

## 3. CANCER IN EXPERIMENTAL ANIMALS

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### 3.1 Studies of components of outdoor air pollution in previous *IARC Monographs*

#### 3.1.1 Introduction

Outdoor air pollution is a complex mixture of multiple pollutants originating from a myriad of natural and anthropogenic sources. This includes transportation-related pollution – the overall mixture of various exhaust emissions, which themselves are source-specific complex mixtures of particulate matter (PM), gases, and volatile and semivolatile substances, and very often contain organic and inorganic substances classified as IARC Group 1 or Group 2 carcinogens (Section 1, Table 1.2) – as well as contributions from other sources. The evaluation of the carcinogenic potential of outdoor air pollution should be based on investigations using polluted outdoor air or on studies of emissions that contribute substantially to air pollution.

In experimental animals, only inhalation experiments are able to investigate complex mixtures of airborne gases, volatile substances, and aerosols, and the primary target organ of air pollutants is the respiratory tract. Therefore, studies in experimental animals using inhalation, intratracheal instillation, or lung implantation were the most informative studies to evaluate the carcinogenicity of air pollutants or of fractions thereof collected using special devices. Skin application or subcutaneous injection of PM, condensates, and extracts of PM, all contained

in outdoor air and in exhaust emissions, were also used to assess the carcinogenic potential of exhaust emissions and therefore also of outdoor air pollution.

One class of carcinogens that has been widely identified in outdoor air pollution – because it is generated by high-temperature incomplete combustion of organic material like oil, oil-derived products, coal, and wood – comprises non-heterocyclic polycyclic aromatic hydrocarbons (PAHs), nitroarenes, and some related compounds. In outdoor air, these compounds are attached primarily to outdoor PM. Some examples of PAH-containing emissions are those from household combustion of coal or wood and exhaust emissions from diesel and gasoline engines (see Section 1).

The carcinogenic effects in experimental animals of PAHs, of related compounds, and of mixtures containing two or more representatives of these agents have been evaluated previously in the *IARC Monographs*, in Volume 3 ([IARC, 1973](#)), Volume 32 ([IARC, 1983](#)), Volume 92 ([IARC, 2010a](#)), and Volume 100F ([IARC, 2012b](#)), as well as in Volume 95 ([IARC, 2010b](#)) and Volume 100E ([IARC, 2012a](#)) (both of which deal with emissions from combustion of coal or wood) and Volume 105 ([IARC, 2013](#)) (which deals with diesel and gasoline engine exhausts and some nitroarenes).

#### 3.1.2 Emissions from household combustion of coal or wood

See [Table 3.1](#), [Table 3.2](#), [Table 3.3](#), [Table 3.4](#), [Table 3.6](#), and [Table 3.7](#).

In two studies in mice and one study in rats, inhalation exposure to emissions from incomplete combustion of coal caused high incidences of malignant lung tumours ([IARC, 2012a](#)). In one study in mice, exposure to high emissions from incomplete combustion of wood caused an increased incidence of lung tumours (mainly adenocarcinomas) after 15 months of exposure. A study in rats with a similar design was negative after 19 months of exposure ([IARC, 2010b](#)).

The previous *IARC Monographs Working Groups* concluded that there was *sufficient evidence* in experimental animals for the carcinogenicity of emissions from combustion of coal ([IARC, 2012a](#)) but only *limited evidence* in experimental animals for the carcinogenicity of emissions from combustion of wood ([IARC, 2010b](#)).

In addition, four skin application or subcutaneous injection studies using coal-derived soot extracts in mice ([IARC, 2012a](#)) and two subcutaneous injection studies using wood smoke extracts in mice ([IARC, 2010b](#)) showed increased incidences of lung cancers or skin tumours.

The *IARC Monographs Working Groups* concluded that there was *sufficient evidence* in experimental animals for the carcinogenicity of coal-derived soot extracts ([IARC, 2012a](#)) and *sufficient evidence* in experimental animals for the carcinogenicity of wood smoke extracts ([IARC, 2010b](#)).

