the parent compound and, as discussed above, these metabolites can produce cellular injury to human cells in vitro. It is clear that naphthalene requires metabolism by the cytochrome P450 monooxygenases for lung toxicity *(84)* and that glutathione plays a major role in protecting the cells from injury *(85,165)*. There is some evidence that metabolites generated in the liver can enter the bloodstream, causing downstream toxicities in extrahepatic tissues either directly or through depletion of glutathione, with increased susceptibilities to metabolites generated in situ in the respiratory system *(108,166)*. Studies in isolated murine Clara cells *(167)* and in isolated perfused murine lung *(62,168)* demonstrated that target tissues were capable of generating sufficient metabolite from the parent compound to produce cytotoxicity in the airway epithelium. When tested in isolated Clara cells, naphthalene oxide and 1,4-naphthoquinone produced similar losses in cell viability at both 2 and 4 hours. The remaining metabolites were either less potent or did not cause a loss of cellular integrity (1-naphthol or dihydrodiol) at either point in time. Interestingly, preincubation

of cells with the cytochrome P450 monooxygenase inhibitor piperonyl butoxide inhibited the cytotoxic effects of naphthalene but not of naphthalene oxide *(167)*, a finding that suggests that metabolites downstream of the epoxide may not be keys to naphthalene toxicity. Likewise, naphthalene oxide produced selective injury to Clara cells in perfused lungs and the 1,2- and 1,4-quinones were approximately 10-fold less potent *(62)*. These studies need to be interpreted with caution, because isolated cells may or may not be a good model for the Clara cell in its normal microenvironment within the airway. Similarly, the toxicity of various metabolites in isolated perfused lungs would be strongly influenced by the amounts of these reaching the target cell from the perfusate, and there is no indication that the amounts of these were the same for the metabolites tested.

Short-term mutagenicity assays. Naphthalene and a number of naphthalene metabolites have been tested in a variety of mutagenicity assays. These have been reviewed thoroughly by IARC *(115)* and Schreiner *(169)* and will be addressed only briefly here. In all of the Ames assays using various *Salmonella typhimurium* strains, with and without activating enzyme, naphthalene is negative. As stated above, both 1,2- and 1,4-naphthoquinone were found to be positive in SCE assay *(131)*. Other short-term tests evaluating neoplastic transformations with γ-glutamyltranspeptidase-positive liver foci and in vitro cell transformation assays were, likewise, negative. Micronucleus assays for chromosome breakage were positive, as were assessments of chromosome aberrations in Chinese hamster ovary cells. Overall, the preponderance of evidence suggests that naphthalene is not a genotoxic carcinogen and that any DNA damage associated with the compound may derive from the cytotoxic actions of the naphthalene metabolites *(170)*.

The cytotoxicity associated with naphthalene exposure may play an important role in the overall effects observed in the chronic bioassay. 1,2-Naphthoquinone has been shown to bind to DNA, forming adducts at the N3 position of adenine and the N7 position of guanine that depurinate *(82)*. Recent work has disclosed formation of depurinating DNA adducts following a four-hour dermal exposure of female SENCAR mice to naphthalene, 1-naphthol, 1,2-DDN, 1,2-DHN or 1,2-NQ *(171)*. The relevance of these data is unknown, since markers of DNA reactivity associated with naphthalene in target tissues of animals and of biomarkers for evaluating these processes in humans have still to be developed *(172)*.

Health risk evaluation

Critical health outcomes

The principal health concerns of exposure to naphthalene are respiratory tract lesions, including respiratory tract carcinogenicity demonstrated in animal studies and haemolytic anaemia in humans. Regarding cataract formation seen in experimental animals after high oral exposure to (but not after inhalation of)

naphthalene, there is only suggestive evidence of an association with exposure to naphthalene in humans, if at all.

Most of the reports on haemolytic anaemia in humans refer to dermal uptake of naphthalene from clothes treated with naphthalene mothballs or unintentional or suicidal ingestion of mothballs. Many of the cases were in infants. For this end-point, data on dose–response relationships are insufficient. Since experimental rodents or rabbits do not disclose haemolytic anaemia following exposure to naphthalene, there is no relevant information from animals to extrapolate to human exposures for this effect.

No reliable data in humans are available for long-term inhalation toxicity of naphthalene, and evaluation of the risk to health of inhaled naphthalene has to rely essentially on animal studies and in vitro results. Evidence is sufficient to infer that naphthalene is a respiratory toxicant in rats and mice following acute and chronic exposure to rather low concentrations. Epithelial cells in the proximal airways are the primary target cells for naphthalene toxicity. In rats, a pronounced susceptibility of the olfactory region of the nasal mucosa was confined to the high air flow area of the medial meatus *(50)*. With increasing naphthalene concentrations, the proximal airway lesions became more severe and proceeded to the distal airways.

In two rat strains, olfactory epithelium necrosis occurred at a single six-hour whole-body exposure to the lowest naphthalene concentration of 5 mg/m^3. In a recent brief communication by Dodd et al. *(135)*, exposure to 0.5–2 mg/m^3 revealed very weak effects in a few animals, indicating a NOAEL for acute inhalation exposure. In mice, Clara cell injury was seen following a four-hour exposure to 11 mg/m^3 *(134)*.

In two reports that were not peer reviewed but have been examined and found to be of good quality, mild lesions of the nasal olfactory epithelium with signs of proliferative repair were observed following subacute or subchronic exposure down to 5 or 11 mg/m^3, respectively. This was the LOAEL after subacute or subchronic inhalation exposure (D. W. Coombs et al., unpublished data, 1993).

Compared to the acute (some hours), subacute (4 weeks) or subchronic (13 weeks) exposure studies, long-term (104 weeks) inhalation studies were performed only with relatively high naphthalene concentrations *(164)*. Chronic exposure of mice to naphthalene at 53 or 159 mg/m^3 resulted in nasal inflammation, metaplasia of the olfactory epithelium and hyperplasia of the respiratory epithelium in almost all exposed male and female animals. Alveolar/bronchiolar adenomas were seen in both exposed males and females. A statistical significance of an elevated incidence of adenomas was achieved only in the female high-dose group. A small, statistically insignificant incidence of alveolar/bronchiolar carcinomas occurred in male mice. The LOAEL for chronic respiratory tract inflammation seen with almost all mice in this study was 53 mg/m^3.

Similar chronic inhalation studies were performed in rats exposed to naphthalene at 53, 159 or 318 mg/m^3 *(123)*. In the nasal olfactory epithelium, hyperplasia, chronic inflammation and hyaline degeneration were seen in almost all animals, even in the lowest-dose group. A statistically significant dose-dependent increase in olfactory epithelial neuroblastomas occurred in females. The nasal respiratory epithelium was less sensitive, about half of the cells showing signs of hyperplasia, inflammation and hyalinization in both exposed males and females. The incidence of nasal respiratory adenomas increased dose-dependently in male rats. Again, the LOAEL for severe lesions in the olfactory region and, less pronounced, respiratory epithelium of rats chronically exposed to naphthalene was 53 mg/m^3.

The mechanisms responsible for the toxicity and carcinogenicity of naphthalene in the rodent respiratory tract and the gender differences in these responses are not fully understood. Target site cytotoxicity associated with naphthalene exposure is assumed to play a crucial role in the development of tumours observed in the inhalation studies. Studies indicate that metabolism is necessary for naphthalene to develop its cytotoxic effects. In rats, naphthalene metabolism rates are approximately 40-fold higher in the olfactory than in the septal non-olfactory mucosa *(93)*. The neuroblastomas observed in the rat olfactory epithelium are highly malignant and should be considered of relevance to humans, since P450 isoenzymes able to metabolically activate naphthalene in the rodent nose are also present in humans.

In mice, the particular susceptibility to naphthalene injury of Clara cells of the distal bronchiolar epithelium does not seem of high relevance to humans owing to the special nature of metabolism in mice *(173)*.

The possible involvement of a genotoxic mechanism in tumour formation in rodents cannot be ruled out owing to the metabolic activation of naphthalene to an epoxide, which may also be generated in the olfactory and respiratory epithelia of the rodent respiratory tract. There have been positive results in some in vitro tests for mutagenicity, but results of in vivo tests are consistently negative *(115)*. Although depurinating naphthalene-DNA adducts were identified in mouse skin *(171)*, naphthalene is not carcinogenic in this tissue.

Overall, naphthalene is considered a non-genotoxic carcinogen in the rodent respiratory tract, chronic inflammation (eventually resulting in secondary genotoxicity) being the key action in the formation of tumours.

Health relevance of indoor exposure

Indoor air levels of naphthalene may exceed outdoor concentrations manyfold owing to a variety of potential indoor sources, including tobacco smoke, indoor combustion and consumer products. Indoor air levels vary from a few to tens of μg/m^3, with levels markedly higher when mothballs are used.

Conclusions of other reviews

Naphthalene has been classified by IARC in Group 2B as "possibly carcinogenic to humans" on the basis of sufficient evidence of its carcinogenicity in experimental animals and inadequate evidence of carcinogenicity in humans *(115)*. The classification of naphthalene into carcinogenicity group Carc. 2 by the EU *(174)* and into group C by the USEPA *(16)* are compatible with the IARC evaluation.

Guidelines

The principal health concerns of exposure to naphthalene are respiratory tract lesions, including tumours in the upper respiratory tract demonstrated in animal studies and haemolytic anaemia in humans.

Lesions in the nasal olfactory and, at higher concentrations, also in the respiratory epithelia of rats appear to be the critical non-neoplastic effect. At concentrations about 100-fold higher than the lowest lesion level, severe inflammation and tumours have been reported to occur at these sites.

Increased cell proliferation due to cytotoxicity (cell damage) is considered a key element in the development of airway tumours. The likely involvement of cytotoxic metabolites in the carcinogenic response and the apparent primary non-genotoxicity of naphthalene favour the assumption of the existence of a threshold. Therefore, the use of a LOAEL/NOAEL as a threshold, combined with safety factors, is considered to be an appropriate approach for setting indoor air guidelines to minimize the carcinogenic risk to the respiratory tract of naphthalene exposure.

Associated with repeated inhalation exposure of 6 hours/day, 5 days a week for 104 weeks, severe effects in terms of inflammation were observed in almost all rats exposed to the lowest, but still relatively high, naphthalene dose of 53 mg/m^3 *(123)*. In the absence of adequately published data in relation to less severe effects, this can be taken as a LOAEL, even though it is related to severe effects.

Taking this LOAEL as a starting point and adjusting for continuous exposure (dividing by a factor of 24/6 and 7/5), a value of about 10 mg/m^3 is obtained. Further, incorporating a factor of 10 for using a LOAEL rather than a NOAEL, a factor of 10 for interspecies variation and a factor of 10 for inter-individual variation, a guideline value of 0.01 mg/m^3 is established. This guideline value should be applied as an annual average.

Extensive use or misuse of naphthalene mothballs may lead to haemolytic anaemia. Knowledge of the impact of exposure to naphthalene on the risk of haemolytic anaemia in susceptible individuals (glucose 6-phosphate dehydrogenase deficiency) cannot be used to define a guideline owing to the lack of adequate exposure data.

In the absence of mothballs or other sources such as combustion of biomass, indoor air concentrations of naphthalene are just above the typical limit of detec-

tion of about 0.001 mg/m^3. Since the concentration of naphthalene in the residential environment increases up to 100-fold when mothballs are used, the most efficient way to prevent high exposures would be to abandon (ban) the use of naphthalene-containing mothballs.

The guidelines section was formulated and agreed by the working group meeting in November 2009.

Summary of main evidence and decision-making in guideline formulation

Critical outcome for guideline definition

Respiratory tract lesions leading to inflammation and malignancy in animal studies.

Source of exposure–effect evidence

Nasal inflammation and olfactory epithelial metaplasia in nearly all rats chronically exposed to 53 mg/m^3 was considered as the LOAEL, even though related to severe effects *(157–159)*. This was adjusted for continuous exposure (dividing by a factor of 24/6 and 7/5). Further, a factor of 10 for using a LOAEL instead of a NOAEL, a factor of 10 for interspecies variation and a factor of 10 for inter-individual variation were incorporated, leading to a guideline value of 0.01 mg/m^3.

Supporting evidence

- Dose-dependent respiratory tract cytotoxicity following acute to chronic exposure in rats *(123)*.
- Airway toxicity was seen in several strains of rats and mice over a wide range of concentrations *(93,134–146,148,156,157)*.
- Human cells are susceptible to naphthalene metabolites in vitro *(69,131–133)*.

Results of other reviews

- IARC: Group 2B (possibly carcinogenic to humans) *(115)*.
- EU: Group 2 (suspected human carcinogen) *(174)*.
- USEPA: Group C (possible human carcinogen) *(16)*.
- EC INDEX project: guideline 0.01 mg/m^3 (annual average concentration) *(12,13)*.

Guidelines

0.01 mg/m^3 (annual average concentration).

Comments

The long-term guideline is also assumed to prevent potential malignant effects in the airways. No reliable human data for long-term inhalation toxicity are available.

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5. Nitrogen dioxide

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General description

There are seven oxides of nitrogen that may be found in the ambient air. Nitrous oxide (N_2O) is a greenhouse gas with significant anthropogenic sources contributing to its worldwide abundance (~ 0.3 ppm). However, nitric oxide (NO) and nitrogen dioxide (NO_2) are the two principal nitrogen oxides associated with combustion sources. Ambient concentrations of these two gases vary widely according to local sources and sinks, but can exceed a total concentration (NO + NO_2) of 500 μg/m^3 in dense urban areas. Nitrous acid (HONO) is a common pollutant in ambient and indoor environments, produced by the reaction of nitrogen dioxide with water.

Nitric oxide is oxidized in air to form nitrogen dioxide. In its liquid form, nitrogen dioxide is colourless to brown. While the boiling point of nitrogen dioxide is 21.15 °C, in normal ambient conditions its low partial pressure in the atmosphere (908 mmHg at 25 °C) prevents condensation so that it exists in the air in its gaseous form. In that form, nitrogen dioxide is volatile, reddish-brown in colour and heavier than air, and has a characteristic pungent odour perceptible from a concentration of 188 μg/m^3 (0.1 ppm). It is a strong oxidant, corrosive and poorly soluble in water *(1)*. Its molecular weight is 46.01 g/mol, melting point –11.2 °C, boiling point 21.15 °C and density 1.59 (air = 1). It reacts with water and is soluble in sulfuric and nitric acids.

Conversion factors

At 760 mmHg and 20 °C, 1 ppm = 1.914 mg/m^3 and 1 mg/m^3 = 0.523 ppm; at 25 °C, 1 ppm = 1.882 mg/m^3 and 1 mg/m^3 = 0.531 ppm.

Sources and pathways of exposure

In ambient air, the oxides of nitrogen are formed by various combinations of oxygen and nitrogen at high temperatures during the combustion process. The higher the combustion temperature, the more nitric oxide is generated. Indeed, 90–95% of the nitrogen oxides are usually emitted as nitric oxide and only 5–10% as nitrogen dioxide, although substantial variations from one source type to another have been observed. In ambient conditions, nitric oxide is rapidly oxidized in air to form nitrogen dioxide by available oxidants (such as oxygen, ozone and

VOCs) and this rapid oxidation velocity is such that it is nitrogen dioxide that is usually considered as a primary pollutant. In indoor air, however, this oxidation process is generally much slower *(2)*.

Road traffic is the principal outdoor source of nitrogen dioxide. The most important indoor sources include tobacco smoke and gas-, wood-, oil-, kerosene- and coal-burning appliances such as stoves, ovens, space and water heaters and fireplaces, particularly unflued or poorly maintained appliances. Outdoor nitrogen dioxide from natural and anthropogenic sources also influences indoor levels. Occupational exposures can be elevated in indoor spaces, including accidents with silage and in ice arenas with diesel- or propane-fuelled ice resurfacing machines *(3)* and underground parking garages *(4)*.

In ambient conditions, both outdoors and indoors, nitrogen dioxide exists in its gaseous form, and inhalation is therefore the major route of exposure at room temperature. Exceptionally, direct contact with the eyes and associated membranes may lead to eye irritation, although this is more likely to occur in industrial settings after accidental contact with relatively high gaseous nitrogen dioxide concentrations *(1)*.

Indoor levels and relationship with outdoor levels

Indoor air levels in various countries

In the INDEX report *(5)*, nitrogen dioxide concentrations were in the range of 13–62 μg/m^3 indoors, 27–36 μg/m^3 at the workplace, 24–61 μg/m^3 outdoors and 25–43 μg/m^3 for personal exposure. Maximum levels associated with the use of gas appliances (gas cooking and heating) in European homes are in the range 180–2500 μg/m^3. In studies regrouped in the THADE project *(6)*, mean indoor concentrations in Europe ranged from 10–15 μg/m^3 in Scandinavia *(7,8)* to 65 μg/m^3 in Poland *(8)*. Compared to European levels, indoor levels were similar in North America *(8)* but were higher in Asia (43–81 μg/m^3) *(8–10)*, New Mexico, USA *(11)* and Mexico *(8)*.

Levy et al. *(8)* studied nitrogen dioxide concentrations in homes in 18 cities in 15 countries, reporting two-day means ranging from 10 μg/m^3 to 81 μg/m^3 and personal exposures from 21 μg/m^3 to 97 μg/m^3. The use of a gas stove was found to be the dominant activity influencing indoor concentrations. Results showed also the importance of combustion space heaters to elevated nitrogen dioxide concentrations.

Numerous EU studies highlight the importance of key sources in characterizing indoor nitrogen dioxide levels. In an Italian population-based study, the highest weekly indoor concentrations were measured in a rural area of the Po Delta. The weekly mean indoor concentration in the kitchen during winter was higher than that in summer, being 62 μg/m^3 and 38 μg/m^3, respectively. The study also found that the presence of a gas-fired heating furnace was the major factor in the elevated nitrogen dioxide concentrations *(12)*. In a Spanish study of 340 dwell-

ings carried out between 1996 and 1999, average annual indoor concentrations of nitrogen dioxide did not vary significantly, ranging from 12.5 to 14.7 μg/m^3. Respective outdoor air concentrations were slightly higher in 1996 and 1998 and slightly lower in 1997 and 1999; typical indoor : outdoor ratios were close to 1. The principal indoor sources of nitrogen dioxide in Spanish homes were the use of gas cookers, the absence of an extractor fan when cooking, the absence of central heating, and cigarette smoking *(13)*. Consistent risk factors were identified when these data from Barcelona were compared with cohort data from Ashford, Kent (United Kingdom) and Menorca (Spain). In the United Kingdom, studies showed indoor concentrations of nitrogen dioxide in homes without gas stoves ranging from 13 to 40 μg/m^3 and in the presence of gas stoves from 25 to 70 μg/m^3 *(14)*.

Nitrogen dioxide concentrations in indoor air in different countries, the microenvironments and the different fuel sources are summarized in Table 5.1 (see page 268).

Factors influencing nitrogen dioxide levels indoors

Box 5.1 summarizes some of the key factors that influence indoor nitrogen dioxide levels and likely explain much of the variation reported in Table 5.1. Indoor levels of nitrogen dioxide are a function of both indoor and outdoor sources. Thus, high outdoor levels originating from local traffic or other combustion sources influence indoor levels. Annual mean concentrations in urban areas throughout the world are generally in the range of 20–90 μg/m^3 *(15)*. In the European Community Respiratory Health Survey (ECRHS II) covering 21 European cities, annual ambient nitrogen dioxide concentrations ranged from 4.9 μg/m^3 in Reykjavik to 72 μg/m^3 in Turin *(16)*. The maximum hourly mean value may be several times higher than the annual mean. For example, a range of 179–688 μg/m^3 nitrogen dioxide has been reported inside a car in a road tunnel during the rush hour *(15)*.

The distance of buildings from roadways appears to have an impact on indoor nitrogen dioxide levels *(17,18)*. Levels in school classrooms have been found to be significantly correlated with traffic density and distance of the school from the roadway *(19)*.

The air rate exchange between indoors and outdoors affects nitrogen dioxide levels in buildings. Indoor levels vary widely depending on the presence of indoor sources, air mixing within and between rooms, the characteristics and furnishing of buildings, and reactive decay on interior surfaces. Further, it has been shown that car exhausts containing nitrogen dioxide may enter a house from an attached garage *(20)*.

In the absence of indoor nitrogen dioxide sources, indoor levels will be lower than outdoor levels. Under normal ventilation conditions, the indoor : outdoor ratio has been found to vary from 0.88 to 1 *(21)*. This is attributable to the re-

Box 5.1. Factors that influence indoor concentrations of nitrogen dioxide

Indoor sources

Fuel-burning stoves (wood, kerosene, natural gas, propane, etc.)
Fuel-burning heating systems (wood, oil, natural gas, etc.)
Tobacco use

Source characteristics

Flued/unflued sources
Presence of pilot lights

Outdoor sources (via infiltration)

Mobile sources (petrol- and diesel-powered vehicles)
Stationary sources (industrial combustion)

Resident behaviour

Stove usage (for fuel-burning appliances)
Use of heating equipment (including cooking stoves)

Dwelling and indoor environment characteristics

Dwelling size (where there are indoor sources)
Air exchange rates
Distance to roadway
Surface characteristics
Indoor humidity

moval of nitrogen dioxide by the building envelope and its reactions with interior surfaces and furnishing *(22)*. However, in the presence of indoor sources, especially unvented combustion appliances, indoor levels may exceed those found outdoors *(23)* with an increase in the indoor : outdoor ratio from 0.7 without an indoor source to 1.2 in the presence of an indoor source *(3,24,25)*. These ratios, however, reflect average levels over several days of measurement and do not reflect the more extreme indoor/outdoor differences that one would expect to see over shorter periods of time – for example, when a gas appliance is being used inside a home.

The presence and use of indoor sources are the primary determinants of indoor nitrogen dioxide levels within populations. In an inner-city population in the United States, mean nitrogen dioxide concentrations were higher in homes with a gas stove (33.1 ppb or 63.3 $\mu g/m^3$) than in those without a gas stove (16.8 ppb or 32.1 $\mu g/m^3$) *(26)*. In this study, indoor levels were also associated with the presence of a gas heater and the use of a space heater or oven for supplementary heating.

The average nitrogen dioxide concentration over a period of several days may exceed 150 $\mu g/m^3$ when unvented gas stoves are used *(27)*. On the other hand, wood-burning appliances were not related to elevated nitrogen dioxide concentrations in a Canadian study carried out in 49 houses *(28)*. While most studies

of indoor air pollutant exposures from biomass burning in developing countries have focused on airborne particulate matter, nitrogen dioxide levels can also be elevated. In a study in Ethiopia where wood, crop residues and animal dung were the main household fuels, the mean 24-hour concentration of nitrogen dioxide was 97 μg/m^3 *(29)*. A study in rural, urban and roadside locations in Agra, India showed the dominance of outdoor sources (principally diesel generators and traffic) on elevated indoor nitrogen dioxide concentrations (indoors 255 ± 146 ppb; outdoors 460 ± 225 ppb) *(30)*.

Indoor levels are typically higher in winter than in summer, probably owing to increased use of heating, lower ventilation rates and higher outdoor concentrations *(11,14,31,32)*. In the recently published ECRHS II study carried out in 21 European cities, concentrations in winter exceeded summer values with an average winter : summer ratio of 1.50 *(16)*.

There are limited data on nitrogen dioxide peaks. However, it has been shown that, in homes, peak concentrations are typically related to the use of combustion appliances for cooking and heating *(32–35)*. In particular, occurrences of peaks are strongly associated with the use of gas and solid fuel stoves, the highest nitrogen dioxide concentrations coinciding with the time of meal preparation *(36)*. In a study of Australian homes, the mean peak-to-average nitrogen dioxide ratio was 2.9 (1.2–4.6) for homes without gas cookers and 7.8 (2.6–13.0) for those with gas cookers *(37)*. A modelling study of indoor nitrogen dioxide exposures in the United Kingdom concluded that those regularly using gas for cooking would experience 1-hour mean exposures above 287 μg/m^3 (150 ppb) for at least 1 hour on every day of the year *(38)*. Reported maximum measured nitrogen dioxide levels associated with the use of gas appliances in homes are in the range 150–2055 μg/m^3 over 1 hour, with peaks of 400–3808 μg/m^3 for 1 minute *(27,34)*.

In addition to the direct release of nitrogen oxides, indoor combustion sources emit various co-pollutants including ultrafine particles, which are also produced during cooking *(36)*. Secondary reactions, such as the production of nitrous acid from surface chemistry involving nitrogen dioxide, can contribute to indoor pollutant concentrations that directly affect health *(39,40)*. The role of these co-pollutants in the health effects attributed to nitrogen dioxide in field studies is unknown, but abatement measures for nitrogen dioxide, such as improved ventilation, will be beneficial in reducing co-exposures also.

High nitrogen dioxide concentrations are also associated with the use of candles and mosquito coils. In chamber (18 m^3) tests, maximum nitrogen dioxide concentrations up to 92 μg/m^3 were observed during incense burning *(41)*. High values of nitrogen dioxide up to 7530 μg/m^3 were also reported in enclosed ice arenas with inadequate ventilation from the exhaust emissions of propane- and petrol-fuelled ice resurfacing machines *(42,43)*. The link between direct source exposure and high nitrogen dioxide levels was noted in a small study of unvented natural gas fireplaces (mean 688.7 μg/m^3; n = 2) *(33)*.

Indoor concentrations of nitrogen dioxide are also subject to geographical, seasonal and diurnal variations. Differences in the indoor concentrations in various countries are mainly attributable to differences in the type of fuel used for cooking and heating and the rate of fuel consumption. While few studies have included repeated measurements of indoor nitrogen dioxide levels, it is known that within-home variability can be significant owing to the various contributing factors discussed above *(44)*.

Seasonal variability can be significant, owing to variations in source use (e.g. heaters and stoves) and seasonal fluctuations in air exchange rates. This variability results typically in higher indoor concentrations during winter months *(31,45,46)*. This variability, and its principal determinants, should be considered when extrapolating from exposure estimates determined using daily or weekly measurements to estimates of annual exposures. Since few (if any) studies have directly measured annual averages of indoor nitrogen dioxide concentrations, periodic measurements across seasons would be needed to construct representative estimates of long-term exposure.

Kinetics and metabolism – effects observed in experimental studies

In vitro studies

As nitrogen dioxide is a free radical, it has the potential to deplete tissue antioxidant defences and, as a consequence, cause injury and inflammation as shown in a variety of in vitro test systems. Exposure of human blood plasma to 26 230 $\mu g/m^3$ (13.95 ppm) nitrogen dioxide resulted in a rapid loss of ascorbic acid, uric acid and protein thiol groups, in addition to lipid peroxidation and a depletion of alpha-tocopherol (vitamin E) *(47)*.

In another study, exposure to nitrogen dioxide over a lower concentration range (94–1880 $\mu g/m^3$; 0.05–1.0 ppm) resulted in the antioxidant defences, uric acid and ascorbic acid being depleted in human bronchoalveolar lavage (BAL) fluid *(48)*. More recently, Olker et al. *(49)* have shown that superoxide radical release is significantly impaired from BAL cells isolated from rats exposed to nitrogen dioxide (18 800 $\mu g/m^3$; 10 ppm) for 1, 3 or 20 days. This was explained by decreased production as a result of an inhibition of NADPH oxidase and complex III of the respiratory chain, and to a lesser extent increased scavenging brought about by enhanced glutathione peroxidase and CuZn-superoxide dismutase mRNA expression and enzyme activities. Evidence for the role of oxidative stress in the effects of nitrogen dioxide on respiratory virus-induced injury comes from a study that found that pre-treatment of cultured primary human nasal epithelial cells and cells of the BEAS-2B line with the antioxidant *N*-acetylcysteine inhibited the production of IL-8 following exposure to 3160 $\mu g/m^3$ (2 ppm) nitrogen dioxide for three hours in combination with human rhinovirus type 16 (RV16) *(50)*.

Cell culture systems have also been used to describe nitrogen-dioxide-mediated cell injury and inflammation. One system exposed cultured human bronchial epithelial cells to 7520 and 15 040 μg/m^3 (4.0 and 8.0 ppm) nitrogen dioxide and elicited cell membrane damage and increased membrane permeability *(51)*. It should be remembered that confluent airway epithelial cell monolayers in vitro are not fully differentiated and posses a markedly decreased level of resistance to pollutants when compared to the epithelium in the intact human. However, in a more physiologically relevant system, nitrogen dioxide (200 and 800 μg/m^3; 0.1 and 0.43 ppm) has also been shown to trigger inflammation in cultured human nasal mucosa explants, using histamine release into the culture medium as a marker of the inflammatory response *(52)*. The early pro-inflammatory responses following exposure to a brief high concentration of nitrogen dioxide – up to a maximum of 84 600 μg/m^3 (45 ppm) over 50 minutes – have also been assessed using normal human bronchial epithelial (NHBE) cells as an in vitro model of inhalation injury *(53)*. While immunofluorescence studies confirmed oxidant-induced formation of 3-nitrotyrosine, the nitrogen-dioxide-exposed cells exhibited marked increases in the levels of nitrite (used as an index of nitric oxide), IL-8, IL-1β and TNF-α. Further, to simulate a pre-existing "inflammatory" condition of the bronchial epithelium, such as would exist in asthma and other hyperreactive airway diseases, cells were pre-treated with various pro-inflammatory cytokines (IFN-γ, TNF-α, IL-1β and IL-8) for 24 hours prior to exposing them to nitrogen dioxide. The combination of cytokine treatment and nitrogen dioxide exposure consistently enhanced the generation of nitric oxide and IL-8. More recently, further findings have been published on the early changes in NHBE cells on exposure to a brief high dose (84 600 μg/m^3; 45 ppm) of nitrogen dioxide, focusing on the nature and time-course of nitrogen-dioxide-mediated cell death and, more generally, on the cellular mechanisms by which the various pro-inflammatory mediators affect the target cells *(54)*. Cells were found to undergo apoptotic cell death during the early post-nitrogen-dioxide period, independent of any significant increase in caspase-3 activity, while necrotic cell death was more prevalent at later time intervals. Exposed cells also exhibited increased expression of heme oxygenase-1 (HO-1), a redox-sensitive stress protein, at 24 hours and increased adhesion to neutrophils, which in turn resulted in an increased NHBE cell death. Earlier reports of an involvement of nitric oxide *(53)* were supported by the significant decrease in cell death and neutrophil adhesion in the presence of nitric oxide synthase inhibitors (L-NAME and 3-aminoguanidine) *(54)*.

Effects on experimental animals

Nitrogen dioxide per se, but not specifically in relation to indoor sources/exposure patterns, has been shown to exert a range of biological effects on experimental animals, including changes in lung metabolism, structure, function, inflam-

mation and host defence against infectious pulmonary disease. Such effects vary widely, however, depending on the species and strain exposed, the concentration and duration applied, and the age and sex of the animals *(55)*.

In extrapolating the aforementioned data to humans, it is the anatomical and physiological differences between animals and humans that represent a particular challenge. For example, we have known for some time from mathematical modelling that the distribution of nitrogen dioxide deposition within the respiratory tract of rats, guinea-pigs, rabbits and humans appears to be similar *(56–58)*. More recently however, Tsujino et al. *(59)*, using mathematical airway models of rats, dogs and humans, demonstrated that interspecies variations in anatomy and respiratory patterns do cause significant differences in the concentration of nitrogen dioxide in the airways and alveoli. Despite some limitations, owing to many simplifications and assumptions necessary to construct the airway model and carry out calculations, intra-airway nitrogen dioxide concentrations were higher in the upper and lower airways of humans compared with rats and dogs, while those in the alveolar regions were lowest in humans.

Pulmonary metabolism

The majority of biochemical studies show effects only after acute or subchronic exposure to high levels of nitrogen dioxide exceeding 3160 μg/m^3 (2 ppm) *(60–62)*. A notable exception is the effect on lung lipid metabolism. Continuous exposure of rats to concentrations as low as 752 μg/m^3 (0.4 ppm) for 18 months increased lipid peroxidation when thiobarbituric acid reactants were used as an indicator, while lipid peroxidation was raised by 75 μg/m^3 (0.04 ppm) for 9 months when ethane exhalation was the indicator *(63,64)*. Effects on both lipid and antioxidant metabolism showed a response pattern that depends on both concentration and duration of exposure *(65)*. Frequently observed features at higher nitrogen dioxide levels include the induction of lung oedema, an increase in antioxidant metabolism, an increase in lung enzymes associated with cell injury, and changes in lung lipids. On investigating the basis of oxidative stress elicited in rats exposed to 18 880 μg/m^3 (10 ppm) nitrogen dioxide for 3 and 20 days (inducing acute and chronic lung injury, respectively), Hochscheid et al. *(66)* reported an imbalance of glutathione status (by analysing the activity and mRNA expression of a host of enzymes involved in glutathione metabolism) in type II pneumocytes following both types of lung injury.

In relation to nitrogen-dioxide-induced oxidative stress and perturbations in antioxidant metabolism, a potential protective role that antioxidant status may play in influencing the lung response to pollutant exposure has been explored *(67)*. The effects of a low-selenium diet (1.3 μg/day) with or without selenium supplementation in rats exposed to either acute (62 000 μg/m^3 (50 ppm) for 30 minutes), intermittent subacute (5 ppm, 6 hours/day for 5 days) or intermittent long-term nitrogen dioxide (1 or 10 ppm, 6 hours/day, 5 days/week for 28 days)

on a host of markers were examined and the majority of these (particularly those indicating increased permeability of the lung epithelial barrier) indicated the protective role of normal selenium status.

Although still not fully understood, alterations in pulmonary metabolism may be early signs of cell lesions, which become manifest only at higher concentrations or upon longer exposure *(60–62,68,69)*.

Pulmonary structure

In the tracheobronchial and alveolar regions, nitrogen dioxide at concentrations down to 640 μg/m^3 (0.34 ppm) results in replacement of the type I alveolar epithelial and ciliated epithelial cells with the more oxidant-resistant type II and nonciliated bronchiolar (Clara) cells, respectively. Furthermore, the replaced cells exhibit alterations of their cytoplasm and hypertrophy after short exposure (10 days) to concentrations of nitrogen dioxide above 940 μg/m^3 (0.50 ppm), the significance of which is not known (*60–62,69)*. We do, however, appreciate that both the exposure regimen used and the time of exposure are important. In a subchronic study of lung lesions in rats, Rombout et al. *(70)* showed that the concentration (C) of inhaled nitrogen dioxide had more influence on epithelial metaplasia than exposure duration (time, T) when C × T was constant, and that the effect of C was greater with intermittent exposure than with continuous exposure. Other experiments have addressed the temporal pattern of nitrogen dioxide effects and found them to be complex *(60)*. For example, over a 7-day period, a wave of epithelial hyperplasia occurs, peaking by about day 2 *(71)*. Rombout et al. *(70)* showed that even 2 months after a 1-month exposure ceased, some nitrogen-dioxide-induced interstitial changes were still present.

Long-term exposure to nitrogen dioxide leads to emphysema-like structural changes in animals, in addition to thickening of the alveolar capillary membrane, loss of ciliated epithelium and increases in lung collagen. Such changes have been observed in mice, rats, dogs and monkeys (*60–62,68)*. In 1993, the USEPA reviewed 23 research reports on nitrogen dioxide exposure and emphysema to determine whether the effects reported met the US National Heart, Lung, and Blood Institute definition for human emphysema *(72)*. This can be important because the animal studies were of interest for the purposes of extrapolation to humans, whether or not the more rigorous definition of human emphysema (which includes destruction of alveolar walls) is met. Many of the reports contained insufficient detail to permit an independent judgement as to whether "human-type" emphysema had occurred. Nevertheless, three studies reported convincing evidence of human-type emphysema following exposure to very high nitrogen dioxide levels relative to ambient concentrations: Haydon et al. *(73)* exposed rabbits to 15 040–22 600 μg/m^3 (8–12 ppm) for 3–4 months; Freeman et al. *(74)* exposed rats to 37 000 (reduced to 28 200 or 18 800) μg/m^3 (20, reduced to 15 or 10 ppm) for up to 33 months; and Hyde et al. *(75)* exposed dogs for 5½ years to a

mixture containing 1210 μg/m^3 (0.64 ppm) nitrogen dioxide and 310 μg/m^3 (0.25 ppm) nitric oxide, respectively. These animals exhibited several decrements in pulmonary function, which continued to deteriorate compared to controls during a 2½-year post-exposure period in clean air. After this post-exposure period, lung morphometry studies showed changes analogous to human centrilobular emphysema.

Studies undertaken to localize collagen deposition within the lung have exposed ferrets to 940 or 18 800 μg/m^3 (0.5 or 10 ppm) of nitrogen dioxide for 4 hours a day for 8 or 15 weeks. Increased lung collagen deposition was identified within the respiratory bronchiolar submucosa, although this was only significant in the higher-dose group *(76)*. The onset of emphysema-like changes, together with the major features characteristic for human chronic obstructive pulmonary disease (COPD), have also been reported in another study in which mice were exposed to 31 160 μg/m^3 (20 ppm) nitrogen dioxide for 14 hours a day for up to 25 days *(77)*. The main findings were progressive airway inflammation with a marked influx of neutrophils and macrophages, goblet cell hyperplasia indicative of increased mucus hypersecretion in the central airways, progressive airflow obstruction and focal parenchymal inflammation associated with airspace enlargement. Exposure of rats to 18 880 μg/m^3 (10 ppm) nitrogen dioxide for 23 hours per day for 3, 7 or 21 days, or 21 days followed by 28 days in room air, resulted in increased alveolar septal cell turnover, indicated by an 8-fold increase in alveolar septal cell apoptosis at day 3 and a 14-fold increase in proliferation *(78)*. These changes led to accelerated lung growth, characterized by an imbalance in the relative composition of the extracellular matrix, but failed to induce emphysema. Indeed, although airspace enlargement was evident, nitrogen dioxide resulted in an increase in the total surface area and absolute volume of alveolar walls comprising all compartments.

Pulmonary function

Exposure to nitrogen dioxide at concentrations of 376–18 800 μg/m^3 (0.2–10 ppm) has been shown to affect pulmonary function in several animal species (rats, mice, guinea-pigs and ferrets). The extent of an effect may be influenced by the mode of exposure, in that compared to continuous exposure (376 μg/m^3; 0.2 ppm) alone, a greater reduction in end-expiratory volume, vital capacity and respiratory system compliance was shown in mice chronically exposed to 1-hour spikes (twice a day) of 1504 μg/m^3 (0.8 ppm) of nitrogen dioxide superimposed on the baseline exposure *(79)*. Adult rats exposed for 6 weeks to 940 μg/m^3 (0.5 ppm) nitrogen dioxide and daily 1-hour spikes of 2820 μg/m^3 (1.5 ppm) experienced reduced lung compliance that returned to normal 3 weeks post-exposure *(80)*. An increase in lung volume and compliance was seen (at 3 weeks but not at 6 weeks) in neonatal rats exposed to an identical regimen *(80)*. A chronic (78-week) exposure study assessed a number of pulmonary function parameters in

rats exposed to nitrogen dioxide (940 μg/m^3 (0.5 ppm) background with a daily peak rising to 2820 μg/m^3 (1.5 ppm)). No changes in compliance, lung volume or diffusion capacity of carbon monoxide were seen, while a decrease in the delta forced expiratory flow at 25% of forced vital capacity disappeared soon after the end of exposure *(81)*. In guinea-pigs exposed to 112.8, 940 or 1880 μg/m^3 (0.06, 0.5 or 1.0 ppm) nitrogen dioxide for 6 or 12 weeks, significant effects were limited to an increase in pulmonary specific airway resistance in 13% (2 of 15) of animals following the 12-week 1.0-ppm exposure regimen *(82)*. The effect of exposure to either 940 or 18 800 μg/m^3 (0.5 or 10 ppm) nitrogen dioxide on tracer particle clearance from the airways of ferrets during postnatal respiratory tract development has also been examined. Thoracic clearance was reduced in both exposure groups, but was not significantly different in the 940-μg/m^3 (0.5-ppm) group compared to that of the control animals exposed to clean air *(76)*.

Airway inflammation and responsiveness

The effects of short-term (24-hour) exposure to nitrogen dioxide on airway eosinophilic inflammation and bronchial hyperreactivity have been examined using a standard murine model of antigen-modified broncho-constriction and airway inflammation *(83)*. BALB/c mice were sensitized to ovalbumin and exposed to 3760 μg/m^3 (2.0 ppm) nitrogen dioxide prior to being challenged with aerosolized ovalbumin on days 13 and 14. Nitrogen dioxide was found to enhance epithelial damage, reduce mucin expression and increase baseline smooth muscle tone. Although a modest increase in airway neutrophilia was detected, exposure was not associated with airway eosinophilia or with an increase in bronchial hyperresponsiveness. In contrast, Poynter et al. *(84)* reported no changes in the inflammatory response in C57BL/6 mice immunized and challenged with ovalbumin before exposure to 3 days of 9400 μg/m^3 (5 ppm) nitrogen dioxide. A 5-day inhalation of 31 000 μg/m^3 (25 ppm) nitrogen dioxide was, however, found to prolong ovalbumin-induced inflammation and airway hyperresponsiveness. Findings included acute damage associated with inflammation and lesions in the alveolar duct region and an influx of macrophages and neutrophils into the lavageable air spaces. Moreover, 20 days after cessation of the inhalation regimen, eosinophilic and neutrophilic inflammation, pulmonary lesions and airway hyperresponsiveness were still present.

A hypothesis that nitrogen dioxide acts as an effective inhaled adjuvant that accentuates the adaptive immune response to otherwise innocuous antigens prompted a study in which mice were exposed first to 18 880 μg/m^3 (10 ppm) nitrogen dioxide and then to aerosolized 1% ovalbumin *(85)*. Mice were subjected to the same sensitization regimen one week after and challenged with 1% ovalbumin alone an additional week later. Following the final challenge with ovalbumin, mice developed eosinophilic inflammation, mucus cell metaplasia, airway hyperresponsiveness and antigen-specific IgE and IgG1, and Th2-type cytokine

responses. The authors likened these changes to the phenotypic alterations in allergic asthma and those elicited following ovalbumin challenge in antigen-sensitized mice.

The inflammatory response to nitrogen dioxide, with particular focus on the activation state of alveolar macrophages, has been studied in a rat inhalation model using continuous exposure to 18 800 μg/m^3 (10 ppm) for 1, 3 and 20 days *(86)*. Whereas the number of inflammatory cells and total protein concentration in BAL were increased, TNF-α was markedly reduced with increasing exposure time. In contrast, IL-10, IL-6 and suppressor of cytokine signalling-3 protein were elevated. Furthermore, in vitro lipopolysaccharide stimulation of BAL cells revealed reduced capability to produce TNF-α, IL-1 β and nitric oxide, but showed markedly increased transcription and protein release for IL-10. In addition, elevated levels of IL-6, scavenger receptor B and suppressor of cytokine signalling-3 mRNA were detected in BAL cells from exposed animals. Analyses of highly purified alveolar macrophages indicated that changes in the activation state of these cells were most likely responsible for the observed effects.

To increase our understanding of the contribution of nitrogen dioxide to the development of COPD, Brandsma et al. *(87)* studied the effects of combined exposure to nitrogen dioxide and cigarette smoke on pulmonary inflammation and emphysema. Mice were exposed to either 31 160 μg/m^3 (20 ppm) nitrogen dioxide for 17 hours a day, 24 puffs of cigarette smoke twice a day or both, 5 days a week for 4 weeks. Cigarette smoke exposure increased eosinophil numbers and levels of TNF-*α*, KC (mouse IL-8), monocyte chemoattractant protein (MCP)-1, and IL-6. Nitrogen dioxide exposure increased goblet cells, eosinophils and the levels of IL-6, while it reduced the levels of IL-10. Four weeks of nitrogen dioxide, cigarette smoke or both was not sufficient to induce significant emphysema, nor did it lead to lower numbers of lymphocytes, neutrophils or macrophages in lung tissue. Instead, nitrogen dioxide exposure dampened the cigarette smoke-induced increases in the inflammatory cytokines TNF-*α*, KC and MCP-1. The authors suggested that these attenuating effects may be due to modulating effects of nitrogen dioxide on cytokine production by macrophages and epithelial cells. Clearly, cigarette smoke contains a range of radical species, including nitrogen dioxide, and these data may simply reflect the induction of similar pathways by both challenges.

Host defence

Several types of animal study have indicated that nitrogen dioxide increases susceptibility to respiratory infections *(60,61,88–90)*. An extensive set of data was collected using the infectivity model, which measures the total antibacterial defences of the lungs of mice. For long-term exposures, the lowest concentration tested that increased mortality when challenged with *Klebsiella pneumoniae* was 940 μg/m^3 (0.5 ppm) for 3 months of exposure *(91)*. After a 3-hour exposure, the

lowest concentration tested that affected resistance to *Streptococcus pneumoniae* was 3760 μg/m³ (2 ppm) *(92)*. Continuous exposure to concentrations ranging from 52 640 to 940 μg/m³ (from 28 to 0.5 ppm) resulted in linear, concentration-related increases in mortality due to pulmonary infection *(93)*. Other studies have shown that peak and patterns of nitrogen dioxide exposure are important in determining response *(60,79,94)*. For example, Miller et al. *(79)* found that infectivity, mortality and pulmonary function deficits in mice were significantly greater following a spiked exposure regimen (up to 52 weeks of continuous baseline 376 μg/m³ (0.2 ppm) plus spikes of 1504 μg/m³ (0.8 ppm) compared to the baseline exposure alone). In mice exposed to nitrogen dioxide at 8460 μg/m³ (4.5 ppm) for 1, 3½ or 7 hours and challenged with *Streptococcus* sp. either immediately or 18 hours after exposure, the mortality rate was directly related to the length of peak exposure when the streptococcal challenges were immediately after nitrogen dioxide exposure but this was not the case when the challenge was delayed for 18 hours *(94)*. In summary, the body of work shows that the effects of nitrogen dioxide are due more to concentration than to duration of exposure or to total dose (expressed as C × T), that differences in species sensitivity exist, that the lowest effective concentration of nitrogen dioxide also depends on the microbe used in the test, and that low levels only cause effects after repeated exposures *(60–62,68)*. The extrapolation of these findings to humans cannot be made directly, because most of the studies used pneumonia-induced mortality as an end-point. However, the infectivity model reflects alterations in the defence mechanisms of mice that are shared by humans. Nevertheless, the quantitative relationship between effective nitrogen dioxide levels in animals and in humans is unknown. Although numerous studies provide evidence of the effects on the systemic humoral and cell-mediated immune systems, these studies are difficult to interpret *(60,61)*.

In mice, an exposure of 9400 μg/m³ (5 ppm) nitrogen dioxide (following the bacterial challenge) was required to impair the intrapulmonary killing of *Staphylococcus aureus (90)*. The same effect, however, was found at 3100 μg/m³ (2.5 ppm) or less in lungs immunosuppressed with corticosteroids, while the adverse effect of the pollutant was only evident at 18 800 μg/m³ (10 ppm) when exposure preceded the bacterial challenge *(90)*.

The association between exposure to common air pollutants, including nitrogen dioxide, and altered host immunity to respiratory viral infections has recently been reviewed *(95)*. Two studies by Rose et al. *(88,89)* exposed mice to nitrogen dioxide at 9400 μg/m³ (5 ppm) for six hours per day for two days prior to infection with murine cytomegalovirus, followed by another four days of nitrogen dioxide. The mice exposed to nitrogen dioxide not only required 100-fold less virus to become infected (possibly due to reduced phagocytosis and macrophage destruction of the virus in the pollutant-exposed mice) but were more likely to be re-infected with murine cytomegalovirus, suggesting that exposure

can adversely affect the development of virus-specific immunity. Enhanced susceptibility to infection was not found after exposure to 3100 or 1800 μg/m^3 (2.5 or 1 ppm) nitrogen dioxide. In another study using mice and the Sendai virus, while exposure to 9400 μg/m^3 (5 ppm) nitrogen dioxide for four hours per day did not alter infection it did enhance lung damage, which was suggested to have been caused by increased proliferation of the virus *(90)*.

Mutagenic/genotoxic/carcinogenic effects

There are no reports among the limited number of carcinogenicity studies that nitrogen dioxide causes malignant tumours or teratogenesis *(60,61,96,97)*. High (11 280–28 200 μg/m^3; 6–15 ppm) concentrations of nitrogen dioxide have been shown to be mutagenic in bacterial (*S. typhimurium*) test systems *(98)*. In vitro genotoxicity studies have reported chromosomal aberrations, sister chromatid exchanges or DNA single strand breaks at concentrations ranging from as high as 31 160 μg/m^3 (20 ppm) to as low as slightly over 1800 μg/m^3 (1 ppm) but not 940 μg/m^3 (0.5 ppm) *(99–101)*. Genotoxicity studies in vivo have produced mixed results. While lung cells of rats exposed to nitrogen dioxide for three hours exhibited increased mutation to ouabain resistance at 28 200 μg/m^3 (15 ppm) and increased chromosome aberrations at 15 040 μg/m^3 (8 ppm) *(102)*, no genotoxic effects were reported in alveolar macrophages of rats exposed to 2256 μg/m^3 (1.2 ppm) for three days *(103)*, in bone marrow after inhalation by mice of 31 160 μg/m^3 (20 ppm) for 23 hours *(104)* and in spermatocytes or lymphocytes of mice following a six-hour exposure to 180–18 000 μg/m^3 (0.1–10 ppm) *(105)*. Numerous studies of the interaction of nitrogen dioxide with other air pollutants, predominantly ozone, show that the effects are due to ozone alone, are additive or are synergistic, depending on the end-point and exposure regimen *(60)*.

Reproductive effects

A recent study has examined effects in the rat of fetal exposure to diesel-engine exhaust containing nitrogen dioxide at 1504 or 188 μg/m^3 (0.80 or 0.10 ppm) with or without particulate matter (1.71 or 0.17 mg/m^3) on testicular cell numbers and daily sperm production in adulthood *(106)*. The mature rats that were exposed to diesel exhaust from gestational day 7 to delivery showed a decrease in the daily production of sperm due to an insufficient number of Sertoli cells. All exhaust-exposed groups showed almost the same reactions to the inhalation, indicating that the gaseous phase must have included the responsible toxicants; these were not identified, although nitrogen dioxide would be a major constituent.

Experimental studies – summary

Experimental animal work on the health effects, and mechanisms thereof, of nitrogen dioxide has not focused on indoor sources or exposure patterns

of the pollutant (other, of course, than to have conducted all studies in an indoor environment). As such, in addition to newly published work, the studies reviewed in this section include those described in WHO's latest guidelines for ambient nitrogen dioxide *(15)*. Acute exposures (hours) to low (75–1880 μg/m^3; 0.04–1.0 ppm) levels of nitrogen dioxide have rarely been observed to cause effects in animals. Subchronic and chronic exposures (weeks to months) to low levels, however, cause a variety of effects, including alterations to lung metabolism, structure and function, inflammation and increased susceptibility to pulmonary infections. Emphysema-like changes (destruction of alveolar walls and airspace enlargement), features characteristic of human COPD (increased mucus production and progressive airway obstruction), generation of an atopic immune response and airway hyperresponsiveness have been reported only at high (15 040–47 000 μg/m^3; 8–25 ppm) nitrogen dioxide concentrations. It is apparent from both in vitro and animal toxicology studies which toxic effects of nitrogen dioxide *might* occur in humans. Nevertheless, owing to (*a*) the frequent use of extremely high exposure concentrations in experimental studies, (*b*) the inherent differences between mammalian species and (*c*) the dearth of information available on tissue response of different species to a given dose of nitrogen dioxide, it is difficult to extrapolate quantitatively, with any degree of confidence, the effects that are *actually* caused by a specific inhaled dose or concentration.

An important point, worthy of consideration, is the possible interaction between nitrogen dioxide and other indoor pollutants. It is increasingly acknowledged by indoor environmental scientists that it is the reactions between primary pollutants, creating secondary pollutants indoors, that are probably responsible for adverse health effects.

Health effects

A plethora of outdoor studies have examined the health effects of exposure to outdoor nitrogen dioxide. While there are concerns that some of the associations reported for health effects and outdoor nitrogen dioxide may be explained by co-pollutants, extensive reviews have concluded that respiratory health is associated with nitrogen dioxide exposure, independently of these other exposures *(15,107)*.

Outdoor nitrogen dioxide is increasingly being implicated in a wide range of disorders. For example, increased risk of otitis media *(108)*, eczema *(109)* ear/nose/throat infections and sensitization to food allergens *(110)* in children, as well as increased blood coagulability after periods of elevated ambient exposure in adults *(111)* have recently been reported. There is also an increasing interest in the role of outdoor pollution with reproductive outcomes *(112)*. A full review of these is beyond the scope of this report. This section will:

- briefly summarize conclusions from earlier WHO reports on the evidence for health effects based on controlled human exposure studies;

- review the epidemiological evidence for health effects of exposure to indoor nitrogen dioxide where nitrogen dioxide has been directly measured; and
- review the epidemiological evidence for health effects of exposure to gas appliances in the home – a proxy marker for high exposure to indoor nitrogen dioxide.

Controlled human clinical studies

Studies in which exposure to nitrogen dioxide has been carefully controlled in small numbers of selected participants have been reviewed in several previous publications *(15,62,107)*. These studies have examined symptoms, changes in pulmonary function and changes in airway reactivity in healthy volunteers and in those with pre-existing lung disease. Some studies have included bronchoalveolar lavage following exposure and have provided information on the inflammatory changes that may occur.

There are some inconsistencies in the results of these studies but studies on healthy volunteers can be summarized as follows.

- Measurable change in lung resistance, total airway resistance and bronchial responsiveness to acetylcholine and methacholine in healthy volunteers has been seen at exposures in excess of 1880 μg/m^3, although in one study exposures well in excess of this (7520 μg/m^3 for 75 minutes) failed to show any change *(113)*.
- A study in which nitrogen dioxide exposure was followed by bronchoalveolar lavage with assessment of cell profile in lavage fluid and in blood suggested that high exposure (up to 2821 μg/m^3 for three hours) is associated with mild airway inflammation, changes in white blood cells and increased susceptibility of airway epithelial cells to injury from respiratory virus (as assessed in vitro) *(114)*. Two studies (one at 2821 μg/m^3, the other at 7524 μg/m^3 and both for 20 minutes on 6 occasions) found decreased alveolar macrophages and lymphocyte subgroups in bronchoalveolar lavage fluid *(115,116)*. Repeated exposure to 1128 μg/m^3 for two hours on four days did not change the percentage of neutrophils, total lymphocytes or macrophages in blood and lavage fluid, although small increases in the percentage of killer cells in lavage fluid were observed *(117)*. Repeated exposure for four hours to 3760 μg/m^3 daily for four days *(118)* and for four hours to 3760 μg/m^{3}daily for three days *(119)* was associated with evidence of neutrophilic inflammation. Similar patterns of exposure in healthy volunteers have been associated with increased expression of IL-5, IL-10 and IL-13 in bronchial biopsies, suggesting an upregulation of TH2 cytokines consistent with a pro-allergic effect *(120)*, even when pulmonary function changes are not observed. These authors also observed an increase in expression of ICAM-1, which may indicate a mechanism by which nitrogen dioxide exposure could be associated with increased respiratory infections.

- Exposure to nitrogen dioxide at levels of 3600 μg/m^3 in healthy subjects produced changes in bronchoalveolar lavage fluid consistent with oxidative stress (low levels of uric acid and ascorbate), with evidence of a relatively short-lived (< 24-hour) protective response in the form of increased glutathione levels *(121)*. The initial loss of antioxidants in bronchoalveolar lavage fluid may be attenuated with repeated exposures *(118)*.
- Repeated exposures above 1880 μg/m^3 for two hours per day over three days may be associated with increased susceptibility to infection with influenza virus *(122)*. A three-hour exposure to 1128 μg/m^3 may be sufficient to inhibit the alveolar macrophage response to the influenza virus *(123)*.

Results from controlled exposure to nitrogen dioxide in those with pre-existing lung disease can be summarized as follows.

- People with asthma exposed to 560 μg/m^3 for up to 2.5 hours may experience relatively minor changes in pulmonary function *(124–126)* but this is by no means consistent across studies. Studies with much higher exposures have failed to show any effect *(127)* and some studies showing small effects at lower exposures have been difficult to reproduce *(128)*. Similar inconsistencies have been observed when people with COPD have been studied.
- Two meta-analyses of the association of nitrogen dioxide exposure with bronchial reactivity have been conducted. The first, including studies up to the early 1990s, showed that there was a statistically significant increase in airway hyperresponsiveness to a range of constrictor stimuli following nitrogen dioxide exposure (> 200 μg/m^3 in asthmatics and > 1900 μg/m^3 in healthy controls) *(129)*. A more recent systematic review considered peer-reviewed and non-peer-reviewed original research published up to 2009 and included 41 exposure scenarios from 28 studies *(130)*. Provoking agents included methacholine, histamine, carbachol, cold air and allergens. Nitrogen dioxide exposure was considered in categories of 188–375 μg/m^3, 376–563 μg/m^3, 564–751 μg/m^3, 752–939 μg/m^3, 940–1127 μg/m^3 and 1128–1316 μg/m^3. Overall exposure was associated with increases in airway reactivity and in stratified analyses, associations of bronchial reactivity with exposure were seen within each of the two lowest exposure categories. There was no clear dose–response relationship between the categories. The authors felt the lack of a dose–response effect suggested that nitrogen dioxide did not cause these effects, and that the effect size was too small to be of clinical significance. The lack of a clear dose–response effect is difficult to explain and would argue against a causal relationship. However, for some of the analyses conducted the results are remarkably consistent across studies (I^2 = 0% for the fraction of asthmatics with greater airway hyperresponsiveness following nitrogen dioxide exposure in the lowest and second lowest exposure categories). The small significant increase in airway reactivity associated with low-level exposure could, if borne by a large

proportion of the population, be associated with population-level health effects.

- Included in the meta-analysis by Goodman et al. *(130)* are studies suggesting that exposure to nitrogen dioxide may reduce the threshold of responsiveness to inhaled allergen in those who are sensitized. There was no evidence that the effect of nitrogen dioxide on these specific airway challenges was any different from its effect on response to nonspecific agents. One of the earliest studies to look at this showed that asthmatics sensitized to house dust mites, exposed for 60 minutes to 752 μg/m^3 nitrogen dioxide, had significantly larger falls in FEV_1 in response to house dust mite challenge compared to exposure to air *(131)*. A similar effect was observed in pollen-sensitized asthmatics exposed for 30 minutes to 490 μg/m^3 nitrogen dioxide and then exposed to pollen. After exposure, the falls in peak flow were larger (6.6% difference) and more asthmatics experienced a late asthmatic response, although this latter difference was non-significant *(132)*. Repeated daily exposures of 500 μg/m^3 for 30 minutes for 4 days increased the early- and late-phase falls in FEV_1 following low-dose allergen exposure *(133)*. Simultaneous exposure to nitrogen dioxide (752 μg/m^3) and sulfur dioxide for 6 hours has also been demonstrated to increase response to allergen *(134,135)* . Repeated 30-minute exposures to 500 μg/m^3 nitrogen dioxide are associated with a more pronounced eosinophilic response to allergen challenge, as demonstrated by elevated levels of eosinophilic cationic protein in bronchial washings *(136)* and in sputum and blood *(137)*.
- Exposure to 1880 μg/m^3 nitrogen dioxide for three hours followed by analysis of bronchial lavage fluid has shown that markers of airway inflammation were altered (decreased 6-keto-prostaglandin, increased thromboxane B2 and prostaglandin D2) in those with mild asthma. This was not seen in healthy volunteers, and not seen after exposure to filtered air *(138)*.

Another approach is to directly examine health effects following exposure to cooking with gas. In a chamber study, nine adults and eleven children with asthma were exposed to nitrogen dioxide alone and then nitrogen dioxide with combustion products from a gas heater for a one-hour period *(139)*. Symptoms, lung function and airway reactivity were monitored. Small, clinically non-significant increases in airway reactivity were seen on exposure to 1128 μg/m^3, but this was not seen when exposure occurred with combustion products. The authors concluded these exposures were not associated with clinically relevant health effects.

Overall, these controlled human clinical studies, many of which were conducted more than 20 years ago, have examined the health effects of acute and often very high levels of exposure to nitrogen dioxide rather than the chronic, low-dose exposures experienced by most human populations. Notwithstanding

this, however, they suggest that those who are sensitized or who have asthma may be at particular risk of health effects from exposure to nitrogen dioxide at levels that may be experienced for short periods when individuals are near an unvented combustion appliance.

Epidemiological studies

Identification of studies

Epidemiological studies on health effects of indoor nitrogen dioxide exposure were identified from several electronic searches and by hand searching references in former reviews by WHO *(15)* and EPA *(107)*. Electronic searches were made in PUBMED (http://www.ncbi.nlm.nih.gov/pubmed/), in the ISI Web of Science (http://apps.isiknowledge.com/) and in the LUDOK literature database on air pollution and health effects (http://www.ispm-unibasel.ch/ludok/welcome.html) in January 2009.

We intended to identify all studies with original data on health effects with indoor nitrogen dioxide measurements, and the main descriptors used were "air pollution, indoor", "nitrogen oxides", and "morbidity" or "mortality". We excluded studies that referred to solid fuel use, as this source is often also associated with high levels of particulate matter. We also excluded studies with purely descriptive results or in which no attempt had been made to adjust for potential confounders. In December 2009, a similar search strategy was adopted in order to identify reports published during the period of the review.

We found 72 studies with indoor measurements and evaluation of health effects up to January 2009. Three of these were related to the same study, and here we used the newer results or the publication with the analysis of the most complete sample.

An update of the search found two more publications up to December 2009. Among the remaining 71 studies, we focused on 35 studies on respiratory symptoms and disease, because this outcome was most often significantly related to nitrogen dioxide exposure and permitted the evaluation of concentrations for setting guidelines. Of these 35 studies, 20 were in children, 5 in adults and 10 in asthmatics (children and adults).

In addition, studies that examined the health effects of indoor gas appliances were identified through hand searches of earlier reviews of the topic, citations within the papers identified on health effects of nitrogen dioxide, and papers known to the expert group. We also searched for epidemiological studies on health effects of indoor gas combustion without measurements with the terms "air pollution, indoor", "gas" with "cooking" or "heating", and "respiratory tract diseases" or "lung function".

As there has been some concern that susceptibility may vary with age, the epidemiological studies are presented in two sections – studies in children and studies in adults.

Epidemiological studies in children

Estimates of health effects from studies in which direct measurements of indoor nitrogen dioxide have been made are included in Table 5.2 (see page 280).

Health effects in infants: studies measuring indoor nitrogen dioxide. There have been concerns that infants may be at particular risk of symptoms with high indoor nitrogen dioxide levels because of their high minute volume in relation to body size and because they are likely to spend a large proportion of their time indoors.

However, an early longitudinal study of over 1000 infants up to the age of 18 months living in non-smoking homes showed that the incidence of respiratory illness was not associated with two-week average bedroom nitrogen dioxide levels (22% of nitrogen dioxide levels measured were > 37.6 μg/m^3) *(140)*.

A large cross-sectional study of infants aged 3–12 months taking part in a birth cohort study showed no association of two-week average bedroom nitrogen dioxide (median 12.7 μg/m^3) with respiratory symptoms, including cough, breathlessness and wheezing. Of the 20 infant symptoms examined, only diarrhoea was associated with indoor nitrogen dioxide levels (adjusted odds per doubling of 1.38; 95% CI 1.11–1.70) *(141)*.

More recently, a nested case control study of infants taking part in a birth cohort study was conducted in Oslo, where gas appliances are not used indoors for heating or cooking and levels of indoor nitrogen dioxide are low *(142)*. No association of bronchial obstruction (wheezing, chest recession, rhonchi during auscultation of the chest, forced expiration or rapid breathing, with at least one other episode of "obstructive airways disease") in the first two years of life at living room levels of nitrogen dioxide (arithmetic mean 14.7 μg/m^3; range 2–43 μg/m^3) was seen in 153 matched pairs of cases and controls. However, data from a birth cohort study in Sweden (where, again, indoor gas appliances are rare), using a similar nested case control design, suggested an association of recurrent wheezing up to the age of two years with mean four-week living room nitrogen dioxide *(143)*. There was an increased risk (OR 1.48; 95% CI 0.91–2.42) of wheeze comparing the highest quartile (> 15.6 μg/m^3) to the lowest quartile (< 8.4 μg/m^3), with little evidence of an association below 15.6 μg/m^3. However, the reported associations are below conventional levels of statistical significance. Another study in Scandinavia, but this time based in Copenhagen, showed no association of bedroom nitrogen dioxide levels (mean level 8.6 μg/m^3; 5th centile 3.3 μg/m^3; 95th centile 17.0 μg/m^3, based on up to three ten-week periods of monitoring) with symptoms of wheeze in almost 400 infants born to asthmatic mothers in the first 18 months of life *(144)*.

Populations of infants with higher exposure to indoor nitrogen dioxide were examined in a three-centre birth cohort study. Two-week average (median and 75th centile) living room nitrogen dioxide in each of the three centres (Ashford,

Kent, United Kingdom 10.7 and 16.5 μg/m^3; Barcelona, Spain 86.2 and 112.0 μg/m^3; Menorca, Spain 22.2 and 39.9 μg/m^3) was not associated with wheeze, cough, chestiness or doctor-diagnosed respiratory illness in the first year of life *(145)*. Researchers in the Menorca centre went on to examine associations of neurocognitive status in four-year-old children with level of exposure to nitrogen dioxide as measured at three months *(146)*. A negative association of poor cognition with nitrogen dioxide was observed (a decrease of 0.27 points on a standardized McCarthy Scale measure of cognition per 1.88 μg/m^3). Further, children with increased exposure were at a higher risk of having symptoms of inattention (6% increased risk per 1.88 μg/m^3 increase in nitrogen dioxide). This was particularly seen in children with the GSTP1–Val 105 allele, a genetic polymorphism that may lead to reduced antioxidant defences within the developing brain. In this study, over 70% of children lived in homes with a gas cooker, and almost a quarter had gas fires. The authors stated that confounding by other pollutants such as particulates could not be ruled out, particularly as many of the homes were using bottled gas. Unfortunately, the other centres taking part in the study do not have the necessary outcome information to try to replicate this observation.

Two publications based on a longitudinal study of infants in Connecticut examined lung health and its association with indoor gas appliances and indoor nitrogen dioxide levels *(40,147)*. Participants were selected if, at birth, their mother reported she had another child under the age of 11 years who had asthma. In the 850 infants included in the first report, living in a home with a gas stove was associated with an increased risk of persistent cough (OR 1.52; 95% CI 1.06–2.18) after adjustment for maternal education and a range of household factors including allergen levels, mould and mildew, and smoking in the home. These children had asthmatic siblings and might be considered to be a genetically susceptible group, but the associations were only seen in children whose mother did not have asthma. Forty-five per cent of the infants were living in homes with a two-week average living room nitrogen dioxide level greater than 18.8 μg/m^3, and an association of nitrogen dioxide level with persistent cough was also reported (1.21; 95% CI 1.05–1.40 per 18.8-μg/m^3 increase). In the second report, mothers of about 750 newborn infants recorded each day their infants' respiratory symptoms during the first year of life. Two-week mean living area nitrogen dioxide was measured concurrently with nitrous acid (interquartile range 9.6–32.7 μg/m^3 for nitrogen dioxide, 1.1–4.2 ppb for nitrous acid). In single-pollutant models, a dose-dependent association of the number of days with wheeze, cough and shortness of breath was observed in these infants. Observed associations were most marked for shortness of breath. The adjusted rate ratio for shortness of breath in those with nitrogen dioxide levels > 32.7 μg/m^3 compared to those with levels below 9.6 μg/m^3 was 2.38 (95% CI 1.31–4.38) after adjustment for nitrous acid level. No independent association of symptoms with nitrous acid was seen.

Health effects in children: studies measuring indoor nitrogen dioxide. One of the earliest studies to measure indoor nitrogen dioxide was conducted in the United Kingdom *(148)*. Children living in homes that cooked with gas had more ($P > 0.06$) respiratory illness (positive response to any of cough, wheeze, colds that went to the chest, or asthma or bronchitis in the previous 12 months). In the children who lived in gas-cooking homes, the prevalence of respiratory illness increased with increasing bedroom nitrogen dioxide level: 44%, 59% and 71%, respectively in those exposed to 0–37.6 μg/m^3 , 37.6–75.2 μg/m^3 and 75.2 μg/m^3 ($P < 0.05$). No association of $FEV_{0.75}$, peak flow or mean mid-expiratory flow rate with indoor level of nitrogen dioxide was observed.

Cross-sectional studies were conducted in the Netherlands, where there was concern over the combustion products produced by gas water heaters or geysers. In a study of over 1000 children, no association was observed between respiratory symptoms and lung function measurements (FEV_1, FVC, PEFR, MMEF) and household weekly average nitrogen dioxide level (mean levels 23.6 μg/m^3 for homes without geysers, 40.3 μg/m^3 for homes with vented geysers and 71.7 μg/m^3 for homes with unvented geysers) *(149,150)*.This supported earlier work in the Netherlands showing no association of indoor nitrogen dioxide level with respiratory symptoms *(151)*.

Garrett et al. *(152)* studied children living in Victoria, Australia over a one-year period. Indoor nitrogen dioxide was measured on five occasions in three locations in the home and the frequency recorded of eight respiratory symptoms during the year of observation. Respiratory symptoms were associated with the presence of a gas stove but not with any of the other sources of indoor nitrogen dioxide (gas heaters or smoking in the home). Respiratory symptoms, but not peak flow variability, were more frequent in children with higher bedroom levels of nitrogen dioxide but not kitchen or lounge levels (adjusted odds for any of eight possible respiratory symptoms with bedroom average level > 20 μg/m^3 = 3.62 (95% CI 1.08–12.08) compared to < 10 μg/m^3). The association of respiratory symptoms with a gas stove persisted even after adjustment for bedroom nitrogen dioxide, raising the possibility that the association with gas appliances was not explained by exposure to this pollutant. Interestingly, both the presence of a gas stove and bedroom nitrogen dioxide level were non-significantly more strongly associated with respiratory symptoms in atopic children than in non-atopic children.

In some parts of the world, children's exposure to gas combustion products may be determined or at least strongly influenced by their exposure to gas heaters at school. This has been investigated in Australia. School levels of nitrogen dioxide were monitored during the winter, as were the personal levels of nitrogen dioxide in children who lived in homes with gas sources *(27)*. The winter average six-hourly mean nitrogen dioxide levels in classrooms with an unflued gas heating source ranged from 33.8 to 248 μg/m^3 compared to 13.2–43.2 μg/m^3

in rooms without a gas heater. Several symptoms were investigated (hoarseness, cough with phlegm, dry cough, sneeze, stopped up nose, runny nose, wheeze, sore throat, colds and school absence) but only the mean symptom rate for the latter three showed consistent and significant associations with exposure to > 150.4 μg/m^3 compared to 37.6 μg/m^3. There was some evidence of a dose–response relationship.

One of the most comprehensive assessments was conducted as part of the Six City study. The association of respiratory symptoms with indoor nitrogen dioxide level was examined in more than 1500 children *(153)*, who were followed up for one year. About half of the children lived in homes with a major source (gas stove or kerosene heater). Household annual average levels were determined based on summer and winter measurements made in three household locations, and were 16.1 μg/m^3 for homes without a source and 44.2 μg/m^3 for homes with a source. At the end of follow-up, the annual cumulative incidence of any lower respiratory symptom (shortness of breath, chronic wheeze, chronic cough, chronic phlegm or bronchitis) was higher in those children living in homes with a source (29.0% vs 22.8%) and was higher with increasing annual average indoor nitrogen dioxide (1.40; 95% CI 1.14–1.72 per 28-μg/m^3 increase). Household particulate matter ($PM_{2.5}$) was also measured in this study and included as a covariate in the final analysis. The observed association of incidence of symptoms with both the presence of a gas stove and with increasing indoor nitrogen dioxide level persisted after adjustment for the indoor particle level. In this study, no consistent association of lung function with source or measured nitrogen dioxide was observed. Further analyses were conducted later using regression calibration to include information from children who were not directly measured for nitrogen dioxide but who did have information on surrogate factors such as the presence of a gas appliance *(154)*. Although the authors argued that the estimate, now based on more than 2800 children, was 34% more precise, the effect estimate was little different to earlier analyses (risk of lower respiratory illness over one year, OR 1.5; 95% CI 1.2–1.8 per 28 μg/m^3 increase).

A detailed longitudinal study of the health effects of indoor and outdoor nitrogen dioxide was conducted in schoolchildren in Japan, a country in which the use of gas for cooking is almost universal and where some homes use unvented gas appliances for heating *(10)*. Indoor measurements of nitrogen dioxide were made in summer and winter (mean of the two measurements in homes with vented and unvented appliances were 34.5 and 60.9 μg/m^3, respectively) and outdoor nitrogen dioxide measurements were made at a sampling station based at the child's school (three-year average in each of the six areas involved ranged from 13.2 to 58.3 μg/m^3). There was no association of respiratory symptoms with exposure to gas heaters. At baseline in girls (but not boys), significant associations of indoor nitrogen dioxide with wheeze (OR 1.90; 95% CI 1.30–2.83 per 18.8 μg/m^3), asthma (OR 1.63; 95% CI 1.06–2.54 per 18.8 μg/m^3) and a history of

bronchitis (OR 1.42; 95% CI 1.06–1.90 per 18.8 μg/m^3) were observed even after adjustment for outdoor levels. However, over a three-year period there was no evidence that indoor nitrogen dioxide was associated with incidence of disease, although associations were seen with outdoor nitrogen dioxide level.

Health effects in children: studies measuring personal nitrogen dioxide. Some studies have measured personal nitrogen dioxide rather than indoor levels. The extent with which personal nitrogen dioxide reflects exposure to indoor, compared to outdoor, nitrogen dioxide will vary depending on the time-activity patterns of the child and the frequency and duration of use of indoor sources.

Personal exposure to nitrogen dioxide was measured in children in Hong Kong SAR *(155)*, where indoor sources are common. No association of exposure with symptoms was observed. However, personal exposure was strongly influenced by outdoor levels, with significant differences in personal exposure seen in children who wore samplers during a week of high ambient nitrogen dioxide (40.2 μg/m^3) and those who wore them during a week with lower levels (33.4 μg/m^3).

Personal nitrogen dioxide exposure was measured in 3–4-year-old children in Quebec City, Canada during the winter months and a dose-dependent association of exposure with asthma was reported. Only 6 of the 140 children lived in a home with a gas stove (mean personal exposure with gas stove 32.4 μg/m^3; without gas stove 17.3 μg/m^3). The adjusted OR for case status with the highest level of exposure appears unrealistically high, with very wide confidence levels (24-hour mean of 28.2 μg/m^3 compared to "a zero level"). The unmatched analysis OR was 19.9 (95% CI 4.75 –83.03) while the matched analysis OR was 10.55 (95% CI 3.48 –31.89) *(156)*; this was probably related to the small numbers of children in the risk group.

Personal nitrogen dioxide samplers were worn by Australian primary school children living in Canberra, a low pollution area *(157)*. They were worn from the end of the school day till the following morning, and if the child was taught in a classroom with a gas heater, classroom levels were also measured. Average total personal exposure was low (19.0 μg/m^3) and was not associated with changes in lung function, except for a slightly more pronounced response to a cold air challenge (as measured by change in FEV_1/FVC). There was some evidence that this association was more apparent in children who were *not* mite-sensitized.

One-week average personal exposure was measured in 163 preschool children in Finland *(158)* who attended one of eight day care centres. A small proportion (9.2%) lived in homes with gas appliances (study median 21.1 μg/m^3). However, there was an increased risk of reported cough during the same week as measurement (≤ 16.2 μg/m^3 reference group; 16.2–27.2 μg/m^3, RR 1.23, 95% CI 0.89–1.70; and ≥ 27.2 μg/m^3, RR 1.52, 95% CI 1.00–2.31), particularly in the winter. No clear association with peak expiratory measurements in a subsample

of children (n = 53) was seen. Data from the same study were re-analysed using several methods of defining nitrogen dioxide exposure *(159)*. Overall, statistically significant associations of cough were seen only with personal exposure and in winter, although the direction of association with levels of nitrogen dioxide measured inside the day care centre, outside the day care centre and at a local fixed site was consistent with this observation.

Health effects in children with asthma: studies measuring indoor or personal nitrogen dioxide. Several studies have examined nitrogen dioxide exposure in relation to symptoms of asthma in those with established disease. All have observed increases in some symptoms, but some of the observed associations have not been consistent across the whole population under study.

In Adelaide, Australia, 125 asthmatics wore lapel nitrogen dioxide monitors each day for six weeks while they were at home and kept a symptom diary *(160)*. Time-averaged level of personal exposure to nitrogen dioxide in the home was strongly related to the presence of gas appliances, particularly those in homes with unflued heating appliances (mean average exposure 125 $\mu g/m^3$; interquartile range 50.7–310 $\mu g/m^3$) compared to those in homes using all electric appliances (22.6 $\mu g/m^3$; interquartile range 20.7–28.2 $\mu g/m^3$). In participants under the age of 14 years, there was an association of personal exposure level with symptoms of chest tightness (OR 1.29; 95% CI 1.16–1.43 per standard deviation increase in nitrogen dioxide, which in this study was 48.3 $\mu g/m^3$), breathlessness (OR 1.13; 95% CI 1.0–1.28 with a one-day lag) and asthma attacks. In adults, only one isolated association of one of the seven symptoms investigated was associated with nitrogen dioxide, and this was seen only after accounting for a one-day lag.

Personal exposure to nitrogen dioxide was measured in 45 asthmatic children over a 10-day period by Delfino et al. *(161)* in Seattle. The children, aged 9–18 years, were also subject to daily measurements of airway inflammation (exhaled nitric oxide) with a personal active sampling device. Increases in fractional exhaled nitric oxide (FENO) were seen with $PM_{2.5}$, elemental carbon and nitrogen dioxide (for each 32-$\mu g/m^3$ increase in nitrogen dioxide, an increase of 1.6 ppb FENO (95% CI 0.4–2.8)). Positive associations were seen only in those who were taking anti-inflammatory medication, which may reflect the severity of the underlying disease. In this population, there was a wide range of personal exposure (5.1–198.7 $\mu g/m^3$). Daily lung function measurements were also made *(162)*. Personal nitrogen dioxide was associated with decrements in lung function, the change in FEV_1 as a percentage of predicted FEV_1 being 2.45 (95% CI 3.57 –1.33) per 32-$\mu g/m^3$ increase in nitrogen dioxide. Lung function associations were more clearly seen in those children who did not use a bronchodilator. These associations were robust to adjustment for personal $PM_{2.5}$ exposure, but the authors in their discussion suggest that the association with nitrogen dioxide may be due to confounding by another toxic pollutants from traffic.

A larger study conducted in eight inner-city areas in the United States (Bronx, NY; East Harlem, NY; St Louis, MO; Washington, DC; Baltimore, MD; Chicago, IL; Cleveland, OH; and Detroit, MI) recruited children attending accident and emergency departments for the treatment of asthma, measured indoor nitrogen dioxide in the children's bedrooms (median 56.0 μg/m^3; range 0.9–902.4 μg/m^3) and gathered health information at baseline and three, six and nine months later *(23)*. Three respiratory outcomes were considered: (*a*) more than four days in the last fortnight with symptoms; (*b*) unscheduled visits to health care providers; and (*c*) peak flow less than 80% of predicted. Associations of these outcomes with nitrogen dioxide were modified by atopic status and by season. Nitrogen dioxide levels in the highest quartile (cut-off not given) were associated with more than four days with symptoms compared to those exposed to lower levels, but this was only in children who had *negative* skin tests. No association was seen in those who were skin-test-positive to at least one of 16 common indoor and outdoor aero-allergens. The overall difference in indoor nitrogen dioxide level in warm and cold months was relatively small (6.8 μg/m^3) but low peak flow was associated with nitrogen dioxide only in the colder months. The authors postulate that this may reflect increased susceptibility to infections during the colder months. However, as children spend more time indoors when it is cold, the association may have arisen because of the closer correlation of indoor measurements with personal measurements in the winter period.

A total of 150 inner-city children with asthma, predominantly African Americans, were studied over a six-month period in Baltimore, United States *(26)*. Although at baseline the average indoor nitrogen dioxide level was similar in the homes of asthmatic children and a control group of non-asthmatic children *(163)*, there was evidence that indoor nitrogen dioxide was associated with symptoms in the asthmatic children. Assessment of bedroom indoor nitrogen dioxide and indoor $PM_{2.5}$ was made on three occasions (overall mean of average seven-day indoor nitrogen dioxide 56.4 μg/m^3) and caregivers were asked to report symptoms that had occurred in the previous fortnight. There was a significantly increased risk of reporting symptoms with increasing nitrogen dioxide levels (e.g. adjusted incident rate ratio for speech-limiting wheeze 1.17 (95% CI 1.08–1.27) per 37.6-μg/m^3 increase in nitrogen dioxide) and these associations were present even after adjustment for indoor particulates. No association was observed for other outcomes such as medication use or use of health services. About two thirds of the children were atopic as assessed by skin tests, but in general the presence of atopy did not modify the associations observed. However, nocturnal symptoms were more strongly related to nitrogen dioxide levels in atopic children (incidence rate ratio 1.13 per 37.6 μg/m^3 increase in nitrogen dioxide) compared to non-atopic children (incidence rate ratio 1.03 per 37.6 μg/m^3 increase in nitrogen dioxide). About 83% of these homes had gas stoves for cooking and 12% reported they used their stove for heating the home.

The association of symptoms in children with asthma with the use of gas appliances and indoor nitrogen dioxide level may be different in different housing conditions. In Connecticut and south-west Massachusetts in the United States, the reporting of wheeze and chest tightness in the month prior to indoor nitrogen dioxide sampling (mean 10-day average living room nitrogen dioxide 16.2 $\mu g/m^3$ in homes without a source and 48.7 $\mu g/m^3$ in homes with a source) in children with asthma was significantly associated with the presence of a gas stove and with increases in nitrogen dioxide levels as measured in the main living area. However, this association was only seen when analyses were limited to children living in homes that were in "multi-family" housing and were not seen in single-family housing *(164)*. Among these children in multi-family homes, exposure to gas stoves increased the likelihood of wheeze (OR 2.27; 95% CI 1.15–4.47), shortness of breath (OR 2.33; 95% CI 1.12–5.06) and chest tightness (OR 4.34; 95% CI 1.76–10.69) and each 37.6-$\mu g/m^3$ increase in nitrogen dioxide increased both likelihood of any wheeze (OR 1.52; 95% CI 1.04– 2.21) or chest tightness (OR 1.61; 95% CI 1.04– 2.49), and days of wheeze (RR 1.33; 95% CI 1.05–1.68) or chest tightness (RR 1.51; 95% CI 1.18–1.91). These multi-family homes were smaller, the implication being that main living room levels of nitrogen dioxide may better reflect the child's bedroom level. Multi-family homes had higher levels of nitrogen dioxide (45% recording two-week averages of > 37.6 $\mu g/m^3$ compared to only 9.3% in the single-family homes). Children included in this study were the siblings of the infants recruited into the study reported by Belanger et al. *(147)* and Van Strien et al. *(40)* and described earlier in this chapter.

One possible mechanism to explain the association of indoor nitrogen with asthma may be an increased susceptibility to severe or prolonged infection. In England, 112 children with asthma were followed up for almost a year, during which period they kept an asthma diary and measured their personal exposure over each seven-day period using Palmes tubes *(165,166)*. When asthma exacerbations occurred, nasal aspirates were taken to confirm the presence of a viral infection.

The geometric mean exposure to nitrogen dioxide at the time of infection was 10.6 $\mu g/m^3$. Compared with exposures of < 8 $\mu g/m^3$, exposures of > 28 $\mu g/m^3$ were associated with an increased risk of asthma following infection (RR 1.9; 95% CI 1.1–3.4). The risk of experiencing an episode of lowered peak expiratory flow after having experienced symptoms highly suggestive of infection increased in a dose-dependent fashion with increasing nitrogen dioxide levels at the time of the infection. The increase in symptom score and the decrements in peak flow experienced during the laboratory-confirmed viral infection were larger in children who had higher exposures measured in the week prior to the start of the exacerbation. Although the average personal nitrogen dioxide was lower than in some studies, it was strongly related to the presence of gas appliances in the home and 23 of the children had at least one measurement in

excess of 100 μg/m^3 (32 of the measurements of the seven-day average personal exposure were > 100 μg/m^3).

These observations that children with asthma have worse symptoms if exposed to higher levels of indoor nitrogen dioxide suggest that their removal from exposure should lead to amelioration of their symptoms. However, few interventional studies have been reported. In recognition that classroom levels of nitrogen dioxide (largely determined by the use of unflued gas heaters) are an important source of exposure, an intervention study was conducted in Australia. Researchers assessed the effect of changing from unflued gas heaters in school classrooms to flued gas heaters or electric heaters *(167)*. Almost 200 asthmatic children in 10 control schools, 4 schools that had changed to flued gas heaters and 4 schools that had changed to electric heaters were followed for a period of 12 weeks. Following the intervention, the mean rate of symptoms of difficulty breathing during the day and at night, chest tightness and asthma attacks during the day was lower in children attending intervention schools. No change in lung function parameters was observed. Six-hourly average classroom levels of nitrogen dioxide ranged from 13.2–71.4 μg/m^3 (intervention) to 22.5–218.1 μg/m^3 (control) during the period of follow-up.

In further studies, 174 of these children with asthma kept a symptom diary over a 12-week period *(168)*. Home (kitchen) and classroom nitrogen dioxide levels were measured (indoor daily range: classroom 16.9–577.2 μg/m^3; kitchens 5.6–795.4 μg/m^3) and the association of several symptoms with the maximum of three daily time-averaged kitchen and classroom levels was assessed. Difficulty in breathing at night was associated with school (adjusted relative rate 1.11 (95% CI 1.05–1.18) per 18.8- μg/m^3 increase in nitrogen dioxide) and home levels (adjusted relative rate 1.03 (95% CI 1.01–1.05) per 18.8 μg/m^3 increase), while associations with nocturnal chest tightness and nocturnal asthma were seen only for school (adjusted relative rate 1.12 (95% CI 1.07–1.17) per 18.8-μg/m^3 increase in nitrogen dioxide) and home level (adjusted relative rate 1.04 (95% CI 1.00–1.07) per 18.8-μg/m^3 increase), respectively. Decrements in lung function (mean FEV_1 % predicted) were observed (–0.39% per 18.8 μg/m^3 increase in nitrogen dioxide level in the kitchen). At the time of the study, mattress house dust mite levels were assessed; there was no evidence that the association of nitrogen dioxide with symptoms was modified by mattress allergen levels.

Another intervention study was conducted in New Zealand *(169)*. Parents of children with asthma, living in homes heated by an unflued gas heater or a plug-in electrical heater, were invited to alter their current heating system to either a heat pump, a wood pellet burner or a flued gas heater. All eventually received their heater of choice, but they were randomized to receive them immediately (treatment group) or after one year (control group).

Improvements in subjective markers of health as reported by parents (sleep disturbed by wheeze, dry cough at night, overall health) symptoms reporting

in diaries (cough at night, wheeze at night) and health service utilization (visits to the doctor, visits to the pharmacist) were seen in the treatment group but no change in objective markers such as peak flow variability or lung function tests were seen. During the period of the study, the levels of nitrogen dioxide in the bedrooms and living rooms of the intervention group were 8.5 and 7.3 μg/m^3, respectively, compared with the levels in the control group of 15.7 and 10.9 μg/m^3, respectively (both $P > 0.001$). However, the intervention houses were also warmer (0.57 °C and 1.1 °C for the bedroom and living room, respectively) and therefore health status may have been higher owing to increased warmth rather than decreased nitrogen dioxide levels.

The Hasselblad meta-analysis. In the early 1990s, Hasselblad et al. published a meta-analysis of the association of indoor nitrogen dioxide levels with respiratory illness in children *(170)*. This report was one of the earliest examples of the use of meta-analysis for synthesizing evidence from studies of environmental hazards. It included published studies that had measured either indoor nitrogen dioxide or the use of a gas appliance as the exposure metric and combined the results from 11 studies, presented in 15 different publications from the Netherlands *(150,171)*, the United Kingdom *(148,172–178)* and the United States *(153,179–182)*.

In studies in which gas stoves rather than measured nitrogen dioxide represented the exposure, it was assumed that the average nitrogen dioxide exposure was about 30 μg/m^3 higher in homes with a gas stove than in those without one. Respiratory symptoms were any respiratory symptom, with some variation between studies but including wheeze, cough, coughs going to the chest, shortness of breath and bronchitis. In children under the age of 12 years, a 30-μg/m^3 increase was equivalent to a 20% increased risk of symptoms. Exclusion of studies in which gas stoves were the proxy markers for exposure led to an increase in effect size (OR 1.27 per 30-μg/m^3 increase). This analysis is of considerable importance, as it provided the basis for outdoor air quality guideline setting by WHO in 1997 *(183)* and its conclusions have, to date, not been seriously challenged by any new evidence. Extrapolating directly from the Hasselblad meta-analysis, WHO in 1997 reported that "On the basis of a background level of 15 μg/m^3 (0.008 ppm) and the fact that significant adverse health effects occur with an additional level of 28.2 μg/m^3 (0.015 ppm) or more, an annual guideline value [for outdoor nitrogen dioxide] of 40 μg/m^3 (0.023 ppm) is proposed. This value will avoid the most severe exposures."

Epidemiological studies in adults

In comparison to the number of studies in children, there are relatively few studies that have reported associations of adult respiratory health with indoor nitrogen dioxide levels.

Health effects in adults: studies measuring indoor nitrogen dioxide. Indoor nitrogen dioxide measurements were made in a subsample of households taking part in a longitudinal study in the United States (mean 24-hour average 94.0 μg/m^3 in homes using gas for cooking and 37.6 μg/m^3 in those using electricity). No association of respiratory illness in any member of the household (including adult members) with measured nitrogen dioxide level was observed *(180)*.

A cohort of 152 non-smoking women living in Vlagtwedde and Vlaardingen in the Netherlands was studied in 1982. Time-activity patterns showed that the women spent about 25% of their time in their kitchens, 25% in the lounge and 35% in the bedroom *(184)*. Most (97) of the women had also had their FEV_1 measured and, in general, decrements in FEV_1 were associated with increases in indoor nitrogen dioxide levels, the largest estimates being seen for measurements in the bedroom (mean deficit in FEV_1 2.38 ml per μg/m^3 increase in nitrogen dioxide; $P < 0.01$) and the lounge (mean deficit in FEV_1 3.91 ml per μg/m^3 increase in nitrogen dioxide; $P > 0.05$) rather than the kitchen (mean deficit in FEV1 0.69 ml per μg/m^3 increase in nitrogen dioxide; $P > 0.05$) *(185)*. Although there was some evidence that increasing nitrogen dioxide level was associated with the decline in FEV_1 that had been recorded in these women over the previous 13 years, this association failed to reach conventional levels of significance. Effect modification by atopy or asthma was not considered.

In Hong Kong SAR, mothers of children taking part in a large study of respiratory health wore personal nitrogen dioxide badges for a 24-hour period *(155)*. Personal nitrogen dioxide was higher in women if they cooked more frequently, but only among those who did not ventilate their kitchen by the use of an extractor fan. In users of LPG or kerosene, the mean personal 24-hour average exposure was 37.7 μg/m^3 if the kitchen was ventilated during cooking and 41.7 μg/m^3 if the kitchen was unventilated. There was no consistent association of personal nitrogen dioxide with frequency of cooking or presence of ventilation fans in the children living in these homes, probably reflecting their different time-activity patterns. Personal nitrogen dioxide was higher in women who cooked with LPG compared to those using piped gas.

There was some evidence that personal exposure levels may be associated with chronic cough and allergic rhinitis. The mean personal 24-hour average exposure was 42.3 μg/m^3 in women with cough and 35.9 μg/m^3 in those without ($P = 0.05$), while it was 42.4 μg/m^3 in women with allergic rhinitis and 35.5 μg/m^3 in those without ($P = 0.002$).

Women who had recently given birth were recruited into a study in which health information was collected at baseline and the presence of respiratory symptoms regularly collected every two weeks for a year *(186)*. Indoor nitrogen dioxide measurements were made with passive samplers over a two-week period in the winter and this was repeated depending on whether gas or kerosene appliances were present. Even though this was a large study involving 888

non-smoking women, no association of nitrogen dioxide level with symptoms of wheeze, chest tightness, hoarseness or phlegm was seen when nitrogen dioxide was considered as a continuous variable. When it was dichotomized, however, to compare the top quartile (> 150.4 μg/m^3) with the lower three quartiles, household levels were associated with chest tightness (1.94; 95% CI 0.98–3.85) and with wheeze (4.00; 95% CI 1.45–11.0). An association of symptoms with the use of kerosene heaters was reported and this may have been due to the sulfate emissions from these heaters.

Indoor nitrogen dioxide levels were measured for one week in the homes of 148 people with severe COPD living in north-eastern Scotland *(187)*. Half of the homes had at least one active smoker, and nitrogen dioxide levels were higher in homes with smokers (median 16.2 μg compared to 12.9 μg; $P < 0.02$) and no association of symptoms or specific respiratory quality of life was observed.

Kitchen, living room and bedroom nitrogen dioxide levels were measured for one week in the summer and one week in the winter in urban (Pisa) and rural (Po Delta) areas of Italy *(188)*. Mean kitchen levels of nitrogen dioxide were statistically different in the two regions (54.5 vs 62.0 μg/m^3 ($P < 0.05$) in winter and 41.4 vs 37.6 μg/m^3 ($P < 0.01$) in the summer for Pisa and the Po Delta, respectively). An average personal exposure in adults was generated from time-activity patterns. Exposure above the study median nitrogen dioxide index was associated with an increased risk of acute respiratory symptoms (OR 1.66; 95% CI 1.08–2.57) but no association was seen for bronchitic/asthmatic symptoms.

Those who cook in the home and professional cooks are exposed to high levels of nitrogen dioxide as well as to other cooking-related pollutants. There is no evidence from large-scale epidemiological studies that have measured indoor nitrogen dioxide levels that respiratory health is worse in those who regularly use unvented gas appliances for cooking. In a cross-sectional study of 37 professional cooks (mainly women) working in four large hospital kitchens in Brazil *(189)*, average daily levels of nitrogen dioxide in one kitchen reached almost 188 μg/m^3. The authors presented tabulated coefficients from regression models to suggest total exposure to nitrogen dioxide (the product of years working in that kitchen times the level of nitrogen dioxide in the kitchen) was associated with lower levels of forced expiratory flow ($P < 0.031$), even after adjustment for smoking behaviour and the presence of asthma, but it is difficult to interpret the effect estimates from this. Confounding by other cooking-related pollutants cannot be ruled out.

Presence of gas appliances at home as an indicator of nitrogen dioxide exposure

In setting guidelines for indoor air quality, there is clearly a need for direct measurements of nitrogen dioxide levels, and for these levels to be associated with some health impairment. In the indoor setting, however, where the source of indoor nitrogen dioxide may, in the main, be from indoor gas appliances, the pres-

ence of the appliance itself may act as a proxy marker of exposure. Of particular concern is that the use of some appliances, particularly gas cookers (which are unvented) is associated with short-lived peaks of exposure that are not captured or measured by most of the monitoring techniques used in epidemiological studies. Controlled exposure studies suggest that nitrogen dioxide at levels associated with these peaks may be harmful but studies that use "average nitrogen dioxide" may not be able to detect these health effects. Under these circumstances, association of a health effect with the presence and use of the gas appliance may provide stronger evidence of health effects of indoor nitrogen dioxide than measurements of the gas itself.

However, there are three major pitfalls with this approach. First, a simple exposure metric of "exposed to a gas appliance" does not capture potential variation in the nature or intensity of the exposure, which may vary with frequency of use, intensity of use, use of extractor fans, household ventilation, the proximity to the appliance and the contribution of this source to the individual's total exposure. This may explain the heterogeneity in the results of studies. Second, examination of dose–response effects may be difficult as "frequent use" of a gas cooking appliance also implies frequent exposure to a range of other cooking-related pollutants. Third, and related to the previous point, the group selected for comparison should ideally comprise a group that uses electricity. This may be difficult in countries where gas is used almost universally – for example, until more recently in Hong Kong SAR and Italy. In these circumstances, studies often report comparisons of "frequent users" with "less frequent users".

There are many more studies on the association of health with the presence of gas appliances than with measured nitrogen dioxide and they are of varying quality. While cross-sectional studies are of interest in making a causal inference, greater weight would naturally be placed on those that are longitudinal in design. Further, in older children and adults, objective markers of disease such as lung function may be used and these measurements, in well-designed studies, could be argued to provide better evidence for associations than those that are based on self-reported symptoms. However, respiratory disease may occur in the absence of measurable change in lung function parameters.

Health effects: studies measuring acute health effects of exposure to gas appliances. The acute effects of direct exposure to gas combustion have been studied in people's homes. An early pilot study that used this approach reported change in FVC in asthmatic and non-asthmatic women in whom continuous measurements of nitrogen dioxide were made during cooking at home over a five-day period *(190)*. Spirometric measurements were made before, during and after each cooking period. The highest average nitrogen dioxide level reached was about 1500 $\mu g/m^3$ (0.8 ppm) and the highest five-minute average level was about 1692 $\mu g/m^3$ (0.9 ppm). No formal statistical analyses were presented but in the asth-

matic women, decrements in FVC were seen when cooking with gas on many of the cooking occasions, and when nitrogen dioxide levels reached over 564 μg/m^3 (mean or five-minute average) nearly all asthmatic women showed a drop in FVC. This was not seen in the non-asthmatic women, but it should be noted that the peak nitrogen dioxide levels for the non-asthmatic group were not as high (no reason was given for this). The magnitude of the change in FVC reported in the asthmatic women was substantial, some individuals showing a 10% change in FVC and one individual on one occasion showing a change of 20%.

In another study, 16 adult non-smoking women with asthma had their peak flow measured before and after cooking with gas in their own homes. The fall in peak flow after cooking was related to the level of nitrogen dioxide recorded during cooking (highest peak exposure 500 μg/m^3) and over a two-week period their personal exposure to nitrogen dioxide (range 37.3–135.6 μg/m^3; mean 80.49 μg/m^3) was associated with increased use of salbutamol *(191)*.

Health effects in children: cross-sectional studies looking at exposure to gas appliances. Cross-sectional studies conducted more than 25 years ago suggested that the use of gas cooking was associated with increased hospital admissions for respiratory disease in preschool children in the United States *(179)* and more respiratory symptoms in schoolchildren in England *(174)* and the United States *(192–194)*. Participants in the United Kingdom study were followed for about five years and there was some evidence that the association became less apparent as the children became older *(177)*. Other cross-sectional studies conducted at a similar time did not observe these associations *(180,182,195)* and a longitudinal study in which children were studied for one year to identify episodes of respiratory illness also found no association *(181)*. Many, but not all, of these early studies made attempts to adjust for potential confounding by social class, parental smoking and other household factors, recognizing that the use of gas appliances, in some communities at least, was strongly related to lower socioeconomic status and increased rates of parental smoking *(179)*.

In cross-sectional studies conducted more than 25 years ago, non-significant ($P > 0.05$) decrements in $FEV_{0.75}$ were reported in children living in homes that cooked with gas *(193)*. Hosein et al. *(196)* reported decrements in FEV_1 with the use of a gas stove in 1357 children living in the United States, but this study collected information on eight household factors (pet keeping, hobbies that exposed residents to gases/vapours/dust, cooking fuels, heating system, presence of a fireplace, use of humidifiers or air conditioning, domestic crowding and smokers in the household) and there were significant interactions between some of them, making the overall effect of gas stoves difficult to interpret. Ekwo et al. *(179)* administered isoprenaline to children and examined differences in response in children with different household exposures. Greater bronchodilator responses were observed in children exposed to tobacco smoke than in those who were not

exposed, but no such difference was seen in children exposed to gas cooking appliances, even though an association of symptoms with the use of gas had been observed.

In the past, unvented gas water heaters were relatively common indoor gas appliances in the Netherlands, and researchers have examined their effect on children's health. Lung function was measured by spirometry and a forced oscillation technique in 470 primary school children *(197)*. Respiratory symptoms were non-significantly ($P > 0.05$) more common in children living in homes with unvented gas water heaters. There was no clear, consistent association of spirometric indices or of measurements of impedance with the use of these appliances, although the small observed differences were in the expected direction and were greater for measurements of resistance and impedance in girls.

Large-scale cross-sectional studies have been conducted more recently. In Canadian schoolchildren (n = 10 819), the presence of a gas cooking stove in the home was associated with current asthma (OR 1.95; 95% CI 1.4– 2.68) but not with "wheezing syndrome" after adjustment for a variety of household (e.g. damp, environmental tobacco smoke) and socioeconomic (parental education) confounders *(198)*. A study in eastern Germany conducted in 1992/1993 showed an increased risk of "cough without a cold" (OR 1.63; 95% CI 1.23–2.04) and other cough symptoms in over 2000 children aged 5–14 years living in homes with a gas cooker *(199)*. The lifetime prevalence of other symptoms was not increased. White blood cell counts were increased in children in homes with gas cookers, particularly in those likely to be exposed to high levels of gas-cooking-derived pollutants (those in homes with no extraction fans or smaller homes, and children who spent more time indoors). There was a suggestion that this latter association may be stronger for bottled gas than for the town gas that was in use at the time. In 4–5-year-old Australian children, the use of a natural gas stove was associated with an increased risk of wheeze, asthma and colds *(200)*. In the Third National Health and Nutrition Examination Survey, use of a gas stove for cooking or for heating was associated with an increased risk (OR 1.8; 95% CI 1.02–3.20) of physician-diagnosed asthma in children under the age of six years *(201)*. However, in a large (n ~ 28 700) study of children aged 6–9 years living in Austria and taking part in the International Study of Asthma and Allergies in Childhood (ISAAC, Phase 1), the 12-month period prevalence of wheeze was not associated with gas cooking, although associations with other indoor risk factors such as environmental tobacco smoke, dampness and mould were seen *(202)*.

Effect modification by allergic predisposition, as shown by total IgE levels, on the association of lung function with exposure to gas has been observed in a cross-sectional study of adolescents. Corbo et al. *(203)* studied teenagers in Italy, where the use of gas for cooking is almost universal, and asked them how much time they spent in the kitchen. More girls than boys reported they were "often in the kitchen while their mother cooked" and in girls but not boys those who

reported being "often in the kitchen" had worse lung function as measured by forced expiratory flow rate than those who were in the kitchen only "some of the time". This association was observed mainly in girls with total IgE above 48.6 IU/ml (the median value in girls), suggesting the effect may be greater in girls who are atopic. Effect modification by total IgE level persisted even if children with positive skin tests were excluded. This study suggests a dose-dependent association of exposure to gas cooking with airway function that is modified by both atopy and gender in this age group. It also suggests that proximity to the gas cooker at the time of its use rather than "living in a home with a gas cooker" is the exposure associated with symptoms. However, confounding by exposure to cooking fumes or other pollutants from gas combustion cannot be ruled out.

An analysis of information collected from asthmatic children taking part in the National Health and Nutrition Examination Survey showed that girls who lived in a home with a gas stove (about 45% of the sample) had lower lung function (FEV_1, FEF_{25-75}, FEV_1/FVC) than those who did not *(204)*. However, this association was only seen in girls who did not take asthma medication and was not seen in boys.

The association of respiratory symptoms with gas for cooking may be modified by levels of outdoor nitrogen dioxide. This was examined in a small study in Hong Kong SAR, where cooking with gas (piped town gas and LPG) is almost universal. Children living in two contrasting areas were examined *(205)*. In an area with relatively low background pollution (nitrogen dioxide annual mean 45 $\mu g/m^3$), current doctor-diagnosed "respiratory illness" was more common in children living in homes that cooked most frequently with gas. This dose–response relationship to gas cooking was not observed in a nearby area with higher outdoor background levels of particulate matter and oxides of nitrogen (nitrogen dioxide annual mean 59 $\mu g/m^3$), suggesting that in multicentre studies the association of gas cooking with symptoms may vary with level of ambient pollutants.

Using a case-control design, the association of severe asthma (children whose parents reported that they suffered either 12 or more wheezing attacks in the past 12 months or an attack of wheeze over the same period that limited speech to only one or two words at a time between breaths) with use of gas for cooking was examined *(206)*. Controls were children with no history of asthma or wheezing at any age. There was no evidence that the use of gas for cooking differed between cases and controls (adjusted OR 0.86; 95% CI 0.61–1.23).

Health effects in children: longitudinal studies looking at exposure to gas appliances. A longitudinal study design has been adopted in some studies to examine the health effects of exposure to gas appliances in infancy. Primigravida mothers were interviewed early in pregnancy regarding household characteristics, and the health visitor information on their offspring (n = 1565) was collected at one year of age *(178)*. Although the proportion of children with a respiratory illness

and with an admission to hospital for respiratory illness was higher in homes with gas cooking, the difference was not significant.

The association of exposure to gas appliances in infancy to later respiratory health was examined in Australia, where gas heaters are the main source of indoor combustion products. As part of the Tasmanian Infant Health Survey, the type of heating appliance in use in infancy was recorded and, at the age of seven years, information on respiratory symptoms was collected *(207)*. Only a small proportion of the children lived in homes with a gas heater (most likely to have been fuelled by LPG) but this small group had a substantially increased risk of asthma in later life (1.92; 95% CI 1.33–2.76), even after adjustment for some markers of socioeconomic status (maternal education) and household smoking. In the same publication, the authors presented results from an extended analysis involving more than 6000 children, in which they noted a cross-sectional association of recent wheeze (OR 1.41; 95% CI 1.17–1.71) and asthma (OR 1.33; 95% CI 1.12–1.57) with current use of gas heaters.

Another publication using data from the same children looked at the association of gas use in infancy with lung function *(208)*. Those who had lived in a home with either a gas heater or a gas cooker in infancy were more likely to be sensitized to house dust mites and had a lower $FEV_{1.}$ In the children included in this analysis, the association of asthma with the use of gas was below conventional levels of significance. However, airway obstruction was more strongly associated with current gas cooking in children sensitized to house dust mites than in those not sensitized.

The long-term health effects of exposure to gas appliances was also examined in a United Kingdom study that measured prevalence of wheeze in teenagers in relation to exposure to gas cookers as a child *(209)*. Almost 2000 children provided information on symptoms at ages 7–8 and 15–17 years and, in addition, at the age of 16–18 years reported their use of gas appliances (for cooking or heating) in their current home and in their home when they were a child. Childhood wheezing was associated with childhood exposure to any gas appliance and with childhood exposure to a gas hob (OR 1.47; 95% CI 1.05–2.04). However, childhood exposure to gas appliances was not associated with wheeze that persisted into the teenage years. Wheeze in adolescence was not associated with current teenage exposure and, surprisingly, persistent wheeze was less frequent in those exposed to gas in the teenage years. The authors argued that this latter observation might be explained by selective avoidance.

A similar age group was studied as part of a longitudinal study in southern California *(210)*. Participants aged 9–16 years were recruited and followed for five years or until graduation. Excluding those with "ever physician-diagnosed asthma" at baseline, the association of indoor factors with doctor-diagnosed asthma was examined in the remaining 3535 children. In this group, at baseline, the use of gas for heating and for cooking was common and more commonly

reported by those who had wheeze (78% of those with wheeze and 73.8% of those without wheeze used gas for heating ($P = 0.004$), while 79.4% of those with wheeze and 77.7% of those without wheeze had a gas stove ($P = 0.27$)). Over the five-year period, there was no evidence that the presence of these appliances was associated with the incidence of physician-diagnosed asthma in children with or without wheeze at baseline.

Longitudinal studies have been used to examine whether exposure to gas appliances has a deleterious effect on lung growth in children. In Arizona, United States, a four-year study was conducted to determine whether living in a home where gas was used for cooking was associated with poor lung growth in children aged eight years at baseline. Despite strong cross-sectional associations of the use of gas for cooking with symptoms of wheeze, cough and sputum production at baseline, there was no evidence that exposed children had lower rates of lung growth than unexposed children *(192)*.

These findings are supported by work conducted as part of the Six Cities Study in the United States. First reports from the study suggested that exposure to gas stoves was associated with reduced lung function in children *(194)*. Later work suggested that a reduction of 0.7% in mean FEV_1 and 0.6% in mean FVC seen in the first examination was not observed after three years of follow-up, where a non-significant reduction of 0.3% in both measurements was seen *(182)*. After further examination, analyses based on 7834 children aged between six and ten years who had between two and five annual measurements of lung function showed no effect of the use of a gas stove on pulmonary function level at the end of the study *(211)*.

As part of the meta-analysis conducted by Hasselblad et al. *(170)* in the early 1980s, the association of childhood respiratory illness with the use of gas for cooking was determined to be 1.15 (95% CI 1.09–1.22).

Health effects in adults: cross-sectional studies looking at exposure to gas appliances. If we hypothesize that the health effects of combustion products such as nitrogen dioxide from unvented gas appliances depend on repeated high exposures, those that actually use the appliances and are therefore exposed to these peaks would be expected to be at greatest risk. In most communities, cooking remains a task largely performed by women and particularly by young and middle-aged women with large families. This being so, we might expect women to be at particular risk. However, one of the first studies examining the association of the use of gas with respiratory health suggested the opposite. In a community-based representative sample of almost 2000 adults in Maryland, United States, men living in homes with a gas cooker had more chronic cough and wheeze with breathlessness than those living in homes with electric cookers. No association was seen in women. The authors hypothesized that women have, over thousands of years, been exposed to pollutants generated by cooking and heating and have

an evolutionary advantage over men in being resistant to the health effects of exposure to fumes from cooking and cooking appliances *(212,213)*.

A small case-control study was conducted shortly afterwards. The type of cooker that was used by 102 non-smokers with FEV_1 in the highest quartile of the distribution was compared with that used by 103 non-smoking women with FEV_1 in the lowest quartile *(214)*. Exposure to gas appliances was non-significantly higher in those with low lung function (30.4% vs 22.3%; OR 1.82; P = 0.076) and cooking with gas for more than 10 years was more common in those with low lung function. Effect modification by atopy or frequency of use was not examined.

Large-scale cross-sectional studies conducted more recently in the United States suggest little association of symptoms with use of gas in either men or women. For example, in the National Health and Nutrition Examination Survey III (NHANES III), the association with gas stove use was examined in 7630 lifelong non-smokers (mean age 42 years) *(215)*. There was no association of current gas stove use with symptoms of phlegm, wheeze or dyspnoea, although an association with chronic cough was seen (OR 1.6; 95% CI 1.1–2.3). In fact, those who had a gas stove appeared to have *better* lung function than those who did not. These analyses were extensively adjusted for other household and sociodemographic factors. Effect modification by gender (and by atopy as measured by skin tests) was tested and was not observed.

Nevertheless, an analysis of data collected as part of the ECRHS multicentre study presented evidence that respiratory symptoms suggestive of asthma, and lung function changes suggestive of airway obstruction, may be associated with the use of gas cooking in some communities *(216,217)*. A strong cross-sectional association of respiratory symptoms with the use of gas for cooking was seen in women, but not men, living in three towns in England. The association was particularly strong in women (P for interaction < 0.05). Women who were sensitized to one of four common aero-allergens were particularly at risk, although formal tests for effect modification by atopy in women were not significant (P for interaction > 0.05). These observations were supported by decrements in FEV_1 and FEV_1/FVC. As part of the ECRHS, the same protocol was followed in other research centres in Europe. However, when the same statistical approach was extended to include these centres, considerable heterogeneity was observed between centres with the strongest effects being seen in the United Kingdom centres. No explanation for this heterogeneity was found but it may have been due to the varying exposure suggested by reporting that a participant "mainly uses gas for cooking". In this early study no information on frequency of use, use of natural ventilation, type of gas used and maintenance of appliances was collected.

Researchers in the Netherlands *(218)* looked for effect modification by atopy on the association of gas cooking with bronchial reactivity. The protocol adopted for the study was that of the ECRHS but the age range studied was larger (20–70

years). Atopy was defined by the presence of specific IgE to common aero-allergens and measured bronchial reactivity to methacholine. There was no evidence that atopics had greater bronchial reactivity if they used gas for cooking, but the associations were much stronger in both men and women who had high total IgE compared to those with low total IgE. The authors argued that total IgE was a superior way of identifying the susceptible "atopic" subgroups but to date their findings have been supported only by the study of adolescents in Italy *(203)*. Total IgE is higher in smokers *(219)* and in those exposed to "gas, dust, fumes or mists" in the workplace *(220)*. None of these authors considered whether total IgE was higher in those who cooked with gas and was a surrogate marker for greater exposure to gas.

Older adults may not use their gas cooking appliances as frequently as those living with small children. In a questionnaire survey of men and women aged 65 years or older in Bristol, United Kingdom, the presence of a gas hob or gas oven was associated with a small, non-significant risk of respiratory symptoms suggestive of COPD *(221)*. The risk of respiratory symptoms appeared to be higher in women than in men for gas hobs, although the gender interaction was not significant ($P > 0.05$). Many people in this age group will no longer be preparing meals for their families and no information was collected on the frequency of use of gas hobs and ovens. Associations may have been weak owing to infrequent cooking in this age group.

Many of the remaining cross-sectional studies in adults have been restricted to women. In Polish women over 65 years of age, chronic cough, chronic phlegm and shortness of breath on exertion were more common in those who cooked with gas for more than three hours a day compared to those who cooked with gas less frequently *(222)*. The authors reported that "decline in FEV_1" was also greater in this group, but this association was assessed by comparison of the age coefficient for regression equations of FEV_1 on height and age, stratified by those with short or medium/long daily exposure, and was not derived from repeated measurements of FEV_1 .

In a study of 1282 women in Singapore (223), there was a non-significant increased risk of respiratory symptoms among non-smoking women who cooked frequently and a significantly reduced adjusted FEV_1 noted in those who described themselves as housewives and who cooked frequently (0–2 times a week 1.82 litres; 3–14 times a week 1.62 litres; ≥ 15 times a week 1.61 litres). In Singapore, as in Poland, the use of gas for cooking was universal and greater exposure to "cooking with gas" also implies greater exposure to other substances generated by the cooking process. Stir frying with spices and chillies is a common method of cooking, and regular cooking is most likely to be associated with greater exposure to oil mists and frying fumes that may themselves cause respiratory symptoms. There was a strong association of chronic cough and phlegm with the frequency with which the kitchen was "filled with cooking fumes". This could

reflect poor ventilation and greater exposure to the products of gas combustion, but could also reflect greater exposure to pollutants created by cooking.

A cross-sectional study of asthmatics recruited into NHANES III *(215)* did not show an association of the use of gas cooking with asthma severity. Nearly half of the 445 asthmatics studied cooked with gas but they had only a non-significant increased prevalence of symptoms of wheeze and dyspnoea and had the same lung function (FEV_1, FVC or $FEF_{25–75}$) as those who cooked with electricity. The authors of this study concluded that their results "should be reassuring to adults with asthma and their health care providers". In another report, the same research group followed asthmatics living in California over an 18-month period. Those who cooked with gas had similar health service utilization rates (hospital admissions and emergency department visits) as those who cooked with electricity. There was no evidence that the reporting of use of a gas stove at baseline (or changing the use of that gas stove over the period of the follow-up) was associated with asthma severity or with SF-12 or asthma-specific quality of life scores, even though associations with exposure to ETS were observed *(224)*.

Health effects in adults: longitudinal studies looking at exposure to gas appliances. Longitudinal studies over several years of follow-up to examine the chronic respiratory health effects of gas cooking in adults are less common than cross-sectional studies, but some have been published. Keller et al. *(180)* examined incident lower respiratory illness in members of 441 families living in Ohio, United States by contacting the families every two weeks over a period of a year. About half of the families used electricity to cook and the rest used gas, but overall the rates of respiratory illness in mothers (and children) were lower in families with gas cookers than in families with electric cookers.

The longest follow-up has been of residents of households in Chesterfield, England, that were included in a housing survey in 1936. They were followed through the National Health Service Central Registry *(225)*. The presence of a gas cooker in the home at the time of the survey was not associated with overall mortality in children or adults and was negatively associated with death ascribed to COPD (RR 0.8; 95% CI 0.6–1.2). This result is unsurprising, as positive results would have implied a very strong association of gas cooking at one point early in life with development of severe respiratory disease many years later, without adjustment for smoking.

The largest longitudinal study with the most comprehensive analysis to examine respiratory outcomes in adults, based on exposures in earlier life, was from the 1958 birth cohort in the United Kingdom *(226)*. In a sample of 1500 adults enriched with people who had a history of wheezing in earlier assessments, information on the fuel currently used for cooking and the fuel used for cooking when the participant was 11 years old was collected at age 35 years. Of those who first reported symptoms indicative of asthma at the age of 7 years, those

who reported using gas for cooking at age 35 years were more likely to report current wheeze than those who currently used electricity for cooking (OR 1.26; 95% CI 0.84–1.88). This increased risk was greater in women (OR 1.82; 95% CI 1.02–3.26) than in men (OR 0.86; 95% CI 0.48–1.58) and in non-atopics (OR 1.84; 95% CI 1.05– 3.22) than in atopics (OR 0.76; 95% CI 0.39–1.49), but the interaction of gender and atopy with gas cooking for persistence of symptoms was not significant ($P > 0.05$). Cross-sectional analyses of the 243 people with asthma or wheeze in the previous year showed little evidence that the current use of gas for cooking was associated with current asthma severity as measured by number of attacks of asthma or the reporting of sleep disturbed by wheezing in the previous 12 months.

At variance with the results for symptoms is that decrements in lung function were associated with the use of gas for cooking in men (FEV_1 –141 ml; 95% CI –234 to –48 ml) but not in women, and current asthmatics showed worse lung function if they cooked with gas than if they cooked with electricity (FEV_1 –129 ml; 95% CI –234 to –14 ml). This latter association was not examined in men and women separately. The authors concluded that "past and current use of gas for cooking is unlikely to be a major influence on respiratory morbidity in young adults".

A longitudinal design was used to assess acute health effects of exposure to gas appliances in a panel study of 164 asthmatics living in Denver, United States *(227)*. Participants recorded symptoms, medication use and their use of indoor gas appliances. The reporting of moderate or severe cough and shortness of breath was associated with gas stove use on that day, the "non-users" comprising people with either a gas or an electric stove.

Studies conducted in developing countries

In developing countries, many people cook with either biomass or with gas (usually LPG). Use of biomass is associated with a range of health effects, which were reviewed in 2004 *(228)*. Solid fuel use was shown to be associated with acute lower respiratory tract infection, COPD, asthma, cataracts, tuberculosis and lung cancer. Association of nitrogen dioxide with disease in these settings is likely to be confounded by the high particulate counts. There has, however, been one study in Ethiopia where about 90% of homes cook with biomass (wood or charcoal) and the remainder cook with a modern fuel such as kerosene, gas or electricity *(229)*. Those who used kerosene had a significantly increased risk of IgE sensitization to aero-allergens, eczema and rhinitis compared to those who did not. Those who used gas had some increased risk and those who used electricity had an increased risk for eczema only. The authors argued that the increased risk of allergic outcomes in those using modern fuels producing nitrogen dioxide was unlikely to be explained by factors associated with socioeconomic status, as they had adjusted for this (family occupation, household crowding).

Health effects: type of gas

Some of the variation in the associations of gas cooking with symptoms may be explained by the type of gas used. In areas where there is no piped gas, LPG is often used. In a study of more than 25 000 children in the United Kingdom, there was no association of gas used for cooking or heating with wheeze symptoms *(230)*. Children who lived in homes that used bottled gas (which in this study also included paraffin) were at an increased risk of wheeze compared to those using electricity for heating (OR for speech-limiting wheeze 1.38). One of the few studies in developed countries to examine the association of bottled gas compared to mains gas on respiratory health in adults was conducted in Italy, where cooking with gas is almost universal. In a population-based study in the Po Delta *(231,232)*, 30% of people used bottled gas and 67% used natural gas for cooking. The type of gas used for cooking was closely associated with the type of heating in the home, and exposure status was defined by both heating and cooking appliances. The lowest prevalence of symptoms was in those with natural gas central heating and gas cooking. Dyspnoea was more common in men and women who used bottled gas for cooking compared to those who used natural gas. No effect modification by atopy was examined or reported. Viegi et al. *(231)* hypothesized that the use of bottled gas may produce more pollutants from gas combustion owing to inefficient burning that went unnoticed. Bottled gas appliances were not subject to the mandatory regulation and official inspection imposed on natural gas appliances.

Health effects: studies conducted in ice arenas

High concentrations of nitrogen dioxide are observed in indoor ice arenas that use resurfacing machines powered by combustion engines, and studies have been conducted to assess the effect of exposure to nitrogen dioxide in this setting. A review of studies in which measurements were made of both nitrogen dioxide and carbon monoxide in indoor ice arenas was published in 2002 *(233)*. Exposure to nitrogen dioxide in this setting is accompanied by exposure not only to carbon monoxide and particles but also to cold air, and cross-sectional studies suggesting a high prevalence of respiratory symptoms in ice hockey players and figure skaters *(234)* may reflect a response to cold air. This interpretation is supported by evidence that the prevalence of asthma is higher in those who participate in outdoor winter sports such as cross-country skiing *(235)*.

To overcome this problem, one study in Sweden compared respiratory symptoms in children who played ice hockey in ice arenas with propane-fuelled and electric resurfacing machines *(236)*. Some 1500 children aged between 10 and 16 years who had played ice hockey in the previous three years and had trained in one of 15 indoor ice arenas were identified. Nine of the arenas used a propane-fuelled resurfacing machine and the median nitrogen dioxide level obtained from three consecutive days of monitoring in each arena during opening hours was 190

μg/m^3, although there was considerable range in the daily measurements (28–1016 μg/m^3). The remaining six arenas used electric resurfacing and the equivalent indoor nitrogen dioxide levels were 9 μg/m^3 (4–31 μg/m^3). Symptom prevalence (wheezing in the last 12 months, exercise-induced wheeze, physician-diagnosed asthma or current rhinitis) was non-significantly higher in children who trained in arenas with an electric resurfacing unit rather than a propane unit. The authors then looked at children attending the propane arenas only. They considered exposure as "high nitrogen dioxide" if levels were above the median and low if below the median. All symptoms were more common in those attending high nitrogen dioxide propane arenas and this reached statistical significance for those who had ever had symptoms of rhinitis (OR 1.7; 95% CI 1.3–2.3), rhinitis in the last 12 months (OR 1.7; 95% CI 1.2–2.4) and those who had ever had wheezing (OR 1.4; 95% CI 1.0–1.9). The vast majority of the children (over 80%) had played ice hockey for more than three years in these arenas.

Chronic health effects of exposure were also examined in Finland *(43)*. Junior ice hockey players were asked to complete a questionnaire. Information on the weekly average nitrogen dioxide in the ice arenas in which they trained was collected (range 21–1176 μg/m^3; mean 228 μg/m^3). For each increase of 100 μg/m^3, the risk of reporting rhinitis and cough was significantly increased (OR 1.54; 95% CI 1.05–2.26 for rhinitis and OR 1.62; 95% CI 1.06–2.47 for cough) and that of mucus production and sore throat was non-significantly increased (OR 1.41; 95% CI 0.96–2.08 for mucus production and OR 1.43; 95% CI 0.88–2.35 for sore throat), although in the discussion the authors intimate that the relationship is non-linear.

There are case reports of acute pneumonitis in adult ice hockey players in the 24 hours after a match *(237,238)*, but these studies lacked indoor nitrogen dioxide measurements. However, as part of an investigation into acute onset of cough, haemoptysis and/or chest pain during or shortly after playing hockey in an indoor ice arena among the members of four ice hockey teams *(239)*, the mean nitrogen dioxide level during 30 minutes of resurfacing was found to be 7520 μg/m^3. In 1994, a similar incident occurred in Stockholm, Sweden, probably associated with exposures of up to 2358 μg/m^3 *(240)*, and five years later the prevalence of self-reported shortness of breath, wheezing, cough and rhinitis was higher in those exposed to the high levels of nitrogen dioxide than in the control group (ice hockey players who had trained in electric resurfacing arenas) *(241)*. Longer-term health effects of high-dose exposures were also reported from Philadephia, United States *(242)*. Six months after an incident in which 16 previously healthy hockey players developed symptoms following exposures thought to be between 658 and 2068 μg/m^3 for around 3 hours, 50% remained symptomatic. Impulse oscillometry tests before and after bronchodilator use suggested increased airway resistance and small airway disease in those reporting more symptoms.

Nitrous acid

Nitrous acid is present as a gas in indoor and outdoor air. In the indoor environment it is produced both directly by combustion processes, such as by the use of unvented gas appliances, and indirectly by absorption of nitrogen dioxide and then release of nitrous acid from water-containing surfaces in the home *(243)*. One chamber study in healthy volunteers showed that exercising in 650 ppb nitrous acid for three hours is followed by minor reductions in airway conductance *(244)* and another small study in asthmatics, again conducted in a chamber, showed that similar levels of exposure are associated with minor reductions in forced vital capacity *(245)*.

It has been established that the presence of nitrous acid will interfere with accurate measurement of nitrogen dioxide by most commonly used methods, and it has been proposed that adverse health outcomes that have been attributed to nitrogen dioxide (or to exposure to appliances producing nitrogen dioxide, such as gas stoves) could be confounded (or explained) by exposure to nitrous acid *(243,246)*. It has been argued that the variations in the reported association of indoor nitrogen dioxide with respiratory health may be explained by failure to measure this co-pollutant *(247)*. The dearth of studies in which both have been measured has been identified as a gap in our understanding of the health effects of gas appliances *(247)*.

In a study of infants at high risk of developing asthma because they had an older sibling with physician-diagnosed asthma, both indoor nitrous acid and nitrogen dioxide were measured and lung function assessed *(40)*. As referred to earlier in this chapter, the authors concluded that nitrogen dioxide, not nitrous acid, was more closely associated with lower lung function. Nevertheless, in a small study of British adults, decrements in lung function were associated with exposure to nitrous acid in the kitchen (predicted decrease in FEV_1 0.96% (95% CI 0.09–1.82) and decrease in FEV_1/FVC 0.45% (95% CI 0.06–0.83) per 1-ppb increase in indoor nitrous acid) and the association persisted after adjustment for nitrogen dioxide *(248)*.

Health risk evaluation

The main health outcomes of interest are respiratory symptoms, bronchoconstriction, increased bronchial reactivity, airway inflammation, and decreases in immune defence leading to increased susceptibility to respiratory infection. No other health effects have been consistently associated with exposure to nitrogen dioxide in the indoor environment.

Quality of exposure and effect assessment

Controlled exposure studies in humans that assess the health effects of short-term exposures give no cause for concern regarding exposure assignment/measurement. In epidemiological studies, exposure assignment is less precise, being

based on passive samplers (which provide average levels over several hours or weeks) or on proxy measurements (e.g. the presence of a gas appliance). The latter is an imprecise estimate of actual exposure but, at group level, is associated with elevated long-term indoor exposure to nitrogen dioxide and with short-term peaks of exposure. No biomarker is available.

Controlled exposure studies in humans assessing the health effects of short-term exposures have used well-standardized, objective methods for the assessment of health effects. In epidemiological studies, self- (or maternally) reported symptoms have been widely used as the health metric. These measurements are susceptible to over-reporting by those who perceive their exposure to be high. Although lung function measures provide more objective estimates of health status, inflammatory changes and symptoms may occur in the absence of lung function changes.

Controlled exposure studies in humans have shown acute respiratory health effects of short-term exposures in healthy volunteers or those with mild pre-existing lung disease. Mechanical failure of resurfacing machines in ice arenas leading to short-term, high-dose exposures demonstrates similar effects and suggests there may be long-term sequelae. Population-based studies have shown health effects of chronic indoor nitrogen dioxide exposure in infants, children and adults.

Levels and duration of exposure

Controlled exposure studies in humans have assessed acute health effects to short-term exposure (maximum of several hours) at levels consistent with peak exposures experienced when gas appliances are used, but well above the average levels in most indoor environments. The majority of epidemiological studies have examined populations with average indoor levels that can be considered representative of longer-term population exposures.

Exposure–response relationship

There is evidence of a dose–response effect in controlled exposure studies (particularly at high levels) and in epidemiological studies. There is no evidence for a threshold in epidemiological studies. The exposure–response effect of repeated daily peak exposures to nitrogen dioxide is not known.

Susceptible population or response modifiers to consider in guideline setting

Controlled exposure studies assessing the health effects of short-term exposures show health effects at lower levels more consistently in asthmatics than in non-asthmatics, and both chamber studies and some epidemiological studies suggest that exposure enhances the response to allergens in those who are sensitized. Some epidemiological studies suggest stronger associations of respiratory health

with indoor nitrogen dioxide in females compared to males, but it is not clear whether this is due to women spending more time indoors or an underlying biological basis.

Quality of evidence

Although associations of exposure with health are well-documented, there is unexplained heterogeneity in results from well-conducted studies. This may reflect underlying variations in the nature of the exposure or in host susceptibility.

There is sufficient evidence of a causal relationship between controlled exposure to nitrogen dioxide concentrations as low as 380–560 μg/m^3 for periods of one hour or longer and a range of responses within the lung that suggest airway inflammation and alteration in lung immune defences in asthmatics. Recent systematic review and meta-analysis provides suggestive evidence that controlled exposures to as low as 188–360 μg/m^3 are associated with small increases in airway reactivity to a range of stimuli in asthmatics. Studies that have examined health effects of repeated short exposures to 500 μg/m^3 provide suggestive evidence that this is associated with exaggerated/prolonged response to allergen challenge in asthmatics/atopics. There is limited or suggestive evidence of an association between indoor nitrogen dioxide at levels currently occurring in populations and (*a*) reported respiratory symptoms in children, (*b*) increased reporting of symptoms in children with asthma, (*c*) increased asthma severity following respiratory viral infection and (*d*) reported respiratory symptoms in adults.

Health relevance of current indoor exposures in various regions of the world

Epidemiological studies conducted in several countries show that a proportion of homes and classrooms have indoor nitrogen dioxide levels exceeding the WHO ambient guidelines for outdoor air.

Indoor studies suggest that those using gas cookers, particularly in poorly ventilated spaces, can experience peak nitrogen dioxide exposures in excess of 500 μg/m^3. Important factors that may increase indoor exposure are the use of unvented gas appliances, poor ventilation, proximity to major highways and the use of propane- and petrol-fuelled ice resurfacing machines in indoor ice arenas.

Guidelines

A 1-hour indoor nitrogen dioxide guideline of 200 μg/m^3, consistent with the existing WHO air quality guideline, is recommended.

At about twice this level, asthmatics exhibit small pulmonary function decrements. Those who are sensitized may have small changes in airway responsiveness to a variety of stimuli already at this level. Studies of the indoor environment provide no evidence for an indoor guideline different to the ambient guideline.

An annual average indoor nitrogen dioxide guideline of 40 μg/m^3, consistent with the existing WHO air quality guideline, is recommended.

The ambient annual average guideline of 40 μg/m^3 was initially based on a meta-analysis of indoor studies. It was assumed that having a gas stove was equivalent to an increased average indoor level of 28 μg/m^3 compared to homes with electric stoves, and the meta-analysis showed that an increase in indoor nitrogen dioxide of 28 μg/m^3 was associated with a 20% increased risk of lower respiratory illness in children. Homes with no indoor sources were estimated to have an average level of 15 μg/m^3. Several exhaustive reviews to further develop ambient guidelines have not challenged these findings.

Recent well-conducted epidemiological studies that have used measured indoor nitrogen dioxide levels support the occurrence of respiratory health effects at the level of the guideline.

The guidelines section was formulated and agreed by the working group meeting in November 2009.

Summary of main evidence and decision-making in guideline formulation

Critical outcome(s) for guideline definition

Respiratory symptoms, bronchoconstriction, increased bronchial reactivity, airway inflammation and decreases in immune defence, leading to increased susceptibility to respiratory infection.

Source of exposure–effect evidence

- Short-term exposures: human controlled exposure experimental studies indicating minor changes in pulmonary function in people with asthma exposed to 560 μg/m^3 nitrogen dioxide for up to 2½ hours *(124–126)*. Small increases in airway reactivity to a range of stimuli in asthmatics at repeated short exposures to 500 μg/m^3 *(131,133,136,137)*.
- Long-term exposures: meta-analysis of studies on association of lower respiratory illness in children showing that an increase in indoor nitrogen dioxide of 28 μg/m^3 above the background of ca. 15 μg/m^3 was associated with a 20% increased risk of lower respiratory illness in children *(170)*.

Supporting evidence

- Significant association of various respiratory symptoms or lung function indices with nitrogen dioxide measured indoors or as personal exposure in all identified epidemiological studies of asthmatics *(23,26,160–162,164,165,168,191,268)*. Lowest measured levels were ca. 5 μg/m^3.
- Associations also found in half of the studies of non-asthmatic children *(10,27,40,146,147,148,153,154,156,158,208)*.

Results of other reviews

WHO *Air quality guidelines: global update 2005 (15)* and EC INDEX project *(5)* agreed on the same set of guidelines.

Guidelines

– 200 μg/m^3 – 1 hour average.
– 40 μg/m^3 – annual average.

Comments

No evidence from epidemiological studies for an exposure threshold.

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Table 5.1. Levels of nitrogen dioxide in various countries

Reference	Project / programme	Country	Averaging time / survey year(s) / methods	Location of measurement
Baxter et al. *(24,25)*		United States (Boston, MA)	3–4 days from 2003 to 2005 in 2 seasons (May–October, December–March)	
Belanger et al. *(164)*		United States	Palmes tubes	
Berglund et al. *(249)*	INDEX	Sweden	24 hours Urban area Rural area	Schoolchildren, personal exposure
Bernard et al. *(250)*		France	14 days (passive monitors)	
Blondeau et al. *(21)*	PRIMEQUAL	France (La Rochelle)	2 weeks	
Brauer et al. *(251)*	International survey NO_2 indoor ice skating	9 countries	1 week average	
Breysse et al. *(252)*		United States (Baltimore, MD)	72 hours	
Chao *(253)*		China (Hong Kong SAR)		
Cyrys et al. *(254)*	THADE	Germany (Hamburg) Germany (Erfurt)	1 week mean	Living room
Diette et al. *(163)*		United States (East Baltimore, MD)	72 hours	Bedroom
Dutton et al. *(33)*		United States (Boulder, CO)	4 hours in 1999	
Emenius et al. *(143)*	BAMSE birth cohort	Sweden (northwest Stockholm): Urban area Semi-urban area Suburban area	4 weeks (passive samplers)	Living room
Franklin et al. *(37)*		Australia	During summer (passive samplers)	Kitchen

Emission source	Number of homes / volunteers	NO_2 measurement results ($\mu g/m^3$) [a]	Comments
	43	37.5 (mean)	Low socioeconomic status households (I/O ratio 0.99 +/– 0.63)
Electric stoves	728 children	16.45 (SD 17.41)	
Gas stoves		49.55 (SD 34.62)	
		13 (median) 7 (median)	Most important source of exposure = indoor ice skating (levels up to 8000 $\mu g/m^3$)
	107 volunteers	31.9 (mean) / 12.7 (SD)	Personal exposure
	8 schools		I/O ratio 0.88–1
Type of fuel used in the resurfacing machine	332 ice arenas	436.16 (AM) (breathing height to the ice surface); 422.77 (spectators' area)	
	100 (bedroom)	60.45 / 76.9 (SD)	25% of the samples below the limit of detection
	60 people (personal exposure)	46 (mean) (60 personal exposures)	When cooking 59.7 $\mu g/m^3$; when not cooking 41.8 $\mu g/m^3$
	12 people (homes: living room, bedroom, kitchen)	47.3 (mean) (12 personal exposures; 55.2 (12 indoor measurements)	
	201	17 (median)	
	204	15 (median)	
	300 Inner-city preschool children (150 with asthma, 150 controls)	41.32 (median, children with asthma); 40 (median, controls)	
	2	688.68 (mean)	Measurements during operation of unvented natural gas fireplaces
Few gas combustion appliances	540		Mean outdoor levels:
(8.52% of homes with gas stove)		18.3 (mean) (8 – 45.1) 12.2 (mean) (4.4 – 25.1) 8.1 (mean) (2.3 – 21.1)	31.5 (17.9–46.7) 21.6 (8.7–36.4) 13.7 (6–29)
Gas cooker	53	Average 16.2 (12.7–20.6); peak 45.3 (36–57.1)	

[a] AM = arithmetic mean, SD = standard deviation, GM = geometric mean, max = maximum value.

Reference	Project / programme	Country	Averaging time / survey year(s) / methods	Location of measurement
Gallelli et al. *(255)*	THADE	Italy (Genoa)	2 months in 2000 (passive diffusion tubes)	Kitchen Bedroom
Garcia Algar et al. *(256)*		United Kingdom (Ashford) Spain (Menorca) Spain (Barcelona)	Between 7 & 15 days (passive filter badges)	
Garcia Algar et al. *(13)*		Spain (Barcelona)	Over 7–30 days between 1996 & 1999 (passive filter badges)	
Garrett et al. *(31)*	THADE	Australia (Latrobe Valley, Victoria)	4 days in 1994/1995 (passive samplers)	
Gee et al. *(257)*	THADE	United Kingdom (Manchester)	5 days	Living room Bedroom
Gilbert et al. *(258)*		Canada (Quebec City)	7 days between January & April 2005 (passive monitors)	
Guo et al. *(259)*	INDEX	China (Hong Kong SAR)	15 minutes 15 minutes	Ice skating arenas
Hagenbjork-Gustafsson et al. *(7)*	THADE PEACE	Sweden (Umeå, suburban control)	2 x 24 hours between January & March 1994	
Hazenkamp-von Arx et al. *(16)*	ECRHS II	21 European study centres of ECRHSII (from northern Italy to Iceland)	14 days in 2000 (passive sampler)	
Kattan et al. *(23)*	NCICAS	United States (8 inner city areas)	7 days (Palmes tubes)	Child's sleeping area

Emission source	Number of homes / volunteers	NO_2 measurement results ($\mu g/m^3$) [a]	Comments
	89	47 (indoor mean) 24.78 (indoor mean) 24.9 +/– 7.8 (students, personal exposure); 44.3 +/–10.1 (workers); 40 +/– 13.4 (housewives)	
Gas combustion appliances, cigarette smoking	1421 (living room wall)	11.07 (median) 11.59 (median) 45.66 (median)	
	340	45 (mean in 1996); 53.22 (mean in 1997); 51.69 (mean in 1999)	
Gas stoves, vented gas heaters, smoking	80	11.6 (median, ranging from < 0.7 to 246)	Highest levels recorded in winter
	69	27.2 (AM) 20.3 (AM)	
	96	8.3 (3.3–29.1)	
Gasoline fuelled Propane fuelled		58 – 91 (AM) 242 (AM)	
No gas appliances	23 (urban area) 20 (suburban control area)	11 (mean in urban area) 6 (mean in control area)	I/O ratio: 0.44 I/O ratio: 0.67 Heavier traffic density in Umeå
		Annual mean from 4.9 to 72.1	
Gas stoves	469 (1444 children)	57 (median)	Indoor levels considerably higher than US national outdoor median value (34.43)

[a] AM = arithmetic mean, SD = standard deviation, GM = geometric mean, max = maximum value.

Reference	Project / programme	Country	Averaging time / survey year(s) / methods	Location of measurement
Kousa et al. *(260)*	EXPOLIS	Switzerland (Basel) Finland (Helsinki) Czech Republic (Prague)	48 hours in 1996/1997 (passive samplers)	
Lambert et al. *(11)*	THADE	United States (Albuquerque, NM)	2 weeks mean in 1988–1991 (passive diffusion samplers)	Kitchen Living room Bedroom
Lawrence et al. *(30)*		India (Agra)	October 2002 to February 2003 (multigas monitor)	
Lee & Wang *(41)*	INDEX			Chamber test
Lee et al. *(39)*		United States (2 communities: Upland & San Bernardino County, southern California)	6 days in April & May 1996 (passive samplers)	
Leung et al. *(9)*	THADE	China (Hong Kong SAR)	1 week mean (stationary samplers)	Kitchen Bedroom Lounge
Levy et al. *(3)*	THADE	Finland (Kuopio) Norway (Kjeller) Switzerland (Geneva) Germany 1 (Erfurt) Canada (Ottawa) Germany 2 (Berlin) Croatia (Zagreb) United States (Boston, MA) United Kingdom 3 (London) Japan 2 (Sapporo) Philippines (Manila) China (Beijing) Poland (Sosnowiec) Republic of Korea 1 (Taejon) India (Bombay)	48 hours in 1996	

Emission source	Number of homes / volunteers	NO_2 measurement results ($\mu g/m^3$) [a]	Comments
	262 adults	27/13 (SD in residential indoor) to 36/24 (SD in workplace) 18/11 (SD in residential indoor) to 27/15 (SD in workplace) 43/23 (SD in residential indoor) to 30/18 (SD in workplace)	Mean outdoor levels: 36 +/– 13 24 +/– 12 61 +/– 20
Gas cooking stoves	1205 children / homes	65.04 (AM) 55.48 (AM) 40.17 (AM)	Lower indoor concentration observed during the summer
		487.81/279.3 (SD)	Measurements conducted in rural, urban and roadside
Mosquito coils and candles burning		17–91 (AM)	NO_x most abundant gas pollutants relating to candle burning
	119 homes (57 in Upland, 62 in San Bernardino)	53.56 (mean)	Average indoor NO_2 concentration (38.26 $\mu g/m^3$) was significantly higher than outdoor concentrations
	40 homes	93.16 (AM) 58.1 (AM) 59.6 (AM)	
	30 30 33	10.34 (mean) 14.66 (mean) 15.60 (mean)	
	29 29 31 15 20	16.97 (mean) 20.12 (mean) 23.12 (mean) 31.58 (mean) 36.10 (mean)	
	117	40.42 (mean)	
	59 14	43.43 (mean) 45.12 (mean)	
	44 15	47.75 (mean) 64.67 (mean)	
	40	72.76 (mean)	
	20	76.70 (mean)	

[a] AM = arithmetic mean, SD = standard deviation, GM = geometric mean, max = maximum value.

Reference	Project / programme	Country	Averaging time / survey year(s) / methods	Location of measurement
		Japan 3 (Tokushima) Republic of Korea 2 (Seoul) Mexico (Mexico City)		
Lindgren *(261)*				Cabins
Mi et al. *(262)*		China (Shanghai)	7 days (diffusion samplers)	30 classrooms
Monn et al. *(263)*	THADE SAPALDIA	Switzerland (4 urban, 2 rural & 2 alpine areas)	1 week in 1993/1994 (passive sampler)	Personal exposure
Mosqueron et al. *(264)*		France (Paris)	48 hours (passive samplers)	Personal exposure Living room Indoor office
Nakai et al. *(18)*		Japan (Tokyo)	10 seasons over 3 years (personal exposure)	
Noy et al. *(265)*	INDEX	Netherlands	3 measurements of 1 week within a year (Palmes tubes)	Kitchen
Osman et al. *(187)*		United Kingdom(North East Scotland)	1 week	Living room
Pennanen et al. *(42)*	INDEX	Finland	Max 15 minutes Max 1 hour	Indoor ice arenas
Pennanen et al. *(266)*	INDEX	Finland	1 week	

Emission source	Number of homes / volunteers	NO_2 measurement results ($\mu g/m^3$) [a]	Comments
	30	78.77 (mean)	
	31	81.22 (mean)	
	30	117.88 (mean)	
	26; intercontinental flights (Boeing 767-300)	14.1 (mean) 37 (max)	
	10 naturally ventilated schools	33–85	Outdoor : 45 – 80 $\mu g/m^3$ I/O ratio : 0.63 – 1
Gas cooking Smoking	more than 500 subjects	27 (average personal exposure) 21 (average indoor measurements)	Personal exposure correlated best with indoor; outdoor average 31 $\mu g/m^3$
	62 office workers	43.6 (mean personal exposure) 35.1 (mean in home) 44.9 (mean in office)	
Gas cooking stoves, heaters	50 residents from 3 residential zones A: 0–20 m from the roadside B: 20–150 m from the roadside C: residential district, suburban area	 121.28 (mean) 116.7 (mean) 105.79 (mean)	Outdoor concentrations in zone A were always the greatest
Gas stoves		2500 (max)	
Smoking (39% of the 148 patients)	148 patients (home measurements)	14.92 (mean)	
Ice resurfacing machines with combustion engines		320 – 7530 (max) 270 – 7440 (max)	Highest levels with propane-fuelled ice resurfacing machines and insufficient ventilation
Ice resurfacing machine: propane petrol electric	69 Indoor ice arenas	2–1838 396 (AM) 283 (AM) 25 (AM)	

[a] AM = arithmetic mean, SD = standard deviation, GM = geometric mean, max = maximum value.

Reference	Project / programme	Country	Averaging time / survey year(s) / methods	Location of measurement
Piechocki-Minguy et al. *(267)*		France	2–24-hour sampling periods (1 during a working day, 1 during the weekend) (personal measurements, diffusion samplers)	4 categories of microenvironment
Pilotto et al. *(27)*		Australia	Hourly peak level (personal exposure)	
Pilotto et al. *(268)*		Australia	9 days (classrooms) 3 days (each household)	Classrooms Dwellings
Ponzio et al. (269)	ECRHS II	Italy (Pavia)	2001–2002	
Raw et al. *(270)*	National representative survey	England	2 weeks in 1997–1999 (Palmes tubes)	Kitchen Bedroom
Riedeker et al. *(271)*		United States (California)	25 work shifts (3 p.m. to midnight) in autumn 2001	
Ross *(272)*		United Kingdom	7-day average and maximum 1-hour average (Palmes samplers)	12 homes
Sabin et al. *(273)*		United States (Los Angeles, CA)	24 morning and afternoon commutes & 10 additional runs	
Saintot et al. *(274)*	THADE SUVIMAX	France	2 periods of 5 consecutive days (1 in winter, 1 in autumn of 1998)	
Sakai et al. *(275)*	INDEX	Sweden (Uppsala) Japan (Nagoya)	24 hours in February through May 1998, 24 hours in February 1998 (diffusion sampler)	Urban dwellings Urban dwellings
Salonen et al. *(43)*			Personal exposure	

Emission source	Number of homes / volunteers	NO_2 measurement results ($\mu g/m^3$) [a]	Comments
		From 17 on summer weekend to 38 on winter weekday	Indoor environments contributed more than 78% to total personal exposure
Unflued gas appliances at home	41 classrooms (388 children)	153 (max)	
Unflued gas heating	10 control schools (unflued gas heating) 8 intervention schools (replacement flued gas or electric heaters installed)	89.91/51.26 (SD, control schools) 29.65/12.62 (SD, intervention schools)	
	114	47.1/24.5 (SD)	
	845 homes	21.8 (GM) 11.9 (GM)	
	Patrol cars	41.7 (mean)	
Kitchen, living room, bedroom		191–1148 (hourly mean)	
	Conventional diesel school buses	64–220; 370 (max)	
	294	43/26.1 (SD) in winter 43.8/20.6 (SD) in autumn	
	27 37	6.7 (GM); 11 (max) 98 (GM); 369 (max)	
Ice resurfacing machines	793 young ice hockey players	21–1176	

[a] AM = arithmetic mean, SD = standard deviation, GM = geometric mean, max = maximum value.

Reference	Project / programme	Country	Averaging time / survey year(s) / methods	Location of measurement
Shima et al. *(10)*	THADE	Japan (Chiba)	24-hour mean (2 × 24 hours, 1 in January or February 1993, 1 in June or July 1993 (passive samplers)	
Simoni et al. *(12)*	INDEX	Italy (Po Delta)	2 weeks (1 in winter, 1 in summer)	
Simoni et al. *(188)*	THADE	Italy (Pisa, urban) Italy (Po Delta, rural)	1 week (summer or winter) in 1991–1994	
Son et al. *(276)*	THADE	Korea (Asan & Chuna)	Passive samplers; house measurements & personal exposure	
Thunqvist et al. *(236)*		Sweden	3 consecutive days (passive diffusion samplers)	
Yang et al. *(46)*		Australia (Brisbane) Republic of Korea (Seoul)	30 consecutive days 21 consecutive days (passive filter badges)	
Zhao et al. *(277)*		China (Taiyuan)		
Zipprich et al. *(278)*		United States (Richmond, VA)	48 hours (personal exposure & indoor concentrations, July to September, passive samplers)	
Zmirou et al. *(279)*		France (Paris, Nice, Toulouse, Clermont-Ferrand, Grenoble)	48 hours (personal exposure & indoor measurements) between 1998 and 2000	Personal exposure & indoor measurements
Zota et al. *(32)*		United States (Boston, MA)	Palmes tubes	Kitchen Living room

Emission source	Number of homes / volunteers	NO_2 measurement results ($\mu g/m^3$) [a]	Comments
	842 schoolchildren	Homes with vented heaters 35.2 (mean) in winter; homes with unvented heaters 32 (mean) in winter	Concentrations in winter very much higher in homes with unvented heaters than in those with vented heaters
	383 adults (homes)	38.26 (median in winter); 26.78 (median in summer); 63.13 (kitchen in winter); 38.26 (kitchen in summer)	
	282 139	28.7 (mean) 42.09 (mean)	
	31 taxi drivers (houses)	47.25 /20.47 (SD in houses); 57.96 /18.56 (SD personal exposure)	Personal exposure more strongly correlated with interior of vehicle
Combustion engine-powered resurfacing machine	9 propane machines 6 electric machines	276 (28–015) 11 (2–30)	
	28 houses 37 houses	15.8 / 18.2 (SD) 44.7 / 38.1 (SD)	I/O ratio 0.88 +/– 0.32
	10 schools	39.4 (mean)	Outdoor levels were 2–3 times higher
	39 adults 9 children in 23 households	30.6 (personal exposure) 24.87 (personal exposure) 34.43 (bedrooms) 36.34 (living rooms)	
	217 pairs of matched 4–14-year-old cases & controls	36.1/21.4 (SD, indoor concentrations); 31.4/13.9 (SD, personal exposure)	Indoor levels higher in the heating season
Gas stove use Reduced air exchange rate during the heating season	77 homes	82.26/38.26 (SD) 68.87/32.52 (SD)	

[a] AM = arithmetic mean, SD = standard deviation, GM = geometric mean, max = maximum value.

Table 5.2. Studies that have examined associations between respiratory symptoms and indoor measurements of or personal exposure to nitrogen dioxide

Reference	Participants	NO_2 exposures in the study
Florey et al. *(148)*	Children	One week mean bedroom (mean): electric cooker 5.64–69.6 µg/m^3; gas cooker 7.52–317.7 µg/m^3
Hoek et al. *(151)*	Children aged 6 years	One-week average (range): kitchen 110–789 µg/m^3; living room 17–277 µg/m^3; bedroom 10–146 µg/m^3
Dijkstra et al. *(150)*	Children aged 6–12 years	Given in Fig. 2 of paper
Koo et al. *(155)*	Children aged 7–13 years	24-hour personal mean: children: 35.9 µg/m^3; mothers: 36.5 µg/m^3
Neas et al. *(153)* (supported by Li et al. *(154)*)	Children aged 7–11 years	Household annual average: without an NO_2 source 16.1 µg/m^3; with an NO_2 source 44.2 µg/m^3
Infante-Rivard et al. *(156)*	Children aged 3–4 years	24-hour personal average: without an NO_2 source 17.3 µg/m^3; with an NO_2 source 32.3 µg/m^3
Samet et al. *(140)*	Infants	Two-week average bedroom: 22% of measures greater than 37.6 µg/m^3
Pilotto et al. *(27)*	Children aged 6–11 years	Winter six-hour average in classrooms: electrically heated, range 13.2–43.2 µg/m^3; gas heated, range 33.8–248.2 µg/m^3
Garret et al. *(152)*	Children aged 7–14 years	Bedroom, living room and kitchen: "indoor" median 11.6 µg/m^3 (5.01–27.9 as 10th & 90th centiles)

Results
Prevalence of respiratory illness: 0–37.6 μg/m^3, 44%; 37.6–75.2 μg/m^3, 59%; > 75.2 μg/m^3, 71%; *P* for trend < 0.05
No association of levels in any of the three locations with symptoms
Odds ratio of symptoms comparing > 60 μg/m^3 Cough 0.80 (0.21–3.05) Wheeze 0.94 (0.37–2.40) Asthma 0.56 (0.15–2.06) No association with lung growth
Unadjusted comparison of mean levels in those with and without symptoms: cough *P* = 0.99 children; asthma *P* = 0.42 children; allergic rhinitis *P* = 0.75 children
Cumulative incidence of respiratory illness: adjusted OR 1.4 (1.14–1.72) per 28.2 μg/m^3
Adjusted OR for asthma: not detectable, 1.00; <18.8 μg/m^3, 0.95 (0.31–2.95); 18.8–28.2 μg/m^3, 3.85 (0.92–16.09); ≥ 28.2 μg/m^3 19.87(4.75–83.03)
No association
Adjusted difference in mean symptom rates for high (≥ 75.2 μg/m^3) compared to low (<75.2 μg/m^3): Sore throat *P* = 0.03 Cough with phlegm *P* = 0.06 Dry cough *P* = 0.9 Wheeze *P* = 0.2 Absent from school *P* = 0.01 Adjusted OR for having at least one day with symptom for high (≥ 75.2 μg/m^3) compared to low (< 75.2 μg/m^3): Sore throat 1.39 (0.80–2.41) Cough with phlegm 1.28 (0.76–2.5) Dry cough 1.08 (0.62–1.90) Wheeze 1.41 (0.63–3.15) Absent from school 1.92 (1.13–3.25)
Adjusted OR per 10 μg/m^3: Cough 1.47 (0.99–2.18) Wheeze 1.15 (0.85–1.54) Asthma attacks 1.06 (0.77–1.46)

Reference	Participants	NO_2 exposures in the study
Magnus et al. *(142)*	Infants from birth to 2 years	Mean living room level 14.7 μg/m^3 (2–43 μg/m^3)
Mukala et al. *(158)*	Pre-school children	Study median 21.1 μg/m^3
Shima et al. *(10)*	Children aged 9–10 years	Mean annual living room: Vented appliances 34.5 μg/m^3 Unvented appliances 60.9 μg/m^3 Winter mean: Vented appliances 45.1 μg/m^3 Unvented appliances 141.1 μg/m^3
Ponsonby et al. *(208)*	Primary school children	Mean personal exposure 19.0 μg/m^3
Emenius et al. *(143)*	Children from birth to 2 years	Mean living room: Urban, no gas stove 16.4 μg/m^3 Urban, gas stove 22.6 μg/m^3 Semi-urban 12.2 μg/m^3 Suburban 8.1 μg/m^3
Belanger et al. *(147)*	Infants who had a sibling with asthma	Two-week mean living area: interquartile range 9.6–32.7 μg/m^3
van Strien et al. *(40)*	Infants from birth to 1 year who had a sibling with asthma	Two-week mean living area: interquartile range 9.6–32.7 μg/m^3
Sunyer et al. *(145)*	Infants	Median in each of three centres: 10.7, 22.2 & 86.2 μg/m^3
Diette et al. *(163)*	Children aged 2–6 years	
Raaschou-Nielsen et al. *(144)*	Infants from birth to 18 months	Mean ten-week average bedroom 8.6 μg/m^3 (95% range 3.3–17.6 μg/m^3)

Results
No association with symptoms of bronchial obstruction and physical signs as identified by physician
Adjusted risk ratio of reporting cough during the same week as measurement: < 16.2 μg/m^3, 1.00; 16.2–27.2 μg/m^3, 1.23 (0.89–1.70); ≥ 27.2 μg/m^3, 1.52 (1.00–2.31) *Adjusted for allergy, stove type, smoking, parental education, day care centre, season*
Adjusted OR per 18.8 μg/m^3 increase: *Boys* Wheeze 0.98 (0.68–1.39) Asthma 0.77 (0.48–1.20) *Girls* Wheeze 1.90 (1.30–2.83) Asthma 1.63 (1.06–2.54)
No association with incidence of wheeze or asthma over 3-year period Difference in post-cold-air challenge FEV_1/FVC ratio per 1.88 μg/m^3: –0.12 (–023 to –0.01) *Adjusted for height, sex, technician, smoking exposure, days between NO_2 measurements, spirometry*
Recurrent wheezing: < 8.4 μg/m^3, 1.00; 8.4–11.7 μg/m^3, 0.97 (0.42–2.24); 11.7–15.6 μg/m^3, 1.17 (0.46–2.99); > 15.6 μg/m^3, 1.48 (0.91–2.42) *Adjusted for gender, family history of asthma, smoking, breastfeeding, age of building*
Persistent cough per 18.8 μg/m^3 increase in living room NO_2: 1.21 (1.05–1.40) *NB: most of the pertinent data from this study presented in paper by van Strien (40)*
Adjusted relative rate of symptoms (1st, 2nd, 3rd, 4th quartiles of NO_2): Wheeze 1.00; 1.15 (0.79–1.67); 1.03 (0.69–1.53); 1.45 (0.92–2.27) Persistent cough 1.00; 0.96 (0.69–1.36); 1.33 (0.94–1.88); 1.52 (1.00–2.31) Shortness of breath 1.00; 1.59 (0.96–2.62); 1.95 (1.17–3.27); 2.38 (1.31–4.34) *Quartile cut-offs for analyses 5.1, 9.9, 17.4 μg/m^3* *Adjusted for season, parental asthma, mother's ethnicity, mother's education, smoking in the home, day care, living in an apartment, presence of siblings, gender, nitrous acid*
Adjusted OR for lower respiratory tract illness: < 9.4 μg/m^3, 1.00; 9.4–18.8 μg/m^3, 0.88 (0.63–1.23); 18.8–56.4 μg/m^3, 0.99 (0.69–1.43); ≥ 56.4 μg/m^3, 1.31 (0.75–2.26)
Median (IQR) three-day bedroom NO_2 (μg/m^3): children without asthma, 39.3 (26.3–58.3); children with asthma, 40.6 (26.3–63.9); $P = 0.84$
Adjusted odds of wheezing in first 18 months: < 5.2 μg/m^3, 1.00; 5.2–6.8 μg/m^3, 0.66 (0.27–1.61); 6.8–8.6 μg/m^3, 0.80 (0.32–2.01); 8.6–11.7 μg/m^3, 1.15 (0.40–3.32); ≥ 17.7 μg/m^3, 0.43 (0.15–1.18) *Adjusted for sex, area, mother's education, lung function*

Reference	Participants	NO_2 exposures in the study
Morales et al. *(146)*	Children aged 4 years	Median two-week average living room 21.6 µg/m^3; range 0.8–185.9 µg/m^3
Adults		
Keller et al. *(180,181)*	Adults	Mean 24-hour average. cooking with electricity 37.6 µg/m^3 ; cooking with gas 94.0 µg/m^3
Fischer et al. *(185)*	Women aged 40–60 years	Weekly mean: kitchen 18.8 –735 µg/m^3 ; living room 15.1–372.4 µg/m^3 ; bedroom 15.1–99.6 µg/m^3
Koo et al. *(155)*	Mothers	Personal NO_2: user of LPG/kerosene (no fan) 37.7 µg/m^3; user of LPG/ kerosene (fan) 41.7 µg/m^3
Simoni et al. *(188)*	Population-based sample	Seven-day mean indoor (average of kitchen, bedroom and living room): Pisa summer 24.4 µg/m^3; Po Delta summer 28.2 µg/m^3; Pisa winter 28.2 µg/m^3; Po Delta winter 41.4 µg/m^3
Triche et al. *(186)*	Mothers	Two-week average No source: median 25.3 µg/m^3; max 276 µg/m^3; source: median 22.7 µg/m^3; max 312.8 µg/m^3
Studies in asthmatics		
Smith et al. *(160)*	Asthmatics (children and adults)	Personal indoor daily mean 6.9–275.6 µg/m^3
Chauhan et al. *(165)*	Asthmatics	Geometric mean personal exposure 10.6 µg/m^3
Pilotto et al. *(268)*	Children with asthma (randomized controlled trial)	Six-hourly average levels: intervention classes 13.2–71.4 µg/m^3; non-intervention classes 22.5–218.1 µg/m^3

Results
Change in cognitive function score (95% CI) per 1.88-μg/m^3 increase; GSTP genotype: Ile/Ile 0.10 (– 0.22 to 0.42); Ile/Val or Val/Val –0.55 (– 0.86 to –0.25); *P* for interaction = 0.04
Adjusted OR (95% CI) of inattention symptoms; GSTP genotype: Ile/Ile 0.98 (0.88–1.09); Ile/Val or Val/Val 1.11 (1.03–1.20); *P* for interaction = 0.26
Adjusted for maternal social class, maternal education, school age, observer, maternal smoking in pregnancy, number of smokers in home, maternal alcohol consumption during pregnancy, home location
No association with respiratory illness observed
Adjusted difference in annual decline in FEV_1 (se) per unit change in NO_2: kitchen –0.025 ml (0.021); living room –0.048 ml (0.043); bedroom –0.164 ml (0.165)
Personal exposure level Without chronic cough 35.9 μg/m^3; with chronic cough 42.3 μg/m^3; *P* = 0.05 Without allergic rhinitis 35.5 μg/m^3 ; with allergic rhinitis 42.4 μg/m^3; *P* = 0.002
Generated personal exposure as product of hours per day indoors and measured indoor levels Divided exposure into two categories (above and below the median) Adjusted OR for acute respiratory symptoms with fever (high vs low): 1.66, 95% CI 1.08–2.57 No association with asthma/bronchitic symptoms without fever, or with peak flow, or with irritant symptoms, or with non-specific symptoms (values not given) *Adjusted for sex, age, active smoking and area of residence*
Adjusted OR of symptom comparing > 150.4 μg/m^3 with other measures: wheeze 4.00 (1.45–11.0); chest tightness 1.94 (0.98–3.85)
Adjusted OR in under 14-year-olds: wheeze 1.04 (0.89–1.12); cough 1.07 (0.89–1.29); daytime attacks of asthma 1.13 (1.02–1.26) No associations seen in other age groups
Risk of asthma exacerbation following infection
Mean rate ratio (intervention vs control) Difficulty breathing in day 0.41 (0.07 –0.98) Difficulty breathing at night 0.32 (0.14–0.69) Chest tightness 0.45 (0.25–0.81) Asthma attacks during the day 0.39 (0.17–0.93)

Reference	Participants	NO_2 exposures in the study
Nitschke et al. *(168)*	Children with asthma aged 5–13 years	Indoor daily mean (range): classrooms 16.9–577.2 μg/m^3; kitchens 5.6–795.4 μg/m^3
Belanger et al. *(164)*	Children with asthma aged < 12 years	Median average 10-day living room: homes without a source 16.2 μg/m^3; homes with a source 48.7 μg/m^3 Mean average 10-day living room: single-family homes 19.2 μg/m^3; multi-family housing 43.1 μg/m^3
Delfino et al. *(161)*	Children with asthma aged 9–18 years	Personal exposure: range 5.1–198.7 μg/m^3
Delfino et al. *(162)*	Children with asthma aged 9–18 years	Personal exposure: range 5.1–198.7 μg/m^3
Kattan et al. *(23)*	Children with asthma aged 4–9 years	Bedroom: median 56.0 μg/m^3 (0.9–902.4 μg/m^3)
Hansel et al. *(26)*	Children with asthma aged 2–6 years (same asthmatic children as included in Diette et al. *(163)*)	Mean three-day average bedroom: without gas stove 31.6 μg/m^3; with gas stove 62.3 μg/m^3
Ng et al. *(191)*	Adults with asthma	NO_2 levels measure while cooking: highest peak seen 500 μg/m^3; personal exposure 37.6–135.6 μg/m^3

Results
Adjusted relative rates of symptoms per 18.8 μg/m^3 increase NO_2 Classroom: Nocturnal wheeze 0.99 (0.93–1.06) Nocturnal cough 1.01 (0.98–1.04) Nocturnal asthma attacks 1.00 (0.93–1.08) Nocturnal difficulty breathing 1.11 (1.05–1.18) Kitchen: Nocturnal wheeze 1.00 (0.90–1.11) Nocturnal cough 0.99 (0.96–1.02) Nocturnal asthma attacks 1.04 (1.00–1.07) Nocturnal difficulty breathing 1.03 (1.01–1.05) Mean FEV_1 predicted –0.39% per 18-μg/m^3 increase in NO_2 No consistent evidence of interaction of NO_2 with Der p 1
Adjusted OR of any symptom per 37.6 μg/m^3 increase Single-family home: wheeze 0.99 (0.71–1.38); cough 1.07 (0.84–1.35); chest tightness 1.10 (0.78–1.57) Multi-family housing: wheeze 1.52 (1.04–2.21); cough 1.06 (0.75–1.49); chest tightness 1.61 (1.04–2.49)
Increase in FeNO: 1.6 ppb per 32-μg/m^3 increase in personal NO_2
Change in FEV_1 as percentage of predicted FEV_1: –2.45 (–1.33 to –3.57)
Highest quartile compared to lowest three quartiles: More than 4 days of symptoms in last fortnight 1.75 (1.10–2.78) in non-atopics; 1.12 (0.86–1.45) in atopics
Adjusted incidence ratio for symptoms per 37.6 μg/m^3 increase: limited speech due to wheeze 1.17 (1.08–1.27); coughing without a cold 1.15 (1.07–1.23); nocturnal symptoms 1.12 (1.04–1.19)
Fall in peak flow was related to level of NO_2 measured while cooking

6. Polycyclic aromatic hydrocarbons

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General description

The term polycyclic organic matter (POM) defines a broad class of compounds that generally includes all organic structures containing three or more fused aromatic rings. These structures can contain the elements carbon, hydrogen, oxygen, nitrogen and sulfur.

POM containing up to seven fused rings has been identified, and theoretically millions of POM compounds could be formed; however, only about 100 species have been identified and studied. The most common subclass of POM is the polycyclic aromatic hydrocarbons (PAHs). These compounds contain only carbon and hydrogen *(1)*.

PAHs are a large group of organic compounds with two or more fused aromatic (benzene) rings *(2)*. Low-molecular-weight PAHs (two and three rings) occur in the atmosphere predominantly in the vapour phase, whereas multi-ringed PAHs (five rings or more) are largely bound to particles. Intermediate-molecular-weight PAHs (four rings) are partitioned between the vapour and particulate phases, depending on the atmospheric temperature *(3)*. Particle-bound PAHs are considered to be very hazardous to human health. Benzo[*a*]pyrene (B[*a*]P) is often used as a marker for total exposure to carcinogenic PAHs, as the contribution of B[*a*]P to the total carcinogenic potential is high (in one study reported as being in the range 51–64%) *(4)*.

B[*a*]P (CAS Registry Number, 50-32-8; $C_{20}H_{12;}$ molecular weight = 252.31 g/mol) is a pale yellow monoclinic crystal with a faint aromatic odour. It has a melting point of 179 °C, a high boiling point of 496 °C at 1 atm, a Henry's Law constant of 4.8×10^{-5} kPa.m^3/mol and a low vapour pressure of 7.3×10^{-7} Pa at 25 °C. As a consequence of these physical properties, B[*a*]P is predominantly particle phase rather than gas phase.

PAHs have a relatively low solubility in water (e.g. solubility in water of B[*a*]P at 25 °C is 3.8 µg/l) but are highly lipophilic (e.g. B[*a*]P log K_{ow} = 6.04)[1] and are soluble in most organic solvents. Once adsorbed on to soil, PAHs have low mo-

[1] The octanol–water partition coefficient (K_{ow}) is a measure of the hydrophobicity of a compound. It is a measure of the distribution of a compound between water and an organic (octanol) with which is in contact *(6)*.

bility (e.g. B[*a*]P log K_{oc} = 6.6–6.8).[2] Therefore, once released into the environment and owing to their low aqueous solubility, elevated octanol–water and organic carbon coefficients as well and high melting and boiling points, PAHs have a tendency to be associated with particulate matter, soils and sediments *(2,5)*.

In the atmosphere, PAHs may be subject to direct photolysis, although adsorption to particulates can retard this process. PAHs can also react with pollutants such as ozone, hydroxyl radicals, nitrogen dioxide and sulfur dioxide, yielding diones, nitro- and dinitro-PAHs, and sulfonic acids, respectively *(2)*. PAHs may also be degraded by some fungi and microorganisms in the soil and can be metabolized by a wide variety of terrestrial and aquatic organisms *(7)*, although they are expected to bioconcentrate in organisms (aquatic and terrestrial) that cannot metabolize them *(2,8)*.

Conversion factors

At 760 mmHg and 20 °C, 1 ppm of B[*a*]P = 10.494 mg/m^3 and 1 mg/m^3 = 0.095 ppm; at 25 °C, 1 ppm of B[*a*]P = 10.318 mg/m^3 and 1 mg/m^3 = 0.097 ppm *(9)*.

Sources and pathways of exposure

Sources

PAHs are widespread environmental pollutants that are formed in the combustion process of carbonaceous materials at high temperature *(10)*. Indoor air is contaminated by PAHs, which come not only from infiltration or intrusion of outdoor air but also from indoor emission sources such as smoking, cooking, domestic heating with fuel stoves and open fireplaces, as well as from incense and candle emissions *(11–16)*.

For lower-molecular-weight PAHs, the impact of house characteristics and indoor activities tends to be greater than the influence of the penetrating outdoor air. On the other hand, while indoor sources may exist for PAHs with two or three rings, outdoor air may contribute significantly to the indoor PAHs, especially those with four or more rings *(17)*.

Emissions from traffic have been found to be the main outdoor source for the indoor PAH concentration at urban and suburban locations in many industrialized countries *(18)*. Motor vehicle emissions account for around 46–90% of the mass of individual PAHs in ambient air particles in urban areas *(19)*, while domestic heating can account for some 16% of PAHs in outdoor air in the United States, 29% in Sweden and 33% in Poland, as reported in the early 1980s *(20)*. Other outdoor sources of PAHs are industrial plants, power generation plants, waste incinerators and open burning. The age of a house or building, since it reflects its condition, affects PAH concentrations indoors. For example, the older

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[2] The organic carbon coefficient (K_{oc}) is a measure of a chemical compound's mobility in soil and the prevalence of leaching from soil *(6)*.

a house the higher the PAH concentrations will be, as outdoor sources have a greater impact owing to higher air exchange through such routes as poorly fitting windows *(21)*.

In industrialized countries, ETS appears to have the greatest impact on the total PAH concentration indoors and it is identified as the single largest source of PAHs in the indoor environment, with significant emission factors associated with smoking *(22)*. Although reductions in the emission of PAHs in mainstream cigarette smoke have been reported (Table 6.1), the concentration of B[*a*]P in a room extremely polluted with cigarette smoke could still be as high as 22 ng/m^3 *(23)*. In smokers' homes, more than 87% of the total PAHs may be attributable to this source. On the other hand, background sources are the largest contributor to PAHs in non-smokers' homes *(24)*.

Cooking and heating with solid fuels such as dung, wood, agricultural residues or coal, especially in unvented or flueless stoves, is likely to be the largest source of indoor air pollution globally owing to the high level of use of these fuels in developing countries. More than 75% of people in China, India and nearby countries, and 50–75% of people in parts of Africa and South America, use solid fuel for cooking *(25)*.

Concerning data reported on emission factors for B[*a*]P (Table 6.1) and PAHs (Table 6.2) from different fuels, these can be ranked as briquettes < wood < wood/root-fuel mixtures according to their polluting potential, and natural gas < coal < briquettes < wood according to their B[*a*]P and PAH emission factors, respec-

Table 6.1. Benzo[*a*]pyrene emission factors

Source	Emission factor	Unit	Comment	Reference
Cigarettes	35	ng/cigarette	Average content in mainstream smoke before 1960	WHO *(23)*
	18	ng/cigarette	Average content in mainstream smoke, 1978–1979	WHO *(23)*
Fuel	0.8 1.6–8.2 5.3–13.2	mg/kg mg/kg mg/kg	Peat briquettes Wood Mixture of wood and root-fuel	Kakareka et al. *(26)* Kakareka et al. *(26)* Gupta et al. *(27)*; Venkataraman et al. *(28)*
Candles	n.d.–0.13[a]	ng/g of wax burned	Candles	Lau et al. *(16)*
Creosote	58–749	µg/g	Creosote-impregnated wood products	Ikarashi et al. *(29)*

[a] n.d. = not determined.

Table 6.2. PAH emission factors

Source	Emission factor	Units	Comment	Reference
Fuel	1–2000	pg/kg	Natural gas	Rogge et al. *(30)*
	0.95–2.0	mg/kg	Coal	Oanh et al. *(31)*
	2.8–3.0	mg/kg	Briquettes	Venkataraman et al. *(28)*
	2.0–114	mg/kg	Wood	Venkataraman et al. *(28)*; Oanh et al. *(31)*; Schauer et al. *(32)*; Ravindra et al. *(33)*
Candles	4.75–156	ng/g of wax burned	Candles	Lau et al. *(16)*

Note: Different authors might report different groups of PAHs.

tively. However, caution should be exercised, as different studies report different ranges of compounds, which might not be comparable. Data on emission factors from burning candles show that this source emits less than cigarettes and fuels *(16)*.

Wood burning in fireplaces and wood/solid fuel stoves is used as the main source of heating in developing countries and as a secondary heating source in countries with a cold winter climate. The burning of fossil fuel, solid fuel and biomass has been recognized as an important source of airborne PAHs as it releases a wide range of air pollutants, including PAHs, which are emitted to the indoor atmosphere in unvented or flueless combustion and also to the outdoor air *(34)*. Even in airtight stoves with a flue, elevated indoor levels of PAHs can result from intrusion of outdoor air and/or leakage from wood-burning appliances *(35)*.

High concentrations of particulate PAH compounds have been reported in indoor environments during the burning of fossil fuels and biofuel for cooking *(36)*, generally in unvented stoves, suggesting that exposure during the cooking period is 2–10 times higher than ambient exposure *(37)*. Concentrations of PAHs and B[*a*]P indoors, using different types of cooking fuel, increased in the order LPG < kerosene < coal < wood < dung cake/wood mixture < dung cake as reported in Tables 6.3 and 6.4, respectively. Transient high concentration peaks were reported in measurements performed during cooking *(38)*.

Apart from cooking fuel being a source of PAHs, generated particularly in unvented stoves, cooking practice (e.g. charring meat, deep frying) is another source of PAHs generated during cooking. The emissions from cooking practice depend greatly on the cooking method used, the fat content of the food and the quantity of food being cooked. Food with a higher fat content emits more PAHs than low-fat food *(41)*. Also, an increase in cooking temperature generally increases the production of most PAHs *(3)* because there is an increase firstly in the evaporation of PAHs from heated oils into the air and secondly there is an increase in the PAHs generated by pyrolysis from partially cracked organic compounds in food and cooking oils *(3,42)*. A comparative study of cooking

Table 6.3. Indoor PAH concentrations associated with different sources

Source	Concentration (μg/ m^3)	Comment	Reference
ETS	0.02–0.84	Pubs and restaurants (16 PAHs) [a]	Harrison et al. *(39)*; Bolte et al. *(40)*
Fuel	0.11	LPG for cooking	Raiyani et al. *(36)*
	0.27–0.31	Kerosene for cooking	Raiyani et al. *(36)*
	1.22–1.9	Cattle dung and wood as cooking fuel	Raiyani et al. *(36)*
	2.01	45–60 minutes, 16 PAHs,[a] wood as cooking fuel	Raiyani et al. *(38)*
	3.46	45–60 minutes, 16 PAHs,[a] wood dung cake as cooking fuel	Raiyani et al. *(38)*
	3.56	45–60 minutes, 16 PAHs,[a] dung cake as cooking fuel	Raiyani et al. *(38)*
Cooking	7.6	Chinese domestic cooking, 12 PAHs [b]	Zhu & Wang *(41)*
Heating	0.164	Kerosene stoves in Indian homes, 12 PAHs [b]	Pandit et al. *(37)*

[a] The 16 PAHs comprised naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, indeno[1,2,3-*cd*]pyrene, dibenz[*a,h*]) anthracene and benzo[*ghi*]perylene.

[b] The 12 PAHs comprised naphthalene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz[*a*]anthracene, chrysene, benzo[*k*]fluoranthene, benzo[*e*]pyrene and benzo[*a*]pyrene.

Note: Different authors might report different groups of PAHs.

practices showed that boiling produced the least PAHs, while broiling and frying produced most PAHs *(43)*.

Varying amounts of PAHs are present in creosote, which has been traditionally used as a wood preservative in the foundations of buildings, in fences and in the manufacture of garden furniture and outdoor recreational facilities in parks. B[*a*]P levels of 58–749 μg/g were found in creosote-impregnated wood products *(29)*.

The EU restricts creosote applications inside buildings *(44)* and Japan restricts the B[*a*]P content in creosote *(45)*, but creosote-treated wood might be an indoor source in other parts of the world.

Finally, mothball storage is associated with significant levels of naphthalene *(39,46)*, acenaphthalene, phenanthrene and fluorene indoors *(46)*.

Routes of exposure

Humans are exposed to PAH through several routes, namely inhalation of air and re-suspended soil and dust, consumption of food and water, and dermal contact with soil and dust *(65)*. All these sources are relevant to global human exposure. However, while soil contact generally occurs outdoors and food and water consumption is usually indoors, inhalation leads to exposure both indoors and outdoors. Yet people spent 80–93% of their time indoors, and hence indoor air would be the most relevant source contributing to the inhalation route *(66)*.

Table 6.4. Indoor benzo[*a*]pyrene concentrations associated with different sources

Source	Concentration (ng/m^3)	Comment	Reference
ETS	22	Extremely ETS polluted	WHO *(23)*
	0.23–1.7	Homes with ETS in industrialized countries	Chuang et al. *(22)*; Mitra & Ray *(24)*; Harrison et al. *(39)*; Fromme et al. *(47)*
	0.01–0.58	Homes without ETS in industrialized countries	Chuang et al. *(22)*; Mitra & Ray *(24)*; Harrison et al. *(39)*; Fromme et al. *(47)*
	1.45–4.1	Pubs and discotheques	Harrison et al. *(39)*; Bolte et al. *(40)*
Fuel	0.2–17.6	Kerosene as cooking fuel, geometric mean	Aggarwal et al. *(48)*
	33	Coal as cooking fuel, geometric mean	Aggarwal et al. *(48)*
	120–186	Cattle dung and wood as cooking fuel, geometric mean	Aggarwal et al. *(48)*
	1300–9300	Cattle dung and wood as cooking fuel, peak values over 15–30 minutes	Aggarwal et al. *(48)*
Cooking	6–24	Chinese domestic cooking, 12-PAH	Zhu & Wang *(41)*
Heating	2–490	Use of non-airtight stoves	Traynor et al. *(49)*
	0.63	Use of airtight stoves burning wood	Traynor et al. *(49)*; Daisey et al. *(50)*
	70	Unvented fireplaces in Burundi homes	Viau et al. *(51)*
	6.9	Kerosene stoves in Indian homes	Pandit et al. *(37)*
	33–166	Heating using coal, wood and cattle dung	Aggarwal et al. *(48)*
Unspecified source	0.05–0.44	American homes	Chuang et al. *(22)*; Mitra & Ray *(24)*; Van Winkle & Scheff *(46)*; Turpin et al. *(52)*; Naumova et al. *(53)*; Chuang et al. *(54)*
	0.01–0.65	European homes	Gustafson et al. *(34)*; Harrison et al. *(39)*; Fromme et al. *(47)*; Kingham et al. *(55)*; Fischer et al. *(56)*; Minoia et al. *(57)*
	1.42	Italian homes, max	Menichini et al. *(58)*
	0.09–25.52	Polish homes	Choi et al. *(59)*
	0.21–3.4	Asian urban homes	Li & Ro *(14)*; Sugiyama et al. *(60)*; Chao et al. *(61)*; Saito et al. *(62)*; Ohura et al. *(63)*; Azuma et al. *(64)*
	0.3 (0.01–1.25)	United Kingdom offices	Harrison et al. *(39)*
	0.03–0.07	Libraries and museums	Harrison et al. *(39)*

Air

The potential doses of carcinogenic PAHs[3] were estimated using the standard EPA recommendation for an individual's respiration rate *(67)* and applying this factor to the range of concentrations reported in the section of this chapter dealing with indoor levels and their relation to outdoor levels (page 301). The recommended value for the average inhalation rate of the general population is 11.3 m^3/day for women and 15.2 m^3/day for men *(67)*. Considering the different B[*a*]P indoor air concentrations reported, and using the adult male inhalation rate as a worst-case scenario, the daily intake dose due to inhalation spans the range of 0.15–32 ng/day. However, higher daily levels of inhaled B[*a*]P can be experienced during exposure to specific indoor sources such as cooking with different fuels (91–2523 ng/day) or using non-airtight stoves for heating (30–7448 ng/day) *(36)* (Table 6.5).

Table 6.5. Benzo[*a*]pyrene inhalation daily dose

Source	Daily dose (ng/day)	Comment	Reference
General	0.15–21	Homes in industrialized countries	See methodology described in the text
	3–26	Asian homes	
	6–21	Public indoor spaces in the United Kingdom and the United States	
Cooking	91–365	Chinese kitchens	
	105	Cooking with kerosene	Raiyani et al. *(38)*
	502	Cooking with wood	Raiyani et al. *(38)*
	2523	Cooking with cattle dung	Raiyani et al. *(38)*
Heating	30–7448	Indoors using non-airtight stoves	Raiyani et al. *(38)*
ETS	4–15	ETS-polluted indoors	See footnote 4
	1.3–6.7	Non-ETS-polluted indoors	
	26–62	Pubs and discotheques	

ETS is an important contributor to the inhalation source of PAHs. Using the same methodology as describe above,[4] daily inhalation of B[*a*]P in indoor environments would range from 4 to 15 ng/day in ETS-polluted compared with 1.3–6.7 ng/day in homes not exposed to ETS. The daily (24-hour) inhalation can be as high as 26–62 ng/day in pubs and discotheques. Children's daily exposures, expressed as urinary cotinine levels (a biomarker of tobacco smoke) were 8.1 µg/l urine in ETS-exposed children compared to 2.7 µg/l in children not exposed to ETS *(68)*.

[3] Carcinogenic PAHs include benz[*a*]anthracene, benzo[*a*]pyrene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, chrysene, dibenzo[*a,h*]anthracene, benzo[*ghi*]perylene and indeno[1,2,3-*cd*]pyrene.

[4] Calculated using the concentrations reported by Mitra & Ray 1995 and Fromme et al. 1995 and applying the USEPA 1997 male individual's breathing rate.

Drinking-water

Several studies performed in the United States reported values of carcinogenic PAHs for drinking-water in the range 0.1–61.6 ng/l, although most of the values fell between 1 and 10 ng/l. In the case of B[*a*]P, all the values were below the limit of detection (0.1 ng/l) *(65,69)*. Similarly, the examination of a number of drinking-water supplies for six PAHs (fluoranthene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, B[*a*]P, benzo[*g,h,i*]perylene and indeno[1,2,3-*c,d*]pyrene) indicated that the collective concentrations generally did not exceed 100 ng/l. The concentrations of these six PAHs were between 1 and 10 ng/l in 90% of the samples and higher than 110 ng/l in 1% *(23,70)*.

As regards the concentrations of the 16 PAHs, these span the range of 106.5–150.3 ng/l in several European and Canadian cities *(71)*, while lower values of 85.2–94.6 ng/l have been reported in Taiwan, China *(72)*. Studies performed in Europe have reported levels of B[*a*]P in the range of < 1 ng/l in Germany *(73)* to 10 ng/l in Poland *(74)*. Values of B[*a*]P in the same range (1.4–2.5 ng/l) have also been reported in Taiwan, China *(72)*.

Assuming an average drinking-water consumption of 2 l/day, the potential dose of carcinogenic PAHs via drinking-water ranged from 0.2 to 123 ng/day, 170–300 ng/day for the 16 PAHs and < 2–20 ng/day for B[*a*]P .

Food

PAHs are found in substantial quantities in some foods, depending on the method of cooking, preservation and storage, and intake is influenced by personal eating habits *(75)*. PAHs are detected in a wide range of meats, fish, vegetables and fruits, fluoranthene and B[*a*]P being the two PAHs detected at highest levels in food with fluoranthene levels exceeding those of B[*a*]P *(76,77)*. Food groups that tend to have the highest levels of PAHs and B[*a*]P include charcoal-broiled or smoked meats, fats and oils, and some leafy vegetables and grains. For these food groups, concentrations of 16 PAHs were typically in the tens of micrograms per kilogram (Table 6.6) *(78–81)*. However, the PAH load on leafy vegetables and grains can be removed by washing. As regards B[*a*]P, recent studies report that food containing fat show the highest levels of B[*a*]P, with maximum levels of 60 μg/kg (Table 6.7) *(65,75,82)*. Lower levels of B[*a*]P in the range of hundreds of nanograms per kilogram have been reported in more recent studies for fruits and vegetables, sweets, dairy products, beverages, bread, cereals, grains and seafood *(83,84)*.

A Dutch "market basket" study of dietary components for 18-year-old males, involving the determination of 17 different PAHs,[5] revealed that all of

[5] The 17 PAHs comprise naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*j*]fluoranthene benzo[*k*]fluoranthene, benzo[*a*]pyrene, indeno[1,2,3-*cd*]pyrene, dibenz[*a,h*])anthracene, benzo[*ghi*]perylene.

these compounds were detected. The most frequently occurring were benzo[*b*] fluoranthene, fluoranthene and benzo[*k*]fluoranthene in 59%, 48% and 46% of the samples, respectively. The highest concentration of a single PAH was found for chrysene, at 36 μg/kg in the commodity group "sugar and sweets". The mean daily intake of the total PAH fraction (17 PAHs) analysed ranged between 5 and 17 μg/day. The intake of the carcinogenic PAH fraction was roughly half of these

Table 6.6. The 16-PAH content of foods

Location	Concentration (μg/ kg)	Comment	Reference
General	8–71	Fish and seafood	Bordajandi et al. *(80)*; Douabul et al. *(81)*
	13.4–25.56	Meat products	Marti-Cid et al. *(78)*; Falco et al. *(79)*
	23.48	Oils and fats	Marti-Cid et al. *(78)*; Falco et al. *(79)*
	14.5–44	Cereals	Marti-Cid et al. *(78)*; Falco et al. *(79)*

Table 6.7. Benzo[*a*]pyrene content of foods

Location	Concentration (μg/ kg)[a]	Comment	Reference
General	0.001–0.22	Potatoes	Jakszyn et al. *(82))*
	0.003–0.48	Vegetables	
	n.d.–3.37	Fruit	
	n.d.–1.60	Milk and dairy products	
	n.d.–5.4	Cereals	
	n.d.–4.60	Meat	
	n.d.–11.2	Fish and seafood	
	n.d.–58.2	Fats	
	0.01–0.44	Sweets and desserts	
	0.009–0.36	Beverages	
United States, 1990	0.1–15	Smoked food	Lioy & Greenberg *(75)*
	3–29.3	Charcoal-broiled food	
	0.1–48.1	Vegetables, fruit and cereals	
United States, 2001	0.01–4.86	Beef and chicken	Kazerouni et al. *(83*
	0.01–0.13	Pork	
	0.01–0.24	Seafood	
	0.01–1.75	Restaurant/fast-food meat	
	0.01–0.18	Dairy products, fat products and beverages	
	0.02–0.56	Bread, salty snacks, cereals and grains	
	0.01–0.47	Sweets and desserts	
	0.01–0.48	Fruit and vegetables	
Republic of Korea, 2007	5.4	Fried chicken or dried beef	Lee & Shim *(84)*
	0.36	Sesame oil	
	0.44	Peanuts	

[a] n.d. = not determined.

amounts. The largest contribution to the daily PAH intake came from sugar and sweets, cereals, oils, fats and nuts *(85)*.

For the average American diet, the intake of carcinogenic PAHs was estimated to be 1–5 μg/day, with unprocessed grains and cooked meats the greatest sources of the compounds *(65)*. This is lower than in a recent study in Spain, where the dietary intake of carcinogenic PAHs[6] ranged from 723 to 969 ng/day and the 16 PAHs ranged from 8.57 to 13.81 μg/day *(78,86)*.

The dietary intake of B[*a*]P ranged between 0.002 and 1.1 μg/day in the United States in the late 1980s *(69)*. However, lower levels were reported in a recent study, similar to those reported in Asia and Europe, ranging from 4.2 to 320 ng/day (Table 6.8). The lowest daily intake for B[*a*]P and 16 PAHs has been reported in Yemen (1.7 and 167 ng/day, respectively) based on the fish consumption of the Yemeni population *(81)*.

Table 6.8. Benzo[*a*]pyrene daily dietary intake dose

Location	Daily dose (ng/day)	Comment	Reference
United States	0.002–1100	United States, 1988	Lioy et al. *(69)*
	0.5–305	United States, 2005	Anderson et al. *(87)*
Asia	70–190	Islamic Republic of Iran and Republic of Korea	Lee & Shim *(84)*; Hakami et al. *(88)*
	1.7	Yemen	Douabul et al. *(81)*
Europe	160–320	Italy	TurrioBaldassarri et al. *(89)*
	73–140	Spain	Marti-Cid et al. *(78)*; Ibanez et al. *(86)*
	4.2–35.0	Czech Republic	Kulhanek et al. *(90)*

Soil

Carcinogenic PAHs are found in all surface soils *(65)*. Typical concentrations in forest soil range from 5 to 100 μg/kg (Table 6.9). Substantial amounts of PAHs are transferred to forest soil from vegetative litter because the compounds are adsorbed from air onto organic matter such as leaves and pine needles. Rural soil contains carcinogenic PAHs at levels of 10–100 μg/kg, originating mainly from atmospheric fallout. For both forest and rural soil, values as high as 1000 μg/kg may occasionally be found *(65,91,92)*.

Metropolitan areas have higher PAH concentrations than forest and agricultural areas because of the many sources of fossil fuel combustion. The majority of urban soil concentrations fall in the 600–3000-μg/kg range *(65,93,94)*. Higher

[6] Carcinogenic PAHs from this study include benz[*a*]anthracene, benzo[*a*]pyrene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, chrysene, dibenzo[*a,h*]anthracene and indeno[1,2,3-*cd*]pyrene.

Table 6.9. PAH content of soils

Location	Concentration (mg/kg)	Comment	Reference
General	0.01–0.1	Rural soil	Menzie et al. *(65)*
	0.05–0.1	Forest soil	Menzie et al. *(65)*
	1.0	Forest and rural soil peaks	Nam et al. *(91)*
	0.6–3.0	Urban soil	Menzie et al. *(65)*
	1.0–3.0	Heavy transportation and industrialized	Menzie et al. *(65)*; Trapido *(93)*; Mielke et al. *(94)*
Europe	14.6–99.6	United Kingdom, 17 PAHs, roadside soil	Harrison et al. *(95)*
	2.02	United Kingdom, 17 PAHs, urban soil	
United States	8–336	Road dust in cities	Menzie et al. *(65)*
Asia	0.20–1.15	Lahore, Pakistan, 17 PAHs	Smith et al. *(92)*

values near areas of heavy transportation and industrialization range from 8 to 336 mg/kg *(65,95)*. Values in the order of 1000–3000 μg/kg are regarded as being in the upper range.

As regards B[*a*]P levels in topsoil (Table 6.10), the lowest concentrations are found in tropical rural and urban soils (0.3–5.5 μg/kg) and the highest in arable and forest areas in temperate latitudes (18–39 μg/kg) *(96)*. The highest concentrations were found in urban areas, with values ranging from 5.5 to 379 μg/kg *(96–100)* and from 971 to 1600 μg/kg in large United Kingdom cities and Chicago *(97)*. Levels in industrialized areas across the world ranged between 18 and 360 μg/kg *(99,101,102)*.

Incidental ingestion of soil by adult males was estimated to be of the order of a few milligrams per day. Soil ingestion rates of the order of 100 mg/day are more typical for small children *(103)*. Therefore, the potential dose of carcinogenic PAHs for urban populations ranged from 0.2 to 96 ng/day (median 7 ng/day).

Relative importance of different routes of exposure

Human exposure will be from both inhalation of contaminated air and consumption of contaminated food and water. Especially high exposure will occur through the smoking of cigarettes and the ingestion of certain foods (e.g. smoked and charcoal-broiled meats and fish) *(2)*. Food ingestion is likely to be a larger route of exposure compared to inhalation for a large section of the general population exposed to PAHs. Drinking-water and soil are generally minor sources of these compounds in the daily intake dose *(65)*.

In an earlier American study, diet was reported to make a substantial contribution (generally more than 70% in non-smokers) to PAH intake other than occupational PAH exposure. For a non-smoking reference male (70 kg body weight),

Table 6.10. Benzo[*a*]pyrene content of soils

Location	Concentration (μg/ kg)	Comment	Reference
General	18–19	Arable and grassland areas	Wilcke *(96)*
	39	Forest areas	
	350	Urban areas	
	0.3–2.0	Tropical topsoil for rural forest in the Amazon and Ghana	
	5.2–5.5	Urban tropical areas, Brazil and Thailand	
Europe	43–236	Urban areas, Baltic countries	Saltiene et al. *(97)*
	76–229	Urban areas, southern Europe	Morillo et al. *(98)*
	971–1600	Large cities, United Kingdom	Saltiene et al. *(97)*
	7–379	Rural and urban areas, United Kingdom	Wild & Jones *(100)*
	18–100	Spanish industrial areas	Nadal et al. *(99)*
	56–22	Spanish residential areas	Nadal et al. *(99)*
	360	Polish heavy industrialized areas	Bodzek et al. *(74)*
	22	Polish urban areas	Maliszewska-Kordybach *(101)*
United States	1600	Chicago	Saltiene et al. *(97)*
Asia	5.5	Urban Bangkok	Wilcke et al. *(104)*
	55	Suburban Beijing	Wang et al. *(105)*
	317–154	Industrialized area, China	Ma et al. *(102)*

a mean carcinogenic PAH intake of 3.12 μg/day was estimated, of which dietary intake contributed 96.0%, air 1.6%, water 0.2% and soil 0.4% *(65)*. In the early 1990s, the potential dose of carcinogenic PAHs for American adult non-smoking males was estimated to be 3 μg/day up to a maximum of 15 μg/day. Smokers of unfiltered cigarettes might have had a potential dose twice that of non-smokers *(65)*.

Recent studies conducted on human exposure to B[*a*]P for non-smokers in developed countries revealed that nowadays, the range and magnitude of dietary exposures (0.5–320 ng/day) *(87)* are generally larger than for inhalation (0.15–26 ng/day). In certain cases where indoor air contains high concentrations of PAHs, however, air could be a major contributing source. This could be the case if a person spent the day in an ETS environment (4–62 ng/day) or in microenvironments fitted with non-airtight stoves (30–7448 ng/day)[7] or cooked food in the Chinese style (91–365 ng/day).[8]

[7] Calculated using the concentrations reported by Traynor et al. *(49)* and applying the USEPA *(67)* male individual's breathing rate.

[8] Calculated using the concentrations reported by Zhu & Wang *(41)* and applying the USEPA *(67)* male individual's breathing rate.

In developing countries where biomass is generally used for cooking in homes without a flue or with a deficient flue, the contribution of inhalation to the B[*a*]P exposure could be as high as 138–3320 ng/day[9] and therefore inhalation would be the main contributor to the total daily intake.

Indoor concentrations and their relation to outdoor concentrations

About 500 PAHs and related compounds have been detected in air, but most measurements have been made on B[*a*]P *(2)*. Indoor levels have been generally found to be influenced by seasonal variations, with higher levels in winter than in summer *(39,63)*. The levels of B[*a*]P in United States homes were found to be between 0.05 and 0.44 ng/m^3, which were within the range of B[*a*]P in European homes (0.01–0.65 ng/m^3) (as shown in Table 6.4). The highest B[*a*]P levels (0.09–25.52 ng/m^3) were found in Polish homes *(59)*.

The levels of B[*a*]P in Asian cities ranged between 0.21 and 3.4 ng/m^3 *(14,60–64)*. Higher levels of B[*a*]P were found in Chinese domestic kitchens. The average concentration of 12 PAHs[10] in Chinese domestic kitchens was 7.6 μg/m^3 and was dominated mainly by 3- and 4-ring PAHs. The B[*a*]P levels in domestic kitchens were 6–24 ng/m^3, which was associated with conventional Chinese cooking methods *(41)*. Lower concentrations were found in domestic kitchens in other Asian cities *(12,14,106)*.

The use of non-airtight stoves was found to increase the levels of B[*a*]P by up to 2–490 ng/m^3 *(49)*, while the mean indoor level of B[*a*]P in homes with airtight wood-burning stoves was 0.63 ng/m^3 *(49,50)*, which in turn is higher than those levels recorded in non-wood-burning homes *(34)*. High levels of B[*a*]P (70 ng/m^3) and other PAHs have been measured in traditional rural houses with unvented fireplaces in Burundi *(51)*.

High levels of 12 PAHs[11] (164.2 ng/m^3 geometric mean) have also been measured when kerosene stoves were used in Indian homes, with B[*a*]P geometric mean levels of 6.9 ng/m^3 *(37)*. However, the highest PAH levels were measured when using other solid fuels such as coal, wood and cattle dung, with B[*a*]P levels ranging from 33 to 166 ng/m^3. Homes in industrialized countries with ETS presented higher B[*a*]P levels (0.23–1.7 ng/m^3) than homes without the presence of ETS (0.01–0.58 ng/m^3) *(22,24,39,47)*.

The sum of all 16 gaseous and particle-bound PAHs measured in pubs, restaurants and discotheques varied between 22 and 840 ng/m^3 (B[*a*]P 1.45–4.1 ng/m^3)

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[9] Calculated using the concentrations reported by Raiyani et al. *(36,38)* and applying the USEPA *(67)* male individual's breathing rate.

[10] The 12 PAHs comprised naphthalene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz[*a*]anthracene, chrysene, benzo[*k*]fluoranthene, benzo[*e*]pyrene and benzo[*a*]pyrene.

[11] The 12 PAHs comprised naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz[*a*]anthracene, chrysene, benzo[*k*]fluoranthene and benzo[*a*]pyrene.

(39,40,107), with discotheques/clubs the locations with the highest mean concentrations in a study carried out in Germany *(40)*.

PAH concentrations measured in public indoor spaces ranged from 0.4–0.6 ng/m^3 in hospitals, libraries and coffee shops and 1.2–1.4 ng/m^3 in food courts and shopping malls in the United States *(108)* to 2.1–18.2 ng/m^3 inside Czech kindergartens *(109)*.

Indoor : outdoor ratios

The concentrations of low-molecular-weight PAHs (two and three rings) are usually higher indoors than outdoors, whereas those of high-molecular-weight PAHs (four rings and larger) are normally higher outdoors than indoors *(63)*, suggesting that the indoor concentrations of the high-molecular-weight PAHs are dominated by outdoor sources *(53)*. However, a study found that the 95th percentile of the I : O ratios of several four-ring PAHs were much higher than unity (> 3) *(53)*, suggesting than in some homes the influence of indoor sources (generally tobacco smoking, heating or cooking sources) may be considerable *(110)*.

The I : O ratios of individual PAHs varied from 0.3 to 10.5. Similarly, the I : O ratio of B[*a*]P ranged from 0.09 to 3.34 *(13,17,47,53,56,60–63,108,111)*. This variety in I : O concentration ratios suggest that the ratios fluctuate substantially across different settings, particularly those with smokers or indoor combustion sources and cooking activities *(61)*. The differences in I : O ratios are affected by variables such as differences in combustion sources and heating systems, climatic conditions and ventilation habits. Nevertheless, smoking is generally the most relevant factor in determining I : O ratios in homes in industrialized countries *(58)*.

Several studies found I : O ratios for PAH species of 1.4 ± 0.6 (B[*a*]P 1.6) in non-smokers' homes and much greater than unity (4.3 ± 3.3 for PAHs, B[*a*]P 5.5) in smokers' homes *(13,22,47)*.

The I : O levels in homes using kerosene stoves for cooking and heating were 4.5 for 12 PAHs (see footnote 11) and 7.6 for B[*a*]P. These high values for the I : O ratio show the impact of indoor combustion sources on indoor levels of PAHs *(37)*.

Toxicokinetics and metabolism

The kinetics and metabolism of PAH(s) have been addressed previously in several WHO documents *(2,8,70)*. The emphasis below is on aspects relevant particularly to exposure in indoor air. Moreover, the most recent data on PAHs are reviewed.

Identification of studies

Studies on pharmacokinetics, metabolism and toxicology were identified by hand searching references in former reviews by WHO (*2,8,70*) and other authors

(*112*) and by electronic searches in PubMed and the ISI Web of Science. As to the description of toxic effects, the focus was on in vivo studies but for the mechanisms of toxicity and metabolism all relevant studies were reviewed. Altogether, 320 original papers were selected from the literature searches with wide scope for review of contents, and 114 papers were included as relevant references for this work.

Toxicokinetics

Owing to differences in the physicochemical properties of PAHs, their toxicokinetics differ widely. In this section, the focus is on the kinetics of lipophilic high-molecular-weight PAHs, such as B[*a*]P, because they cause the main health concern.

Absorption

The major route of exposure to PAHs in the indoor environment is through the lungs and respiratory tract after inhalation of PAH-containing aerosols and particles. Data on the fate of PAHs in lungs are mainly based on animal and in vitro studies.

After deposition in the airways, the structure of the PAH and the dimensions and the chemical nature of the particles define the fate of the PAH. PAHs may dissolve from particles, the remainder in particles may be eliminated by bronchial mucociliary clearance of particles (to be swallowed), or the PAH in particles may remain in the lungs for a longer time.

B[*a*]P is rapidly absorbed in the lungs from solutions. After intratracheal instillation of radiolabelled B[*a*]P in rats, the peak concentration in the liver was attained in 10 minutes *(113)*. The B[*a*]P-associated radioactivity was cleared from lungs with elimination half-lives of 5 and 116 minutes, respectively.

PAH in particles follows biphasic absorption kinetics in the lungs. The absorption kinetics depends on the site of deposition in the respiratory tract. A fraction of B[*a*]P in diesel particles was quickly desorbed and absorbed into circulation through type I epithelial cells in the alveolar region *(114–116)* and systemically rapidly metabolized *(116)*. The fraction deposited in the tracheobronchial region was more slowly absorbed into circulation and intensely locally metabolized *(116)*. The release rates of B[*a*]P from particles decreased drastically after the initial burst and a notable fraction of B[*a*]P (up to 30%) remained unaffected on the surface of particles in lungs and in lymph nodes for several months *(116)*.

In perfused rat lung, the absorption kinetics of B[*a*]P is dose-dependent *(117)*. At low exposure levels, absorption of B[*a*]P in the mucosa followed the first-order kinetics with substantial local metabolism. At high exposure levels, the capacity of epithelium to dissolve and metabolize B[*a*]P became saturated and the absorption rate turned constant (zero-order kinetics). In the indoor environment, human exposure most likely follows the low-dose, first-order kinetics.

No data are available on exact quantitative estimates of PAH absorption in human lungs.

The kinetics of lipophilic PAHs in lungs suggest that, after deposition in lungs, (*a*) there is a rapid systemic exposure to B[*a*]P after inhalation of PAH-containing particles, (*b*) the intracellular B[*a*]P is higher in the tracheobronchial region than the alveolar region and in the epithelium lining the airways, and (*c*) there is a sink of B[*a*]P in particles to cause long-term exposure in lungs and local lymph nodes after inhalation exposure.

B[*a*]P and other PAHs (phenanthrene and pyrene) efficiently penetrate the skin in animals. Absorption of up to 84% of the B[*a*]P-associated radioactivity has been observed in mice *(118)* and 46% in rats *(119)*. Absorption through human skin may be less efficient than in animals.

PAHs are ingested in house dust (as a non-dietary source) and swallowed in particles that are transported by mucociliary transport from the lungs. PAHs are readily absorbed in the gastrointestinal tract by passive diffusion *(120)*. The composition of the diet may increase or decrease the absorption *(8)*. Bioavailability from particles limits the absorption. From soil particles, up to 50% of total PAHs was absorbed from the gastrointestinal tract in vitro *(121)*. The absorption was highest for small-molecule PAHs (naphthalene, acenaphthene, anthracene). The bioavailability from particles, however, probably varies depending on the content of organic carbon in dust particles.

Distribution

PAHs are rapidly and widely distributed in the body. Lipophilic compounds easily pass biological membranes. Detectable levels of B[*a*]P can be observed in most tissues in minutes to hours after exposure, irrespective of the exposure route. PAHs undergo hepatobiliary clearance *(122)* and high concentrations of PAHs and their metabolites are detectable in the gastrointestinal tract *(8,122)*.

PAHs do not accumulate in the body. Fat tends to contain more PAHs than other tissues *(8)*. Fat and PAH contents, however, did not correlate well in lungs *(123)*.

PAHs are generally detectable in most human tissues, typically at the sub-μg/kg level *(8)*. The reactive metabolites are bound covalently to proteins and nucleic acids and the turnover rate of adducts defines the half-life in tissues.

Particles may cause high concentrations of PAHs in lungs. A 100-fold higher radioactivity occurred in lungs of rats after inhalation of labelled B[*a*]P adsorbed on carbon black particles than after inhalation of pure B[*a*]P. The half-time of decline also lengthened from 6 weeks to 34 weeks *(124)*.

B[*a*]P and other PAHs can readily cross the placental barrier *(8)*. The concentrations in animal embryo tissues have been, however, at one to two orders of magnitude lower than in maternal organs *(125–127)*. PAHs, including B[*a*]P, are detectable in maternal milk *(128)*.

Excretion

The faeces are the main route of excretion of high-molecular-weight PAHs and their metabolites *(8)*. Biliary secretion and enterohepatic circulation are significant *(122,129)* and increase the concentrations of metabolites and parent compounds in the gastrointestinal tract. PAHs in bile are nearly completely present as metabolites. Less than 1% was detected as B[*a*]P in bile after intravenous administration of B[*a*]P to mice *(122)*.

Urine is the other main excretion route. Some 4–12 % of B[*a*]P was excreted in urine in rats *(122)* compared with 60% of pyrene as metabolites *(130)*. The role of urine as an excretion route is compound-specific; for large-molecule PAHs, it is a minor route.

Metabolism

Metabolism is crucial for toxicity of PAHs. Reactive intermediates and metabolites are formed that cause the toxicity and carcinogenicity. The metabolism pathways of B[*a*]P are best known *(2,8,70,112)*. Most other large-molecule PAHs probably follow the same metabolism patterns *(131)* but the metabolic activation of sterically nonalternant PAHs, such as benzo[*b*]fluoranthene, may differ *(2)*.

Three principal pathways activate PAHs for toxic intermediates and further metabolism: that via (dihydro)diol-epoxide formation, that via radical cation formation, and the *o*-quinone pathway *(112,131)*. Several enzymes interplay in the metabolism.

The key enzymes in PAH metabolism are CYPs (cytochrome P450s) and epoxide hydrolase. CYPs activate PAH to optically active oxides, which rearrange to phenols. Epoxide hydrolase converts the oxides (epoxides) to optically active dihydrodiols (diols) *(8,112)*. CYPs also metabolize PAHs to a series of quinones. For B[*a*]P, three quinones have been identified in vitro and in vivo: B[*a*]P-1,6-quinone, B[*a*]P-3,6-quinone and B[*a*]P-6,12-quinone *(132)*. The diols can be converted to four optically active isoforms of diol-epoxides by CYPs. The diol-epoxides are highly reactive towards DNA and form a series of stable DNA adducts *(112)*. The (+)-*anti*-B[*a*]P-7,8-diol-9,10-epoxide (*anti*-B[*a*]PDE) is suggested to be the ultimate carcinogenic form of B[*a*]P *(112,131)*.

The catalytic property, mode of regulation and tissue specificity of CYPs vary and there are species differences. One or more members of the CYP family are capable of metabolizing one or more PAHs. The highest metabolism capacity is in the liver, followed by the lung, intestinal mucosa, skin and kidneys *(8)*. Toxic metabolites producing CYPs are expressed and induced in a number of other tissues, including cardiovascular tissues *(133,134)*. The key enzymes for PAH metabolism are CYP1A1 and CYP1B1 but several other CYPs (CYP1A2, CYP2B, CYP2C and CYP3A) also metabolize PAHs *(8,112)*.

PAHs, especially B[*a*]P *(135)*, stimulate their own metabolism by inducing CYP enzymes *(8)*. CYP1A and CYP1B are induced via the Ah-receptor *(8)*. En-

zyme induction results in lower tissue levels of PAHs and more rapid excretion of PAHs as metabolites.

The site of induction is important for toxicity. Strong induction of the metabolism in the liver decreases PAH levels in peripheral tissues and levels of toxic metabolites by local CYP metabolism. Clear differences in PAH toxicity have been demonstrated in mice strains of different CYP induction capacity *(8,136–138)*. PAHs also inhibit CYP enzymes, and even their own metabolism *(139)*. On the basis of toxicokinetics, PAHs may be expected to be relatively more toxic through inhalation and dermal exposure (owing to focal toxicity at the site of entry) than after oral exposure, because inhalation and dermal exposure bypass the first-pass metabolism in the liver.

The diol-epoxides have been regarded as principal toxic metabolites *(70)* but recent data suggest that two other routes of PAH metabolism produce toxic metabolites. In the radical cation metabolism pathway, radical cations are formed from PAH by CYPs or peroxidases and these form depurinating DNA adducts *(140)*. In the *o*-quinone pathway, *o*-PAH diols are converted by aldo-keto reductases to catechols, which autoxidize to *o*-quinones. These *o*-quinones undergo redox cycling and form reactive oxygen species *(131,141)*. Other B[*a*]P quinones have also been associated with reactive oxygen species and mutagenesis. In vivo, both mice and rats metabolize B[*a*]P to B[*a*]P-1,6-quinone, B[*a*]P-3,6-quinone and B[*a*]P-6,12-quinone and these quinones redox cycle and induce mutations *(132,142)*. Reactive oxygen species have been associated with carcinogenesis *(131,141)*.

Although B[*a*]P-diol epoxides, B[*a*]P-radical cations and B[*a*]P-*o*-quinones can form DNA adducts in vitro, only B[*a*]P-diol epoxide- and B[*a*]P-depurinating-DNA adducts have been measured in vivo in experimental animals and in humans *(131,140,143,144)*. The relative importance of each activation pathway of metabolism depends on several factors, including the tissue level and stability of each activated form and the levels of expression of the activation and detoxification enzymes. For B[*a*]P, based on the wealth of data, the diol epoxide metabolic activation mechanism seems to be the dominant mechanism in the induction of lung carcinogenesis in rodents and humans. This conclusion is based on toxicological and mechanistic data obtained from experimental animals and from the many human biomarker studies.

PAHs and their reactive metabolites are finally converted to more polar and detoxified metabolites for excretion by the phase II metabolism enzymes, including glutathione *S*-transferase, UDP-glucuronosyltransferase, sulfotransferase, NAD(P)H-quinone oxidoreductase 1 and aldo-keto reductase *(112)*. Though some of them may also be induced by PAHs, the induction is not as strong as CYP induction *(145)*.

Genetic polymorphism may contribute to capacity to metabolize PAHs and affect toxicity. Genetic polymorphism has been described in CYP1A1, CYP1A2,

CYP1B1, some CYP2C and CYP3A *(8)* and phase II detoxification enzymes *(112,146)*.

Metabolism in the respiratory tract has particular relevance for toxicity of inhaled PAHs. Macrophages are actively metabolizing cells of PAHs in the lung *(8)*. Macrophages can engulf PAH-containing particles and transport them to bronchi. It has been hypothesized that ultimate carcinogenic metabolites released from macrophages contribute to cancer development in the lung *(8)*.

Health effects

DNA adducts

The formation of DNA adducts is a key event in mutagenicity and carcinogenicity by PAHs. Owing to the many stereoisomeric forms of B[*a*]P-diol epoxides (BPDE), their reactivity to covalently bind to nitrogen atoms on guanine (and to a lesser extent on adenine) bases, and epoxide ring opening yielding both *cis* and *trans* adducts, a potential total of eight unique B[*a*]P-diol epoxide stereoisomeric DNA adducts can be formed for each site on the nucleic acid base *(131)*. However, far fewer stable DNA adducts are observed in vitro or in vivo. Only one diol epoxide B[*a*]P-DNA adduct (anti-B[*a*]PDE-deoxyguanosine) was observed in the lungs of mice treated with B[*a*]P *(147)* and the same adduct was found in human diploid lung fibroblasts in vitro *(148)* and in mononuclear white blood cells from exposed coke oven workers *(149)*.

In heavily PAH-exposed workers, the anti-B[*a*]PDE-DNA adducts in peripheral blood lymphocytes were associated with increased micronuclei in cells *(150)*. Radical cations produce a series of B[*a*]P adducts on guanine and adenine that are unstable (depurinating) and cleave from the DNA *(131,140)*. *o*-Quinones, another metabolite of B[*a*]P, also form both stable and unstable adducts in vitro *(131,144)*. PAH-DNA adduct formation blocks DNA replication and induces base and nucleotide excision repair activities *(151)*. Errors in DNA replication (misreplication) and in DNA repair (misrepair) can create mutations that are fixed after cell division.

DNA adducts display tissue- and compound-specific qualitative and quantitative differences *(152,153)*. B[*a*]P formed DNA adducts in rat lungs and liver in a dose- and time-dependent way *(153)*. In rats and mice, the adducts reach maximal levels in tissues within a few days after a single dose, after which they gradually decrease but persist for several weeks *(147,154–156)*. In the rat lung, two adducts predominated equally (adduct with B[*a*]P-diol epoxide and 9-OH-B[*a*]P-derived adduct, about 40% of each) after intraperitoneal administration in the liver, the B[*a*]P-diol epoxide adduct dominated. These same adducts have also been detected in the lungs of mice, B[*a*]P-diol epoxide predominating *(157)*. A comparative study with different PAHs in A/J mice indicated that the formation and persistence of DNA adducts determined the potency to induce adenomas in lungs after a single intraperitoneal administration *(147)*.

DNA adducts have been observed postnatally in thymocytes and splenocytes of pups after in utero exposure of mice to B[*a*]P *(158)*, indicating rather long persistence of the DNA adducts and vulnerability of pups to gestational exposure to B[*a*]P. PAH-DNA adducts have been detected in human fetal umbilical cord blood and maternal blood after exposure to ambient air PAHs at different levels *(159)*. Prenatal exposure may increase the cancer risk of PAHs.

Mutagenicity

A number of PAHs are mutagenic and genotoxic, and induce DNA adduct formation in vitro and in vivo *(8)*.

The potential to cause mutations is compound-dependent. Dibenzo[*a,l*]pyrene-diol-epoxide was over 60-fold more reactive towards DNA, induced over 200 times more mutations and yielded a fourfold higher yield of mutations per adduct than B[*a*]P-diol-epoxide in V79-derived XEM2 cells *(160)*. Moreover, dibenzo[*a,l*]pyrene-diol-epoxide-induced adducts were less efficiently repaired.

Some PAHs probably cause mutations in a number of genes that contribute to cancer development. The *anti*-diol-epoxide of B[*a*]P ((±)-*anti*-BPDE) causes adducts at several hotspots of the *p53* gene *(161)*, especially in codons 157, 248 and 273 *(162)*. The mutations by this epoxide are predominantly G to T transversions *(163,164)*. Diol epoxides of several other PAHs cause adducts in these and other codons *(165)*. PAH *o*-quinones have more potently caused similar *p53* mutations in yeast reporter gene assay than (±) *anti*-BPDE *(161)*. PAH-induced DNA damage stimulates cellular p53 accumulation and up-regulates the p21 protein *(148,166)* as typical cellular responses to DNA damage. In experimental animals, tumours induced by a series of PAHs have harboured mutations in K-*ras* (lung tumours) and H-*ras* oncogenes (skin, liver and mammary tumours). B[*a*]P has induced K-*ras* codon 12 mutations in mouse lung tumours almost exclusively at guanine, consistent with the detection of *anti*-BPDE-deoxyguanosine-DNA adducts in the lung tissues *(167)*.

In human studies, lung tumours from non-smokers exposed to PAH-rich coal combustion emissions had mutations at guanine in K-*ras* codon 12 and *p53* genes *(168)*.

In addition to base-pair substitutions, PAHs cause other mutations to a lesser extent (exon deletions, frame-shift mutations) *(163,169)*.

Ambient air particles have variably caused genotoxicity in vitro and DNA adduct formation *(170,171)*. PAHs, especially B[*a*]P *(170,172)* and nitro- and oxy-PAHs, are major active components *(171)*. B[*a*]P levels have indicated well the presence in ambient air of compounds causing DNA adducts *(172)*.

In a limited data set, PAHs were assessed to contribute 3–23% of the mutagenicity in settled house dust *(173)*. On the mass basis, settled house dust was considered on average more mutagenic than contaminated soils but less mutagenic than suspended particles in indoor and outdoor air *(173)*.

Because some PAHs cause mutations and genotoxicity, they may generally be regarded as genotoxic carcinogens. However, PAHs also promote tumour development (see below).

Carcinogenicity

B[*a*]P and a number of 4- to 7-ring PAHs are carcinogenic in experimental animals *(8,70)*. Several small-molecule PAHs, such as anthracene, perylene and fluorene, have not been carcinogenic and the carcinogenicity of some compounds (acenaphthene, phenanthrene, pyrene) is, as yet, questionable *(8)*. Inhalation of naphthalene has induced respiratory tract tumours in mice and rats at high cytotoxic concentrations but not at non-cytotoxic concentrations *(174,175)*.

Carcinogenic PAHs such as B[*a*]P have induced tumours through dermal, oral, intraperitoneal, intramamillary and respiratory tract routes *(8)*. The species that have developed tumours after exposure to PAHs include mice, rats, rabbits, hamsters and monkeys *(8)*. Tumour induction is not restricted to the site of administration. After oral exposure to PAHs, tumours have been observed typically in the liver, forestomach, lungs and mammary glands *(8)*. PAHs painted onto skin have caused skin papillomas and carcinomas but also lung and liver tumours *(8,70)*. Administration of B[*a*]P into the respiratory tract has consistently caused lung tumours in mice, rats, hamsters and monkeys *(8)*. Fewer data exist for other PAHs after exposure via the respiratory tract, but acenapthene, benzo[*b*]fluoranthene, benzo[*j*]fluoranthene, benzo[*k*]fluoranthene, chrysene, dibenz[*a,h*]anthracene and indeno[*1,2,3-c,d*]pyrene have caused lung tumours in rats and dibenz[*a,h*]anthracene and dibenzo[*a,i*]pyrene in hamsters *(8)*.

The potencies of the carcinogenic PAHs differ by three orders of magnitude *(8)*. In comparative studies with PAHs, B[*a*]P was repeatedly a potent carcinogen after dermal application *(8)* but in an initiation–promotion model in SENCAR mice, dibenzo[*a,l*]pyrene and 7,12-dimethyl-benz[*a*]anthracene were more potent initiators of skin tumorigenesis than B[*a*]P *(176,177)*. Based on the administered dose, dibenzo[*a,l*]pyrene was also more potent than B[*a*]P in inducing mouse lung adenomas *(178)*. Since dibenzo[*a,l*]pyrene was also the most potent in inducing mammary tumours after intramamillary injection *(176)*, it may be regarded as the most potent carcinogenic PAH known.

B[*a*]P *(8)* and dibenzo[*a,l*]pyrene *(179,180)* have been shown to be transplacental carcinogens in mice, causing lung and liver tumours (B[*a*]P and dibenzo[*a,l*]pyrene) and lymphoma (dibenzo[*a,l*]pyrene) in progeny after in utero exposure.

Limited data are available on the potency of specific PAHs to induce lung cancer following inhalation exposure. Data are inadequate for ranking the potency of specific PAHs to induce lung cancer.

The Ah-receptor-mediated pathways are crucial for carcinogenicity of PAHs: Ah-receptor-deficient mice are resistant to B[*a*]P- and dibenzo[*a,l*]pyrene-

induced skin cancer *(181,182)*. Organic extracts of airborne particulate matter, where PAHs are suggested to be the primary carcinogens, have not caused lung tumours in AhR -/- mice either *(183)*.

Although genotoxic effects (mutations in cancer genes and DNA damage) are likely to be the primary events in PAH-induced carcinogenesis, in vitro studies have indicated that PAHs have also non-genotoxic effects that could contribute to carcinogenesis. B[*a*]P-diol epoxide induces gene promoter hypermethylation in immortalized bronchial epithelial cells *(184)* and several PAHs inhibit gap-junctional intercellular communication *(185)*, a typical mechanism of tumour promotion. Several small-molecule PAHs have been more potent in inhibiting gap-junctional intercellular communication in liver cells than high-molecular-weight PAHs *(185)*. *Anti*-B[*a*]P-7,8-diol-9,10-oxide has been shown to increase cell proliferation in human cell lines, including lung cancer cells *(186)* and to induce apoptosis in H460 human lung cancer cells *(187)*. B[*a*]P has induced apoptosis in human lung fibroblast MRC-5 cells via the JNK1/FasL and JNK1/p53 signals *(188)*. It is possible that PAHs with low genotoxic potential also promote tumour development by non-genotoxic mechanisms.

PAHs have induced the expression of a number of genes in cells in vitro with compound-specific profiles *(189–191)*. Little can be interpreted as yet, however, about the mechanisms of toxicity from the gene and protein expression data. In addition to induction of PAH-metabolizing CYP genes, only oxidative stress pathway genes were induced by carcinogenic PAHs in rat liver slices *(190)*. In human mammary carcinoma-derived cells (MCF-7), cytoskeletal proteins, heat-shock proteins, DNA-associated proteins and glycolytic and mitochondrial proteins were altered *(191)*. Dibenzo[*a,l*]pyrene and B[*a*]P, two potent carcinogenic PAHs, have consistently displayed different gene expression patterns *(189–191)*. Diol epoxides of carcinogenic PAHs, but not the parent PAHs, have increased intracellular Ca^{2+} in human small-airway epithelial cells in vitro *(192)*. Increased intracellular Ca^{2+} is likely to be one mechanism contributing to the toxicity of diol epoxides.

General toxicity

There are limited data on the toxicity of individual PAHs in experimental animals. Data on mixtures containing PAHs as principal toxic components (coal tar, coal tar pitch and creosote) complement the information. In general, the acute toxicity of PAHs in animals is low to moderate *(8)*. B[*a*]P causes eye irritation and skin sensitization in animals *(8)* and PAH mixtures are phototoxic in both skin and eyes *(193)*.

On repeated exposure, the main target organs of toxicity are the liver or kidneys in animals, depending on the PAH *(8)*. Typically, the weight of the liver increases owing to enzyme induction. Nephropathy and decreased kidney weight have been caused by pyrene in mice *(8)*.

High oral doses of B[*a*]P have caused bone marrow depression in mice, decreasing especially proliferating haematopoietic cells *(137)*. B[*a*]P has also impaired in vitro proliferation and differentiation of human haematopoietic CD34+ stem cells and caused their apoptosis *(194)*. By destroying these cells, PAHs may down-regulate cell lineages (lymphocytes, macrophages, neutrophils) important for immunoresponse.

PAHs are immunotoxic and cause immunosuppression. Immunotoxicity of PAHs has been demonstrated in several different cells in vitro *(194–197)*. Immunotoxicity requires focal PAH metabolic activation in cells *(194,197)*. Immunosuppression in B6C3F1 mice has followed the structure–activity relationship observed for the carcinogenicity of PAHs, with benz[*a*]anthracene, B[*a*]P, dibenz[*a,c*]anthracene and dibenz[*a,h*]anthracene being more potent than anthracene, chrysene, benzo[*e*]pyrene and perylene *(136)*. The greatest immunosuppression was observed with 3-methylcholanthrene and 7,12-dimethylbenz[*a*]anthracene.

PAHs, including B[*a*]P, dibenz[*a,h*]anthracene, dibenz[*a,c*]anthracene and 7,12-dimethylbenz[a]anthracene, have accelerated atherosclerosis plaque formation in Ah-responsive mice, chickens and pigeons *(198,199)*. The atherogenic effect of PAHs may not be associated with their mutagenic and carcinogenic capacity *(200)*. PAHs are thought to cause adverse cardiovascular effects by metabolites through activation of the Ah-receptor: by increased production of reactive oxygen species, induction of inflammatory-mediated *(201)* and hypertrophic genes, and increased disruption of endogenous substances such as prostaglandins *(134)*. Therefore, induction of inflammation might be a crucial process in PAH-enhanced atherogenesis.

B[*a*]P has decreased fertility and caused embryotoxicity. It increased primordial oocyte destruction, decreased the number of corpora lutea, caused resorptions, decreased the number of pups and decreased fetal weight in rats and mice *(8)*. Sterility associated with alterations in gonadal tissues has also been observed in mice after prenatal exposure, in both females and males *(202)*. In male rats, B[*a*]P reduced testis weight *(203)* and decreased the testosterone level in the blood and sperm motility *(203,204)*, probably contributing to reproduction toxicity.

PAHs are also teratogenic in mice and rats *(8,205)*. Reproduction toxicity has also been noted by the dermal and parenteral exposure routes. Inhalation exposure of rats to B[*a*]P during pregnancy decreased plasma estrogen, progesterone and prolactin levels in dams in association with decreased pup survival and development, thus partly explaining the effects *(206)*. B[*a*]P exposure before conception caused Ah-receptor-dependent fetal intrauterine growth restriction in mice, which was associated with altered vasculature in the placenta and a decreased placental cell death rate *(207)*. Maternally toxic doses of B[*a*]P and 7,12-dimethylbenz[*a*]anthracene caused necrosis in the placenta and haemor-

rhages in fetuses in rats *(208)*, suggesting that the vascular system in general is one target of PAHs.

B[*a*]P, benz[*a*]anthracene and fluoranthene displayed weak estrogenic activity in rat immature uterotropic assay *(209)*, with 3–4 orders of magnitude lower potency than endogenous estrogen. Some PAHs (including B[*a*]P), and especially their hydroxylated metabolites, interact with estrogen receptors *(210,211)*. PAHs have also indicated anti-estrogenic effects *(212)*. Contrasting effects may be explained by a complex crosstalk between Ah-receptor and estrogen receptors and induction of estrogen metabolism by PAHs *(209)*.

PAH mixtures

In indoor air, exposure to PAHs occurs mostly in mixture. Since the toxicity of PAHs depends nearly exclusively on their biotransformation to toxic metabolites, interactions at the level of key metabolism enzymes are highly relevant to the associated health risk. Induction of metabolism by one PAH may enhance the toxicity of another; inhibition of the metabolism may decrease the toxicity.

Gene expression data with B[*a*]P and dibenzo[*a,h*]anthracene in binary mixture with dibenzo[*a,l*]pyrene, benzo[*b*]fluoranthene and fluoranthene in precision-cut rat liver slices in vitro indicated that (*a*) each of them induced an altered expression of genes, (*b*) the genes affected considerably by the combination of PAHs were slightly different from those altered by either of the constituents, (*c*) total altered expression of the genes by a mixture was less than that induced by individual PAHs and (*d*) the interactions were mostly antagonistic, leading to decreased altered expression of genes by the mixture compared to single PAHs *(213)*. Fewer DNA adducts were formed by the mixtures than by individual PAHs. In contrast, in human hepatoma cells (HepG2), equimolar and equitoxic mixtures of these same PAHs have shown an additive effect on apoptosis and cell cycle blockage, an additive or antagonistic effect on gene expression, and a synergistic effect on DNA adduct formation *(214)*. Since the interactions depend on PAH composition, concentrations and cell types, contrasting results may be expected. However, the majority of in vitro studies have shown an antagonistic effect on DNA adduct formation by a mixture of PAHs *(172,189,215)*.

The PAH metabolites have been shown to interfere with each other. The major B[*a*]P metabolite 3-hydroxybenzo(*a*)pyrene inhibits both the mutagenic and tumorigenic activity of the carcinogenic metabolite *anti*-B[*a*]PDE *(216)*. When applied topically to mouse skin, different binary and tertiary mixtures of carcinogenic PAHs formed DNA adducts at levels that were additive, less than additive and greater than additive relative to the levels formed when the compounds were applied individually *(217)*. Complex PAH mixtures have also decreased skin tumorigenicity of B[*a*]P *(218)* and that of dibenzo[*a,l*]pyrene in mice *(219)*. Decreased tumorigenesis was associated with decreased DNA adduct formation. In contrast, mixtures of five PAHs both enhanced and inhibited mouse lung tu-

morigenesis, depending on the composition of the PAH mixture and the dose *(220)*.

Altogether, the experimental data indicate that mixture effects of PAHs may be complex in vitro and in vivo. Attenuation of the toxicity rather than synergism has been observed in several studies. These observations imply that the effects of a PAH mixture may not be reliably predicted from single PAH components.

Biomarkers for evaluation of exposure

Internal exposure to PAHs has been assessed mostly by urine 1-hydroxypyrene or aromatic bulky DNA adducts in peripheral lymphocytes in humans *(8)*.

1-Hydroxypyrene, a metabolite of non-carcinogenic pyrene detectable in urine, may be used as a general biomarker of exposure to PAH mixtures *(8,221)*. Because urinary 1-hydroxypyrene displays all possible sources of PAHs (including food and ambient air) and all exposure routes, it indicates total exposure to pyrene-containing PAH mixtures. A good correlation between the PAH concentration in air and urine 1-hydroxypyrene has been observed in several occupational environments *(8)*. In single studies, a significant correlation has also been observed with exposure to residential indoor air sources of PAHs, such as ETS, cooking practices and the burning of coal for heating *(222,223)*. A review on environmental exposure to PAHs in ambient air indicated that urine 1-hydroxypyrene may serve as a qualitative indicator of excess exposure to PAHs at the group level but a large inter-individual variation limits its use for personal exposure *(224)*.

The levels of DNA adducts reflect not only the exposure to PAHs but also the body's ability to metabolize them. In general, exposures that have led to increased excretion of 1-hydroxypyrene have led to elevated adduct levels *(8)*. This is demonstrated best on occupational exposure to PAHs. However, the inter-individual variation in DNA adducts has been large (up to 50–100-fold) and the correlation between measured/estimated exposure to PAHs and adduct levels variable (from clear to no correlation, for example *(225)*). The correlation may be better when exposure is measured personally *(226)*. As to the general population, elevated DNA adduct levels in blood leucocytes have been detected in populations living in industrialized areas *(227–229)*. Consumption of charcoal-grilled food increases their levels *(230,231)*, as does high indoor air exposure *(232)*. Total PAH-DNA adduct levels and BPDE-DNA adduct levels were significantly higher in smokers than among non-smokers *(233)*. Altogether, PAH-DNA adducts can be used as a qualitative biomarker of exposure to combustion emissions, most reliably on a group basis. DNA adducts are considered to be a less sensitive parameter for exposure assessment than excretion of 1-hydroxypyrene in urine *(8,224)*.

Environmental exposure to PAHs may not be assessed reliably on the basis of exposure biomarkers such as urinary 1-hydroxypyrene concentration or aro-

matic bulky PAH-DNA adducts in blood cells. This is particularly true in indoor environments, where assessment at individual level is often needed. There are no proper risk functions, the individual variation in biomarkers is large and the biomarkers measure exposure to all possible sources of PAHs (including ambient air and food).

Human health effects

While the risks of occupational exposure to PAHs are not the focus of this chapter, indoor exposure to smoke from biomass and coal burning for the population in developing countries could be comparable to the levels of pollutants in the occupational setting *(234)*. For example, indoor particulate matter (< 10 μm) levels in solid-fuel-burning households reach up to several milligrams per cubic metre. An estimated half of the global population depends on solid fuel for cooking and heating, often in inadequately ventilated spaces *(234)*. Women and children are particularly vulnerable because of the longer time spent at home. Timing of exposure in children could influence disease risk owing to the sensitive window of development, as well as exposure levels that are often higher relative to their body size. Indoor smoke exposure in such settings remains one of the top ten risks in the global burden of disease *(235)*.

Identification of studies

PubMed was searched in English, the search being restricting to human studies. For non-carcinogenic effects, the following search terms were used: "polycyclic aromatic hydrocarbons AND indoor AND birth weight", "polycyclic aromatic hydrocarbons AND smoke", "polycyclic aromatic hydrocarbons AND biomass", "polycyclic aromatic hydrocarbons AND coal burning", "polycyclic aromatic hydrocarbons AND indoor AND intrauterine growth restriction", "polycyclic aromatic hydrocarbons AND indoor AND low birth weight", "polycyclic aromatic hydrocarbons AND indoor AND small-for-gestational age", "polycyclic aromatic hydrocarbons AND indoor AND birth length", "polycyclic aromatic hydrocarbons AND indoor AND birth head circumference", "polycyclic aromatic hydrocarbons AND indoor AND fetal growth", "polycyclic aromatic hydrocarbons AND indoor AND neurodevelopment", "polycyclic aromatic hydrocarbons AND indoor AND PAH-DNA adducts", "polycyclic aromatic hydrocarbons AND indoor AND bronchitis" and "polycyclic aromatic hydrocarbons AND indoor AND asthma".

For carcinogenic risk, the following search terms were used: "polycyclic aromatic hydrocarbons AND 1-hydroxypyrene", "polycyclic aromatic hydrocarbons AND PAH-DNA adducts", "polycyclic aromatic hydrocarbons AND DNA", "polycyclic aromatic hydrocarbons AND chromosom*", "polycyclic aromatic hydrocarbons AND cancer", "polycyclic aromatic hydrocarbons AND occupational", "polycyclic aromatic hydrocarbons AND ischaemic heart disease", "poly-

cyclic aromatic hydrocarbons AND cognitive" and "polycyclic aromatic hydrocarbons AND neurodevelopment*".

The search identified 455 papers. Moderate to large population-based prospective cohort studies using a quantitative assessment of PAH exposure were given first priority. Studies that did not adjust for known and potential confounders were not considered. Clinical trials, risk assessments, reviews of the literature, future studies, case reports, diagnostic guidelines and studies that lacked quantitative assessment of exposure were also excluded from the review. Subsequently, 178 papers were chosen for full review and 56 papers are included in the present report. In addition, earlier reviews by WHO *(2,8)*, IARC *(70,92,260)* and other authors *(130)* were considered.

Non-carcinogenic effects

Intrauterine growth restriction. Intrauterine growth restriction has been operationalized as low birth weight (< 2500 g), low birth weight at full term and small for gestational age (SGA), defined as < 10th percentile of population mean weight at a given gestational age and gender.

Prenatal exposure to PAHs has been associated with reduction in birth weight, an increased likelihood of low birth weight in Europe and the United States *(236)* and SGA *(237,238)* in a dose-responsive manner, after controlling for region- and cohort-specific sets of confounders. In Teplice and Prague (Czech Republic), PAHs isolated from respirable particulate matter during winter induced the highest genotoxicity and embryotoxicity *(239)*. High ambient concentrations of PAHs, PM_{10} and $PM_{2.5}$ during the first month of gestation were associated with a significantly elevated risk of SGA in the industrial city of Teplice *(237)*. A study in Poland showed that neonates with high levels of PAH-DNA adducts in the leucocytes had significantly lower birth weight, length and head circumference *(240)*.

Two parallel prospective cohort studies in Krakow and New York City enrolled non-smoking pregnant women with no known risks of adverse birth outcomes and monitored their personal PAH exposure concentrations *(241)*. Mean personal PAH exposures to eight carcinogenic PAHs[12] differed more than 10-fold between the two cities (Krakow mean 39.0 ng/m^3, range 1.8–272.2 ng/m^3; New York mean 3.3 ng/m^3, range 0.3–36.5 ng/m^3). In the Krakow cohort, prenatal exposure to the summed eight carcinogenic PAHs was significantly associated with reduced birth weight (68.75 g),[13] birth length (0.48 cm) and birth head circumference (0.21 cm) *(241)*. In the New York cohort, however, prenatal exposure to PAHs was associated with reduced birth weight (177.57 g) among New York African Americans but not among New York Dominicans *(241)*. Furthermore, a natural log-unit in-

[12] Sum of benz[*a*]anthracene, chrysene/isochrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, B[*a*]P, indeno[*1,2,3-c,d*]pyrene, dibenz[*a,h*]anthracene and benzo[*g,h,i*]perylene.

[13] Per natural-log unit of the carcinogenic PAHs.

crease in prenatal PAH exposure was associated with a two-fold increase in the likelihood of being born SGA among African Americans but not Dominicans in New York *(238)*. SGA is one of the most clinically predictive markers of fetal growth impairment. SGA has been associated with a significantly greater risk of delayed neurodevelopment *(242,243)*, shorter stature, cardiovascular disease, insulin resistance and diabetes during adulthood *(244,245)*. Among the New York African Americans, a natural log-unit of prenatal exposure to PAHs[14] was associated with a five-fold greater risk of preterm delivery (< 37 weeks at birth) *(238)*. While residual confounding remains a possible alternative explanation, African American neonates and children appear to be more vulnerable.

Bronchitis, asthma and asthma-like symptoms. In the same New York City cohort, prenatal exposure to the particle-bound PAHs may increase the risk of asthma symptoms by the age of 1–2 years *(246)*. In Teplice and Prachatice in the Czech Republic, unit exposure to ambient 100 ng/m^3 PAHs, based on a 30-day average, and a unit exposure to 25 μg/m^3 $PM_{2.5}$ were respectively associated with 56% (95% CI 22–100) and 23% (95% CI –6 to –62) increases in the risk of bronchitis in children between the ages of 2 and 4½ years *(247)*.

Fatal ischemic heart disease. A multinational cohort of male asphalt workers was followed for fatal ischemic heart disease (IHD) for 17 years (SD = 9 years). Mean personal exposure to B[*a*]P for the cohort was 273 ng/m^3. For those exposed to ≥ 273 ng/m^3 B[*a*]P, the risk of IHD mortality was 1.64-fold greater (95% CI 1.13–2.38) than in those exposed to ≤ 68 ng/m^3 *(248)*. The risk increased in a dose-responsive manner. At the highest PAH exposure category, cigarette smoking by the workers did not explain the significant increase in IHD mortality risk, thus supporting the etiological role of B[*a*]P.

Neurodevelopmental index

In the same New York City birth cohort, those neonates with a higher than median prenatal PAH exposure (range 4.16–36.47 ng/m^3) had a significantly lower Bayley Mental Development Index as well as a greater likelihood of cognitive developmental delay at the age of three years compared to children exposed to 0.27–4.15 ng/m^3, controlling for ethnic background, gender, gestational age, level of nurturing provided at home, ETS and chlorpyrifos exposure *(249)*.

In a cross-sectional investigation in Tongliang, China, babies born close to a coal-fired power plant in 2002 were associated with 0.32 ± 0.14 B[*a*]P DNA adducts per 10^8 nucleotides *(250)*. Such a level of B[*a*]P adducts at birth was associated with a decreased motor development quotient at two years of age *(250)*. A

[14] Sum of benz[*a*]anthracene, chrysene/isochrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, B[*a*]P, indeno[*1,2,3-c,d*]pyrene, dibenz[*a,h*]anthracene and benzo[*g,h,i*]perylene.

0.1-unit increase in B[*a*]P DNA adducts per 10^8 nucleotides measured at birth was associated with two-times greater likelihood of developmental delay in motor dimension at the age of two years *(250)*. However, following the shut-down of the Tongliang power plant in 2004, lower (0.20 per 10^8 nucleotides) B[*a*]P adduct levels in cord blood were observed in a new cohort of children *(251)*. Subsequently, in 2005, cord blood B[*a*]P adducts were not associated with a reduction in developmental status at two years of age *(251)*.

Human carcinogenic risk

Early biological effects of PAH exposures have been examined in children in a small number of studies. A group of rural Indian children had significantly higher blood PAH concentrations (125.55 ± 26.99 ppb) than those from an urban region (23.96 ± 13.46 ppb), consistent with higher home exposure to the burning of wood, coal, cow dung and kerosene *(252)*. The extent of lipid peroxidation in whole blood was positively, albeit weakly, correlated with their total PAH and carcinogenic PAH levels in whole blood *(252)*. Among school-age children in Bangkok, the major source of exposure was road traffic.

When a group of Bangkok children were compared to a group of rural children, those from Bangkok had 3.5-fold higher exposure to B[*a*]P-equivalent compounds (mean 1.50 ± 0.12 vs 0.43 ± 0.05 ng/m^3). The Bangkok children's corresponding urinary 1-hydroxypyrene levels were significantly higher than those of the rural children. Furthermore, the levels of lymphocyte PAH-DNA adducts in the Bangkok children were 5-fold higher than those for the rural schoolchildren (0.45 ± 0.03 vs 0.09 ± 0.00 adducts/10^8 nucleotides). The frequency of DNA strand breaks was significantly higher, while the DNA repair capacity was significantly impaired in the Bangkok children compared to the rural children *(253)*.

In Prague, the mean personal exposure to 1.6 ng/m^3 of B[*a*]P during winter was associated with an elevated level of PAH-DNA adducts in a group of policeman *(254)*. Compared to the non-smoking controls (mean B[*a*]P = 0.8 ng/m^3), the frequency of chromosomal translocations for the policemen was significantly higher, based on fluorescence in situ hybridization (FISH) *(255)*. Within each individual subject, the level of "B[*a*]P-like" DNA adducts was a significant predictor of genomic frequency of translocations as detected in terms of FISH, percentage of aberrant cells and aberrations per cell *(255)*. In other groups of city policemen in Prague, DNA adducts were also positively correlated with genomic frequency of translocations *(254,256)*. Corresponding personal exposure to B[*a*]P was 1.6 ng/m^3 vs 0.4 ng/m^3 (in controls) *(254,256)*. Personal B[*a*]P exposure > 1.0 ng/m^3 also increased the micronuclei measured by automated image analysis *(257)* as well as DNA fragmentation in sperm *(268)* in the group of city policemen. All these results from biomonitoring studies indicate that exposure to B[*a*]P concentrations > 1.0 ng/m^3 induce DNA damage.

Carcinogenic effects of exposure to PAHs

In 2005, an IARC working group evaluated a number of occupational exposures to PAH-containing complex mixtures for their potential to induce cancer in humans. Occupational exposures during chimney sweeping, aluminium production, coal gasification, coke and steel production, coal-tar distillation, and paving and roofing with coal-tar pitch have been classified as carcinogenic to humans (Group 1). Occupational exposures to PAH-containing complex mixtures have been strongly associated with lung cancer and some of these exposures have a highly suggestive association with bladder and urinary tract tumours *(259)*. In addition, B[*a*]P was upgraded to a human carcinogen, based on sufficient evidence of carcinogenic activity in animals and strong evidence that the mechanisms of carcinogenicity in animals operate in humans *(259)*. The working group also updated a list of probable carcinogens to humans (Group 2A) and possible carcinogens to humans (Group 2B), as shown in Table 6.11. Cigarette smoke contains PAHs and cigarette smoking has also been classified as carcinogenic to humans *(260)*.

Table 6.11. IARC Classification of agents and occupations

Group 1: carcinogenic to humans	Group 2A: probably carcinogenic to humans	Group 2B: possibly carcinogenic to humans
Occupational exposure during coal gasification	Occupational exposure during carbon electrode manufacture	Benz[*j*]aceanthrylene
Occupational exposure during coke production	Creosote	Benz[*a*]anthracene
Occupational exposure during coal-tar distillation	Cyclopenta[*cd*]pyrene	Benzo[*b*]fluoranthene
Occupational exposure during chimney sweeping	Dibenz[*a*,*h*]anthracene	Benzo[*j*]fluoranthene
Occupational exposure during paving and roofing with coal-tar pitch	Dibenzo[*a*,*l*]pyrene	Benzo[*k*]fluoranthene
Occupational exposure during aluminium production		Benzo[*c*]phenanthrene
B[*a*]P		Chrysene
Diesel exhaust		Dibenzo[*a*,*i*]pyrene
ETS		Dibenzo[*a*,*h*]pyrene; indeno[1,2,3-*c*,*d*]pyrene; 5-methylchrysene

Mutagenic and carcinogenic risk of PAH-DNA adducts in adults

PAH-DNA adduct formation represents one of the key first steps in carcinogenesis *(261)*. PAH/aromatic DNA adducts have been associated with increased lung cancer risk in some molecular epidemiological studies. In a nested case-control study, healthy volunteers with detectable adduct levels in their leukocytes at the outset of the study were at two-fold greater risk of lung cancer than those with non-detectable levels *(262)*. In particular, never-smokers with a detectable adduct level were at four-fold higher lung cancer risk than those with a non-detectable level *(262)*. When the exposure was dichotomized at the median (0.6 DNA adducts per 10^8 nucleotides), the non-smokers with greater than median adduct levels were at seven-fold greater risk of lung cancer than those with non-detectable adducts *(262)*. In a small cross-sectional case-control study, those within the highest quartile of leukocyte PAH-DNA adduct levels (> 1.52 PAH-DNA adducts per 10^8 nucleotides) were at three-fold greater risk of colorectal adenoma than the lowest quartile of the adducts (≤ 0.71 adducts per 10^8 nucleotides) *(263)*.

In population-based case-control studies, the detectable level of PAH-DNA adducts has been associated with a 29–35% elevation in breast cancer risk *(261)*. Several genetic variants have been examined for their role in cancer development. A variant allele (GA or AA) in the FAS1377 gene was associated with a 36% increase in breast cancer risk among those with detectable PAH-DNA adduct levels *(264)*. However, variants of other genes, including GSTA1, GSTM1, GSTP1 and GSTT1, did not modify the risk *(265)*, while any dose–response relationship was consistently absent in several molecular epidemiological investigations *(261,266,267)*. In a large population-based cohort, detectable PAH-DNA adducts, measured at the time of the patient's breast cancer diagnosis, were not associated with subsequent all-cause or breast-cancer-specific mortality *(268)*. In a prospective follow-up study of prostate cancer, the risk of biochemical recurrence one year after surgical removal of the tumour was twofold greater in those with higher than the median level of prostate-specific adducts *(269)*.

Quantitative estimates of carcinogenic effects at occupational exposure range

In a combined meta-analysis of the aluminium production, coal gasification, coke production, iron and steel, coal tar, carbon black and carbon electrode production industries, cumulative B[*a*]P exposure concentrations over the working years ranged from 0.75 to 805 μg/m^3-years, equivalent to a concentration range of 0.04–40 μg/m^3 *(270)*. The mean relative risk of lung cancer increased by between 20% and 168% per 100 μg/m^3-years *(270,271)*. The mean risk remained robust when study design, smoking status and exposure measurement were accounted for *(270)*. While risk sizes were consistent within each occupation, large variability in lung cancer risk was observed across the occupations. Such heterogeneity in relative risk was attributed to variability in occupation-related exposure range.

Another meta-analysis estimated the industry-specific relative risk of respiratory and urinary tract cancers *(272)*. Risk of lung cancer was significantly elevated for all examined industries *(272)*. In particular, within the aluminium smelting industry, 100 μg/m^3-years exposure to B[*a*]P (equivalent to 3.3 μg/m^3 for 30 years) was associated with a 2.68-fold greater likelihood of developing lung cancer *(271)*. While a significant departure from linearity was observed in this analysis, indicating that the unit risk might be smaller at the highest exposure range, this pooled estimate sufficiently accounted for confounding by smoking *(271)*.

The risk of bladder cancer was significantly elevated only for those involved in aluminium production.

Conclusions

- Sources of airborne PAHs indoors are infiltration by PAHs in outdoor air and indoor emissions from smoking, domestic cooking and heating with fuel-burning stoves, open fireplaces, and incense and candle burning.
- In the absence of indoor sources, indoor concentrations of PAHs are lower than those outdoors.
- When indoor sources are present, indoor concentrations are likely to exceed those outdoors.
- In industrialized countries, ETS appears to have the greatest impact on indoor PAH concentrations, while in developing countries it is cooking and heating with solid and biomass fuels.
- In industrialized countries, inhalation is a minor route of exposure for non-smokers compared with dietary ingestion.
- In developing countries, inhalation is at least as important a route of exposure as dietary exposure.
- A sufficient body of evidence supports the causal role of PAH/aromatic DNA-adducts in lung cancer occurrence among non-smokers.
- B[*a*]P at levels above 1.0 ng/m^3 predicted greater genomic frequency of translocations, micronuclei and DNA fragmentation.
- Prenatal exposure to PAHs is associated with an increase in the likelihood of low birth weight.
- B[*a*]P and many other PAHs induce cancer by a mutagenic mechanism that involves metabolic activation to reactive diol-epoxides that covalently bind to DNA. These PAH-DNA adducts have been detected in tissues from experimental animals exposed to PAHs, and B[a]P-DNA adducts have been found in human lung tissues.
- PAH-DNA adducts are converted into mutations after cell replication, and mutations in critical tumour oncogenes and tumour suppressor genes have been identified in lung tumours from both experimental animals and humans exposed to PAHs or PAH-containing mixtures.

- Sufficient evidence exists of a link between the prenatal exposure to mixtures of carcinogenic PAHs and intrauterine growth restriction in humans.
- There is a robust body of evidence supporting a strong association between occupational exposure to PAH-containing mixtures and lung cancer in humans.

Health risk evaluation

Critical health outcomes

Biomarkers of exposure and effects

A growing body of evidence suggests that exposure to B[*a*]P at levels over 1.0 ng/m^3 induces DNA damage. Personal exposure to B[*a*]P over 1.0 ng/m^3 predicted greater genomic frequency of translocations, micronuclei and DNA fragmentation in sperm. In children in various developing countries, a number of markers for cytotoxic and oxidative stress have been positively correlated with either monitored personal PAH concentration or carcinogenic PAH levels in whole blood. Further, elevated exposure to B[*a*]P has been associated with higher levels of PAH-DNA adducts, urinary 1-hydroxypyrene, DNA strand breaks and impaired DNA repair capacity.

Intrauterine growth restriction

Intrauterine growth restriction has been defined in terms of low birth weight (< 2500 g), low birth weight at full-term (≥ 37 weeks) or SGA (< 10th percentile of population mean weight at a given gestational age and gender). A strong body of data demonstrates a significant role of prenatal exposure to particle-bound PAHs in reduced or low birth weight in Europe and the United States *(236)*. The direction and size of birth weight reduction are consistent overall. In addition, prenatal exposure to several PAHs induced severe fetotoxic effects in several animal species. Thus, it is concluded that sufficient evidence exists of a relationship between prenatal exposure to mixtures of carcinogenic PAHs and intrauterine growth restriction in humans.

Lung cancer

Occupational exposure to complex mixtures containing PAHs has been strongly associated with lung cancer. Studies on experimental systems have shown many PAHs to be genotoxic and carcinogenic. A detectable level of leukocyte PAH/bulky DNA adducts in non-smokers was correlated with an increased risk of lung cancer compared to those with a non-detectable level. When coded as a continuous variable, each unit increase in DNA adducts led to a 25 % increase in the risk of lung cancer, without a threshold. In occupational settings, with B[*a*]P exposure ranging between 0.04 and 40 μg/m^3, the risk of lung cancer increased by 20–168% per 100 μg/m^3 B[*a*]P-years. As a result, sufficient evidence of a causal relationship is considered to exist between exposure to mixtures of airborne

PAHs containing B[*a*]P and lung cancer. There is a sufficient body of evidence to support the critical role of PAH/bulky DNA adducts, mutations in tumour oncogenes and tumour suppressor genes in the development of lung cancer following exposure to PAHs.

Bladder cancer

Risk of bladder cancer has often been examined along with that of lung cancer in investigations of occupational exposures to PAHs. The risks were significantly elevated for some industries involving exposure to complex mixtures containing PAHs, namely aluminium production, paving and roofing, and chimney sweeping. Experimentally, PAHs have not been shown to induce bladder cancer. It is concluded that insufficient evidence exists of a relationship between exposure to mixtures of airborne PAHs containing B[*a*]P and bladder cancer.

Breast cancer

Risk of breast cancer has been examined mostly in terms of detectable levels of PAH-DNA adducts. A modest (29–35%) elevation in the likelihood of breast cancer diagnosis has been observed for those with detectable levels of PAH-DNA adducts compared to non-detectable levels, without an apparent dose–response relationship. However, when the follow-up was continued to the point of death, the detectable PAH-DNA adducts did not increase the risk of all-cause or breast cancer mortality. It is concluded that insufficient evidence exists for a relationship between exposure to mixtures of airborne PAHs containing B[*a*]P and breast cancer.

Fatal ischaemic heart disease

In experimental animals, PAHs including B[*a*]P, dibenz[*a,h*]anthracene, dibenz[*a,c*]anthracene and 7,12-dimethylbenz[*a*]anthracene have accelerated atherosclerosis plaque formation *(198,199)*. Following several inconclusive studies, a multinational cohort of male asphalt workers suggested that risk of ischemic heart disease (IHD) was associated with B[*a*]P exposure in a dose–responsive manner. However, the authors could still not rule out the possibility of confounding by exposure to fine particulates in the occupational setting *(248)*. It is concluded that limited evidence exists for a relationship between exposure to mixtures of airborne PAHs containing B[*a*]P and IHD. If these findings are corroborated, the IHD mortality from PAH exposure would be higher than that for lung cancer.

Health relevance of indoor exposure

Since there is sufficient evidence that some PAHs, including B[*a*]P, are genotoxic carcinogens, no threshold can be determined and all indoor exposures are considered relevant to health.

Indoor airborne levels of PAHs are influenced not only by infiltration of outdoor PAHs but also by lifestyle-related indoor emissions. In particular, children and women in developing countries are exposed to multiple and season-dependent sources of PAHs, including domestic burning of wood, coal, cow dung and kerosene, as well as industrial coal-burning and road traffic. Indoor B[*a*]P levels in homes that use biomass and coal for heating and cooking range from 33 to 186 ng/m^3 (range of geometric means) compared to B[*a*]P levels generally less than 1 ng/m^3 in non-smoking homes in developed countries (typically based on a 24-hour mean). The indoor B[*a*]P concentrations in developing countries increase the inhalation doses (105–2523 ng/day; range of geometric means). Hence, in these situations, the inhalation of particle-bound PAHs is at least as important a route of exposure as dietary exposure.

Conclusions of other reviews

IARC *(273)* concluded, based on occupational studies, that there is sufficient evidence that coal gasification, soot (as found in occupational exposure of chimney sweeps), aluminium production, coal tar pitch (as encountered in paving and roofing), iron and steel founding and coke production cause human lung cancer *(259)*. There is sufficient evidence that aluminium production causes bladder cancer in humans.

There is limited evidence in humans for a causal association of soot and coal tar pitch with bladder cancer *(259)*. Indoor emissions from household combustion of coal are carcinogenic to humans (Group 1), inducing lung cancer. Indoor emissions from household combustion of biomass fuel (mainly wood) are probably carcinogenic to humans (Group 2A), inducing lung cancer. Emissions from high-temperature frying are probably carcinogenic to humans (Group 2A) *(259)*.

B[*a*]P was reclassified by IARC *(260)* as a human carcinogen (Group 1) based on sufficient evidence of carcinogenic activity in animals and strong evidence that the mechanisms of carcinogenicity in animals operate in humans *(259)*. Cigarette smoke contains PAHs and ETS has also been classified as carcinogenic to humans (Group 1) *(260)*.

WHO concluded in 2000 *(2)* that occupational epidemiology data should serve as the basis for the risk estimate. Based on epidemiological data from studies in coke-oven workers, a unit risk for B[*a*]P as an indicator in ambient air constituents was estimated to be 8.7×10^{-5} per ng/m^3, which is the same as that established by WHO in 1987 *(23)*.

Guidelines

Some PAHs are potent carcinogens and, in air, are typically attached to particles. The primary exposure to carcinogenic PAHs found in air occurs via inhalation of particles. PAHs occur in indoor air as complex mixtures, the composition of

which may vary from site to site. Experimental data on metabolism, gene expression and DNA adducts suggest that interactions between PAHs in mixtures may be complex and highly unpredictable for various PAH compositions (inhibitory, additive, synergistic).

In view of the difficulties in developing guidelines for PAH mixtures, B[*a*]P was considered to represent the best single indicator compound. Its toxicology is best known, most single PAH concentration data in ambient and indoor air are for B[*a*]P, and B[*a*]P has widely been used as an indicator compound for exposure in epidemiological studies.

The health evaluation data suggest that lung cancer is the most serious health risk from exposure to PAHs in indoor air. B[*a*]P is one of the most potent carcinogens among the known PAHs.

In its evaluation of PAHs as ambient air pollutants in 2000, WHO *(2)* expressed a unit cancer risk as a function of the concentration of B[*a*]P taken as a marker of the PAH mixture. Use of the same unit risk factor for indoor air implies that B[*a*]P represents the same proportion of carcinogenic activity of the PAH mixture as in the occupational exposure used to derive the unit risk. This assumption will not always hold, but the associated uncertainties in risk estimates are unlikely to be large.

Reducing exposure to B[*a*]P may also decrease the risk of other adverse health effects associated with PAHs.

Based on epidemiological data from studies on coke-oven workers, a unit risk for lung cancer for PAH mixtures is estimated to be 8.7×10^{-5} per ng/m^3 of B[*a*]P. This is the guideline for PAH in indoor air. The corresponding concentrations for lifetime exposure to B[*a*]P producing excess lifetime cancer risks of 1/10 000, 1/100 000 and 1/1 000 000 are approximately 1.2, 0.12 and 0.012 ng/m^3, respectively.

The guidelines section was formulated and agreed by the working group meeting in November 2009.

Summary of main evidence and decision-making in guideline formulation

Critical outcome for guideline definition

Lung cancer is the most serious health risk from exposure to PAHs in indoor air. B[*a*]P is one of the most potent carcinogens among the known PAHs.

Source of exposure–effect evidence

There is sufficient evidence that some PAHs, including B[*a*]P, are genotoxic carcinogens. Based on epidemiological data from studies in coke-oven workers, a unit risk for B[*a*]P as an indicator of PAH in ambient air was estimated to be 8.7×10^{-5} per ng/m^3 *(2,23)*.

Supporting evidence

Studies on early biological effects of PAH exposure based on biomarkers in general populations of children and adults *(252–257)*, on carcinogenic effects in the occupational setting *(259)* and on mutagenic and carcinogenic risk of PAH-DNA adducts *(261–269)*.

Results of other reviews

IARC: B[*a*]P and PAH-containing indoor emissions from household combustion of coal have been classified in Group 1 (human carcinogens) *(259,260)*.

Guidelines

- No threshold can be determined and all indoor exposures are considered relevant to health.
- Unit risk for lung cancer for PAH mixtures is estimated to be 8.7×10^{-5} per ng/m^3 of B[*a*]P.
- The corresponding concentrations for lifetime exposure to B[*a*]P producing excess lifetime cancer risks of 1/10 000, 1/100 000 and 1/1 000 000 are approximately 1.2, 0.12 and 0.012 ng/m^3, respectively.

Comments

B[*a*]P is taken as a marker of the PAH mixture. Use of the B[*a*]P unit risk factor assumes that B[*a*]P represents the same proportion of carcinogenic activity of the PAH mixture in all indoor environments as in the occupational setting. This assumption will not always hold, but the associated uncertainties in risk estimates are unlikely to be large.

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7. Radon

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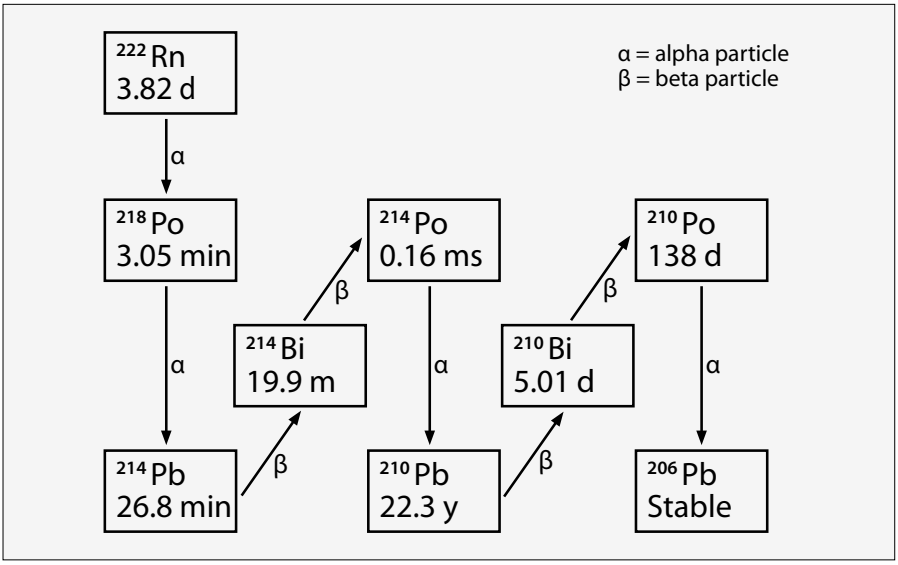
General description

Radon gas is an important source of ionizing radiation of natural origin and a major contributor to the ionizing radiation dose received by the general population. It comes mainly from exposure to radon and its airborne decay products in the homes of the general population *(1,2)*. Radon, which has a number of isotopes, is a naturally occurring colourless and odourless radioactive noble gas. The most stable of the isotopes is radon-222 (^{222}Rn) (half-life 3.826 days), which is universally and henceforth here referred to simply as "radon" or "radon gas". It is a member of the uranium-238 (^{238}Ur) decay series (half-life 4.5 × 10^9 years) and its immediate parent is radium-226 (^{226}Ra) (half-life 1620 years). Radon formed by the decay of radium in soil and rocks and entering the indoor air spaces of buildings or other enclosed locations (such as mines, tunnels or other underground workplaces) may reach concentrations of concern for health. Fig. 7.1 is a simplified decay scheme of radon-222 showing its principal short-lived progeny of radiological importance.

Fig. 7.1. Simplified decay scheme for radon, with isotpes and half-lives



Conversion factors and units

The SI unit for the activity of a radioactive substance is the becquerel (Bq), which is one radioactive decay per second. In indoor air, the degree of radioactive equilibrium between its airborne short-lived progeny and radon gas depends on several factors, principally on the aerosol concentration and its size distribution, the surface-to-volume ratio of the room and the air exchange rate. The degree of equilibrium is usually expressed in terms of the equilibrium factor (F factor), whereby an F factor of 1 means full radioactive equilibrium between radon and its airborne short-lived progeny. The F factor is important for determining the dose to the lungs from radon progeny. Measurements in several countries have shown F factors in dwellings to generally lie between 0.2 and 0.8. The United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR) and the International Commission on Radiological Protection (ICRP) have adopted a typical worldwide F factor of 0.4 for indoor air *(1,3)*. The F factor in outdoor air is usually somewhat higher, in the range 0.6–0.8.

Owing to these considerations, there are several measures used to describe airborne radon decay products. A commonly used measure is the equilibrium equivalent radon concentration, which is the activity concentration of radon (given in Bq/m^3) in equilibrium with its short-lived decay products that would have the same potential alpha energy concentration as the actual non-equilibrium mixture present in the air being measured. The potential alpha energy concentration is the sum of the potential alpha energy per unit volume of the short-lived radon progeny in the decay chain down to ^{210}Pb (half-life 22.3 years); its SI unit is J/m^3.

The potential alpha energy exposure of workers was historically expressed in terms of the working level month (WLM). Here one working level (WL) is any combination of short-lived radon progeny that will result in the ultimate emission of 1.3×10^5 MeV of potential alpha energy. This is approximately the alpha energy released by the decay of radon progeny in equilibrium with 3.7 Bq of radon. The WLM exposure unit was introduced and is still used to specify occupational exposure to 1 WL for a working month of 170 hours.

Analytical methods

Most of the radiation dose and hence the risk from radon is due to its short-lived alpha-particle-emitting polonium decay products (polonium-218 and polonium-214). Radon gas itself contributes a much smaller dose than these decay products. As an equilibrium factor of 0.4 is taken as being representative of indoor air in most homes, a measurement of radon gas is considered in general to be a good surrogate for estimating the concentrations of these decay products. Radon gas measurement is also technically much simpler and cheaper than measuring the decay products. Owing to both the effects of building usage practices (i.e. ventilation and heating) and meteorological variables, indoor

radon concentrations may exhibit quite large diurnal and seasonal variations. In the case of meteorological variables, their effects on radon exhalation from the ground and entry to dwellings is quite complex, with changes in atmospheric pressure, wind speed and precipitation being most important in this regard. As a consequence of these weather effects, in addition to diurnal and seasonal variations, indoor radon concentrations show substantial year-to-year variability.

To make a reliable estimate of the radon risk, it is thus necessary to make long-term (three months to a year) measurements of radon *(4)*. For measurements of a few months, a seasonal adjustment factor, if available, may be applied to obtain an estimate of the annual average value. Measurements made during the course of a single year also need correction for the aforementioned year-to-year variation *(5)*. From a health assessment perspective, short-term measurements of radon (duration of some days) are of limited use but may be of use in radon screening surveys to identify locations with a potential for high radon concentrations or when remediating a dwelling with a radon problem.

Owing to their low unit cost and reliability, the most popular devices used for making long-term radon measurements in European countries are small, passive devices using alpha-particle-sensitive material. These solid state nuclear track materials record the damage in the form of sub-microscopic latent tracks caused by alpha particles from radon and its decay products striking their surface. The latent tracks caused by the alpha particles striking the detector material are enlarged and made visible for optical microscopy by chemical or electrochemical etching *(6)*. These radon detectors are very simple and rugged in construction. They consist of a small piece of the alpha track material mounted inside a pill-box-sized chamber into which radon gas may diffuse. These detectors are passive as they do not require electrical power. The most commonly used alpha-particle-sensitive materials used in these detectors are polyallyl diglycol carbonate (CR-39), cellulose nitrate (LR-115) and polycarbonate (Makrofol). After exposure to radon and subsequent processing, the measured alpha track density is directly proportional to the integrated radon exposure in Bq/hour per m^3. The conversion from track density to the mean radon concentration over the exposure period is achieved by controlled exposure of the detector to a calibrated concentration of radon in a sealed exposure chamber. Comparisons between laboratories in Europe measuring radon take place regularly at the Radiation Division of the Health Protection Agency in the United Kingdom (formerly the National Radiological Protection Board), having originally been organized jointly with the European Commission *(7)*.

In addition to the alpha track passive detectors described above, passive charged electret radon detectors are also available, as well as a range of electronic continuous monitors of both radon gas and its decay products *(4,8,9)*.

These techniques are useful in determining contemporary indoor radon levels. In residential radon epidemiological studies, contemporary radon levels in

the present and in previous dwellings of a person are generally used as surrogates for the unknown radon levels in these dwellings in the past. Indoor radon levels can, however, be quite variable on diurnal, seasonal and annual timescales and can also be affected by changes in indoor living habits over time. Because of this, using contemporary indoor radon measurements as surrogates for indoor radon levels in past decades poses challenges to the assessment of long-term past exposure to radon.

Retrospective techniques based on the measurement of the build-up in a dwelling over many years of long-lived radon progeny such as polonium-210 in glass (the surface trap technique) or in porous media (the volume trap technique) can give some insight into the indoor radon concentration in a dwelling in past decades *(10,11)*. Direct in vivo measurements of lead-210 in the human skeleton have also been used to assess exposures in the past *(12)*.

Sources, occurrence in air and exposure

Indoor air

All rocks contain some uranium, typically at concentrations of 1–3 ppm. The uranium content of a soil will be about the same as the uranium content of the rock from which the soil was derived. As radium-226, the immediate parent of radon, is a decay product of uranium, the higher the uranium content of the soil the greater the radium concentration and the higher the chance that houses built on such soil will have high levels of indoor radon. The main source of indoor radon is the radon produced by the decay of radium in the soil subjacent to a house. Soil gas containing radon enters a house through cracks and fractures in the foundations by pressure-driven flow, as the air in a house is generally warmer and therefore at a lower pressure than the subjacent soil gas *(13)*. Radon concentrations in soil air/gas typically range from less than 10 000 Bq/m^3 up to 100 000 Bq/m^3. Most houses draw less than 1% of their indoor air from the soil; the remainder comes from outdoor air, which is generally quite low in radon. Houses with poorly sealed foundations, built on high-permeability ground and with several entry points for soil gas may draw more than 10% of their indoor air from the soil. Even if the soil air has only moderate levels of radon, levels inside such houses may be very high.

In comparison to soil gas, the radon exhaling from building materials in most cases does not significantly contribute to indoor radon levels. The uranium and radium content of building materials will be similar to the rock or clay from which they are made. While this is generally low, there are some building materials that may have high concentrations of radium. Examples of these are alum shale concrete and building materials made of volcanic tuff, by-product phosphogypsum, and some industrial waste materials *(14)*.

Water supplies can also contribute to indoor radon levels. River and surface reservoir water supplies usually contain very little radon but groundwater may

contain high concentrations, depending on the uranium/radium content of the aquifer formation. Public waterworks using groundwater and private domestic wells often have closed systems and short transit times that do not remove radon from the water or permit it to decay. This radon is out-gassed from the water to the indoor air when the water is used for washing, cooking and other purposes in a house. The areas most likely to have problems with radon in groundwater are those that have high levels of uranium in the underlying rocks. Radon concentrations can reach several thousand Bq/l in water from drilled wells in regions with granite rock or other uraniferous rocks and soils *(15)*. This contributes to indoor radon and to exposure via ingestion but the dose to the lung per unit exposure arising from inhalation is much higher than that owing to ingestion *(16)*. A very rough rule of thumb for estimating the contribution of radon in domestic water supplies is that house water with 10 000 Bq/m^3 radon contributes about 1 Bq/m^3 to the level of radon in the indoor air.

The range and distribution of indoor radon levels in many countries have been determined both by national surveys and in other investigations. Table 7.1 gives a summary of indoor radon surveys that have been carried out in a number of European countries *(17)*. It should be noted, however, that the survey design was not the same for each country. In some countries, dwellings were selected on the basis of population density. In this approach, more measurements are made in large centres of population than in sparsely populated rural areas. This enables estimates to be made of the collective exposure and health risk of the general population in a country. Such information is useful for developing national radon control strategies by the relevant authorities. Some national surveys were made on a geographical basis, where the strategy was to achieve the same density of dwelling sampling per unit area irrespective of the national population density distribution. Notwithstanding these differences, the data presented here give a reasonably accurate overview of average radon concentrations in contemporary European dwellings. It should also be noted that the maximum radon concentration values quoted in Table 7.1 are the maximum values found in national survey data. In many countries, much higher indoor radon concentrations have been found in targeted surveys carried out in areas where high radon levels were expected to be present on the basis of geological characteristics. The results of such surveys yield an erroneously high average radon concentration when extrapolated to the whole country. Table 7.2 gives a summary of indoor radon data for a number of large non-European countries *(1)*. It should be noted that representative national surveys of indoor radon have not yet taken place in countries with the largest populations, such as China and India.

The only other radon isotope that can occur indoors in significant amounts is radon-220 (half-life 55.6 seconds). Radon-220 is referred to as thoron and is a member of the thorium-232 (half-life 1.4×10^{10} years) decay series. Its immediate parent is radium-224 (half-life 4.6 days). It should be noted that there has

Table 7.1. Radon surveys in dwellings in some European countries

Country and population (millions)	No. of dwellings sampled	Period and approximate duration of measurement per dwelling
Austria (8.2)	16 000	1991–2002 3 months
Belgium (10.4)	10 447	1995–present 3 months
Croatia (4.5)	782	2003–2005 1 year
Czech Republic (10.2)	> 150 000	1984–present 1 year
Denmark (5.5)	3120	1995–1996 1 year
Finland (5.2)	3074	1990–1991 1 year
France (62.2)	12 261	1980–2003 3 months
Germany (82.4)	> 50 000	1978–2003 1 year
Greece (10.8)	1277	1994–1998 1 year
Ireland (4.2)	11 319	1992–1999 1 year
Italy (58.0)	5361	1989–1998 1 year
Luxembourg (0.49)	2619	1993–2002 3 months
Netherlands (16.6)	952	1995–1996 1 year
Norway (4.6)	37 400	1990–1999 2 months
Poland (38.5)	2886	1992–1994 3 months
Portugal (10.7)	3317	1988–1991 2.5 months
Slovenia (2.0)	892	1993–1995 3 months
Spain (40.5)	5600	1990–2005 3 months
Sweden (9.0)	1360	1991–1992 3 months
Switzerland (7.6)	55 000	1980–2005 3 months
United Kingdom (61.0)	450 000	1980–2005 3–12 months

Source: compiled mainly from National Summary Reports at http://radonmapping.jrc.ec.europa.eu/.

been an increasing interest in indoor thoron in recent years. Owing to its short half-life, thoron in soil gas beneath a building, in most situations, cannot survive long enough to enter a house and thereby contribute to the level of thoron in indoor air. Indoor thoron is due to the exhalation of thoron from thorium that may be present in the materials forming the internal surfaces of the building. Some building materials, such as volcanic tuff in Italy, have been found to have a high thoron exhalation rate. While in general indoor thoron levels are low, research in recent years has identified uncommon situations, such as cave dwellings, where the doses from airborne thoron decay products can be significant and can even exceed those from the radon decay products in the same location *(18)*. In this context, it should be noted that for the same exposure (i.e. concentration by time) the dose from thoron decay products is estimated to be about four times that of radon decay products *(1,19)*. From the perspective of radiation dose to lung tissue due to inhalation, the most important airborne thoron decay product is lead-210 (half-life 10.64 hours). While lead-210 itself is a beta particle emitter when it decays in the lung, it gives rise to the alpha-emitting decay products bismuth-212 (half-life 60.5 minutes, 36% alpha particle energy $E\alpha$ = 5.5–6.1 MeV) and polonium-212 (half-life 3×10^{-7} seconds, alpha particle energy $E\alpha$ = 8.68 MeV).

Mean Bq/m³	Geometric mean Bq/m³	Percentage > 200 Bq/m³	Maximum Bq/m³	Percentage > 400 Bq/m³
97	61	12	8325	4
69	76	2.4	4500	0.5
68	n/a	7.2	751	1.8
140	110	12–18	25 000	2–3
53	64	2.9	590	0.2
120	84	12.3	33 000	3.6
89	53	8.5	4964	2
49	37	1.6	> 10 000	0.45
55	44	3.1	1700	1.1
89	57	7.5	1924	1.5
70	52	4.1	1036	0.9
115	n/a	n/a	2776	3
30	25	0.3	382	<0.0001
89	n/a	9	50 000	3
49	n/a	2	3261	0.4
86	39	n/a	3558	n/a
87	n/a	7.7	1890	2
90	45	6	15 400	2
108	56	9–13	3904	3–4
77	n/a	17	29 705	7
20	n/a	0.5	17 000	0.1

Outdoor air

Land masses are the sources of outdoor radon while sea waters, having minimal radium concentrations, act as sinks. As a consequence, outdoor air radon levels are much lower (circa 0.1 Bq/m³) over oceans and seas than over a continental land mass such as mainland Europe *(20)*. Outdoor radon levels are determined mainly by the soil characteristics (uranium/radium content, porosity

Table 7.2. Radon concentrations in dwellings from surveys in some non-European countries

Country and population (millions)	Mean Bq/m³	Geometric mean Bq/m³	Geometric standard deviation Bq/m³	Maximum value Bq/m³
Argentina (39)	35	25	2	211
Australia (18)	11	8	2.1	420
Canada (30)	34	14	3.6	1720
China (1316)	44	34	—	596
India (945)	57	42	2.2	210
Japan (125)	16	13	1.8	310
Republic of Korea (49)	53	43	1.8	1350
United States (269)	46	25	3.1	—

Source: UNSCEAR *(1,21)*.

and the consequent radon exhalation rate), local topology and meteorological conditions. In some situations, such as atmospheric temperature inversions in valleys with high radon fluxes from the soil, short-term elevated outdoor radon levels have been observed. High outdoor radon levels are rare but could be of local health significance in areas such as former uranium mining districts, where elevated radon exhalation from tailing ponds combined with meteorological and topological conditions could give rise to high outdoor radon levels of seasonal duration. A direct proportionality in risk between indoor and outdoor radon exposures based simply on radon concentration and duration of exposure cannot, however, be assumed. This is because factors that influence the lung dose, such as the equilibrium factor between radon and its decay products (which are generally higher outdoors than indoors) and also aerosol characteristics, will be different indoors than outdoors.

National data on average outdoor radon levels are quite limited. It seems that they lie between 5 and 20 Bq/m^3 *(21)*. The ratio of the radon concentration in outdoor air to the mean indoor radon concentration in European countries (see Table 7.1) would appear to be in the range of about 7% (Czech Republic) to 20% (United Kingdom).

Routes of exposure

The most important route of exposure to radon and its decay products is inhalation. It is also possible to be exposed to radon by ingestion of water containing high radon concentrations but the dose and risk in this case are very small in comparison to those due to inhalation. In indoor air, radon produces a series of short-lived decay products that may attach to aerosol particles present in the air or deposit on room surfaces *(22)*. It is the inhalation and deposition in the airways of short-lived airborne radon decay products that give rise to irradiation by alpha particles of sensitive cells in lung tissue, such as the basal cells of the bronchial epithelium *(15)*. From considerations of their respective radioactive half-lives as well as their physical and chemical properties, the radiation dose delivered to the lung from inhaled radon decay products is dominated by the alpha particles emitted by the short-lived radon decay products polonium-218 (half-life 3.05 minutes, alpha particle energy E_α = 6.00 MeV) and polonium-214 (half-life 1.64×10^{-4} seconds, alpha particle energy E_α = 7.68 MeV). Because these alpha particles have respective ranges of only 48 μm and 71 μm in tissue, they deliver a high density of DNA damage to cells in these short distances.

Kinetics and metabolism

Absorption and doses

Dosimetry of inhalation of radon and its decay products is important in understanding the biological mechanisms and in estimating the effects of different factors that contribute to carcinogenesis *(21)*. Estimates of the absorbed dose per

unit of radon exposure to various organs and tissues can be derived from information on, for example, the unattached fraction, the activity/size distribution of the radon progeny aerosol, breathing rate, fractional deposition in the airways, mucus clearance rate, location of the target cells in the airways, and lung-to-blood absorption parameters. The doses to various lung tissues may be calculated using the ICRP human respiratory tract model *(23)* and other models. Dose estimates strongly depend on the choice of input parameters and other model assumptions, thus leading to some uncertainty in estimated absorbed doses *(24)*. The various dose calculation procedures and assumptions have been reviewed in detail in several reports *(1,21)*.

Kendall & Smith (*16*) estimated the doses to various organs and tissues from radon and its decay products, either by inhalation or ingestion or by deposition on the skin. With respect to inhalation, about 99% of the lung dose arises from radon progeny and not from the gas itself, as almost all of the gas that is inhaled is subsequently exhaled *(25)*. Radon decay products are largely deposited on the surface of the respiratory tract. Because of their relatively short half-lives, they decay mainly in the lung before being cleared either by absorption into the blood or by particle transport to the gastrointestinal tract. Thus, in the case of inhalation, the highest doses are to the lung and to the extra-thoracic airways (i.e. the nose, pharynx and larynx), while dose estimates to other organs and tissues were at least one order of magnitude lower. Outside the respiratory tract, the kidney is the organ most exposed to radon decay products. In general, the doses from radon gas are much lower than those from radon decay products. However, radon is more soluble in tissues with a higher fat content. As fat receives the highest dose of all tissues outside the lung, the doses to the red bone marrow and the female breast are somewhat higher. Kendall & Smith *(16)* also investigated the dose to the fetus. As the fat content of the fetus is low, its dose is assumed to be similar to that of the maternal muscle, which is estimated to be about 3–4 orders of magnitude smaller than the dose to the lung.

Kendall & Smith *(26)* also considered doses from radon and decay products when inhaled by one-year-old infants and ten-year-old children. They found that the general pattern of doses to organs is broadly similar to that of adults. The largest dose is received by the respiratory tract. Even though dose coefficients for children are generally larger than those for adults, the total annual doses are more similar because of the smaller intake of air by children. Radon decay products deposited on skin may be able to induce skin cancer. However, the location of the sensitive cells is not known with certainty and they may lie too deep to receive significant doses. On irradiation, it is likely that doses to children would be larger than those to adults.

Marsh et al. *(25)* recently provided a mathematical model to calculate the individual annual absorbed doses arising from radon and its decay products to regions of the lung, red bone marrow, liver and kidney among uranium miners of

the Czech, French and German cohort studies. Several exposure scenarios (wet/dry drilling, good/medium/poor ventilation, diesel engines, underground/surface, etc.) and levels of physical activity had been evaluated. For example, the scenario of underground work with wet drilling, medium ventilation and medium physical activity was estimated with the following annual absorbed doses in mGy/WLM: bronchial region 7.3, bronchiolar region 7.3, alveolar-interstitial region 0.45; red bone marrow 0.031, kidney 0.02 and liver 0.0065. As expected, the dose to the lung is the highest, as most of the short-lived radon progeny decay before they leave the lung. For the red bone marrow, the dose arising from the radon gas is greater than that from the radon progeny. Overall, the doses to red bone marrow, liver and kidney were appreciably lower than those to the lung.

Experimental animal studies

Animal studies have been conducted for several decades to evaluate the biological effects of inhaled radon and its decay products, mainly in rats but also in mice and beagle dogs. These studies systematically examined the pathogenic role of radon and its decay products, either alone or in various combinations with uranium ore dust, diesel-engine exhaust and cigarette smoke. In the late 1960s and early 1970s, it was proved that radon and its decay products, either alone or in combination, produce lung tumours *(21)*. Only a few laboratory animal studies investigated the risk of non-respiratory neoplasms, producing inconsistent results *(1,21,27,28)*.

A number of experimental animal studies examined the effects of exposure rate on induction of lung cancer, particularly at low cumulative exposures comparable to current underground mining exposures or to lifetime exposure in houses with high radon levels *(29–33)*. The results indicate that at low cumulative exposures, the risk of lung cancer increased with increasing exposure rate, while at high cumulative exposures (> 100 WLM), the reverse was observed (decreasing risk with increasing exposure rate). These data are consistent with that of underground miners showing an inverse exposure-rate effect at high cumulative exposures but a reduction of this effect at cumulative exposures lower than 50–100 WLM *(28,34–36)*. When biologically based models were applied to the various animal experimental data, the obtained set of significant model parameters appeared to compare reasonably well with that from similar models derived from studies on uranium miners *(37–40)*.

Molecular and cellular studies

Molecular and cellular radiobiology studies are important in understanding the mechanisms involved in carcinogenesis caused by ionizing radiation. In 1996, Jostes *(41)* provided an overview of the genetic, cytogenetic and carcinogenic effects of radon. He reported that radon and radon progeny cause cell transformation, changes in chromosome structure and gene mutations containing a wide

range of deletions, as well as base-pair changes. It is thus possible that even exposure to low radon concentrations such as in homes adds to the genetic load for cancer risk. Since then, a comprehensive review on cellular and molecular responses to various forms of radiation has been given by the Committee on the Biological Effects of Ionizing Radiation (BEIR VI) *(28)* and UNSCEAR *(1)*. The UNSCEAR report *(1)* includes specific annexes on DNA repair and mutagenesis, biological effects of low doses of ionizing radiation and the combined effects of exposure to radiation and other agents. An extensive update of this report, with specific focus on radon, is given in UNSCEAR's 2008 report *(21)*.

A number of in vitro studies of cells exposed to alpha-particle radiation demonstrated not only direct effects in irradiated cells but also non-targeted effects such as the bystander effect *(21)*. Bystander effects occur when irradiated cells emit signals that result in damage to nearby non-irradiated bystander cells. Brenner et al. *(42)* suggested that bystander effects can result in non-linear dose–response relations and inverse dose-rate effects, and thus make it difficult to extrapolate risks based on linear models of miner studies to the risk from residential radon *(43–45)*.

Chromosomal aberrations are among the most useful biomarkers of effects and doses from radon exposure *(1,21)*. Associations between chromosomal aberrations and cancer incidence have been observed in radon-exposed miners, while correlations between radon exposure and chromosomal aberrations have been found in radon-exposed miners and to some extent also in the general population through residential radon exposure *(21)*.

BEIR VI *(28)* and WHO *(4)* argued that it is possible that radon-related DNA damage can occur at any level of exposure to radon, since even a single alpha particle can cause major genetic damage to a cell. Therefore, it is unlikely that there is a threshold concentration below which radon does not have the potential to cause lung cancer.

Health effects

Identification of studies

Health effects of radon were identified by hand searching references in former reviews by UNSCEAR *(1,21)*, the National Research Council *(15,28)*, IARC *(46)* and WHO *(4)*. All these reports were published between 1988 and 2009. The detailed UNSCEAR report *Sources-to-effects assessment for radon in homes and workplaces* from 2008 *(21)* and the WHO radon handbook from 2009 *(4)* formed the major basis for the text. Next to that, electronic searches were made in PubMed in January 2009, with an update in December 2009 in order to identify newly published papers. The keywords were: "radon" and "cancer" or "health effects" or "mortality". Moreover, recent papers known to the experts were included. For the present review, next to the above-mentioned summary reports, approximately 50 publications on health effects in relation to radon exposure

were selected. About 70% of them concerned studies on miners with occupational underground radon exposure and 30% concerned indoor radon studies in the general population.

Effects on humans: lung cancer

Studies on miners

Since the 1960s, studies on underground miners have consistently demonstrated an increased risk of lung cancer caused by radon and its progeny *(15)*. Based on this evidence, IARC classified radon as a human carcinogen in 1988 *(46)*. Since then, several reviews on radon-related risk among miners have been published *(1,4,21,28)*.

In 1999, BEIR VI reported on the joint analysis of 11 miner cohort studies *(28)*. This collaborative study included a total of 60 000 miners, mainly miners of uranium but also of tin, fluorspar and iron from Asia, Australia, Europe and North America. Overall, a total of 2600 deaths from lung cancer had occurred. Lung cancer rates increased approximately linearly with increasing cumulative radon exposure in each study, but the magnitude of risk varied 10-fold between the individual studies. Based on the joint analysis of the 11 cohorts, the average excess relative risk (ERR) per WLM was estimated to be 0.44% (95% CI 0.20–1.00). The ERR/WLM decreased with increasing time since exposure and increasing attained age. In addition, the risk was modified by either exposure rate or duration of exposure. There was an inverse exposure rate effect, i.e. miners exposed at relatively low radon concentrations had a larger ERR/WLM than those exposed at higher radon concentrations. For some of the studies, information on smoking was available. When separate analyses for ever-smokers and never-smokers were performed, the ERR/WLM for never-smokers was higher than that for ever-smokers (1.02%; 95% CI 0.15–1.38 vs 0.48%; 95% CI 0.18–1.27), although this difference was not statistically significant. A potential limitation of the pooled cohort study concerns heterogeneity between the 11 cohorts with respect to differences in exposure quality, other occupational risk factors, lifestyle factors, etc.

Later, several more methodological papers were published based on existing miner cohorts *(47–51)*. To achieve some insight into the mechanisms involved in the genesis of cancer, various biologically based models have been applied to the data of the Czech, French, Colorado and Chinese miner cohort studies *(32,52–54)*. A detailed summary of these mechanistic studies is given the 2008 report from UNSCEAR *(21)*. The Czech *(55,56)*, French *(57–60)* and Newfoundland *(61)* cohort studies have been updated. Moreover, in comparison to data in BEIR VI (*28*), four new studies of radon-exposed miners have been established in Brazil *(62)*, the Czech Republic *(56)*, Germany *(34,36,63,64)* and Poland *(65)*.

The German Wismut cohort study *(34,36)* is similar in size to the pooled BEIR VI study *(28)*. It includes around 59 000 men who had been employed by the Wismut uranium mining company in eastern Germany. In the second mortal-

ity follow-up by the end of 2003, a total of 3016 deaths from lung cancer had occurred. Using a linear relative risk model, the average ERR/WLM was 0.19% (95% CI 0.16–0.22) *(36)*. The ERR/WLM was modified by time since exposure, attained age and exposure rate, but not by duration of exposure. When the exposure-age-concentration model of BEIR VI *(28)* was applied, there was a decrease in the ERR/WLM with time since exposure and attained age, as in the BEIR VI study, although the decrease with attained age was less pronounced. In both studies, a strong inverse exposure-rate effect above cumulative radon concentrations of more than 100 WLM was present. Information on smoking in the cohort was limited. A case-control study on incident lung cancer among German uranium miners, including detailed information on lifelong smoking habits *(66)*, found a somewhat larger ERR/WLM for never-smokers (0.20%; 95% CI 0.07–0.48) than for ex-smokers (0.10%; 95% CI 0.03–0.23) and current smokers (0.05%; 95% CI 0.001–0.14). The data pointed to a sub-multiplicative effect of the two factors, with no significant deviation from the multiplicative or the additive interaction model.

Tomasek et al. *(35)* investigated radon-associated risk, particularly at low exposure rates, based on a pooled analysis of the Czech and French cohorts, including a total of 10 100 uranium miners. These miners were characterized by low levels of exposure (average cumulative WLM < 60) over a long time (mean duration ~ 10 years) and by good quality of exposure (95% of the annual exposures are obtained by radon measurements). The overall ERR/WLM related to measured values was 2.7% (95% CI 1.7–4.3). It was strongly modified by time since exposure and age at exposure. No inverse exposure rate effect below 4 WL was observed. This result was consistent with estimates of the BEIR VI report *(28)* using the age-concentration model at an exposure rate below 0.5 WL.

Residential radon studies

There is substantial uncertainty in the extrapolation of the risk of lung cancer from the miners studies to the risk of lung cancer from radon exposure in the home *(28)*. For this reason, a series of epidemiological studies directly investigated the association between indoor radon and risk of lung cancer since the 1980s *(1,4,21,28)*. The first generation of these studies were ecological studies, in which average radon concentrations were correlated with average lung cancer rates at an aggregated geographical level. This type of study is known to be prone to bias because of several methodological problems *(1,21,67)*. Later, a number of case-control studies were carried out that gathered detailed information on smoking history and other risk factors for lung cancer and assessed the radon exposure retrospectively by measuring radon in the current and previously occupied homes of the study participants.

A detailed review of the results of these individual case-control studies is given in the 2008 report by UNSCEAR *(21)*. The majority of the studies showed

a positive association between radon exposure and risk of lung cancer; however, the estimated risk coefficients often did not reach statistical significance in the individual studies. Moreover, there was a substantial variation in the estimated radon-related risk as published in the individual studies. Several meta-analyses had been undertaken to summarize the findings *(68–70)*. Differences in the methodology used to analyse the different studies, such as adjustment for smoking and exposure quantification, however, limit the interpretation of these meta-analyses. For this reason, the original data of the individual studies were brought together and collaborative analyses were performed on the individual data of 13 European studies *(71,72),* 7 North American studies *(73,74)* and 2 Chinese studies *(75)*.

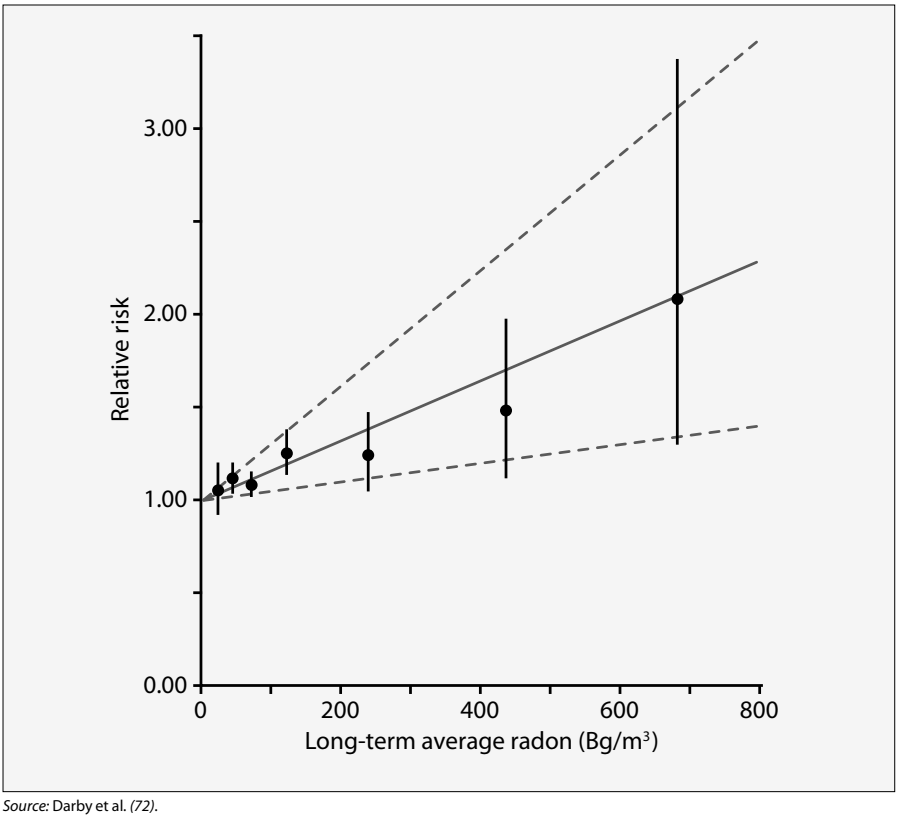
The largest of these pooled studies is the European pooling study published by Darby et al. *(71,72)*. It includes 7148 cases and 14 208 controls from 13 European indoor radon case-control studies on lung cancer, all with detailed information on smoking histories and radon measurements in homes that the individual had occupied for the past 15 years or more. The available radon measurements covered a mean of 23 years in the relevant radon exposure period 5–34 years prior to interview. Individual exposure to radon (called "measured" radon concentration) was calculated as the time-weighted average of the radon concentrations in all the homes occupied over the past 5–34 years, with missing radon values substituted by the mean concentration of the controls in that region. A statistical model was fitted in which the additional risk of lung cancer was proportional to measured radon concentration. In addition, radon exposure was subdivided into categories and the relative risk across categories of measured radon concentrations was plotted against the mean level in these categories. In both models, detailed stratification was performed for study, age, sex, region of residence and 25 smoking categories. Since no statistically significant heterogeneity in the radon-associated risk between the studies was present, the data were pooled.

In the pooled analysis, the excess relative risk of lung cancer per 100 Bq/m^3 "measured" radon concentration was 8% (95% CI 3–16). This proportionate increase did not differ significantly by study, age, sex or smoking history. The corresponding risk estimates for lifelong non-smokers, ex-smokers and current cigarette smokers were 11% (95% CI 3–28), 8% (95% CI 3–21) and 7% (95% CI –1 to –22), respectively. The exposure–response relationship appeared to be approximately linear, with no evidence for a threshold below which there was no risk. In particular, the results were incompatible with a threshold exposure below which there is no risk above 150 Bq/m^3. Even when the analysis was restricted to individuals with measured radon concentrations below 200 Bq/m^3, the exposure–response relationship remained statistically significant. The risk of lung cancer was 1.2-fold (95% CI 1.03–1.30) higher among individuals with measured radon concentrations of 100–199 Bq/m^3 than in those with concentrations < 100 Bq/m^3 and the increase was statistically significant.

Analysis based on the so-called "long-term average radon concentration", which takes into account the random year-to-year variability in measured radon concentration in the homes, led to a doubling of the excess relative risk of 16% (95% CI 5–31) per 100 Bq/m^3. Again, the risk did not differ significantly by study, age, sex or smoking status, and the exposure–response relationship was approximately linear (Fig. 7.2).

Darby et al. *(72)* also reported in detail on the combined effects of smoking and radon within the European pooled study. Table 7.3 gives information on the cumulative risk of death from lung cancer by the age of 75 years for lifelong non-smokers and continuing smokers of 15–24 cigarettes a day ("current smokers"). For these calculations, the estimated excess relative risk of 16% per 100 Bq/m^3 of long-term average radon concentration, which was independent of smoking status, was used. The relative risk for current smokers of 15–24 cigarettes per day compared to lifelong non-smokers was estimated as 25.8-fold. For lifelong non-smokers, it was estimated that living in a home with a long-term average radon concentration of 0, 400 or 800 Bq/m^3 was associated with a cumulative

Fig. 7.2. Relative risk of lung cancer vs long-term average residential radon concentration



Source: Darby et al. *(72)*.

Table 7.3. Cumulative risk of death from lung cancer by the age of 75 years for lifelong non-smokers and continuing smokers of 15–24 cigarettes per day at various radon concentrations [a]

Radon concentration (Bq/m³)	Risk of death per 1000 current smokers of 15–24 cigarettes per day	Risk of death per 1000 lifelong non-smokers
0	101	4.1
100	116	4.7
200	131	5.4
400	160	6.7
800	216	9.3

[a] After correction for uncertainties in the assessment of radon concentrations.

Source: Darby et al. *(72)*.

Note. Absolute risk of lung cancer for lifelong non-smokers taken from the prospective study of the American Cancer Society. Relative risk of lung cancer for continuing smokers of 15–24 cigarettes per day compared to lifelong non-smokers assumed to be 25.8. Relative risk of lung cancer assumed to increase by 0.16 per 100 Bq/m³.

risk of death from lung cancer of 41, 67 or 93 per 1000. For current smokers, the corresponding values would be 101, 160 or 216 per 1000, respectively. For those having stopped smoking, the radon-related risks are substantially lower than for those who continue to smoke, but they remain considerably higher than the risks for lifelong non-smokers.

Krewski and co-workers *(73,74)* reported on the results of the pooled analysis of seven indoor radon case-control studies in Canada and the United States, which included a total of 3662 cases and 4966 controls. Residential radon levels were measured for one year by alpha-track detectors. For each individual, the time-weighted average of the radon concentrations in the homes was calculated, with a focus on the period 5–30 years prior to the date of interview. Because no statistically significant heterogeneity of radon-related risk was found between the studies, a combined analysis was performed. Based on this joint analysis, the risk of lung cancer increased by 11% (95% CI 0–28) per 100 Bq/m³ increase in measured radon concentration. The trend was consistent with a linear exposure–response relationship. There was no apparent difference in the proportionate increase in risk by sex or smoking history, although there was some evidence of decreasing radon-associated lung cancer risk with age. Analyses restricted to individuals with presumed "more accurate dosimetry" resulted in increased risk estimates. For example, for individuals who lived in only one or two homes in the 5–30-year period and for which alpha-track measurements covered at least 20 years of this period, the proportionate increase in lung cancer risk was 18% (95% CI 2–43).

The Chinese pooled study published by Lubin et al. *(75)* included 1050 cases and 1996 controls from two studies in two areas, Gansu and Shenyang. As in the North American pooled study, the time-weighted average of the radon concentration in homes was calculated within the exposure period 5–30 years. No significant heterogeneity in the associated risk was present between the two stud-

ies. For the pooled data, the increase in risk per 100 Bq/m^3 increase in measured radon concentration was 13% (95% CI 1–36) and the results were consistent with a linear exposure–response relationship with no threshold. When analyses were restricted to individuals resident in only one home and with complete measurement coverage in the relevant period, the proportionate risk per 100 Bq/m^3 increased to 33% (95 CI 8–96).

In the *WHO handbook on indoor radon (4)*, a review and comparison of the risks are provided from all three pooled residential radon studies and the miner studies (Table 7.4). The radon-related risk estimates in the three pooled indoor radon studies were very similar. In each study, the exposure–response relationship appeared linear, without evidence of a threshold, and there was no statistically significant evidence that the radon-related risk varied by age, sex or smoking status. A weighted average of the three pooled risk estimates was provided, with weights proportional to their variances, resulting in a joint estimate of 10% proportionate increase in lung cancer risk per 100 Bq/m^3 measured radon concentration. WHO *(4)* estimated that, based on long-term average radon concentration instead of measured radon concentration, this 10% estimate could even increase to 20% per 100 Bq/m^3 if it is assumed that the effect of adjusting for year-to-year random variation in the three pooled studies combined is the same as in the European study.

In general, a direct comparison of the risks of lung cancer between residential and miner studies is difficult. This is due to the higher average radon exposures among miners and the time-dependent modifying factors, but primarily the inverse exposure rate effect. Thus, the summary estimates of the joint 11 miner studies and the German miner study are somewhat lower, with 5% and 3% per 100 Bq/m^3, respectively, than in the residential studies *(4)*. When analyses in the BEIR VI study were restricted to cumulative exposures below 50 WLM, which is comparable to living in a house with a radon concentration of around 400 Bq/m^3 for 30 years, the estimated risk coefficient increased to 14% per 100 Bq/m^3 *(76)* and even to 30% per 100 Bq/m^3 after additional restriction to miners with radon concentrations lower than 0.5 WL (i.e. < ~ 2000 Bq/m^3). No such risk analyses had been performed within the German study; assuming that the same restriction as in the BEIR VI study has the same effect, however, then the corresponding risk would be around 18% per 100 Bq/m^3. Based on the joint analysis of the Czech and French cohorts, which is characterized by low levels of cumulative exposures, an increase of about 29% per 100 Bq/m^3 would be expected in the exposure window 5–34 years.

Based on these comparisons, WHO *(4)* concluded that, in summary, the radon-related risk estimates for lung cancer from residential radon studies and studies of underground miners with relatively low cumulative exposures accumulated at low concentrations are in good agreement.

Table 7.4. Summary of risks of lung cancer from indoor radon based on international pooling studies that have combined individual data from a number of case-control studies and on studies of radon-exposed miners

	No. of studies included	No. of lung cancer cases	exposure window (years) [a]
Pooled residential studies			
European *(71,72)*	13	7148	5–35
North American *(73,74)*	7	3662	5–30
Chinese *(75)*	2	1050	5–30
Weighted average of above results			
Studies of radon-exposed miners [e,f]			
BEIR VI *(28,76)*	11	2787	≥ 5
		468	
		250	
German cohort study *(34)*	1	2388	≥ 5
Czech and French cohort studies *(35)*	2	574	≥ 5
			5–35

[a] Considering radon concentrations during the period starting 35 years before and ending 5 years before the date of diagnosis for cases of lung cancer, or a comparable date for controls.
[b] Adjusting for year-to-year random variability in indoor radon concentration.
[c] Estimate corresponding to higher coverage of measurements.
[d] Informal estimate, indicating the likely effect of removing the bias induced by random year-to-year variation in radon concentration.
[e] Risks per WLM have been converted to risks per 100 Bq/m^3 by assuming that 1 Bq/m^3 at equilibrium is equivalent to 0.00027 WL, that the "equilibrium factor" in dwellings is 0.40, that people spend 70% of their time at home, that there are 365.25 x 24/170 = 51.6

Effects on humans: diseases other than lung cancer

A number of studies focused on the relationship between radon and leukaemia in children and adults. Laurier et al. *(77)* reviewed 19 ecological studies, 8 residential case-control studies and 6 miner cohort studies published between 1997 and 2001. While the ecological studies suggested a positive correlation between residential radon exposure and leukaemia at a geographical level, the case-control studies and cohort studies did not. Overall, the authors concluded that the available data did not provide evidence of an association between radon and leukaemia *(77)*. Since then, a positive association between leukaemia incidence and exposure to radon has been reported in a case-cohort study among Czech uranium miners *(78)*, in a Danish case-control study on residential radon and childhood leukaemia *(79)* and in a French ecological study on childhood leukaemia *(80)*, while no evidence for an increased risk of leukaemia by exposure to radon was reported in two independent studies among German uranium miners *(81,82)*.

Overall, individual miner cohort studies have provided little evidence for an increased risk of cancers due to radon other than lung cancer *(28,58,83,84)*. However, most of these studies were limited owing to small numbers of cases.

Percentage ERR/100 Bg/m³ (95% CI) observed radon	Percentage ERR/100 Bg/m³ (95% CI) corrected for exposure uncertainties
8 (3–16)	16 (5–31) [b]
11 (0–28)	18 (2–43) [c]
13 (1–36)	33 (8–96) [c]
10	20 [d]
All miners	5 (2–12)
Miners < 50 WLM only	14
Miners < 0.5 WL only	19
Miners < 50 WLM and < 0.5 WL	30
All miners	3
Miners at low exposures and low exposure rates	18 [g]
Measured exposures	32 (14–34)
	29 (16–40)

"working months" in one year, and that the ratio of the dose to lung cells for exposures in homes to that for similar exposures in mines (sometimes referred to as the K-factor) is unity.

[f] Only one study *(48)* specifically addressed the effect of measurement error in the estimates of radon-related lung cancer risk in miners. This concluded that for miners exposed at concentrations below 15 WL, measurement error was of little consequence.

[g] Informal estimate, obtained by multiplying the estimate for all miners in the German cohort by 6, i.e. the ratio of the estimates for all miners and for miners exposed to < 50 WLM and < 0.5 WL from the BEIR VI analysis.

The two largest and most informative studies are the pooled analysis of 11 miner cohorts *(85)* and the German Wismut cohort study *(82)*. In the pooled study, the observed mortality from extrapulmonary cancers combined (O) was close to that expected from national rates (E) (n = 1179; O/E = 1.01; 95% CI 0.95–1.07). The trend with cumulative exposure was statistically significant only in the first decade since start of employment.

Among individual sites examined, a statistically significant excess in mortality was found for leukaemia and cancers of the stomach and liver (in the period less than 10 years since starting work). For none of the examined cancer sites was mortality significantly related to cumulative radon exposure, except for pancreatic cancer, which might be a chance finding.

In common with the pooled analysis of 11 miner cohorts, no excess in the overall mortality from extrapulmonary cancers was observed in the German Wismut cohort when compared to the general population (n = 3340; O/E = 1.02, 95% CI 0.98–1.05) *(82)*, while statistically significant excesses in mortality for cancers of the stomach and liver were present. When the relationship with cumulative radon exposure was considered, a statistically significant relationship was found for all extrapulmonary cancers combined (ERR/WLM = 0.014%; 95% CI 0.006–0.023)

and cancers of the extra-thoracic airways and trachea (ERR/WLM = 0.062%; 95% CI 0.002–0.121) *(64)*. The majority of non-respiratory cancer sites investigated revealed positive exposure–response relationships, which were non-significant however. The authors concluded that the study provides some evidence for an increased radon-related risk of cancers of the extra-thoracic airways and some other non-respiratory cancer sites; this is in line with calculations of organ doses, though chance and confounding cannot be ruled out. Based on a large case-control study on laryngeal cancer among German uranium miners, Mohner et al. *(86)* reported no relationship with cumulative radon exposure.

Epidemiological studies on diseases other than cancer mainly focused on the relationship between radon exposure and cardiovascular disease among miners. None of these studies found any evidence that radon causes cardiovascular diseases *(64,87–91)*. A Norwegian study demonstrated an association between multiple sclerosis and indoor radon, but this study was prone to bias owing to the ecological study design *(92)*.

Overall, the currently available epidemiological evidence indicates that there is only suggestive evidence that radon causes a material risk for diseases other than lung cancer.

Health risk evaluation

Definition of health outcomes

Health effects of radon, most notably lung cancer, have been investigated for several decades. An increase in the risk of lung cancer with increasing exposure to radon was first consistently demonstrated in studies on underground miners *(15,28)*. Based on these results, radon was classified by IARC in 1988 as a Group 1 human pulmonary carcinogen (*46*). In addition, there is direct evidence from epidemiological indoor radon studies that radon in homes increases the risk of lung cancer in the general population *(4,21)*.

The pooled analysis of data from the European *(71,72),* North American *(73,74)* and Chinese *(75)* residential radon studies consistently demonstrated that the risk of lung cancer increases approximately linearly with increasing long-term radon exposure. There is no known threshold below which radon exposure presents no risk. The increase is statistically significant even below 200 Bq/m^3. Risk estimates from epidemiological studies of miners and residential case-control studies are remarkably coherent. There is limited, though inconsistent, evidence of other cancer risks due to radon.

When radon gas is inhaled, densely ionizing alpha particles emitted by deposited short-lived decay products of radon can interact with biological tissue in the lungs, leading to DNA damage. Molecular and cellular studies demonstrated that it is possible that radon-related DNA damage can occur at any level of exposure to radon, since even a single alpha particle can cause major genetic damage to a cell *(1,4,21)*.

Relevance for health of current indoor exposures in various regions of the world

Radon is a major contributor to the ionizing radiation dose received by the general population. Outdoor radon levels are usually very low, with average values in the range of 5–20 Bq/m^3. National indoor radon surveys show that the distribution of radon concentrations in dwellings is approximately log-normal, with average values ranging between 20 and 150 Bq/m^3 *(4,93)*. Published estimates of the proportion of lung cancers attributable to residential radon exposure range from 3% to 14%, depending on the average radon concentration in the country concerned and the calculation methods *(4)*. As most people are exposed to low or moderate radon concentrations, the majority of lung cancers related to radon are caused by these exposure levels rather than by higher concentrations. In many countries, radon is the second cause of lung cancer after smoking. Most of the radon-induced lung cancer cases occur among smokers and ex-smokers owing to a strong combined effect of smoking and radon exposure. Nevertheless, radon exposure is the primary cause of lung cancer among people who have never smoked.

In summary, there is sufficient evidence to conclude that radon causes lung cancer, even at concentrations typically found in indoor air. There is suggestive evidence of an association with other cancers, in particular leukaemia and cancers of the extra-thoracic airways.

Guidelines

Radon is classified by IARC *(46)* as a human carcinogen (Group I). There is direct evidence from residential epidemiological studies of the lung cancer risk from radon. The exposure–response relationship is best described as being linear, without a threshold. The ERR, based on long-term (30 years) average radon exposure is about 16% per increase of 100 Bq/m^3 *(71,72)* and on this relative scale does not vary appreciably between current smokers, ex-smokers and lifelong non-smokers. Therefore, as the absolute risk of lung cancer at any given radon concentration is much higher in current smokers than lifelong non-smokers, the absolute risk of lung cancer due to radon is appreciably higher for current and ex-smokers than for lifelong non-smokers. For ex-smokers, the absolute risks will be between those for lifelong non-smokers and current smokers.

The cumulative risk of death from radon-induced lung cancer was calculated for lifelong non-smokers and for current smokers (15–24 cigarettes per day) *(72)*. The derived excess lifetime risks (by the age of 75 years) are 0.6×10^{-5} per Bq/m^3 and 15×10^{-5} per Bq/m^3, respectively. Among ex-smokers, the risk is intermediate, depending on the time since smoking cessation. The radon concentration associated with an excess lifetime risk of 1 per 100 and 1 per 1000 are 67 Bq/m^3 and 6.7 Bq/m^3 for current smokers and 1670 Bq/m^3 and 167 Bq/m^3 for lifelong non-smokers, respectively.

As part of the management of the radon problem, the WHO International Radon Project has recommended that there should be a reference level as an essential tool in this process *(4)*.

> A national Reference Level does not specify a rigid boundary between safety and danger, but defines a level of risk from indoor radon that a country considers to be too high if it continues unchecked into the future. However, protective measures may also be appropriate below this level to ensure radon concentrations in homes are well below that level. In view of the latest scientific data, WHO proposes a Reference Level of 100 Bq/m^3 to minimize health hazards due to indoor radon exposure. However, if this level cannot be reached under the prevailing country-specific conditions, the chosen Reference Level should not exceed 300 Bq/m^3 which represents approximately 10 mSv per year according to recent calculations by the International Commission on Radiation Protection.

A guide for radon management should include, in addition to the setting of a reference level, building codes, measurement protocols and other relevant components of a national radon programme *(4)*.

The guidelines section was formulated and agreed by the working group meeting in November 2009.

Summary of main evidence and decision-making in guideline formulation

Critical outcome(s) for guideline definition

- Lung cancer (sufficient evidence of causality even at concentrations typically found in indoor air).
- Suggestive evidence of an association with other cancers, in particular leukaemia and cancers of the extra-thoracic airways.

Source of exposure–effect evidence

The pooled analysis of data from the European *(71,72)*, North American *(73,74)* and Chinese *(75)* residential radon studies consistently demonstrated that the risk of lung cancer increases approximately linearly with increasing long-term radon exposure. There is no known threshold below which radon exposure presents no risk. The increase is statistically significant even below 200 Bq/m^3.

Supporting evidence

Risk estimates from epidemiological studies of miners *(15,28)* are consistent with residential studies. Molecular and cellular studies demonstrated that it is possible that radon-related DNA damage can occur at any level of exposure, since even a single alpha particle can cause major genetic damage to a cell *(1,4,21)*.

Results of other reviews

- IARC: Group I (known human carcinogen with genotoxic action) *(46)*.
- WHO International Radon Project: Reference Level of 100 Bq/m^3 *(4)*.

Guidelines

- The excess lifetime risk of death from radon-induced lung cancer (by the age of 75 years) is estimated to be 0.6 × 10–5 per Bq/m^3 for lifelong non-smokers and 15 × 10–5 per Bq/m^3 for current smokers (15–24 cigarettes per day). Among ex-smokers, the risk is intermediate, depending on the time since smoking cessation.
- The radon concentration associated with an excess lifetime risk of 1 per 100 and 1 per 1000 are 67 Bq/m^3 and 6.7 Bq/m^3 for current smokers and 1670 Bq/m^3 and 167 Bq/m^3 for lifelong non-smokers, respectively.

Comments

WHO guidelines provide a comprehensive approach to the management of health risks related to radon *(4)*.

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8. Trichloroethylene

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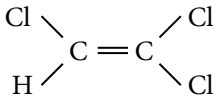
General description

Trichloroethylene (TCE) (CAS Registry Number, 79-01-6; $C_6H_3Cl_3$; molecular weight 131.40 g/mol) is a widely used industrial solvent. It is a volatile, colourless liquid with a sweet ethereal (chloroform-like) smell. It has a melting point of –84.8 °C, a boiling point of 86.7 °C, a Henry's Law constant of 1.03×10^{-2} atm-m^3/mol at 20 °C, a vapour pressure of 7.8 kPa at 20 °C, a water solubility of 1.1 g/l at 20 °C and a log K_{ow} (octanol–water partition coefficent) of 2.29 *(1)*.

The synonyms of TCE include acetylene trichloride, ethinyl trichloride, trichloroethene, TRI, TRIC, 1-chloro-2,2-dichloroethylene, 1,1,2-trichloroethylene, Trilene and Triklone. Its structural formula is *(1)*:



Conversion factors

At 760 mmHg and 20 °C, 1 ppm = 5.465 mg/m^3 and 1 mg/m^3 = 0.183 ppm; at 25 °C, 1 ppm = 5.374 mg/m^3 and 1 mg/m^3 = 0.186 ppm *(2)*.

Production

In 1990, the estimated industrial production of TCE was 131 kilotonnes in western Europe, 79 kilotonnes in the United States and 57 kilotonnes in Japan, compared to 210, 121 and 82 kilotonnes, respectively, in 1980. The estimated annual consumption in these regions is 65–103% of the production levels. No estimates of production and use are available for other parts of the world *(3)*.

Applications and uses

TCE is mainly used for the vapour degreasing and cold cleaning of manufactured metal parts (80–95% of consumption). Other applications include industrial dry cleaning, printing, the production of printing ink, extraction processes, paint production and textile printing *(2–4)*.

In the atmosphere, TCE may react with photochemically produced hydroxyl radicals yielding phosgene, dichloroacetyl chloride, formyl chloride and other

degradation products. Its half-life in the atmosphere varies with latitude, season and concentration of hydroxyl radicals. Reported half-lives for the reaction with hydroxyl radicals range from 1 day to 2½ weeks (in polar regions, the half-life may be as long as several months in winter) *(5,6)*.

Analytical methods in air

Common methods of measuring indoor concentrations of TCE include integrated active and passive sampling methods using tubes packed with a carbon-based sorbent or evacuated SUMMA canisters (passivated canister sampling apparatus), or diffusion samplers such as charcoal badges. Canister sampling involves controlling the flow of air into a pre-evacuated canister. Sorbent tubes and badges retain compounds according to the affinity of the sorbent for that compound. For sorbent or charcoal sampling, the analytes must first be extracted thermally or chemically, then separated using gas chromatography and identified using a detection method such as mass spectrometry *(7)*. Personal exposure studies often use these sorbent-based methods.

Indoor sources and exposure pathways

Inhalation is the main route of exposure for the general population, but ingestion may significantly contribute to total exposure, particularly if drinking-water sources are highly contaminated. Because TCE can volatilize rapidly from surface water *(4,6)*, contaminated water may be an additional source of indoor exposure, through showering or the use of washing machines and dishwashers, for example. Contaminated soil can also contribute to ambient air concentrations of TCE via vapour intrusion (where TCE in soil gas enters homes through cracks in the foundations) *(2,8)*. Dermal exposure can contribute to the total exposure, especially through the use of detergent products or showering *(1,9)*. In a risk assessment study, Fan et al. *(10)* showed that the three most important exposure pathways are water ingestion, dermal absorption when showering and breathing indoor air.

Consumer products

Consumers may be exposed to TCE through the use of wood stains, varnishes, finishes, lubricants, adhesives, typewriter correction fluid, paint removers and certain cleaners, where TCE is used as a solvent *(11)*. Use of these products may result in elevated indoor air concentrations over background, although, as they are expected to be used intermittently rather than constantly, both short-term and long-term average concentrations are likely to be variable.

Groundwater and drinking-water

Groundwater levels are variable and subject to local contamination. In wide-ranging surveys, concentrations have been shown to be mostly low, of the order

of < 0.2–2 μg/l. However, measurements in groundwater related to contaminated sites may show high levels; up to 950 mg/l has been found *(1)*.

Levels of TCE in drinking-water are generally less than 1 μg/l, although higher levels (up to 49 μg/l) have been reported *(1)*. In a survey carried out in Germany during the 1990s, TCE was detected in about 40% of the tested drinking-water samples, with a concentration of more than 1 μg/l for 5.5% of the supplied residents (concentration range < 0.001–21 μg/l) *(6)*. A regular monitoring programme is in place for drinking-water in England and Wales, covering 31 areas. TCE levels were monitored at approximately 2700 sites in 1994. The vast majority of measured TCE levels were below the detection limit (range 0.1–3.0 μg/l), although higher levels (up to 25 μg/l) have occasionally been detected *(1)*. The EPA Groundwater Supply Survey of drinking-water systems nationwide detected TCE in 91 out of 945 water systems in 1984. The median level of the positive samples was about 1 μg/l, with a maximum level of 160 μg/l *(2)*. In Canada, the majority of drinking-water supplies contain less than 0.2 mg/l TCE *(5)*.

Food

The presence of TCE has been demonstrated in a wide range of foodstuffs, and in some exceptional cases high concentrations have been detected. In some countries, the use of TCE as a solvent in the production of foodstuffs has been banned. In total diet studies carried out in the United States, TCE was detected in only a small proportion of the samples *(2,5)*. In the United States, dairy products (particularly butter) and margarine have been found to have high levels of TCE. Levels of 73 μg/kg in butter and margarine were found, while cheese products had an average level of 3.8 μg/kg *(11)*. TCE is lipophilic and has been detected in the breast milk of women living in urban areas, though no quantitative data are available *(2)*. It is possible that this may be leading to a high daily intake for nursing infants.

Indoor air concentrations

Residential concentrations

Indoor concentrations of TCE have been found to be near or below 1 μg/m^3 in many microenvironments where the presence of known sources is uncommon. In several United States cities (New York, Los Angeles, Chicago, Minneapolis, St Paul, Baltimore, Elizabeth and Houston), median indoor residential concentrations ranged from 0.1 to 0.5 μg/m^3; in many cases, standard deviations were of the same order of magnitude as the medians *(12–19)*. Indoor : outdoor ratios in New York and Los Angeles, in a study in a low-income group, were between 1 and 2 at the median and did not have large standard deviations relative to the median (except for New York homes in the winter) *(16)*. This indicates that indoor TCE was probably derived from outdoor sources in the homes in this study. In the United States studies mentioned, outdoor residential median levels ranged

from 0.1 to 0.4 μg/m³ with little variability *(12–19)*. Sampling in different seasons did not show significant differences.

In the European EXPOLIS study, conducted in 1998–1999 using the same methods in six European cities, it was found that in cities with high outdoor residential concentrations such as Athens, Milan and Prague, indoor residential levels were also higher *(20)*. In Athens, the median indoor concentration was 8.2 μg/m³ with a 90th percentile of 22.4 μg/m³, compared with a median of 4.3 μg/m³ outdoors and a 90th percentile of 33 μg/m³. In Milan, the median indoor concentration was 7.7 μg/m³ and the 90th percentile was 21.2 μg/m³, while the median outdoor concentration was 2.3 μg/m³ and the 90th percentile was 8 μg/m³. In Prague, the median indoor concentration was 13.6 μg/m³ and the 90th percentile was 28.9 μg/m³, while the median outdoor concentration was 3.7 μg/m³ and the 90th percentile was 7.7 μg/m³. These indoor levels are likely to be a reflection of the contribution of outdoor sources through infiltration, as the outdoor levels were much higher than in Helsinki, Basel and Oxford. In Helsinki, most of the indoor and outdoor samples were below the detection limit. Basel had similar indoor and outdoor levels to those in Helsinki, while Oxford had slightly higher levels (median 2.1 μg/m³ and 90th percentile 6.6 μg/m³ indoors, and median 2.5 μg/m³ and 90th percentile 4.9 μg/m³ outdoors). The higher indoor (median 7.7–13.6 μg/m³) than outdoor levels (median 2.3–4.3 μg/m³) overall in Milan, Athens and Prague indicate that there may be an additional contribution of indoor sources to indoor TCE concentrations.

A nationwide survey of residences in France (n = 567; monitoring carried out in 2003–2005) found median indoor concentrations of 1 μg/m³; the 95th percentile concentration was 7.4 μg/m³ *(21)*. Indoor concentration in the attached garage (n = 139) was below the detection limit at the median and 12.9 μg/m³ at the 95th percentile, suggesting the presence of indoor sources. Outdoor concentrations (n = 517) were below the detection limit at the median and 2.3 μg/m³ at the 95th percentile.

A study of 25 homes in Shimizu, Japan found indoor geometric mean concentrations of 0.22 μg/m³ (geometric SD = 2.16) in the summer and 0.36 μg/m³ (geometric SD = 1.64) in the winter *(22)*. These were similar to the corresponding outdoor geometric mean concentrations of 0.23 μg/m³ (geometric SD = 2.14) measured in the summer of 2002 and 0.36 μg/m³ (geometric SD =1.61) in winter 2002 *(22)*, indicating that again, infiltration from the outdoors was likely to have been the main source of TCE indoors.

Non-residential microenvironments

A survey of 70 office buildings in the United States (without any reported complaint) found a median TCE concentration of 0.29 μg/m³ with a 95th percentile of 2.6 μg/m³ *(23)*. A study of mechanically ventilated office sector and non-office sector spaces in 20 public buildings in Hong Kong SAR, China found arithmetic

means of 5.6 μg/m³ (SD = 9.6) for offices and 8.8 μg/m³ (SD = 10.7) in non-office areas *(24)*. Other studies of offices, restaurants and stores found TCE median or mean levels below 1 μg/m³, although there was a high degree of variability in some cases *(25–27)*. In general, it appears that, for the most part, TCE in non-residential indoor environments is associated with infiltration from outdoors, as common indoor environments are unlikely to have sources of TCE in amounts that would contribute enough to the indoor concentration to result in significantly elevated levels compared to outdoors. Studies have found that most non-industrial areas do not have significantly high outdoor levels compared to industrial areas.

Modelled air concentrations of TCE near different types of vapour degreasing machine have been estimated to range from around 5 mg/m³ for newer, closed-loop machines to over 1000 mg/m³ for older, open-top machines. In Germany, levels of TCE in degreasing applications based on occupational personal air concentrations in the 1990s were found to be around 20–50 mg/m³ on average *(28)*.

Ambient air

Concentrations of TCE in ambient outdoor air may fluctuate widely over relatively short periods of time, depending on the strength of the emission source, variations in wind direction and velocity, and scavenging and photodecomposition *(5)*. Arithmetic mean rural concentrations in Canada were found to be 0.02 μg/m³ with a maximum value of 4 μg/m³, based on passive month-long measurements in 2001 and 2002 *(29)*.

Short-term measurements made during peak traffic hours on busy roads in industrial, commercial, residential and central business districts in Hong Kong SAR, China gave arithmetic mean TCE concentrations of 48.5 μg/m³ (SD = 77.8), 3.6 μg/m³ (SD = 3.4), 0.4 μg/m³ (SD = 0.5) and 1.3 μg/m³ (SD = 1.8), respectively *(30)*. These patterns indicate that areas of heavy industry have significantly higher, although more variable, concentrations of TCE, probably due to the presence of high-emitting sources. Residential and non-industrial areas where few sources are present have much lower concentrations. Based on results from the European EXPOLIS study in Athens, Milan and Prague, it is likely that small industry sources are either mixed in with residential and business areas or that heavy industry is near these areas. Non-residential indoor environments in Athens, Milan and Prague were found to have similar TCE levels to indoor home environments. Median workplace concentrations were 6.4 μg/m³ in Athens, 4.7 μg/m³ in Milan and 4.4 μg/m³ in Prague *(20)*.

Toxicokinetics and metabolism

Identification of studies

Considering the possible overlaps in the relevant evidence on health effects of trichloroethylene, the search of the literature supporting the "Toxicokinetics and

metabolism" and "Health effects" sections was conducted in one process. The electronic searches were made in PubMed between August and September 2008, with an update in 2009. The keywords used were: "trichloroethylene" and "health effects" or "risk assessment" or "metabolism/biotransformation/kinetics". We selected all relevant papers on this subject. Around 100 publications were selected; 23% concerned the metabolism of TCE and mostly the development of PBPK models, 16% focused on molecular mechanisms and mode of action, and 29% were related to toxicological tests in mammals. One third discussed reproductive and developmental effects while 25% concerned human studies, mostly the exposure of workers and pregnant women in the general population. Finally, two reviews on toxicological effects, three on carcinogenic risk in human and two on risk assessment were also selected.

A complementary Internet search was made in August 2008 on toxicological databases (hazardous substances databank, TOXNET) and on the web site of international or national health assessment agencies, including WHO, the European Commission, IPCS, ATSDR, Health Canada, the French Agency for Environmental and Occupational Health Safety (Affset) and USEPA. Twelve reports from these agencies, published between 1985 and 2009, were selected.

Toxicokinetics

Two update reviews of the pharmacokinetics of TCE have recently been published *(31,32)*.

Absorption

In humans and in animals, TCE is readily absorbed via the oral, inhalation and dermal routes.

Because TCE is an uncharged, nonpolar and highly lipophilic molecule, gastrointestinal absorption is extensive and occurs by passive diffusion *(31)*.

In humans, TCE is known to be highly and rapidly absorbed by inhalation (25–55%) *(33)*, with a high uptake during the first few minutes and steady-state blood levels reached within 2 hours. This high absorption rate is due to the high blood–air partition coefficient, which ranges from 9 to 15 *(34)*. The absorption after inhalation is also high in animals, but significant differences in blood–air partition coefficient exist between species *(31)*.

TCE can also penetrate intact human skin. Four human male volunteers had TCE blood concentrations of 2 mg/l immediately following the immersion of one hand in TCE for 30 minutes. Levels fell to 0.34 mg/l 30 minutes after the end of the immersion period and to 0.22 mg/l after 60 minutes *(35)*.

Distribution

After absorption, TCE is widely distributed in the body via the circulatory system. Because of its high liposolubility, it is predominantly found in adipose tis-

sue and then in the liver, kidneys, cardiovascular system and nervous system *(2)*. TCE crosses the blood–brain barrier and the placenta *(36)*. It has been shown in lactating rats that TCE and its metabolite trichloroacetic acid are excreted in milk. In goats, TCE and its metabolite trichloroethanol were found to be transferred to milk to a slight degree only *(37)*.

Biotransformation

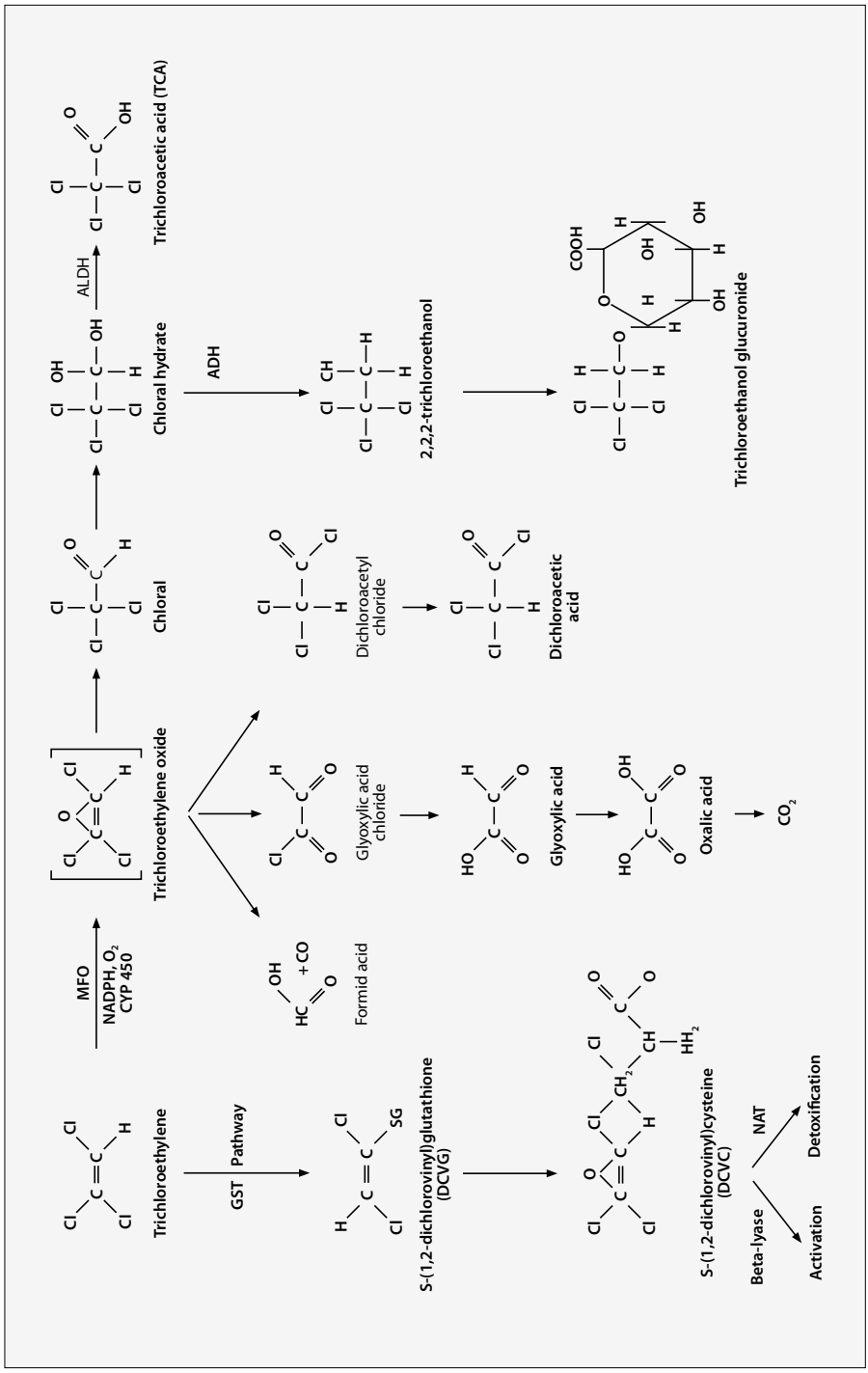
In humans, 40–75% of the retained dose of inhaled TCE is metabolized. The metabolic pathways are qualitatively similar in all species. TCE is metabolized to multiple metabolites either locally or in systemic circulation (e.g. in the liver and by Clara cells in the lung). Many of these metabolites are thought to have toxicological importance *(38)*. The general pattern of enzymatic metabolism occurs through two main pathways: oxidation via the microsomal mixed-function oxidase system (CYP450) and, to a lesser extent, conjugation with glutathione *(32)*.

- TCE is principally and rapidly transformed by CYP 2E1 *(39)* into an epoxide intermediate, which spontaneously rearranges to trichloroacetaldehyde and then chloral hydrate. Chloral hydrate acts as a substrate for alcohol dehydrogenase and chloral hydrate dehydrogenase, leading to the formation of trichloroethanol and trichloroacetic acid, respectively. The main metabolites, which are primarily present in the urine, are therefore trichloroethanol, its glucuronide conjugate and trichloroacetic acid. Metabolism appears to be qualitatively identical, irrespective of the exposure route *(2)*.
- Other minor metabolites of TCE have been identified, including the mercapturic acid N-acetyl-S-(dichlorovinyl)-L-cysteine (DCVC), which is formed in the kidneys from the glutathione conjugate of TCE (previously formed in the liver as a minor biotransformation product). The presence of DCVC in the urine has been demonstrated in rats and also in workers exposed to TCE *(37)* (Fig. 8.1).

The metabolism of TCE is quantitatively dependent on the concentration and species tested: it is concentration-dependent in rats but not in mice. In humans, no saturation has been demonstrated. As a consequence of differences in blood flow and overall metabolic rate, species differences exist in the fraction of administered dose of TCE that is available for conversion to toxic metabolites in the target organs *(31)*. In mice, oxidative metabolites are formed in greater quantities than glutathione conjugate metabolites, and dichloroacetic acid is produced to a very limited extent relative to trichloroacetic acid, while most S-(1,2-dichlorovinyl)glutathione (DCVG) is converted into DCVC *(40)*.

TCE is well metabolized by human hepatocytes in culture, with a K_m of 266 (± 202) ppm and a V_{max} of 16.1 (± 12.9) nmol/hour per 10^6 viable hepatocytes. Lipscomb et al. *(41)* predicted a V_{max} of approximately 1400 nmol/hour per gram of human liver.

Fig. 8.1. Metabolism of trichloroethylene



Sources: ATSDR *(2)*; Chiu et al. *(32)*.

The link between the various metabolites of TCE and diverse types of toxicity is known to be highly complex, making understanding of the toxicological mechanisms of action more complicated. Animal-to-human extrapolation is a source of a high level of uncertainty *(42)*. There is inconclusive evidence suggesting that the glutathione biotransformation route, leading to DCVC production, is more important in humans than in rodents *(43)*.

When ten volunteers were exposed to 250–380 ppm of TCE for 160 minutes, 16% of the retained TCE was eliminated through respiration after exposure. Trichloroacetic acid excretion in females was 2–3 times more than in males for the first 24 hours. However, twice as much trichloroethanol was excreted in males. These observations suggest a sex difference in human metabolism (Nomiyama & Nomiyama 1971, cited in HSDB *(36)*).

Elimination

In humans and animals, non-metabolized TCE can be eliminated via expired air (11–40%). The main metabolites are eliminated by the kidneys: urinary elimination of trichloroethanol and trichloroacetic acid is complete 5 and 13 days, respectively, after the end of exposure *(35,44)*. In humans, the half-times for renal elimination of trichloroethanol and its glucuronide are about 10 hours. Urinary excretion of trichloroacetic acid is slower, with a reported half-time of about 52 hours *(2)*. Male volunteers were administered chloral hydrate in three separate experiments. Chloral hydrate, dichloroacetic acid, trichloroacetic acid, and trichloroethanol and its glucuronide were measured in blood and urine over a 7-day period. Trichloroacetic acid had the highest plasma concentration and the largest area under the curve of any metabolite. The trichloroacetic acid elimination curve displayed an unusual concentration–time profile that contained three distinct compartments. This complex elimination pattern may result from the enterohepatic circulation of trichloroethanol glucuronide and its subsequent conversion to trichloroacetic acid, as shown in rats *(45)*.

Biomarkers of human exposure

Biomonitoring of TCE is possible by measuring levels of the parent compound or its main metabolite, trichloroacetic acid, in expired air, blood and urine.

Several studies have demonstrated a correlation between levels of TCE in ambient air and in exhaled air *(2)*. Following inhalation exposure to TCE, 10–11% of the absorbed dose is found in expired air as TCE and 2% is eliminated as trichloroethanol.

A linear correlation has been reported between the inhalation exposure of TCE and the urinary levels of trichloroethanol and trichloroacetic acid. In a kinetics study in male volunteers, trichloroacetic acid had the highest plasma concentration and the largest area under the curve of any metabolite *(45)*. Because urinary trichloroacetic acid has a longer half-life than trichloroethanol, it bet-

ter reflects long-term exposure, whereas urinary trichloroethanol reflects recent exposure *(2)*. The American Conference of Governmental Industrial Hygienists adopted biological exposure indices for TCE based on blood concentrations of free trichloroethanol and trichloroacetic acid and trichloroethanol in urine *(46)*. It should be noted, however, that there is great inter-individual variability in the concentrations of trichloroethanol and trichloroacetic acid in urine and that trichloroacetic acid is not specific to TCE exposure.

Although some studies have shown that protein and DNA adducts may form with chlorinated hydrocarbons *(37)*, their application has not been validated sufficiently to justify their use as biological markers of exposure *(47)*. Some researchers have developed methods to interpret biomarkers by reconstructing human population exposures *(48,49)*. These methods are based on PBPK models (see below) combined with Monte Carlo or Bayesian analysis and estimate TCE environmental concentrations based on known concentrations in blood. This approach involves the interpretation of human biomonitoring data and a possible comparison with health-based exposure guidelines.

Physiologically based pharmacokinetic modelling

Efforts to develop physiologically based pharmacokinetic (PBPK) models have led to an improved assessment of TCE. Several PBPK models have been proposed. They focus on descriptions of both TCE and its major oxidative metabolites in humans (trichloroacetic acid, trichloroethanol and its glucuroconjugate) (see Chiu et al. *(32)* for additional discussion). Several families of PBPK model are available:

- Fisher models permit the modelling of liver cancer risks following oral and inhalation exposure of TCE and the formation of metabolites in the liver in humans and mice. None of these models consider renal metabolism (and, therefore, the glutathione pathway in particular), which may play an important role in toxic signs in humans *(50–54)*.
- The Clewell model is more complex than the Fisher models, since it includes sub-models for the main metabolites and for the three target organs demonstrated during toxicity studies in animals (lung for trichloroacetaldehyde, kidney for dichlorovinylcysteine and liver for trichloroacetaldehyde, di- and trichloroacetic acids, trichloroethanol and its conjugate). This model takes into account inhalation and oral exposure, along with hepatic and renal metabolism *(55)*.
- The Bois model calibrates the existing models of Fischer and Clewell with new toxicokinetic data and includes a Bayesian statistical framework to bring in issues on variability and uncertainty for each parameter *(56,57)*. More recently, other researchers have shown that a combination of Bayesian approaches and PBPK analysis provides better predictions and yields an accurate characterization of the uncertainty in metabolic pathways for which data are sparse *(58)*.

- Combining the Fischer and Clewell models, and considering the reassessment of the parameters of these models conducted by Bois et al. *(56,57)* using Bayesian methods, the United States Air Force proposed, in 2004, a harmonized model for use in mice, rats and humans *(32,59)*.

PBPK models have largely been applied in risk assessment to predict dose metrics, toxicity or guideline values. Yoon et al. *(60)* found that liver-only metabolism may be a reasonable simplification for PBPK modelling of TCE to predict dose metrics. Simmons et al. *(61)* explored the relationship between measures of internal doses of TCE and neurotoxic outcomes in rats. Another application is based on the time course of TCE in blood and brain of rats and humans to adjust duration for acute guidelines in place of the Haber's law *(62–64)*. Hacks et al. *(65)* used a Bayesian approach to reduce uncertainty in dose metric prediction of TCE and its metabolites (particularly trichloroacetic acid and trichloroethanol). More recently, Evans et al. *(66)* examined the question of whether the presence of trichloroacetic acid in the liver is responsible for TCE-induced hepatomegaly in mice, and concluded that oxidative metabolites, in addition to trichloroacetic acid, are necessary contributors.

Despite their continuous development, none of these PBPK models currently incorporates all exposure routes or all the possible toxic effects. Similarly, the dose measurement to be used in these models is not clearly explained, i.e. whether it should be area under the curve, cumulative dose or maximum concentration. The development of more complex models is notably linked to advances in scientific knowledge concerning understanding of the mechanism(s) of toxic action of TCE.

Health effects

Effects in experimental animals and in vitro test systems

Non-carcinogenic effects

In animals, the major effect of acute exposure includes a state of excitation followed by CNS depression and drowsiness. This depression is marked by a loss of reflexes and motor coordination, potentially progressing to coma. The LC_{50} values are 142 g/m^3 (1 hour) and 71 g/m^3 (4 hours) in rats and 46 g/m^3 (4 hours) in mice, indicating a low acute inhalation toxicity. A transient hepatic toxicity has been observed in rodents. A specific pulmonary toxicity (to Clara cells) has been demonstrated in mice and a transient specific nephrotoxicity is demonstrated when the metabolism is saturated in rats *(35,44)*.

Effects on the CNS have also been demonstrated during subchronic and chronic inhalation exposure. In rats, a NOAEL of 2700 mg/m^3, based on an increase of latency in visual discrimination tasks, was identified after 18 weeks of inhalation exposure *(67)*. In a 16-week study in rats, brainstem auditory-evoked response potentials were depressed at test concentrations as high as 8640 and

17 280 mg/m^3 *(68)*. Electroencephalograph changes have also been reported in rats exposed to up to 50 ppm for 6 weeks *(69)*. In rabbits, neuro-ophthalmological reversible modifications were observed during a 12-week period of inhalation exposure at 1890 and 3780 mg/m^3 *(70)*. Based on the hypothesis that organic solvents can promote noise-induced hearing loss, Vyskocil et al. *(71)* reviewed the effects of low-level exposure to TCE on the auditory system. In rats, TCE affects the auditory function mainly in the cochlear mid- to high-frequency range, with a LOAEL of 2000 ppm. Supra-additive interaction after exposure to noise and TCE has been reported *(71)*. A recent animal study was conducted to determine whether TCE exposure is neurotoxic to the striatonigral dopamine system that degenerates in Parkinson's disease. The study showed that oral administration of TCE for 6 weeks leads to a complex 1-mitochondrial impairment in the midbrain and a striatonigral fibre degeneration and loss of dopamine neurons *(72)*.

Transient hepatic hypertrophy has also been observed, but the results of studies are equivocal and its toxicological significance is not clear. Inhalation exposures to 2000 ppm TCE show elevated plasma alanine and aspartate aminotransferase activities and liver histopathological abnormalities in mice. At the same dose, TCE significantly upregulates PPARα *(39)*. Sano et al. *(73)* demonstrated distinct transcriptional profiles and differences in biological pathways between rats and mice, suggesting species differences in liver toxicity.

An increase in kidney weight has been demonstrated in rats, but without any particular associated histological changes *(74)*. Megalonucleocytosis has been observed and a NOAEL has been defined at 115 mg/m^3 *(75)*. The relative importance of metabolism of TCE by the CYP450 and glutathione conjugation pathways in the acute renal and hepatic toxicity of TCE was studied in isolated cells and microsomes from rat kidney and liver. Increases in cellular glutathione concentrations increased TCE cytotoxicity in kidney cells but not in hepatocytes, consistent with the role of glutathione conjugation in TCE-induced nephrotoxicity. In contrast, depletion of cellular glutathione concentrations moderately reduced TCE-induced cytotoxicity in kidney cells but increased cytotoxicity in hepatocytes, consistent with the different bioactivation pathways in kidney and liver *(76)*. The involvement of CYP450 in TCE-induced hepatotoxicity was also studied in mice and its major role in the hepatotoxicity of TCE confirmed *(39)*. Recently, Khan et al. *(77)* showed that TCE causes altered carbohydrate metabolism and suppresses the antioxidant defence system in rats. These results are consistent with the hypothesis that TCE induces oxidative stress in kidney and other tissues.

Immune disorders have been observed in rats *(78,79)*. A decline of CD4+ in T lymphocytes was observed after intradermic administration of TCE, but no significant concentration differences in IFN-gamma and IL-4 were found between TCE-treated animals and controls *(78)*. Other animal experiments suggest that immunotoxicity is mediated via haptenization of macromolecules and that

haptenized proteins may act as neo-antigens that can induce humoral immune response and T-cell-mediated hepatitis in mice. Further observations suggest that TCE promotes inflammation in the liver, pancreas, lung and kidney, which may lead to SLE-like disease *(80)*. DCA could be involved in the immune disorders and hepatotoxicity induced by TCE *(81,82)*.

Moreover, Tang et al. *(83)* recently found that TCE can induce non-dose-related hepatitis classified as a delayed-type hypersensitivity at low doses in guinea-pigs exposed via intradermal injection (below the dose causing liver injury) and with different histopathological changes. TCE exposure in mice generates a time-dependant increase in antibodies specific for liver proteins in mice, upregulates the methionine/homocysteine pathway in the liver, and alters the expression of selective hepatic genes associated with immunity *(84)*. Moreover, TCE enhances histamine release from antigen-stimulated mast cells and inflammatory mediator production *(79)*. Other researchers suggest that protein oxidation (carbonylation and nitration) could contribute to TCE-induced autoimmune response because an increase in oxydatively modified proteins is associated with significant increase in cytokines. These first results observed in mice require further study *(85)*.

TCE could be skin irritant: a recent study investigating acute and cumulative TCE topical treatment in BALB/c hairless mice showed skin reaction (erythema and oedema) and histopathological changes (hyperkeratosis and inflammatory cell infiltrates) *(86)*.

Based on in vivo and in vitro studies, the US National Research Council *(87)* concluded that exposure to TCE disrupts spermatogenesis, reduces male fertility and the fertilization capacity of spermatozoa, and reduces the capacity of oocytes to be fertilized in females. Studies conducted in male Wistar rats exposed by inhalation to 376 ppm of TCE for 12 or 24 weeks *(88,89)* demonstrated a significant reduction in the number and motility of spermatozoa and in steroid enzyme activity (dehydrogenases), with a reduction of testosterone levels in the sperm. This was associated with a reduction of absolute testicular weight and histopathological changes. The fertility of these male rats was reduced when mating was performed with untreated females. Testicular cholesterol was elevated in exposed rats, suggesting that TCE acts on the biosynthesis of testosterone in the testis. Histological changes have also been shown (in spermatogonia and spermatids, seminal tubes and Leydig cells) but the reversibility of the effects has not been studied. A study in mice exposed to 1000 ppm for 1–6 weeks did not demonstrate any effects on testis or sperm *(90)* but mating with non-exposed females resulted in a significant decrease in the rate of fertilized oocytes after 2 and 4 weeks. Furthermore, an in vitro assay demonstrated a reduction in the number of spermatozoa per oocyte after treatment with 0.1–10 µg/l chloral hydrate or trichloroethanol. This suggests that the metabolites (in particular chloral hydrate) were responsible for the reproductive toxicity of TCE. More recently, Kan

et al. *(91)* showed epithelial damage (vesiculation in the cytoplasm, disintegration of basolateral cell membranes and sloughing of epithelial cells) in the epididymis of mice exposed to 1000 ppm TCE for 1–4 weeks. Further experiments *(92,93)* demonstrated that TCE could also cause reproductive toxicity in female rats, with a decrease in spermatozoon penetration and oocyte fertilization and reduced membrane-binding protein in female rats treated with TCE (2 weeks' administration of drinking-water containing 0.45% TCE). These effects also appear to be dependent on metabolic activation by CYP2E1 (without metabolite(s) being involved) and on glutathione conjugation to DCVC *(94)*. The real impact of the biological effects observed on the reproductive function of the animals is not known, nor is the transposability of these effects to human reproductive function. Finally, these observations suggest that enzyme induction and oxidative metabolism may play a role in the reproductive toxicity of TCE. Oxidative metabolites of TCE are formed in the mouse epididymis, resulting in epididymal damage, and at systemically toxic high doses TCE may adversely affect the maturation of sperm and decrease sperm motility *(95)*. Lamb & Hentz consider that protection against systemic toxicity should also protect against adverse effects, including male reproductive toxicity *(95)*.

One study has suggested the possibility of an increased incidence of malformations in rat pups after oral exposure via drinking-water. An increase in the incidence of cardiac and eye malformations was observed in pups from dams exposed to 0.18 and 132 mg/ kg body weight per day before and during gestation (3 months before or 2 months before and during pregnancy) or only 132 mg/kg body weight per day in dams exposed only during gestation, without maternal toxicity *(33)*. However, a recent evaluation by Williams & Desesso *(96)* pointed out the maternal toxicity associated with this birth defect. The mechanism of action is not known but it may involve metabolism by CYP450 2E1 and dichloroacetic and trichloroacetic acids. No malformation has been reported in inhalation studies in rats and other oral animal studies have not demonstrated conclusive results *(33,87,97)*. A recent study, in which mice were exposed to 31 mg/kg body weight per day via drinking-water from gestational day 1 to postnatal day 42, showed that developmental and early life exposure of TCE could modulate the immune function of pups and may be associated with neurodevelopmental disorders *(98)*.

A summary of relevant animal inhalation studies for subchronic and chronic exposures to TCE, indicating derived NOAELs and LOAELs, is shown in Table 8.1.

Carcinogenic effects

Exposure to TCE was responsible for an increased incidence of liver tumours in male Swiss mice and B6C3F1 mice of both sexes exposed by the inhalation route to 600 ppm for 78 weeks. Pulmonary tumours were also increased in fe-

male B6C3F1 and male Swiss mice at 600 ppm, but not among the male B6C3F1 mice *(75)*. Other studies have demonstrated a significant increase in pulmonary adenocarcinomas in female ICR mice exposed to 150 or 400 ppm for 104 weeks *(100)*. In male Sprague-Dawley rats, inhalation exposure to 600 ppm of TCE for 104 weeks led to a dose-dependent increase in Leydig cell tumours in the testis and a marginal increase in renal tumours (adenocarcinomas of the renal tubules). Finally, in mice, rats and hamsters exposed to 100 and 500 ppm TCE for 18 months, the only significant increase in tumour incidence was for malignant lymphomas in female mice *(75)*. The results of experimental studies are presented in Table 8.2.

The metabolism of TCE doubtless plays a very important role in its mechanism of carcinogenic action. Metabolic pathways have largely been described *(33,101–103)*. The research to date indicates that TCE-induced carcinogenesis is complex, involving multiple carcinogenic metabolites acting in various ways. Past explanations, such as the hypothesis linking mouse liver tumours to peroxisome proliferation, are not consistent with the whole of the data, and more complex hypotheses have been formulated *(38)*. A plausible mode of action is that TCE induces liver tumours through trichloroacetic acid and DCA modifying the cell signalling systems that control cell division rate and cell death *(103–105)*. This hypothesis suggests that humans are likely to be much less responsive than mice and that carcinogenic effects are unlikely to occur at low environmental exposures. The induction of pulmonary tumours in mice may be linked to the fact that Clara cells rapidly metabolize TCE into chloral hydrate, via CYP450 2E1, leading to pulmonary accumulation of this metabolite, which ultimately produces cell changes and compensatory proliferation. But other mechanisms of action may be involved, particularly since chloral hydrate is probably genotoxic and, at high doses, clastogenic. In rats, Clara cells are capable of metabolizing chloral hydrate into trichloroethanol. In humans, the capacity of the lung to transform TCE into chloral hydrate is thought to be negligible and, consequently, the mechanism of pulmonary carcinogenesis demonstrated in mice may be specific to mice.

Finally, renal tumours in male rats may be linked to cytotoxicity and persistent cell regeneration. Conjugation to glutathione and the involvement of beta-lyase in the renal tubules may lead to the formation of nephrotoxic and probably genotoxic reactive metabolites, in particular DCVC and DCVG *(101)*. Studies have demonstrated that TCE induces mutations in the VHL (Von Hippel-Lindau) tumour suppressor gene in the cells of renal carcinomas in patients with this cancer *(106,107)*, but Charbotel et al. *(108)* did not confirm the association between the number and type of VHL gene mutations and exposure to TCE.

A second mechanism may involve increased secretion of formic acid, leading to a disruption in the detoxification mechanism by methionine. The mechanism of carcinogenic action leading to the development of renal tumours in rats is less

Table 8.1. A review of animal inhalation studies for subchronic and chronic exposure

Reference	Species	Duration	Concentrations
Kjellstrand et al. *(99)*	Mice	Subchronic (30 days)	0–37–75–150–300 ppm
Xu et al. *(90)*	Mice	Subchronic (1–6 weeks)	0–1000 ppm
Kan et al. *(91)*	Mice	Subchronic (1–4 weeks)	0–1000 ppm
Arito et al. *(69)*	Rats	Subchronic (6 weeks)	0–50–100–600 ppm
Maltoni et al. *(75)*	Rats	Chronic (104 weeks)	0–100–300–600 ppm
Kulig *(67)*	Rats	Chronic (18 weeks)	0–500–1000–1500 ppm
Rebert et al. *(68)*	Rats	Chronic (12 weeks)	0–1600–3200 ppm
Blain et al. *(70)*	Rabbits	Chronic (12 weeks)	0–350–700 ppm
Kumar et al. *(88)*	Rats	Chronic (12 weeks and 24 weeks)	0–376 ppm
Kumar et al. *(89)*	Rats	Chronic (12 weeks and 24 weeks)	0–376 ppm
Carney et al. *(97)*	Rats	Gestational (gestational days 6–20)	0–50–150–600 ppm

[a] The LOAEL is the lowest dose causing an adverse effect in animals in the experiment (from a statistically significant point of view). The NOAEL is the tested dose just below the LOAEL (the highest dose not causing any adverse effects in the experiment).

clearly determined but its transposability to humans is questionable. There is no tested hypothesis to take into account the mechanistic aspects of the induction of malignant lymphomas in mice and testis tumours in male rats. These considerations, and the effects observed in humans, justify a cautious attitude regarding the extrapolation to humans of results observed in animals.

In conclusion, despite the numerous limitations to confident interpretation of some of the data (e.g. the response of animals differs depending on sex for renal tumours and depending on species for hepatic and pulmonary tumours), animal evidence is deemed sufficient for evaluating the carcinogenic effect of TCE. The European Commission's risk assessment report on TCE concludes that studies provide clear evidence that TCE is carcinogenic in rats and mice through oral and inhalation exposure *(1)*.

Critical effect	LOAEL & NOAEL[a]
Liver and kidney: change in liver and kidney weight (more pronounced in males)	LOAEL = 150 ppm NOAEL = 75 ppm
Significant decrease in the rate of fertilized oocytes after 2 or 4 weeks	LOAEL = 1000 ppm No NOAEL
Epithelial damage in the epididymis	LOAEL = 1000 ppm No NOAEL
CNS: electroencephalograph changes (wakefulness-sleep periods and heart rate)	LOAEL = 50 ppm No NOAEL
Kidney: megalonucleocytosis	LOAEL = 300 ppm NOAEL = 100 ppm
CNS: increase of latency in visual discrimination tasks	LOAEL = 1000 ppm NOAEL = 500 ppm
CNS: depression of brainstem auditory-evoked response potentials	LOAEL = 1600 ppm No NOAEL
CNS: neuro-ophthalmological modifications	LOAEL = 350 ppm No NOAEL
Testicular toxicity: changes in testosterone and testicular cholesterol levels; decrease in 17β-hydroxysteroid dehydrogenase and glucose 6-P-dehydrogenase activity, and sperm (number and motility)	LOAEL = 376 ppm No NOAEL
Testicular toxicity: reduction in absolute testicular weight; changes in testicular enzyme activity associated with spermatogenesis and germ cell maturation; histopathological changes showing depletion of germ cells and spermatogenic arrest	LOAEL = 376 ppm No NOAEL
Maternal toxicity: 22% decrease in body weight (gestational days 6–9) Fetal toxicity: none	LOAEL maternal toxicity = 600 ppm NOAEL maternal = 150 ppm NOAEL fetal = 600 ppm

Genotoxicity

In Europe, TCE has been classified since 2001 as mutagenic category 3 (risk phrase R68) under Directive 67/548/CEE. Several reviews on the mutagenicity of TCE are available in the literature *(2,3,110)*. TCE seems to be genotoxic in vitro: the Ames test and in vitro mouse lymphoma test have shown a weak positive response with activation, but this characteristic is equivocal in vivo. A recent paper by Hu et al. *(111)* assesses the in vitro genotoxic effects of TCE and the underlying mechanisms using human HepG2 cells: TCE exposures (0.5–4 mM) caused positive response in comet assay and micronuclei assay. These results suggest that TCE causes DNA strand breaks and chromosome damage in hepatocytes. In another study evaluating the in vivo genotoxicity of TCE (99.5% purity) by inhalation (500–1000–2000 ppm) and DCVC (> 95% purity) by oral gavage (1–10 mg/kg), using the comet assay to assess DNA breakage in the proximal tu-

Table 8.2. Review of inhalation carcinogenic studies in animals

Reference	Species & strain	Duration
Henschler et al. *(109)*	NMRI mice (2 sexes)	78 weeks, 7 hours/day, 5 days/week
	Syrian hamsters (2 sexes)	78 weeks, 7 hours/day, 5 days/week
	Wistar rats (2 sexes)	78 weeks, 7 hours/day, 5 days/week
Fukuda et al. *(100)*	ICR mice (females)	104 weeks, 7 hours/day, 5 days/week
	Sprague-Dawley rats (2 sexes)	104 weeks, 7 hours/day, 5 days/week
Maltoni et al. *(75)*	Sprague-Dawley rats (2 sexes)	104 weeks, 7 hours/day, 5 days/week
	B6C3F1 mice (2 sexes)	78 weeks, 7 hours/day, 5 days/week
	Swiss mice (2 sexes)	78 weeks, 7 hours/day, 5 days/week

Source: WHO *(37)*.

bules of kidneys, rats were exposed at dose levels in excess of those that produced renal tumours. TCE gave a clearly negative response in the assay at all dose levels. DCVC gave a negative response at the lower dose level. At the higher dose level, there was limited evidence of DNA damage in a small number of animals. The authors suggest that the mechanism for induced renal tumours is non-genotoxic *(112)*.

Finally, while the available data on genotoxicity do not show a consistent pattern, the results indicate that TCE has a weak genotoxic action causing numerical chromosomal aberrations (aneuploidy) in vivo and probably DNA strand breaks in hepatocytes in vitro.

Currently, the various hypotheses suggested do not enable accurate identification of the key events responsible for the development of cancers at different sites (lungs, liver, kidneys, etc.). The ambiguity concerning the role of active metabolites, and the various mechanisms of action and effects, lead to a high level of caution when transposing animal data to humans (differences in sensitivity, quantitative differences in kinetics between species and as a function of exposure levels) *(38)*.

In conclusion, the mechanism of carcinogenic action of TCE can be attributed to numerous mechanisms, involving both non-genotoxic and genotoxic phe-

Concentrations	Tumour incidence
0–100–500 ppm (purified TCE without epoxide)	Female lymphomas: 9/29, 18/28, 17/30
0–100–500 ppm (purified TCE without epoxide)	No increase in tumour incidence
0–100–500 ppm (purified TCE without epoxide)	No increase in tumour incidence
0–50–150–450 ppm (99.8% purity, presence of benzene and epichlorhydrin)	Pulmonary adenocarcinomas: 1/49, 3/50, 8/50, 7/46
0–50–150–450 ppm (99.8% purity, presence of benzene and epichlorhydrin)	No increase in tumour incidence
0–100–300–600 ppm (99.9% purity, presence of benzene and epichlorhydrin)	Male renal tubuli adenocarcinomas only at 600 ppm (4/130 vs 1/130 in control group) Leydig cell tumours: 1/135, 16/130, 30/130, 31/130
0–100–300–600 ppm (99.9% purity without epoxide)	Female pulmonary adenomas: 4/90, 6/90, 10/90, 15/90 Female hepatomas: 3/90, 4/90, 4/90, 9/90 Male hepatomas: 14/90, 19/90, 27/90, 21/90
0–100–300–600 ppm (99.9% purity without epoxide)	Male pulmonary adenomas and carcinomas: 10/90, 11/90, 23/90, 29/90 Male hepatomas: 4/90, 2/90, 8/90, 13/90

nomena. It is thus prudent to consider that TCE can induce a risk of cancer in humans based on a non-threshold hypothesis *(42)*.

Unit risk values based on animal data have been estimated using the linearized multistage model on carcinogenicity data from mice and rats and using the most sensitive tumour type for which there is sufficient evidence. Unit risk values were 9.3×10^{-8} per μg/m^3 and 1.6×10^{-7} per μg/m^3 for pulmonary adenomas in B3C6F1 mice and Swiss mice, respectively, and 4.3×10^{-7} per μg/m^3 for Leydig cell tumours in rats. The most protective unit risk (4.3×10^{-7} per μg/m^3) was used to derive health-based guideline values for TCE in air in Europe *(37)*.

Effects in humans

Non-carcinogenic effects

In humans, the main target following the inhalation of high concentrations of TCE is the CNS, as is observed in animals. Neurological damage, especially affecting the optic and trigeminal nerves, has been reported following accidental exposure.

The acute neurological effects of TCE may be more related to maximum blood concentrations than to "area under the curve". In rats, the peak TCE concentration inducing toxicity appears to be higher than in humans, suggesting

that humans are more sensitive than animals for these neurological effects *(42)*. The neurological effects have been observed at concentrations from 270 mg/m^3 (changes in visual and auditory potentials) to approximately 600–1000 mg/m^3 (decreased psychomotor performances) over several hours *(37)*. Cardiac effects (ventricular fibrillation) may also cause death following massive exposure *(42)*. A recent accidental inhalation of TCE during the cleaning of a metal-degreasing machine produced a reversible kidney injury (urinary proteins and enzymes 7 and 74 hours after exposure) in a 54-year-old man. TCE and trichloroacetic acid had peak blood concentrations at 11 and 62 hours after poisoning, respectively *(113)*. Another case report showed acute liver and kidney failure followed by severe brain oedema and death in a 27-year-old man, probably caused by abuse of glue containing TCE *(114)*.

Effects on the CNS have also been demonstrated during chronic inhalation exposures. The majority of studies in humans describe the symptoms following acute exposure, but these are often of inadequate quality (absence of data on exposure or on confounding factors). More discrete neurological effects such as motor incoordination have also been observed for exposures of 87, 60 and 38 mg/m^3, respectively *(102)* but there is no convincing evidence of TCE-induced hearing losses in workers. No studies on ototoxic interaction after combined exposure to noise and TCE have been identified in humans *(71)*. Analysis of a cluster of 30 workers with neurological disease who were chronically exposed to TCE showed that the 3 workers with workstations nearest the TCE source had Parkinson disease. The authors suggest that TCE is a probable risk factor for Parkinsonism *(72)*.

Renal and pulmonary damage in humans following TCE exposure is absent or very slight, but transient effects on the liver have been observed *(37)*. Levels of total cholesterol and high-density lipoprotein cholesterol increased slightly with dose, but without modification of serum enzyme activity. It was suggested that exposure to TCE can influence hepatic function *(115)*. However, all the studies suffer from major methodological limitations, particularly in terms of characterization of exposures. In addition, individuals exposed to TCE were also exposed to other solvents.

Some authors suggest that TCE induces and exacerbates autoimmunity. This is supported by animal experimentations and observations of SLE and other immunological disorders in occupationally exposed human *(82)*. However, idiosyncratic generalized skin disorders complicated by hepatitis have rarely been observed in populations occupationally exposed to TCE in factories where TCE metabolites could extensively accumulate (urinary trichloroacetic acid concentrations from 318 to 1617 mg/l) *(116)*. This is consistent with a recent study by Xu et al. *(117)* in which TCE induced hypersensitivity dermatitis and liver dysfunction in Chinese workers exposed to 18–683 mg/m^3 for an average of 38.2 days (range 5–90 days). Liu et al. *(118)* found autoantibodies in sera collected

from patients who (had) suffered from TCE-induced dermatitis. These antibodies could perhaps be used to understand underlying mechanisms in the immunotoxicity of TCE.

The effect of inhaled TCE on fertility in humans has not been studied. The most recent studies have demonstrated a number of modifications in endocrine function, revealed by measurement of steroid hormones in particular, with changes observed following exposure to 60 mg/m^3 TCE *(119–121)*, but the toxicological significance of these observations has not been investigated.

Epidemiological studies have been carried out in occupational environments to investigate any link between exposure to degreasing solvents (including TCE) and pregnancy outcomes. Some of these have reported increased risks for cardiac anomalies, with OR ranging from 3.4 (95% CI 1.6–6.9) to 6 (95% CI 1.7–21.3) *(122,123)*. But it is not possible to reach any conclusion with respect to the precise role of TCE. Pregnancy outcomes have been studied in several cohorts in the general population exposed via the oral route (drinking-water) in the United States. Developmental abnormalities (cardiac, neural tubes, cleft palate, eye and ear malformations), perinatal deaths and low birth weights were observed *(42,87,124–128)* but the presence of possible bias or misclassification precludes confident conclusions being made. An interaction between maternal age and TCE exposure in increasing congenital heart defects has been observed by Yauck et al. *(129)*, although the mechanism by which this might occur is unknown. Finally, the National Research Council suggested that epidemiological observations concerning malformations (particularly cardiac ones) and delayed intrauterine growth in humans exposed to TCE are consistent with the animal studies and are supported by mechanistic studies and a relative agreement in the type of malformations.

However, to date, no definitive conclusion has been put forward for humans and it is not possible to extract from these studies either a well-defined dose–response relationship or a LOAEL for assessing the risk of TCE, particularly since the populations are often concomitantly exposed to several toxic substances (halogenated solvents, metals, etc.) *(87)*.

Carcinogenic effects

The results of the Finnish cohort study, in which 2050 men and 1924 women were exposed to TCE and other solvents in the context of their work *(130)*, demonstrated a statistically significant increase in non-Hodgkin's lymphoma and cervical cancer, with a significantly higher risk in the individuals with the highest urinary trichloroacetic acid concentrations (signal-to-interference ratio 4.4; 95% CI 1.4–10.1), and an increase in liver cancers for workers exposed for more than 20 years (RR = 6.1, 95% CI 2.8–17.7). Kidney cancers were not significantly increased. However, the exact exposure duration was not known and the workers were exposed to other solvents (although the estimates were adjusted to the

urinary trichloroacetic acid concentrations). In the same cohort, the risk of liver cancer was increased among male printers, lacquerers and varnishers exposed to chlorinated hydrocarbons (RR = 2.65, 95% CI 1.38–5.11). The authors suggest a role of TCE, which is consistent with previous data *(131)*.

The results of the Swedish cohort study, in which 1670 workers (1421 men and 249 women) were exposed to TCE *(132)*, demonstrated that mortality and morbidity from cancer were not significantly higher in these exposed individuals than in the general population. The majority of workers had urinary trichloroacetic acid levels below 50 mg/l, which may correspond – according to the authors – to an exposure of approximately 20 ppm.

A study conducted on a cohort of 14 457 American aircraft maintenance workers exposed to multiple solvents, including TCE, demonstrated a non-significant increase in mortality due to liver cancer, kidney cancer and non-Hodgkin's lymphoma: a statistically significant increase was observed for multiple myelomas (SMR 236; 95% CI 87–514) and non-Hodgkin's lymphoma (SMR 212; 95% CI 102–390) in white women, and for cancers of the bile duct and liver in white men who died after 1980 (SMR 358; 95% CI 116–836). Exposures were classified according to certain indices (as a function of occupational category) and did not therefore permit a quantitative approach. When only individuals exposed to TCE were examined (6929 people), no significant association was found between the additional risk of cancer and TCE measurements *(133)*.

A recent case-control study in Germany analysed the relationship between exposure to organic solvents (including TCE) and malignant lymphoma in 710 patients. A statistically significant association was found between high exposure to chlorinated solvents and malignant lymphoma (OR 2.1; 95% CI 1.1–4.3). When TCE only is considered, this trend persists (borderline statistical significance) *(134)*.

Other studies conducted in general populations exposed to TCE via drinking-water have demonstrated associations between (increased) incidence of leukaemia or non-Hodgkin's lymphoma and TCE exposures, which is consistent with the data from occupational cohorts. Scott & Chiu *(135)* reviewed recently published scientific literature examining cancer and TCE exposure and suggested that the studies appear to provide further support for the kidney, liver and lymphatic system as targets of TCE toxicity.

All the retrospective cohort studies conducted on TCE have methodological limitations linked either to the absence of quantification of exposures to TCE, to potential co-exposures not taken into account in occupational environments, or to the low number of subjects studied. Nevertheless, some epidemiological studies have measured trichloroacetic acid in urine, which can be directly related to TCE exposure *(130)*. The strongest associations between TCE exposure and human cancer are for the kidney, liver and lympho-haematopoietic system, sites where TCE causes cancer in rats and mice *(102)*. These aspects of biological

plausibility and coherence suggest that a cause-and-effect association between TCE exposure and cancer in humans is credible, even if the interpretation of individual studies may be difficult.

However, several recent meta-analyses have been conducted that did not confirm previous findings. A meta-analysis of 14 occupational cohort and four case-control studies of workers exposed to TCE investigated the relationship between TCE exposure and risk of non-Hodgkin's lymphoma. The comparisons carried out by the authors did not indicate exposure–response trends, suggesting insufficient evidence of a causal link between TCE exposure and non-Hodgkin's lymphoma *(136,137)*. Alexander et al. *(138)* found the same results in analysing occupational studies of TCE exposure and liver/biliary tract cancer. The main conclusions drawn are that exposure to solvents may cause cancer in humans and that TCE is likely to be one of these, but a number of challenging issues need to be considered before concluding clear causal relationships between TCE exposure and cancer *(139)*.

Thus, considering the bias and confounding in epidemiological studies, human evidence of the carcinogenicity of TCE can be considered limited. IARC has classified TCE as probably carcinogenic to humans (Group 2A) based on sufficient evidence in animals and limited evidence in humans *(3)*.

Based on data presented by the USEPA in its health risk assessment of TCE *(102)*, and particularly the cancer potency values, Lewandowski & Rhomberg *(140)* proposed a method for selecting the most appropriate carcinogenic inhalation unit risk estimate for TCE. The method is based on an in-depth analysis of the key studies used to derive unit risks in both animals and humans (protocol, rigour, statistical power, characterization of exposure, confusion factors, critical effects, etc.). The authors evaluated the validity of the studies (suitability of the protocol and dose–response relationships for a quantitative assessment) and the plausibility of the effects (with the use of Hill's criteria). These considerations led to the choice of the unit risk derived from the Finnish cohort study, based on the increase in the incidence of hepatic tumours. The selected unit risk was 9×10^{-7} per $\mu g/m^3$.

Sensitive populations

An extensive review of factors that may affect risk of exposure to TCE, with a particular examination of age (children), genetics, sex, altered health state, co-exposure to alcohol and enzyme induction, was published in 2000 *(141)*.

Since the metabolism of TCE is largely implicated in its toxic mechanisms of action, all the known polymorphisms, and particularly those concerning the CYP2E1, glutathione-S-transferase (GST) and N-acetyltransferase enzymes, are liable to modify individual sensitivity to this substance, although it is currently impossible to accurately quantify the scale of this modification or the number of people affected by these polymorphisms. In the example of polymorphism

of GST, it appears that some subgroups of the population have a risk of kidney cancer that is four times higher than others *(102)*. However, a recent study of about 134 renal cell cancer cases from Brüning et al. *(142)* does not confirm the hypothesis of an influence of the deletion polymorphisms of GST on renal cell cancer development due to exposure to TCE *(143)*. With respect to the polymorphism of CYP450 2E1, Pastino et al. *(141)* reported that a 10- to 50-fold variability in the protein or its activity has been observed in humans.

Merdink et al. *(45)* showed that individuals with an impaired capacity for glucuronidation may be very sensitive to the CNS-depressant effects of high doses of chloral hydrate, which are commonly attributed to plasma levels of TCE.

Individuals with hepatic and/or renal failure may constitute a more sensitive population owing to reduced metabolism of TCE and/or a reduction in the elimination of its toxic metabolites, whether these disturbances be genetic, environmental (alcohol, medicinal products, etc.) or secondary to a disease. Individuals with a history of cardiac arrhythmias may be more susceptible to high-level TCE exposure *(2)*. The provisional version of the 2001 assessment made by the USEPA also cites diabetics among sensitive populations owing to their specific susceptibility to neuropathies and certain cancers, and the specific effects of TCE on the metabolism of carbohydrates and cell signalling *(102)*.

Finally, according to ATSDR *(2)*, people with a high consumption of alcohol or taking disulfiram may be more sensitive to the neurological effects of TCE, owing to an interaction process.

Health risk evaluation

Critical health outcomes

TCE is a chlorinated solvent. Its main health effects are neurotoxic and carcinogenic. Immunotoxic, hepatic and developmental effects are also reported.

Neurotoxic effects

Effects on the CNS (damage affecting the optic and trigeminal nerves) have been observed in humans and animals exposed to high acute (600–1000 mg/m^3) or moderate chronic (38–87 mg/m^3) occupational levels. Sufficient evidence exists to conclude that there is an association between TCE and neurotoxic effects.

Immunotoxic effects

The evidence is suggestive for an association between TCE exposure and the exacerbation or induction of autoimmunity. Several mechanistic hypotheses are suggested in rodents but further research is needed before firm conclusions can be reached. Recent studies in humans confirm the possibility of immune disorders in individuals exposed occupationally to high-to-moderate levels of TCE (18–683 mg/m^3). However, not enough human studies are available to allow a conclusion to be drawn on causality, especially because the human immune re-

sponse varies greatly among individuals. It is concluded that there is limited evidence of an association between immunological effects and TCE exposure.

Hepatic effects

Transient hepatic hypertrophy has been observed in rodents, but the results of studies are equivocal. The human epidemiological studies suffer from methodological limitations, particularly in terms of characterization of exposure. In addition, individuals exposed to TCE were often also exposed to other solvents. It is concluded that there is limited evidence of an association between hepatic effects and TCE exposure.

Developmental effects

Developmental effects, notably cardiac and eye malformations, have been reported in rodents but the results are inconsistent (possible maternal toxicity, positive results only in oral studies in rats). Occupational studies in humans suggest a link between the use of degreasing solvents and adverse pregnancy outcomes. Epidemiological studies in the general population suggest malformations, perinatal death and low birth weight, but possible bias and exposure misclassification prevent firm conclusions being drawn. There is thus insufficient evidence for an association between developmental effects and TCE exposure.

Carcinogenic effects

Animal evidence is sufficient to demonstrate carcinogenic effects of TCE by both oral and inhalation routes, and there is sufficient evidence to conclude that TCE is at least weakly genotoxic. Positive associations have been established between occupational exposure and risks for cancer of the liver, kidney and bile duct and non-Hodgkin's lymphoma. Lung and testis tumours observed in rodents have not been reported in humans but cannot be excluded. The presence of possible exposure misclassification or co-exposure in occupational cohort studies somewhat weakens the confidence in the association. Overall, it is concluded that sufficient evidence exists to suggest an association between TCE exposure and cancer (liver and kidney).

Health relevance of indoor exposures

Since there is sufficient evidence that TCE is a genotoxic carcinogen, all exposures indoors are considered relevant and no threshold can be determined.

Inhalation of TCE is the main route of exposure in the general population. Ambient and indoor air concentrations of TCE are generally less than 1 $\mu g/m^3$ in European and North American countries. Indoor TCE levels of up to 30 $\mu g/m^3$ (90th percentile) have been reported during the EXPOLIS study (1998–1999). More recent studies in French dwellings and American office buildings showed lower levels (95th percentile 7.4 and 2.6 $\mu g/m^3$, respectively).

Consumers may be exposed to TCE by using wood stains, varnishes, finishes, lubricants, adhesives, typewriter correction fluid, paint removers and certain cleaners, where TCE is used as a solvent. Contaminated water or soil may also contribute to indoor pollution through TCE.

Conclusions of other reviews

The previous WHO air quality guideline *(37)* was based on the unit risk estimate of 4.3×10^{-7} $(\mu g/m^3)^{-1}$ derived from the increase of Leydig cell tumours in rats.

IARC has considered TCE a probable carcinogen since 1995 (Group 2A, limited evidence in humans but sufficient in animals). The EU classified it as carcinogenic category 2, risk phrase R45 (may cause cancer) in 2001 for the same reasons. The IARC evaluation is based on experimental data and on three human cohort studies conducted in Finland, Sweden and the United Kingdom, which demonstrated an increased risk of several cancers including liver, kidney and bile duct cancers and non-Hodgkin's lymphoma *(3)*. An additional risk for cervical cancer was observed in two of the studies.

Guidelines

The existence of both positive and negative results has in the past led risk assessors to different interpretations of TCE toxicity and to divergent estimates of human cancer risk *(144,145)*. For a health risk evaluation, bearing in mind recent data on a mechanism of action that is not species-specific, the evidence for weak genotoxicity, and the consistency between certain cancers observed in animals and in humans (in particular liver cancer), it is prudent to consider that the carcinogenicity in animals, the positive epidemiological studies and the plausibility of a human cancer risk leads to the recommendation of a non-threshold approach with a risk estimate rather than a safe level.

Therefore, carcinogenicity (with the assumption of genotoxicity) is selected as the end-point for setting the guideline value. The unit risk estimate of 4.3×10^{-7} $(\mu g/m^3)^{-1}$, derived on the basis of increased Leydig cell tumours (testicular tumours) in rats, is proposed as the indoor air quality guideline. This was also the conclusion of WHO in 2000 *(37)*, the EU in 2004 *(1)* and the French Agency for Environmental and Occupational Health in 2009 *(42)*.

The concentrations of airborne TCE associated with an excess lifetime cancer risk of 1:10 000, 1:100 000 and 1:1 000 000 are respectively 230, 23 and 2.3 $\mu g/m^3$.

The guidelines section was formulated and agreed by the working group meeting in November 2009.

Summary of main evidence and decision-making in guideline formulation

Critical outcome for guideline definition

Carcinogenicity (liver, kidney, bile duct and non-Hodgkin's lymphoma), with the assumption of genotoxicity.

Source of exposure–effect evidence

Increased Leydig cell tumours (testicular tumours) in rats provided the basis for calculation of unit risk, applying a linearized multistage model *(37)*.

Supporting evidence

- Sufficient evidence exists for an association between TCE exposure and cancer (liver and kidney) *(3)*.
- Unit risk derived from a cohort study of occupationally exposed adults *(139)*, based on the increase in the incidence of hepatic tumours, was 9×10^{-7} per $\mu g/m^3$ *(140)*.

Results of other reviews

- IARC: Group 2A (limited evidence in humans but sufficient in animals) *(3)*.
- EU: carcinogenic category 2, risk phrase R45 (may cause cancer) *(1)*.

Guidelines

- Unit risk estimate of 4.3×10^{-7} per $\mu g/m^3$.
- The concentrations of airborne TCE associated with an excess lifetime cancer risk of 1:10 000, 1:100 000 and 1:1 000 000 are respectively 230, 23 and 2.3 $\mu g/m^3$.

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9. Tetrachloroethylene

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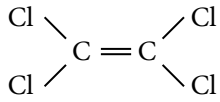
General description

Tetrachloroethylene (PCE) (CAS Registry Number 127-18-4; C_2Cl_4; molecular weight 165.83) is a readily volatile, colourless liquid with an ether-like smell *(1)*. Its main physical and chemical properties are as follows *(2–5)*: molecular weight 165.83 g/mol; density (at 20 °C) 1.6227 g/ml; melting point approximately –22 °C; boiling point 121 °C; water solubility (at 25 °C) 150 mg/litre; vapour pressure 18.47 mmHg at 25 °C *(2)*, 1.9 kPa at 20 °C, 3.2 kPa at 30 °C and 6.0 kPa at 40 °C *(3)*; Henry's Law constant 0.018 atm-m^3/mol at 25 °C; log K_{ow} (octanol/water partition coefficient) 3.40 (measurement value) and 2.97 (estimated value); and log K_{oc} (octanol/carbon partition coefficient) 177~350 (measurement value). The reaction rate constant against the hydroxyl radical is 1.7×10^{-13} cm^3/molecule per second at 25 °C, against ozone 2.0×10^{-23} cm^3/molecule per second at 25 °C and against the NO_3 radical 5.2×10^{-17} cm^3/molecule per second.

Its structural formula is:



Common synonyms include perchloroethylene, PCE, ethylene tetrachloride, tetrachloroethene, 1,1,2,2-tetrachloroethylene, carbon dichloride, perchlor, tetrachloroethane, carbon bichloride, PERC, PCE and perk.

Conversion factors

At 760 mmHg and 20 °C, 1 ppm = 6.897 mg/m^3 and 1 mg/m^3 = 0.145 ppm; at 25 °C, 1 ppm = 6.782 mg/m^3 and 1 mg/m^3 = 0.147 ppm.

Applications and uses

Major industrial applications of PCE are as a synthetic raw material of hydrochlorofluorocarbon, a dry cleaning agent, a degreaser for manufactured metal parts and an industrial solvent. Other applications include the finishing and processing of textiles, the production of paint removers and printing inks, and the formulation of adhesives and specialized cleaning fluids. Consumer products

that may contain PCE include adhesives, fragrances, spot removers, stain removers, fabric finishes, water repellents, wood cleaners, motor vehicle cleaners and dry-cleaned fabrics *(6–8)*. In Japan, 69%, 18%, 12%, and 1.3% of PCE was used as a synthetic raw material of hydrochlorofluorocarbon, a dry cleaning agent, a degreaser and an industrial solvent, respectively *(9)*.

Production

The estimated production volume in the United States in 1990 was 137 kilotonnes, compared to 282 kilotonnes in 1980 *(2)*. In 1994, the total production of PCE in the EU was 164 kilotonnes. Of this amount, 78 kilotonnes was sold for use within the EU, 56 kilotonnes were exported and the remainder used as a chemical intermediate within the chemical industry *(10)*. For Japan and the United States, it was 178 and 102 kilotonnes, respectively *(1)*. In Canada, where PCE is no longer produced, the demand in 1990 was estimated at 14 kilotonnes *(7)*. In Japan, the production and supply of PCE was 25 and 37 kilotonnes, respectively. No estimates of consumption levels are available for other parts of the world.

Analytical methods in air

Common methods of measuring indoor concentrations of TCE include integrated active and passive sampling methods using tubes packed with a carbon-based sorbent or evacuated SUMMA canisters (passivated canister sampling apparatus), or diffusion samplers such as charcoal badges. Canister sampling involves controlling the flow of air into a pre-evacuated canister. Sorbent tubes and badges retain compounds according to the affinity of the sorbent for that compound. For sorbent or charcoal sampling, the analytes must first be extracted thermally or chemically, then separated using gas chromatography and identified using a detection method such as mass spectrometry *(11)*. Personal exposure studies often use these sorbent-based methods.

Environmental fate

There are no known natural sources of PCE. The Henry's Law constant indicates that PCE is expected to volatilize rapidly from water surfaces and that volatilization from moist soil surfaces may occur *(1)*. Concentrations in ambient air may fluctuate widely over relatively short periods of time depending on the strength of the emission source, variations in wind direction and velocity, and scavenging and photodecomposition *(6)*.

The half-life in the atmosphere has been estimated to range from 70 to 250 days *(2)*.Considering the atmospheric concentrations and reaction rate constant of the hydroxyl radical and ozone, this removal from atmosphere is mostly due to reaction with hydroxyl radicals. This process is temperature-dependent, with increased reaction rates in the summer. The results of biodegradability tests sug-

gested that the biodegradability of PCE is extremely low in environmental water *(12,13)*; it may persist in groundwater for several months or more *(7)*.

The main sources resulting in ambient air concentrations are industrial emissions and releases from building and consumer products. Releases are primarily to the atmosphere, but the compound is also released to surface water and land in sewage sludges and in other liquid and solid waste, where it can evaporate rapidly to the atmosphere.

ATSDR has estimated that 80–85% of the PCE used annually in the United States is released into the atmosphere *(2)*. Atmospheric emissions result from evaporation during dry cleaning, metal degreasing, the production of fluorocarbons and other chemicals, and miscellaneous solvent-associated applications *(14)*. However, the percentage is likely to be lower in the United States at present, owing to improved technology and regulatory restrictions. Almost all PCE enters the atmosphere unchanged. A small proportion enters water and wastewater. From surface water, PCE volatilizes owing to its relatively low water solubility and high vapour pressure.

Indoor sources and pathways of exposure

Inhalation is the most common route of exposure to PCE for the general population. Ingestion of contaminated drinking-water may also be important in areas with highly contaminated water. While PCE has been found in some food items, particularly from local source contamination, not enough data are available to estimate exposure through this route *(2)*.

Indoor sources

Consumer products described above are sources of indoor PCE exposure. Contaminated drinking-water may be a source of indoor PCE exposure when taking a shower or washing dishes. Workers from dry cleaning establishments have been found to become sources of PCE at home by exhaling PCE in their breath *(15)*. Dry-cleaned clothes are also possible sources of PCE in a house.

The air in dry cleaning facilities and neighbouring homes or facilities can contain elevated concentrations of PCE. Soil pollution with PCE has resulted in indoor air concentrations of these chemicals in residential homes. These chemicals can evaporate from the soil, permeate through floors and result in elevated indoor air concentrations. PCE can permeate through synthetic water pipes and contaminate drinking-water, thus leading to exposure during bathing or showering.

Drinking-water

Exposure can take place by drinking contaminated water as well as volatilization. In the United States, PCE is not detectable in drinking-water in most cases. The EPA Groundwater Supply Survey of 945 drinking-water systems nationwide reported PCE in 75 out of 945 systems in 1984; the median level of the positive

samples was about 0.75 μg/litre, with a maximum of 69 μg/litre *(2)*. In several studies in Canada, PCE was detectable in 6–60% of the tested samples. Reported mean values ranged from 0.1 to 0.9 μg/litre *(6)*. In several other countries, including Germany and the United Kingdom, similar results have been observed *(8,16)*. In some villages in Finland, concentrations of up to 180 μg/litre were found *(17)*. In Japan, PCE levels in 99.7% of drinking-water (n = 5600) were less than 1 μg/litre with a maximum of 7 μg/litre in 2007 *(18)*.

Food

Data on concentrations of PCE in food are scarce. In some studies from Germany and Switzerland reported in the early 1980s, relatively high total intakes of 87–170 μg/day were found *(16)*. The results of market basket surveys in the United States, reported in 1987 and 1988, indicated lower levels. From the results of these surveys, total daily intake via food has been estimated at 0.12–65 μg/kg body weight *(6)*. Several groups of researchers have reported elevated concentrations of PCE in fatty food products in residences and markets, owing to contamination from dry cleaning establishments nearby. In a supermarket near a dry cleaning shop in Germany, concentrations were 36 μg/kg and 110 μg/kg in cheese and margarine, respectively *(8)*. In one instance in the United States, a very high PCE concentration was found in margarine (up to 50 mg/kg) in a shop next door to a dry cleaning establishment *(2)*. Moreover, food grown on contaminated soil can contain PCE. In Japan, the daily maximum levels of PCE in the diet between 1990 and 1999 (n = 72~81 in each year) were 4.4 μg/kg wet weight *(14)*.

Indoor air concentrations and relationship with outdoor levels

Residential concentrations

In several United States cities (New York, Los Angeles, Chicago, Minneapolis, St Paul, Baltimore, Elizabeth and Houston), median indoor residential concentrations ranged from 0.4 μg/m^3 in spring (Minneapolis) to 3.5 μg/m^3 during winter (New York City) *(19–26)*. The 90th percentiles ranged from 1 μg/m^3 in Minneapolis in the spring to 14 μg/m^3 in New York City during the summer *(23)*.

Geometric mean concentrations of PCE in the homes of dry cleaning workers have been found to be 265 μg/m^3, while non-exposed homes had a mean concentration of 2 μg/m^3 *(15)*. Also, shops, offices or homes that share a building with a dry cleaning establishment have been found to have higher levels of PCE than those that are not near dry cleaners. A study of apartments in neighbourhoods with different income levels and ethnic compositions in New York City between 2001 and 2003 found overall geometric mean levels of 34 μg/m^3, although in low-income neighbourhoods, the geometric mean was 256 μg/m^3. Apartments with no dry cleaners in the building had a geometric mean concentration of 3 μg/m^3. Before 1997, when New York adopted stricter regulations for dry cleaners, PCE

was found to be about 340–360 μg/m^3 in residential buildings with a dry cleaning establishment *(27)*.

In a national survey of dwellings in France, a median concentration of 1.4 μg/m^3 was found, with a 90th percentile of 5.2 μg/m^3 *(28)*. In the European multinational urban air study EXPOLIS *(29)*, median levels of PCE inside homes in Helsinki were not detectable, while they were 0.6 μg/m^3 in Basel. The 90th percentile in Basel was 2.9 μg/m^3. In Oxford, United Kingdom, the median indoor residential PCE concentration was 1.9 μg/m^3, and 6.3 μg/m^3 at the 90th percentile. Homes in Athens had a median indoor level of 4 μg/m^3 and a 90th percentile level of 14.3 μg/m^3. Milan and Prague were found to have median concentrations of 7.4 and 8.7 μg/m^3, respectively, with 90th percentiles of 28.1 μg/m^3 and 26.1 μg/m^3, respectively. Median outdoor residential PCE levels were under the detection limit in Helsinki, 0.7 μg/m^3 in Basel and 1.7 μg/m^3 in Oxford. The 90th percentiles in Basel and Oxford were 1.4 μg/m^3 and 3.4 μg/m^3, respectively. In Athens, Milan and Prague, the median outdoor residential levels were 2.3 μg/m^3, 4.1 μg/m^3 and 5.3 μg/m^3, respectively. Median personal exposure levels were lower than the indoor levels.

In 25 homes surveyed in Shimizu, Japan, geometric mean indoor PCE concentrations were 0.16 μg/m^3 in summer and winter, with geometric standard deviations of 2.34 μg/m^3 in summer and 2.77 μg/m^3 in winter *(30)*. The geometric mean concentration outside these homes was 0.11 μg/m^3 in both summer and winter, with geometric standard deviations of approximately 2 μg/m^3 in both seasons. In a national survey of dwellings in Japan, median indoor PCE concentrations in residential houses in 1997 (n = 180) and 1998 (n = 205) were 0.4 μg/m^3 (mean 1.8 μg/m^3; maximum 83.5 μg/m^3) and 0.3 μg/m^3 (mean 1.9 μg/m^3; maximum 43.4 μg/m^3), respectively *(31)*.

Non-residential microenvironments

Predicted PCE concentrations in the air around metal degreasing machines were found to range from about 10 000 μg/m^3 to above 1 million μg/m^3, depending on the type of machine. In Germany, mean personal air samples in occupational environments with degreasers ranged from 18 000 to 40 000 μg/m^3 *(10)*.

Exposure to PCE from dry cleaning affects both workers and members of the general public. A study in Finland of personal exposure of workers in commercial and industrial dry cleaning operations using dry-to-dry machines found that machine operators had the highest exposure levels (28 000 and 32 000 μg/m^3 at commercial and industrial sites, respectively). Customer service workers had the lowest exposures, with an average of about 800 μg/m^3 *(32)*. Another study of dry cleaning establishments in Chicago found indoor levels of PCE ranging from 12 000 to 355 000 μg/m^3 *(33)*.

A survey of stores in a one-level commercial building in New Jersey found a median concentration of 690 μg/m^3 and a 90th percentile of 4200 μg/m^3 in a

dry cleaning establishment in the building, and a median of 570 μg/m^3 and 90th percentile of 5800 μg/m^3 (8 hour average) in a neighbouring clothes rental shop, which may have also contained dry-cleaned clothes *(34)*. The neighbouring shop on the other side had lower levels, but the median was still nearly 90 μg/m^3, a level much higher than the average indoor environment. Dry cleaned clothes have been shown to emit PCE *(2,32)*.

A survey of 70 office buildings across the United States (in which there were no complaints) found a median indoor PCE level of 1.5 μg/m^3 and a 95th percentile of 18 μg/m^3 *(35)*. A study of mechanically ventilated non-office and office buildings in Hong Kong SAR, China found arithmetic mean levels of 1.4 μg/m^3 (SD = 3.4) and 1.9 μg/m^3 (SD = 9.2), respectively *(36)*. Median indoor concentrations at workplaces of the EXPOLIS participants were under the detection limit in Helsinki, 1.2 μg/m^3 in Basel, 3.5 μg/m^3 in Athens, 5.4 μg/m^3 in Milan and 3.7 μg/m^3 in Prague. The 90th percentile of workplace levels were 3.1 μg/m^3 in Basel, 10.6 μg/m^3 in Athens, 17.5 μg/m^3 in Milan and 10 μg/m^3 in Prague. The workplaces were typically offices. A survey of stores in Boston, MA found that geometric mean levels of PCE in stores and restaurants were near or under 3 μg/m^3 *(37)*.

Ambient air

Residential outdoor median concentrations ranged from 0.3 μg/m^3 in Minneapolis (spring) to 1.7 μg/m^3 in Los Angeles (winter). The median outdoor concentrations of PCE in Shimizu (Japan) were 0.2 μg/m^3 (mean 0.5 μg/m^3; maximum 4.7 μg/m^3) and 0.2 μg/m^3 (mean 0.7 μg/m^3; maximum 10.8 μg/m^3), respectively *(30)*. Outdoor levels in rural Canada were found to have a mean of 0.04 μg/m^3 and a maximum of 7 μg/m^3 *(38)*.

Indoor : outdoor ratios in New York and Los Angeles from a study in a low-income group of teenagers were between 1 and 2 at the median and did not have large standard deviations relative to the median (except for New York homes in the winter) *(23)*. The earlier Total Exposure Assessment Methodology (TEAM) study found higher indoor : outdoor ratios in the cities of Bayonne and Elizabeth, NJ, with arithmetic means ranging from 2 in summer to 7 in winter and 5 in the winter in Los Angeles *(39,40)*. The medians were not reported but are likely to have been lower than the arithmetic means.

Personal exposure

Median personal exposures of the teenagers were 2.2 μg/m^3 in Los Angeles and 3.7 μg/m^3 in New York, although the mean in New York was 8.8 μg/m^3, indicating a more skewed distribution with some higher values *(19–26)*. The New York indoor home distribution was also fairly skewed. The RIOPA study in Los Angeles, Elizabeth and Houston found that adult personal air concentrations had lower medians than means, with a particularly large difference in Elizabeth,

where the median was 0.56 μg/m^3 and the mean was 17.3 μg/m^3 *(26)*. Indoor and outdoor residential mean concentrations in Elizabeth, however, were near 1 μg/m^3 and were detectable in only 24% of outdoor samples and 39% of indoor samples, while in personal samples PCE was detected in 44% of the samples *(26)*.

Personal exposures to PCE are most influenced by indoor concentrations. PCE can reach extremely high levels indoors, even in non-occupational environments such as homes or businesses that are in the same building as or close to dry cleaners. The TEAM studies found that higher personal PCE exposures were associated with visits to dry cleaners *(39,40)*. Unlike other industries that use PCE, such as metal degreasing, dry cleaners are often located in residential, non-industrial commercial and business districts and may affect both the occupants of neighbouring establishments and food. Indoor environments next to dry cleaners have been found to have PCE concentrations of several hundreds of μg/m^3. Indoor environments without sources of PCE have concentrations well below 10 μg/m^3, with median concentrations near 1 μg/m^3 in many areas. Dry-cleaned items can be an occasional source of PCE in some homes.

Toxicokinetics

Absorption

PCE is readily absorbed in the gastrointestinal tract and the lungs. Pulmonary uptake is proportional to ventilation rate, duration of exposure and, at lower atmospheric concentrations, the concentration in the inspired air *(2)*. In both humans and animals, initial uptake following inhalation is rapid, with rates levelling off after a few hours of exposure. The available data suggest that a high proportion is absorbed in humans, but actual percentages have not been reported. In rats, the proportion absorbed was approximately 55–70% after 1 minute, gradually declining to 40–50% after 2 hours *(41)*.

Dermal absorption from the gaseous phase is negligible. However, dermal absorption of significant amounts of PCE is probably possible in some situations. Dermal absorption of PCE solutions resulted in measurable levels of the compound in the breath, reaching a maximum 10 minutes after exposure *(16)*. Experiments in hairless guinea-pigs have shown substantial dermal absorption from dilute aqueous solutions *(21)*. In vitro dermal absorption of PCE in aqueous solution through freshly prepared and previously frozen human skin was measured and compared to absorption of other volatile organic compounds in aqueous solution *(42)*. The dermal absorption of PCE from a soil matrix was compared in rats and humans using real-time mass spectrometric exhaled breath technology and physiologically based pharmacokinetic (PBPK) modelling *(43)*.

Distribution

Repeated inhalation exposure to PCE results in accumulation of the compound in the body, especially in fatty tissue since it is lipid-soluble. PCE crosses the

placenta and can be found in breast milk; the fetus and nursing neonates may therefore be at increased risk of adverse effects from maternal exposure *(2,44,45)*. In rats, distribution to the brain, liver and kidneys has also been demonstrated *(2,46)*. In a study in mice, transplacental transport of unchanged PCE has been observed *(2)*. In rats and goats, it has been shown that excretion of unchanged PCE in milk occurs after intraruminal administration *(23,24)*. Following inhalation exposure of lactating rats to a concentration of 4070 mg/m^3 (600 ppm) for 2 hours, total body burden for the pups was up to about 14 mg/kg body weight *(23)*. Since women have a higher proportion of body fat than men, it is assumed that women retain PCE longer *(47)*.

Biotransformation

In experiments in humans and rats, it was observed that most of the absorbed amount of PCE is exhaled unchanged. The two principal pathways of PCE metabolism that occur in the liver and kidney of experimental animals are cytochrome P450-dependent oxidation and glutathione conjugation. The hepatotoxic, nephrotoxic and carcinogenic effects of PCE depend on its metabolism to reactive metabolites, which may covalently bind to cellular macromolecules.

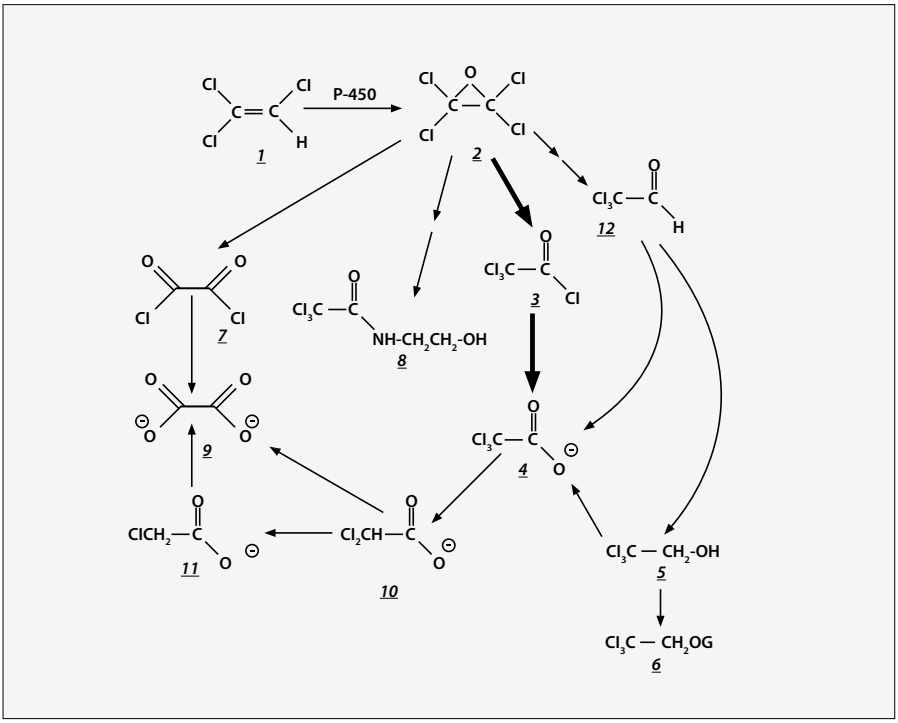
Cytochrome P450 enzymes primarily catalyse PCE oxidation. Presumably, CYP2E1 plays an important role in this process in rodent liver and kidney as well as human liver. This results in the formation of a PCE-epoxide, which further reacts to trichloroacetyl chloride. Trichloroacetyl chloride can react with amino groups in macromolecules, resulting in hepatotoxicity. When it reacts with water it forms trichloroacetic acid.

Trichloroacetic acid is the principal metabolite recovered from urine in both humans and rodents following inhalation exposure to PCE. Another metabolite that can be formed by the cytochrome P450 pathway is dichloroacetate. Di- and trichloroacetate are associated with hepatic toxicity and carcinogenicity. Other metabolites of PCE by the P450 pathway that have been identified are shown schematically in Fig. 9.1 *(48,49)*.

There is a broad range of halogenated hydrocarbons and other small organic molecules that are oxidized by CYP2E1. Several drugs as well as physiological or pathological conditions may lead to the induction of CYP2E1. This implies that under certain conditions, prior or concurrent exposure to chemicals such as ethanol or acetaminophen may influence the response to PCE. Also, individual susceptibility due to genetic polymorphisms in CYP450-dependent metabolism can result in altered toxicity.

PCE is conjugated by glutathione to S-(1,2,2-trichlorovinyl)glutathione (TCVG). No significant effects were found of PCE or TCVG on cytotoxicity or mitochondrial function in isolated hepatocytes from rats or in isolated liver mitochondria from rats or mice. Therefore, it is unlikely that the liver is a major acute target for PCE or TCVG. Glutathione conjugation of PCE, followed by ac-

Fig. 9.1. Metabolism of PCE by the P450 pathway



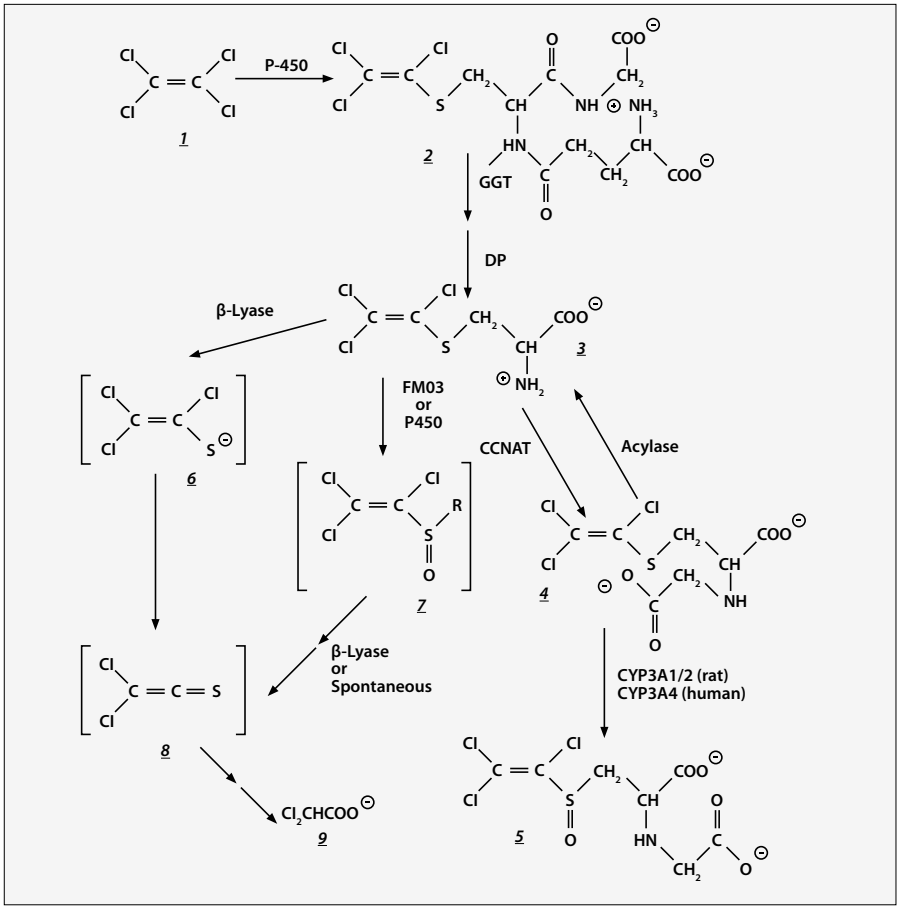
Note. Identified urinary metabolites: 1 = PCE; 2 = PCE epoxide; 3 = trichloroacetyl chloride; 4 = trichloroacetate; 5 = trichloroethanol; 6 = trichloroethanol glucuronide; 7 = oxalate dichloride; 8 = trichloroacetyl aminoethanol; 9 = oxalate; 10 = dichloroacetate; 11 = monochloroacetate; 12 = trichloroacetaldehyde.
Source: Lash & Parker *(48)*.

tivation of trichlorovinylcysteine (TCVC), is responsible for nephrotoxicity and possibly nephrocarcinogenicity *(49,50)*. Fig. 9.2 shows the metabolites of PCE by the glutathione conjugation pathway, so far as they have been identified to date.

Species-dependent differences in biotransformation

Mice metabolize PCE to trichloroacetic acid to a greater extent than rats. In rats, saturation of the oxidative metabolism at exposure concentrations exceeding 678 mg/m^3 (100 ppm) prevents the occurrence of the high trichloroacetic acid concentrations that are observed in mice at these dose levels. The available evidence indicates that in humans too, saturation of the oxidative metabolism of PCE occurs at ≥ 678 mg/m^3 (≥ 100 ppm) *(51)*. In a comparative study, the urinary levels of *N*-acetyl-*S*-(1,2,2-trichlorovinyl)-*L*-cysteine, one of the final metabolites in the glutathione biotransformation pathway, were shown to be higher in rats than in mice after inhalation exposure to PCE. In vitro tests in hepatocytes and kidney fractions of rats, mice and humans suggest that the potential for this biotransformation in the kidneys is also lower in humans than in rats *(2,6,52)*. Experimental data show that rates of TCVG formation in rats and mice

Fig. 9.2. Metabolism of PCE by the glutathione conjugation pathway



Note. Identified urinary metabolites: 1 = PCE; 2 = TCVG; 3 = TCVC; 4 = NAcTCVC; 5 = NAcTCVC sulfoxide; 6 = 1,2,2-trichlorovinylthiol; 7 = TCVCSO; 8 = 2,2-dichlorothioketene; 9 = dichloroacetate. Enzymes: GST, GGT, dipeptidase (DP), ß-lyase, FMO3, CCNAT, CYP3A1/2 and CYP3A4. Unstable, reactive metabolites are shown in brackets.

Source: Lash & Parker *(48)*.

are higher in males than in females, the formation rates in mice being higher than in rats *(48,53)*.

The overall kinetics of PCE oxidative metabolism differs significantly between humans and rodents. The excretion of trichloroacetic acid after inhalation of PCE has been shown to be slower in humans than in rats. The elimination half-time of trichloroacetic acid in the urine was approximately 4.1-fold longer in humans than in rats exposed to 10 or 40 ppm PCE. Maximal trichloroacetic acid concentrations in blood were 3- to 10-fold lower (depending on dose) in humans than in rats *(48,54)*. It is assumed that saturation of PCE metabolism occurs at lower doses in humans *(55,56)*.

While the target organs for toxicity in general are similar for different species, this is not the case for carcinogenicity.

Sex-dependent differences in biotransformation

Regarding renal toxicity, sex differences have been found in experiments on rats and mice. PCE is demonstrated to cause a significant release of lactate dehydrogenase in isolated kidney cells from male but not from female rats. TCVG causes much more lactate dehydrogenase to be released from male than from female rat kidney cells. Examination of effects on mitochondrial respiration in suspensions of isolated mitochondria showed that PCE and TCVG are more toxic in renal mitochondria from male rats than in those from female rats. Respiratory function in (renal) mitochondria from mice was significantly inhibited by PCE and TCVG, but showed little sex dependence *(49)*. Experimental data showed that TCVG formation in kidney and liver subcellular fractions from male rats and mice were invariably higher than corresponding values in female rats and mice *(53)*.

Elimination

In humans and animals, the major part of the absorbed amount of PCE is exhaled unchanged. In humans, 80–100% of the uptake was exhaled as parent compound in the 7 days following a single 4-hour inhalation exposure to 488 or 976 mg/m^3 (72 or 144 ppm) PCE. In rats, the proportion is somewhat lower (68%). Elimination of PCE from adipose tissue is relatively slow (calculated half-life 55 hours) owing to the high adipose/blood partition coefficient and the low rate of blood perfusion to this tissue *(2)*. Excretion of metabolites in urine represents only a small proportion of the inhaled dose. Following single inhalation exposure in humans, only 2% of the uptake was found as the major urinary metabolite, tetrachloroacetic acid. This compound was excreted from the blood with a half-life of 75–80 hours. In another study, its half-life in urine was estimated at 6 days *(16)*.

In a study in which humans were exposed by inhalation to 6.8 mg/m^3 (1 ppm) PCE in the air for 6 hours, the average recovery by exhalation was calculated to be 82%. This implies that about 18% was metabolized. PCE was readily detected in alveolar air and venous blood; trichloroacetic acid was found in venous blood and urine. Recovery was primarily through exhaled air; only a small amount (less than 1% of intake) of trichloroacetic acid was excreted via the urine. Between about 63% and 93% of the intake was exhaled as parent compound 6 days after the exposure; 0–10% was estimated to be exhaled as PCE after the 6-day period *(57)*. An inhalation study in humans and rats exposed for 6 hours at different concentrations of PCE showed excretion of trichloroacetic acid and the acetylated metabolite N-acetyl-TCVC in the urine of both species. Excretion of the N-acetyl-TCVC was significantly higher in male than in female rats *(54)*.

In rat studies, high concentrations in the milk have been observed, peak concentrations being an order of magnitude higher than in maternal blood *(58)*. Experimental data in lactating goats showed that, following intraruminal admin-

istration of an equal volume mixture of PCE, TCE and methyl chloroform, the milk secreted during the 24 hours following the administration contained 6.43 mg PCE (in addition to TCE and methyl chloroform). PCE demonstrated the greatest tissue partitioning compared to TCE and methyl chloroform, being persistently in the blood and secreted into the milk *(59)*. Limited data in humans also show that PCE is excreted in the milk *(60)*.

Biomarkers of exposure

Biological monitoring of exposure to PCE can be carried out by measuring levels of the parent compound in blood, urine or exhaled air or of the metabolites in blood or urine. These methods have been applied for assessing both occupational and non-occupational exposure.

There has been discussion in the literature as to whether measurement of the parent compound in blood or in exhaled air is the preferred biological index to monitor PCE exposure *(47,61–63)*. A study in humans showed that correlation coefficients (r) between environmental PCE and PCE-B (PCE measured in blood), PCE-Alv (PCE measured in alveolar air) and PCE-U (PCE measured in urine) were 0.94, 0.81 and 0.67, respectively. A high correlation was also found among biological indices: the r value was 0.96 between PCE-B and PCE-Alv, 0.95 between PCE-B and PCE-U, and 0.87 between PCE-Alv and PCE-U *(61)*. The examined biological indices proved sensitive enough for biological monitoring of low exposure to PCE and can give substantially similar information in terms of exposure evaluation. PCE measurement in alveolar air offers some practical advantages over the other exposure indices.

Measuring of metabolites (trichloroacetic acid, trichlorethanol) in blood or urine may not necessarily represent exposure to PCE because some related chlorinated hydrocarbons (TCE, 1,1,1-trichloroethane) are converted to the same metabolites *(2)*.

Physiologically based pharmacokinetic modelling

Several groups of researchers have developed PBPK models for PCE and several of the models have been evaluated. Table 9.1 provides an overview of these efforts *(41,46,58,60,64–79)*.

Most PBPK models for PCE share the four-compartment structure (liver, fat, rapidly perfused tissues and slowly perfused tissues) and steady-state description of lung equilibration developed by Ramsey & Andersen *(80)* for styrene. Only one of the published models *(71)* provides a description of the kinetics of trichloroacetic acid, the major metabolite of PCE.

Some studies have focused on the evaluation of these models. In one, the key parameters and predictions for PBPK models for mice, rats and humans developed by different groups of researchers were compared. The amounts of metabolized PCE predicted showed considerable differences (about 12- to 14-fold for

humans). Most of the differences in risk-related model predictions were due to the choice of the data sets used for calibrating metabolic parameters (V_{max}, K_m) *(77,81)*. A statistical approach to some of the PBPK models for PCE in mice, rats and humans showed that the kinetic parameters defining the metabolic rate were the most important parameters for model sensitivity *(82)*.

Health effects

Identification of studies

Epidemiological studies on health effects of PCE exposure were identified from electronic searches and hand searches of references in former reviews by WHO. Electronic searches were made in PubMed in July 2008, with an update in June 2009. We intended to identify all studies with original data on health effects of PCE and the main descriptors used were "tetrachloroethylene" and "perchloroethylene". In addition, studies that examined the health effects of PCE were identified through hand searches of earlier reviews of the topic, citations within papers identified on health effects of PCE and searches on the web sites of international or national health assessment agencies, including WHO, the European Commission, Health Canada, IARC, ATSDR, USEPA, the United Kingdom Committee on Carcinogenicity of Chemicals in Food, Consumer Products and the Environment and the US National Institute for Occupational Safety and Health. Papers known to the expert group in 2009 concerning health effects of PCE were also used.

Of 148 papers identified, 59 were relevant to the health effects section of this chapter. These papers were included in the discussion presented below, addressing human exposure and epidemiological issues (37), children (0), animal studies (10), cell studies (4), reviews (4) and other studies (4). In addition, 11 reports of the above-mentioned agencies were used in the "Health effects" section.

Effects on experimental animals and in vitro test systems

Toxicological effects

PCE has a low acute inhalation toxicity (LC_{50} in rodents ≥ 16.6 mg/litre). Acute oral toxicity is also low (LD_{50} in rodents ≥ 3000 mg/kg body weight) *(6)*.

The liver, kidneys, blood and CNS are the target organs for systemic effects. Hepatic effects occur at lower dose levels in mice than in rats. In a series of experiments in mice, continuous (24 hours/day) inhalation exposure for 30 days at concentrations of 61, 251, 508 or 1017 mg/m^3 produced a dose-related increase in liver weight at all dose levels. Morphological changes in the liver were observed at 251 mg/m^3 and, as stated but not fully reported, at all other dose levels as well. Plasma butyrylcholinesterase activity showed a slight increase at ≥ 251 mg/m^3. The reversibility of the effects was examined at 1071 mg/m^3 only, showing that at 120 days after cessation of treatment, only a slight increase in liver weight remained *(83)*.

Table 9.1. Physiologically based pharmacokinetic (PBPK) models for PCE metabolism

Species	Input parameters and calibration
Mice, humans	Data from single-dose inhalation
Humans	Partition coefficients determined in vitro; metabolic parameters from occupational studies
Mice, rats	Partition coefficients determined in vitro; metabolic parameters from oral and/or inhalation exposure studies in rats and mice
Humans	Animal data and metabolic parameters derived from data on urinary levels of trichloro compounds in urine of workers exposed to PCE
Rats, humans	Partition coefficients determined in vitro; metabolic parameters calculated and optimized from inhalation studies in rats and humans
Rats (male)	Partition coefficients and metabolic parameters from data generated by a single intra-arterial dose study in male rats
Rats (male), dogs (male)	Partition coefficients from AUCs[a] as determined in vivo in single-dose oral tests in rats and dogs; metabolic parameters estimated from blood and liver concentrations
Rats (lactating)	Partition coefficients determined in vitro; metabolic parameters from in vivo gas-uptake tests in lactating rats
Humans (lactating)	Existing model adjusted for prediction of excretion of PCE in mother's milk
Humans	Assess an existing PBPK model; metabolic parameters from in vivo experiment in Orientals and Caucasians
Mice	Partition coefficients determined in vitro; metabolic parameters from in vivo gas-uptake tests and Monte Carlo analysis to estimate variability
Humans	Describe the relationship between individual and population physiological parameters by using an existing PBPK model in a Markov chain Monte Carlo (MCMC) analysis; metabolic parameters from an existing in vivo inhalation study
Rats, mice, humans	Validate an existing PBPK model and develop a refined one; metabolic parameters from in vivo and in vitro experiment
Rats	Develop a PBPK model; metabolic parameters from mixed exposure in vivo experiments
Humans	Validate and calibrate an existing PBPK model; anatomical, physiological, biochemical and physicochemical data from literature
Rats, humans	Percutaneous permeability coefficients determined in dermal exposure experiments (in vivo)
Humans	Calibrate single PBPK models and describe metabolic interactions; metabolic data from inhalation exposure studies in rats and humans
Humans, rats	Evaluate and compare existing PBPK models and develop a refined one; metabolic parameters from oral and inhalation exposure studies in rats and humans
Humans	Describe the relationship between individual and population physiological parameters by using an existing PBPK model in an updated MCMC analysis; metabolic parameters from an existing in vivo inhalation study
Humans	Characterize the uncertainty and variability of metabolized fraction by using a modified PBPK model in an MCMC analysis; metabolic parameters from several existing in vivo inhalation studies.

[a] AUC = area under the curve.

Model application	Reference
Human cancer risks estimated on the basis of the predicted amounts of PCE metabolites in mice and humans, and using liver tumour incidences in mice	Chen & Blancato *(64)*
Simulation of the time-course of PCE concentration in blood for continuous inhalation exposure	Bogen & McKone *(65)*
Simulation of the amount of urinary metabolites in mice or proportion of exhaled PCE following a single inhalation exposure in rats	Ward et al. *(66)*
Simulation of the time-course of exhaled unchanged PCE	Ward et al. *(66)*
Simulation of the proportion of exhaled PCE and body burden in rats and humans	Koizumi *(67)*
Simulation of the time-course of concentrations in tissues, blood and exhaled air	Dallas et al. *(41,46)*
Simulation of the time-course of the fractions exhaled and metabolized in single-dose studies in rats and dogs	Dallas et al. *(68,69)*
Simulation of the time-course of PCE concentrations in maternal blood and milk and in pup tissues	Byczkowski et al. *(58)*
Concentrations in milk and infant doses predicted for inhalation exposure scenarios in and close to dry cleaning shops	Schreiber *(60)*
Study ethnic differences in biological monitoring to improve reliability of bio-monitoring (and setting biological limit values)	Jang & Droz *(70)*
Simulation of variability of PBPK model parameters and their effects on PBPK model predictions in risk assessment	Gearhart et al. *(71)*
Estimate the population variability of human PCE metabolism	Bogen & McKone *(65)*
Carcinogenic risk assessment based on amounts of PCE metabolites formed in the livers of rodents and humans	Reitz et al. *(72)*
Investigate pharmacokinetic interactions among PCE, TCE, methyl chloroform; assessment of occupationally relevant exposures at or below threshold limit values	Dobrev, Andersen & Yang *(73)*
Analyse human exposure data and predict urinary excretion of trichloroacetic acid	Loizou *(74)*
Assessment of dermal absorption from a soil matrix and evaluation of skin compartment models	Poet et al. *(75)*
Evaluate metabolic interactions (and thresholds) between PCE, TCE and 1,1,1,-TCE	Dobrev, Andersen & Yang *(76)*
Simulation of urinary excretion of trichloroacetic acid at low concentration exposures	Clewell et al. *(77)*
Estimate the population variability of human PCE metabolism	Chiu & Bois *(78)*
Estimate the population variability of human PCE metabolism	Covington et al. *(79)*

Within the United States National Toxicology Program (NTP) subchronic inhalation studies were carried out in rats and mice. In mice, liver effects were observed at 1350 mg/m^3 (≥ 200 ppm) or higher. Karyomegaly of the renal tubule epithelial cells was observed at ≥ 1350 mg/m^3 (≥ 200 ppm). At 680 mg/m^3 (100 ppm), no effects were seen but at this level no histopathological examination of the liver was carried out. In rats, mild congestion of the liver was seen at ≥ 1350 mg/m^3 (≥ 200 ppm) (lower concentrations not tested), with congestion of the lungs, decreased survival and growth retardation at higher dose levels *(84)*. In chronic studies, renal tubular cell karyomegaly was observed in female rats and mice at both 1356 and 2712 mg/m^3 (200 and 400 ppm) in rats and 678 and 1356 mg/m^3 (100 and 200 ppm) in mice.

In addition, in rats, renal tubular hyperplasia (males), thrombosis and squamous metaplasia in the nasal cavity (males and females) and hyperlasia in the adrenal medullae (males) or cortices (females) were found. In mice only, liver cell degeneration and necrosis were seen at both concentrations *(84)*. A study exposing mice to 2040 mg/m^3 (300 ppm) perchloroethylene gas for 6 hours daily for 5 days revealed regeneration of olfactory mucosa. Two weeks and three months after exposure, the nasal mucosa was examined histopathologically. After two weeks, ciliated epithelium started to replace the olfactory epithelium. Damage was more persistent in the nasal mucosa of the olfactory region than in the respiratory region *(85)*.

CNS effects have been observed at high dose levels. In the short-term NTP experiment in rats and mice, neurological symptoms were observed only at the highest concentration of 11 860 mg/m^3 (1750 ppm) *(84)*. Biochemical changes in brain tissues have been observed in rats and gerbils at concentrations of ≥ 407 mg/m^3 (≥ 60 ppm). However, the toxicological significance of these changes is unclear *(2)*. In a recent short-term study in rats, decreased weight of brain regions and reductions in neuronal marker proteins were seen at 4080 mg/m^3 (600 ppm) but not at 2040 mg/m^3 (300 ppm) *(86)*. Haematotoxicity was observed in an inhalation study in mice at test concentrations of 915 and 1830 mg/m^3 (no other concentrations tested) in which the animals were exposed for 6 hours/day, 5 days/week for 7.5 or 11.5 weeks *(87)*. Rats that were acutely exposed to a high dose (500 mg/kg oral) of PCE and others subchronically to lower doses (5 and 50 mg/kg oral), showed significant effects on nociception and locomotor activity *(88)*. An inhalation study assessed the effects of PCE on sustained attention in rats performing a visual signal detection task. It showed that inhaled PCE acutely impaired sustained attention in rats, and its potency weakened on repetition of the exposure. Accuracy was significantly reduced at 3390 mg/m^3 (500 ppm), significantly elevated response time was seen at 6780 mg/m^3 (1000 ppm) and significantly reduced trial completions were found at 10 170 mg/m^3 (1500 ppm) *(89)*.

Limited teratogenicity studies in rats, mice and rabbits suggest that PCE produces fetotoxicity and embryotoxicity at high dose levels (≥ 2034 mg/m^3). In a

behavioural teratogenicity study in rats, neuromuscular ability in pups was decreased at 6100 mg/m^3 (900 ppm) but not at 2034 mg/m^3 (300 ppm) *(2)*. In a study on developmental toxicity, embryos from rats were explanted on gestational day 120 and cultured for 46 hours in the presence of PCE. Exposure to PCE ranging from 3.5 to 15 mM caused concentration-related effects on growth and development. The presence of hepatic microsomal fractions in the culture medium partially prevented the effects of PCE on the measured parameters of embryo growth and development *(90)*. A study showed altered behaviour in adult mice orally exposed to PCE as neonates (5 and 320 mg/kg PCE/day between days 10 and 16 postnatally), indicating neonatal susceptibility of brain maturation in achieving long-lasting changes in adult behaviour *(91)*. A developmental study in rats following inhalation exposure to PCE showed maternal responses that were of limited toxicological significance. Developmental effects at 600 ppm consisted of reduced gravid uterus, placental and fetal weights and decreased ossification of thoracic vertebral centra. Developmental effects at 1695 mg/m^3 (250 ppm) were of minimal toxicological significance, being limited to minor decreases in fetal and placental weights. There were no developmental effects at 441 mg/m^3 (65 ppm) *(92)*.

PCE at 68–2043 mg/m^3 (10–300 ppm) was found to increase, in a concentration-dependent manner, ACh- and high K+-induced muscle contraction in tracheal tissue from swine. Swine tissue was used because there are many similarities between swine and human airways. More research is necessary to be able to conclude that these responses play a role in airway impairment and hyperresponsiveness after PCE exposure *(93)*. A study using renal cell suspensions of rats and mice showed that PCE caused acute renal cellular toxicity in rats and mitochondrial toxicity in rats and mice *(49)*.

Carcinogenic effects

Data on the carcinogenicity of PCE have been evaluated in various health assessment documents *(2,6,51)*. PCE-induced nephrotoxicity and nephrocarcinogenicity have been associated with metabolism by the glutathione conjugation pathway to form TCVG *(48,53)*.

Oral and inhalation studies with NTP have been performed in mice and rats. In a gavage study in B6C3F1 mice (time-weighted average (TWA) daily dose levels of 536 and 1072 mg/kg body weight in males and 386 and 772 mg/kg in females), increased incidences of hepatocellular carcinomas were found in males and females (4/37, 32/49 and 27/48 in males and 2/40, 19/48 and 19/48 in females). In Osborne Mendel rats, no increase in tumour incidence was seen. This study has various limitations: numerous dose adjustments during the study, early mortality related to compound-induced toxic nephropathy, and pneumonia due to intercurrent infectious disease in both rats and mice *(2,94)*. In the inhalation study in B6C3F1 mice (test concentrations 100 and 200 ppm, 6 hours/day,

5 days/week for 103 weeks, corresponding to 680 and 1360 mg/m^3), incidences of hepatocellular carcinomas were increased (7/49, 25/49 and 26/50 males and 1/48, 13/50 and 36/50 females). In F344/N rats (test concentrations 200 and 400 ppm, 6 hours/day, 5 days/week for 103 weeks, corresponding to 1360 and 2720 mg/m^3), PCE caused dose-related increases in the incidence of stage 3 mononuclear cell leukaemia in animals of both sexes: in males, 20/50 in controls, 24/50 at the low dose and 27/50 at the high dose; in females, 10/50, 18/50 and 21/50, respectively. The historical control for mononuclear cell leukaemia in rats in the same laboratory was 47% in males and 29% in females. Uncommonly occurring renal tubular cell adenomas or adenocarcinomas were found in male rats (adenomas,1/49 in controls, 3/49 at the low dose and 2/50 at the high dose; adenocarcinomas, 0/49, 0/49 and 2/50, respectively).

Although induction of peroxisome proliferation as a mechanism underlying the hepatocarcinogenic effect of PCE in mice appears attractive, a poor quantitative correlation was seen between peroxisome proliferation and tumour formation in the liver following administration by inhalation.

The induction of kidney tumours observed in male rats provides only weak evidence for (human) carcinogenicity. The observed increase in incidence is not statistically significant. In addition, two different mechanisms of action have been proposed for the induction of these tumours: alpha 2μ-globulin nephropathy, an effect specific for male rats, and formation of genotoxic metabolites in the kidneys as the final step of the glutathione biotransformation pathway. Given the low incidences observed, combined with the data on the mechanism of induction, it can be concluded that the result in male rats is equivocal evidence only for a risk of renal cancer in humans.

A review of six carcinogenicity studies of PCE in rats concluded that evidence of increases in mononuclear cell leukaemia has been seen in two studies in the F344 rat but not in other strains, and that as a rat-strain-specific effect it is not considered to be relevant to the evaluation of human cancer risk *(95)*.

IARC *(96)* has concluded that the results of the available animal bioassays provide sufficient evidence for carcinogenicity in animals.

Mutagenicity

Comprehensive reviews are available of the data obtained from the many studies carried out with PCE *(2,51,96)*. In vitro studies include tests for gene mutations in prokaryotes and eukaryotes and tests for chromosome aberrations in mammalian cell lines. In vivo studies include tests for chromosome aberrations in bone marrow in rats and mice and a dominant-lethal assay in rats. In addition, several studies have been carried out on DNA damage in vitro and in vivo. The body of results shows absence of mutagenicity of PCE in virtually all of the systems tested. The few weakly positive results may be due to the presence of mutagenic stabilizers in the test samples *(2,51)*. Binding of PCE in vivo to unpurified

DNA from several organs was noted in one study, although no covalent binding to purified hepatic DNA could be demonstrated in another. In the other available in vivo studies, it is not known whether the test compound (or its metabolites) reached the target tissues. This observation reduces the value of the negative results observed. Nevertheless, an in vivo study, in which male mice were administered PCE intraperitoneally, assessed the induction of micronuclei in hepatocytes and reticulocytes and showed that PCE might have a clastogenic effect in hepatocytes *(97)*. For the metabolites of PCE that are formed by conjugation with glutathione (a minor biotransformation route demonstrated in rodents), positive results were obtained in in vitro studies in *S. typhimurium* TA 100 (reverse mutation), with metabolic activation with kidney microsomes, in the presence of glutathione and glutathione transferase *(96)*.

Interactions with other chemicals

Results of studies on the influence of ethanol on the metabolism and toxicity of PCE in liver did not show synergism *(2,98)*. Increased urinary excretion of metabolites of PCE and enhanced hepatotoxicity have been observed in rats following pretreatment with polychlorinated biphenyls *(2)*. Simulation of interaction thresholds for human exposure to mixtures of TCE, PCE and 1,1,1-trichloroethane was done with PBPK models. Model simulations indicated that during combined exposures, certain toxicological effects may be expected to occur at lower exposure levels compared with PCE exposure alone *(73,99)*.

Effects on humans

Exposure to PCE can affect the CNS, eyes, kidney, liver, lungs, mucous membranes and skin. CNS effects have been most frequently noted *(44)*.

The odour threshold in air for detection of PCE is 8 mg/m^3. The odour threshold in air for recognition of PCE is 3–4 times higher *(16)*.

Acute poisoning incidents

Acute exposure to PCE at air levels of 100–200 ppm can cause irritation of the skin, eyes and upper respiratory tract. Non-cardiogenic pulmonary oedema, nausea, vomiting and diarrhoea can occur. CNS effects have also been observed with acute inhalation exposure to PCE at 50–300 ppm. At these levels, neuromotor effects may be seen and results of certain coordination and behavioural tests may be abnormal. At higher air concentrations, loss of consciousness can occur *(44)*.

Several acute PCE intoxications have been reported. In one case, up to 16 mg was ingested by a 6-year-old boy weighing 22 kg. He was admitted to hospital one hour after ingestion, where his condition deteriorated from somnolence to coma. The initial blood level was 21.5 μg/ml. He recovered completely without liver, renal or CNS injury and was discharged on day 9 *(100)*. In another report,

a 32-year-old man became semi-comatose and experienced oliguric acute renal failure after accidental ingestion of 75 g PCE. A renal biopsy performed on the 19th day after ingestion showed severe acute tubular necrosis. He regained normal renal function after five haemodialyses and conservative treatment. The amount of PCE in the blood and urine were not measured *(101)*.

Three fatalities due to inhalation of PCE have been reported in the literature. A concentration of 4.5 mg/l was measured in the blood of a man aged 53 years weighing 70 kg, who died while he was cleaning and recycling PCE by distillation *(102)*. A two-year-old boy died due to PCE that had been retained in the curtains of his bedroom. A concentration of 66 mg/l blood was measured *(103,104)*. Finally, a 33-year-old man clearing a line in a dry cleaning establishment died with a blood concentration of 44 mg/l *(105)*. A fatality due to poisoning by both PCE and TCE has been described, a 45-year-old woman being discovered unconscious and in cardiac arrest in a laundry area. Examination revealed that she was deeply comatose and had acute respiratory distress syndrome and severe metabolic acidosis. Cardiovascular instability and acute renal failure occurred and eventually the patient died; the results of the autopsy are described in the paper. The blood levels of PCE 2, 4, 5, 6 and 7 days after hospital admission were respectively 1319, 758, 787, 436 and 656 μg/l *(106)*.

Effects on the CNS

Depending on the concentration, acute exposure can result in loss of coordination, reversible mood and behavioural changes, or potentially anaesthetic effects. People chronically exposed to PCE may experience ataxia, disorientation, irritability, peripheral neuropathy, short-term memory deficits and sleep disturbance. Reversibility depends on the degree of severity of the exposure and associated effects *(44)*.

PCE has been associated with neurobehavioural dysfunction, including reduced attention in humans. In a series of controlled short-term studies in limited numbers of human volunteers, neurological symptoms (including dizziness, drowsiness and decreased functioning in motor coordination tests) and visual system dysfunction were observed at ≥ 678 mg/m^3 (≥ 100 ppm) and 339 mg/m^3 (50 ppm), respectively *(2,16,107,108)*. Limited information on neurological effects following long-term exposure was obtained in occupational studies in dry cleaning workers. In a cross-sectional study with two groups of exposed workers (exposure concentrations 83 ± 53 mg/m^3 and 364 ± 114 mg/m^3, duration of exposure 127 or 141 days), small effects on scores in psychological tests were found. However, the response did not correlate with the exposure level in this study *(2,6,109)*.

In another study in female dry cleaning workers with exposures of 6.8–408 mg/m^3 (4-hour averages, median 102 mg/m^3) and PCE concentrations in the blood of 12–864 mg/l (median 145 mg/l), performance test scores in a test bat-

tery for neuromotor functions were decreased. Neither duration of exposure nor blood concentration of PCE was significantly correlated with performance *(110)*. In cross-sectional studies in dry cleaning workers, effects on blue–yellow colour vision were found at mean PCE concentrations of 42–102 mg/m^3 *(111,112)*. In Italy, PCE-induced impairment of colour vision was studied in 33 dry cleaning workers. Exposure was evaluated with passive samplers and colour vision was assessed. Two years later, the workers were re-examined. The exposure to PCE had increased in subgroup A (median 1.7 ppm vs 4.3 ppm). In subgroup B, exposure was reduced (median 2.9 ppm vs 0.7 ppm). Colour vision worsened in subgroup A but no vision changes were noticed in subgroup B, indicating that an increase in exposure during a 2-year period can cause colour vision to deteriorate *(113)*.

A case report described a 57-year-old woman exposed to supposedly high concentrations of PCE while working at a dry cleaning shop, who suffered from blindness for 9 days in the left eye and 11 days in the right eye with optic neuritis. Blood concentrations of PCE 48 and 80 hours after onset of the optic neuritis were respectively 1.08 mg/g and 0.65 mg/g *(114)*.

In a prospective population-based cohort study on 88 829 children, offspring from dry cleaners (144 children) were followed from birth to age 21–33 years. Preliminary findings suggested an increased incidence of schizophrenia (4 cases) in these children *(115)*. However, it should be mentioned that there was no exposure characterization, there were few cases and few details were provided. These findings need to be corroborated in follow-up studies.

Research from 2002 suggests that chronic, environmental exposure to airborne PCE adversely affects neurobehavioural function in healthy individuals. A significantly lower visual contrast sensitivity was reported in apartment residents exposed to PCE (daytime mean 620 μg/m^3, nighttime mean < 100 μg/m^3) compared to unexposed controls. The reliability of these data was later discussed in the literature. Methodological limitations preclude a definitive attribution of causation, and further research is necessary to draw reliable conclusions *(99,116)*.

Effects on the liver

Case reports of human exposure to PCE show that it can cause hepatotoxic effects in humans, which include abnormal liver function tests *(48)*, cirrhosis, hepatitis, hepatomegaly and liver cell necrosis *(44)*. A dose–response relationship in humans for the effects on the liver is not completely known. In case studies of high accidental exposures, effects on the liver have been reported. In limited studies in dry cleaning workers exposed to a TWA concentration of 143 mg/m^3 (21 ppm) over a 6-year period, serum enzymes indicative of liver function were not affected *(62,112)*. A study in dry cleaners frequently exposed to PCE concentrations of 16 ppm (8-hour TWA) revealed mild to moderate hepatic parenchymal changes as determined by hepatic ultrasonography *(117)*.

Renal effects

Nephrotoxic effects have been described in humans *(48,118,119)*. Symptoms of renal dysfunction, including proteinuria and haematuria, have been associated with accidental exposure to anaesthetic concentrations of PCE vapour *(2)*. In several cross-sectional studies in dry cleaning workers, the effect of PCE on renal function was examined. Female workers exposed for an average of 14 years to an estimated TWA concentration of 68 mg/m^3 (10 ppm) had increased urinary levels of lysozyme and ß-glucuronidase suggestive of mild renal effects *(120)*. No effects were observed in workers estimated to have been exposed to a TWA concentration of 142 mg/m^3 (21 ppm) for 6 years *(62)*. In a cross-sectional study, about 20 markers of early nephrotoxic effects were measured in workers in dry cleaning facilities (n = 50). Exposure was determined by analysing air samples collected during 4-hour periods randomly selected over the working week. The median exposure concentration was 102 mg/m^3 (range, trace–580 mg/m^3). Compared to the control population, the exposed group had significantly higher frequencies of abnormal values for a number of the markers in urine, including albumin, transferrin, tissue-nonspecific alkaline phosphatase and brush-border antigens. The significance of the findings cannot be easily assessed but may represent an early stage of clinically silent but potentially progressive renal disease *(118)*. At an average airborne exposure of 8.4 mg/m^3 (range 2.2–44.6), a PCE-related effect on the tubular reabsorption of retinol-binding protein in urine was observed in exposed workers from a dry cleaning shop *(119)*.

Since there are sex- and species-dependent differences in bioactivation and detoxification pathways, more knowledge about the metabolism of PCE in several target organs and in different species is needed to improve human risk assessment *(50)*.

Reproductive and developmental effects

A few epidemiological studies have reported reproductive or developmental abnormalities due to exposure to PCE in drinking-water. The 1998 Camp LeJeune study reported a weak association between PCE exposure due to contaminated drinking-water and birth weight outcomes for small-for-gestational age births. Stronger associations were observed between PCE exposure and birth weight for infants of mothers who were 35 years of age or older and for infants of mothers with a history of fetal death *(121)*. Another study found oral cleft defects associated with PCE-contaminated drinking-water. The authors indicate that this study cannot resolve whether the drinking-water contaminants caused the adverse birth outcomes; these findings should therefore be followed up utilizing available drinking-water contamination databases *(122)*.

In a comment on this paper, it is suggested that the oral cleft defects found are biologically plausible *(123)*. According to these authors, a study of mothers with pregnancies affected by neural tube defects in Dublin *(124)* suggests a mecha-

nism for the induction of neural tube defects related to a metabolic slowdown of the methionine synthase reaction. Recently, two cohort studies examined the effects of prenatal exposure to PCE-contaminated drinking-water on adverse birth outcome and risk of behavioural disorders. It was concluded that prenatal and early postnatal PCE exposure is not associated with disorders of attention, learning and behaviour *(125)*. Further, it was suggested that prenatal PCE exposure does not have an adverse effect on birth weight or gestational duration *(126)*.

The results of several studies have indicated that occupationally exposed women might suffer higher rates of spontaneous abortion *(127–129)*. Exposure was classified by reported work history. A significant effect was found when the work tasks included dry cleaning for at least one hour daily or the women reported handling of PCE at least once a week *(128,129)*. A significantly higher risk was found in operators compared to non-operators *(127)*, but other studies have not found this association *(130,131)*. Furthermore, a case-referent study performed within two cohorts did not give any substantial support to the hypothesis that exposure to PCE at work during pregnancy enhances the risk of adverse pregnancy outcome *(132)*. A retrospective time-to-pregnancy study among Finnish women indicated a reduced ability to reproduce among 20 women exposed to PCE (concentration not clear) for 1–4 days a week or daily by inhalation. It should be noted that the studies have a number of limitations, primarily that exposure concentrations are not available *(133)*.

The Camp Lejeune study of ATSDR *(134)* was not included in this guideline, since an error was made in the exposure classification; these data will be re-analysed by ATSDR at a later stage.

Mutagenic and carcinogenic effects

IARC concluded in 1995 that there is evidence for consistently positive associations between exposure to PCE and the risks for oesophageal and cervical cancer and non-Hodgkin's lymphoma. These associations appear unlikely to be due to chance, although confounding factors cannot be excluded and the total numbers in the cohort studies combined are relatively small. IARC therefore concluded that there is limited evidence for the carcinogenicity of PCE in humans *(96)*.

PCE is reasonably anticipated to be a human carcinogen on the basis of limited evidence from studies in humans and sufficient evidence of carcinogenicity from studies in experimental animals. PCE has been studied by observing laundry and dry cleaning workers, who may also have been exposed to other solvents, especially TCE but also petroleum solvents. In several cohort and proportionate mortality studies, excesses have been reported of lymphosarcomas, leukaemias and cancers of the skin, colon, lung and urogenital tract. Some excess of lymphomas and of cancers of the larynx and urinary bladder was seen in a large cohort of dry cleaners. A familial cluster of chronic lymphocytic leukaemia has also been related to dry cleaning. Although these studies suggest a possible associa-

tion between long-term occupational exposure to PCE and increased lymphatic malignancies and urogenital cancers, the evidence must be regarded as inconclusive because workers were exposed to petroleum solvents and other dry cleaning agents as well as PCE *(135)*.

The results of a study in differently exposed dry cleaners and launderers indicated a reduction in oxidative DNA damage in exposed dry cleaners compared to launderers. The mean value for PCE in the blood of these dry cleaners was 0.078 mg/l, and the TWA was < 5 ppm in air for 17 out of 18 dry cleaners. However, PCE could not clearly be identified as the source of the effect *(136)*. In two studies for genetic effects (sister chromatid exchanges and/or chromosome aberrations) in lymphocytes of occupationally exposed workers, no clear-cut effects were found *(6)*. Another study showed modulation of the expression of some genes related to cancer induction in human cord blood cells *(137)*.

A population-based case-control study was undertaken to investigate the association between breast cancer and PCE exposure from public drinking-water (drinking-water consumption as well as bathing habits were taken into account). The same author performed a more extensive case-control study a few years later. Both studies suggest that women with the highest PCE exposure levels have a slightly to moderately increased risk of breast cancer. Analyses of the same data using a newly constructed personally delivered dose model to make a more accurate estimation of the exposure did not significantly change the subjects' exposure classification *(138–140)*. An association between PCE exposure from public drinking-water and lung cancer and possibly colorectal cancer was suggested, based on another population-based case-control study *(141)*.

A paper from 2003 assessed (based on specified methodological and scientific quality criteria) 44 epidemiological papers that provided reasonable data on up to 17 cancer sites. The authors concluded that no evidence for an association between breast, prostate, skin or brain cancer and exposure to PCE was demonstrated. A relationship between PCE and cancer of the oral cavity, liver, pancreas, cervix and lung was considered unlikely by the authors. Scientific evidence was considered inadequate for laryngeal, kidney, oesophageal and bladder cancers *(142)*.

In 2006, the epidemiological literature since 1990 on occupational chlorinated solvent exposure was reviewed. The paper reviewed 28 studies of PCE, all but 5 of which focused on workers in the dry cleaning industry with few or no other chemical exposures. Within the study populations with multiple exposures, three studies found some increased risk of biliary and liver or brain cancer; in the other studies, no increased risk for renal cell carcinoma or for any cancer was found. Census records for those working as a "dry cleaner or laundry worker" or from a database of those being monitored for PCE exposure were linked to cancer registries (three studies) and statistically significant increased risks for liver and pancreatic cancer, Hodgkin's lymphoma and leukaemia were found. In general,

findings in occupational cohort studies have not been consistent, even within the same industries *(143)*.

Health risk evaluation

Critical health outcomes

The main health effects of concern are local irritation (eyes, mucous membranes, respiratory tract and skin), effects on the CNS and cancer. Effects on the liver and kidneys have also been reported.

CNS effects and local irritation

The evidence is sufficient to conclude that PCE can cause irritation of mucous membranes and the respiratory tract. These effects generally occur at high concentrations. Acute exposure to PCE at air levels of 680–1360 mg/m^3 (100–200 ppm) can cause irritation of the skin, eyes and upper respiratory tract in humans. Moreover non-cardiogenic pulmonary oedema, nausea, vomiting and diarrhoea can occur. CNS effects have been observed at high dose levels in rats and mice. In humans, CNS effects have also been observed with acute inhalation exposures of 340–2040 mg/m^3 (50–300 ppm) of PCE. The evidence is sufficient to conclude that there is an association between PCE and CNS effects at sufficiently high concentrations. Since high concentrations typically do not occur in indoor environments for a long period of time, these end-points are not taken into account when setting the guideline value.

Liver and kidney effects

The evidence of an association between PCE exposure and effects on the liver is suggestive. Hepatic effects have been observed in rats and mice in subchronic studies. Case reports of accidental exposures of humans to PCE confirm that hepatotoxic effects can occur in humans, including abnormal liver function tests, cirrhosis, hepatitis, hepatomegaly and liver cell necrosis.

There is suggestive evidence of an association between PCE exposure and effects on the kidneys. In chronic studies, renal effects were observed at 1360 and 2710 mg/m^3 (200 and 400 ppm) in rats and at 680 and 1360 mg/m^3 (100 and 200 ppm) in mice. Reversible kidney damage in humans (e.g. proteinuria and haematuria) has been associated with accidental exposure to acutely toxic concentrations of PCE vapour. A long-term exposure study showed that renal effects may develop at lower concentrations, with the reported onset of renal damage occurring following exposure to a median concentration of 102 mg/m^3 (range, trace–576 mg/m^3).

Reproductive and developmental effects

A few epidemiological studies have reported developmental abnormalities due to PCE exposure in drinking-water (reduced birth weight and oral cleft defects).

Other studies concluded that prenatal PCE exposure does not have an adverse effect on birth weight or gestational duration. Some studies have consistently indicated that occupationally exposed women might suffer higher rates of spontaneous abortion, while others found no association. All the above-mentioned studies have limitations, primarily that the exposure concentrations are not available. Therefore the evidence stays suggestive.

Cancer risk

IARC concluded that the results of the available animal bioassays provide sufficient evidence for carcinogenicity to animals. In these studies, an increased incidence of adenomas and carcinomas was observed in the livers of exposed mice. There is suggestive evidence from mechanistic studies that humans are likely to be less sensitive to the development of these tumours following PCE exposure. A low incidence of kidney tumours among male rats has been reported.

It can be concluded from this small and statistically non-significant increase, together with the data relating to the possible mechanism of induction, that the result in male rats is equivocal evidence only for a risk of renal cancer in humans.

The significance for humans of the increased incidence of mononuclear cell leukaemia, as observed in two studies in F344 rats, is unclear. This is due to the lack of understanding of the mechanism underlying the formation of this cancer type (which has a high background incidence) and the suggestion that it might be a rat-strain-specific effect.

IARC concluded in 1995 that there is evidence for consistently positive associations between exposure to PCE and the risks for oesophageal and cervical cancer and non-Hodgkin's lymphoma.

These associations appear unlikely to be due to chance, although confounding factors cannot be excluded and the total numbers in the cohort studies combined are relatively small.

A paper from 2003 assessed (based on specified methodological and scientific quality criteria) 44 epidemiological papers that provided reasonable data on up to 17 cancer sites. The authors concluded that no evidence for an association between breast, prostate, skin or brain cancer and exposure to PCE was demonstrated.

A relationship between PCE and cancer of the following sites was considered unlikely by the authors: oral cavity, liver, pancreas, cervix and lung. Scientific evidence was considered inadequate for laryngeal, kidney, oesophageal and bladder cancers. Since then, new studies have been published, but the evidence concerning the carcinogenicity of PCE is still not conclusive. The evidence for an association therefore remains suggestive.

From the weight of the evidence from mutagenicity studies, there are no indications that PCE is genotoxic. Although several in vitro studies indicate that

conjugation of PCE with reduced glutathione (a minor biotransformation route demonstrated to occur in rodents) produces renal metabolites that are mutagenic in *S. typhimurium* TA 100, the significance of the latter results for humans is doubtful. In two studies investigating genetic effects (sister chromatid exchanges and/or chromosome aberrations) in lymphocytes of occupationally exposed workers, no clear-cut effects were found.

In conclusion, carcinogenicity is not used as an end-point, since there are no indications that PCE is genotoxic and there is some uncertainty about the epidemiological evidence as well as the relevance of the animal carcinogenicity data to humans. However, because of the remaining uncertainty about the carcinogenicity of PCE, it should be kept under review.

Health relevance of current indoor exposures

Inhalation of PCE is the major route of exposure in the general population. Ambient air concentrations of PCE are generally < 5 μg/m^3 in urban areas and typically < 1 μg/m^3 in rural areas. Indoor concentrations are generally well below 20 μg/m^3, with median concentrations ranging from 0.16 to 8.7 μg/m^3 in the described studies. These concentrations are an order of magnitude below concentrations that can be relevant for health.

Indoor PCE air levels may be up to 5.0 mg/m^3 (4–24-hour average) in buildings with dry cleaning operations where PCE is used. There is sufficient evidence to conclude that concentrations in premises next to dry cleaning operations can exceed safe concentrations. This should be the focus of concern.

Conclusions of other reviews

IARC concluded that there is evidence for consistently positive associations between exposure to PCE and the risks for oesophageal and cervical cancer and non-Hodgkin's lymphoma. PCE is classified by IARC as a Group 2A carcinogen (probably carcinogenic to humans) *(96)*.

The EU classified the substance as carcinogenic category 3 (substances that cause concern for humans owing to possible carcinogenic effects but in respect of which the available information is not adequate for making a satisfactory assessment) and risk phrase 40 (limited evidence of a carcinogenic effect) *(144)*.

USEPA does not currently have a classification for the carcinogenicity of PCE but has calculated a provisional inhalation unit risk estimate of 5.8×10^{-7} (μg/m^3)$^{-1}$. A provisional value is one that has not received Agency-wide review *(145)*.

The United Kingdom Committees on Toxicity, Mutagenicity and Carcinogenicity of Chemicals in Food, Consumer Products and the Environment concluded that there was "no satisfactory epidemiological evidence to associate PCE exposure to cancer in the available cohort studies" *(146)*.

The US National Institute for Occupational Safety and Health considers PCE to be a potential occupational carcinogen *(147)*.

ATSDR has derived both an acute inhalation minimal risk level (MRL) of 1.38 mg/m^3 (0.1 ppm) and a chronic inhalation MRL of 0.28 mg/m^3 [1] (0.04 ppm) *(2)*.

Guidelines

Carcinogenicity is not selected as the end-point for setting the guideline value for three reasons: the epidemiological evidence is equivocal, the animal tumours detected are not considered relevant to humans, and there are no indications that PCE is genotoxic. The derivation of a guideline value is at present based on two non-neoplastic effects as the critical end-point: impaired neurobehavioural performance and early renal changes.

On the basis of a long-term LOAEL for kidney effects of 102 mg/m^3 in dry cleaning workers, a guideline value of 0.25 mg/m^3 has been calculated. In deriving this guideline value, the LOAEL is converted to continuous exposure (dividing by a factor of 4.2 (168/40)) and divided by an uncertainty factor of 100 (10 for use of a LOAEL and 10 for intra-species variation). Recognizing that some uncertainty in the LOAEL exists because the effects observed at this level are not clear-cut and because of fluctuations in exposure levels, an alternative calculation was made based on the LOAEL in mice of 680 mg/m^3 and using an appropriate uncertainty factor of 1000. This calculation yields a guideline value of 0.68 mg/m^3.

A chronic inhalation MRL of 0.28 mg/m^3 (0.04 ppm) has been derived by ATSDR based on the LOAEL of 15 ppm identified in the Ferroni study *(110)*. The MRL was calculated from this concentration by expanding to continuous exposure (8/24 hours, 5/7 days) and dividing by an uncertainty factor of 100 (10 for use of a LOAEL and 10 for human variability). This reference found significantly prolonged reaction times in workers occupationally exposed to an average of 15 ppm for about 10 years *(2,110)*.

The value and appropriateness of establishing a short-term guideline value is questionable because acute effects occur only at very high concentrations of 50 ppm (340 mg/m^3) and higher, compared to generally observed levels in close proximity to dry cleaning facilities. Establishing a long-term value is more protective of human health.

On the basis of the overall health risk evaluation, the recommended guideline for year-long exposure is 0.25 mg/m^3. This is the same as the previous WHO guideline *(148)*.

The guidelines section was formulated and agreed by the working group meeting in November 2009.

[1] This figure was calculated and found to be 0.24 mg/m^3 using the information provided by ATSDR. This may be due to a different conversion factor for converting ppm to mg/m^{3}and rounding during the calculation.

Summary of main evidence and decision-making in guideline formulation

Critical outcome for guideline definition

Effects in the kidney indicative of early renal disease and impaired neurobehavioural performance. Carcinogenicity is not used as an end-point as there are no indications that PCE is genotoxic and there is uncertainty about the epidemiological evidence and the relevance to humans of the animal carcinogenicity data.

Source of exposure–effect evidence

- Based on a health surveillance study of dry cleaning workers exposed to PCE, the long-term LOAEL for kidney effects was considered to be 102 mg/m^3. This was adjusted for continuous exposure by dividing by a factor of 168/40. Further, factors of 10 for use of a LOAEL and 10 for intraspecies variation were incorporated, leading to a guideline value of 0.25 mg/m^3 *(62)*.
- Based on a study of 30 female dry cleaning workers exposed to PCE for an average of 10 years *(110)*, a LOAEL of 103 mg/m^3 (15 ppm) has been derived and a chronic-duration inhalation MRL of 0.28 mg/m^3 (0.04 ppm) calculated.

Supporting evidence

- Several epidemiological studies on renal changes and exposure to PCE, including studies in dry cleaning facilities. The significance of these findings cannot be easily assessed, but may represent a relationship between long-term exposure and effects on the kidney in humans *(48, 118,119, 120)*.
- Associations between PCE exposure and neurobehavioural symptoms were suggested in two studies *(108,109)*.

Results of other reviews

- ATSDR derived a chronic inhalation MRL of 0.28 mg/m^3 (0.04 ppm) *(44)*.
- IARC: Group 2A (probably carcinogenic to humans) *(96)*.

Guideline

0.25 mg/m^3 – annual average.

Comments

No change in the guideline as compared to *Air quality guidelines for Europe (148)*.

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This book presents WHO guidelines for the protection of public health from risks due to a number of chemicals commonly present in indoor air. The substances considered in this review, i.e. benzene, carbon monoxide, formaldehyde, naphthalene, nitrogen dioxide, polycyclic aromatic hydrocarbons (especially benzo[*a*]pyrene), radon, trichloroethylene and tetrachloroethylene, have indoor sources, are known in respect of their hazardousness to health and are often found indoors in concentrations of health concern. The guidelines are targeted at public health professionals involved in preventing health risks of environmental exposures, as well as specialists and authorities involved in the design and use of buildings, indoor materials and products. They provide a scientific basis for legally enforceable standards.

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