Since the above-mentioned previous *IARC Monographs* evaluations, only one new skin application study using wood smoke extract in mice [inadequate for the evaluation] ([Lewtas, 1993](#); [Cupitt et al., 1994](#)) and no new studies on emissions from combustion of coal were available to the Working Group.

### 3.1.3 Exhaust emissions from diesel engines and gasoline engines

See [Table 3.1](#), [Table 3.2](#), [Table 3.3](#), [Table 3.4](#), [Table 3.5](#), and [Table 3.6](#).

It has been shown in 11 studies in rats that whole diesel engine exhaust from engines produced before 2000 caused an increased incidence of benign and/or malignant lung tumours after long-term inhalation exposure to sufficiently high concentrations of particles contained in whole diesel engine exhaust. All studies in mice were negative except one, which showed inconsistent results. No increase in the incidence of lung tumours was observed in three studies in hamsters exposed to whole diesel engine exhaust. The gas phase of diesel engine exhaust (i.e. without diesel engine exhaust particles) did not cause an increase in lung tumours in studies in mice, rats, or hamsters. Diesel engine exhaust particles caused malignant lung tumours in rats after intratracheal instillation, and extracts of these particles caused malignant lung tumours in rats after intrapulmonary implantation and caused malignant fibrous histiocytomas in mice after subcutaneous injection ([IARC, 2013](#)).

No lung tumours were observed in rats, hamsters, or dogs after inhalation exposure to whole gasoline engine exhaust. However, gasoline engine exhaust condensates induced malignant tumours of the skin in three skin application studies in mice, malignant lung tumours in one intrapulmonary implantation study in rats, and pulmonary adenomas in one intratracheal instillation study in hamsters ([IARC, 2013](#)).

The Working Group of Volume 105 of the *IARC Monographs* concluded that there was *sufficient evidence* in experimental animals for the carcinogenicity of whole diesel engine exhaust, of diesel engine exhaust PM, and of extracts of diesel engine exhaust particles. With respect to gasoline engine exhaust, the Working Group concluded that there was *sufficient evidence* in experimental animals for the carcinogenicity of only the condensates of the exhaust ([IARC, 2013](#)).

**Table 3.1 Studies of carcinogenicity in experimental animals of components of outdoor air pollution by inhalation or whole-body exposure**

Species, strain (sex) Duration Reference	Test materials/ Type of samples	Dosing regimen Animals/group at start	Incidence of tumours	Statistical significance	Comments
<i>Coal smoke and soot from household combustion of coal</i>					
Mouse, Buffalo (NR) Up to 19 mo <a href="#">Seeling &amp; Benignus (1936)</a> , <a href="#">IARC (2010b, 2012a)</a>	Coal soot as bedding in the cages	Shaking the cage, 2–3 ×/d 50 (controls) 100 (exposed)	Lung adenocarcinoma: 1/50 (control), 8/100	NS	Age at start: 3 mo Unusually high mortality in controls, and lack of reporting on skin tumours
Mouse, NR (M, F) 2 yr <a href="#">Campbell (1939)</a> , <a href="#">IARC (2010b, 2012a)</a>	“Moderate” cloud soot in inhalation chamber	1 h/d, 5 d/wk, 12 mo “moderate” dose Number of animals NR	No increase in the incidence of lung tumours, and no skin tumours	NS	Age at start: 3 mo
Mouse, Kunming (M, F) 2 yr <a href="#">Lin et al. (1995)</a> , <a href="#">IARC (2010b, 2012a)</a>	Amounts of coal chosen to simulate normal indoor air conditions for humans in Harbin City, China. Exposure assumed to be daily exposure	Control, clean air Smoke, 60 g of coal, daily Smoke, 105 g of coal, daily Smoke, 160 g of coal, daily 30 M + 30 F	Lung cancer: 3.6% (control), 9.4%, 12.8%*, 24.3%*	* $P < 0.05$	Purity NR; age at start NR; weight, 13 ± 1 g
Mouse, Kunming (M, F) 15 mo <a href="#">Liang et al. (1988)</a> , <a href="#">IARC (2010b, 2012a)</a>	Bituminous coal was incompletely combusted to simulate indoor air in Xuanwei County, China Total suspended particles: 0.91 mg/m <sup>3</sup> (control, clean air), 14.38 mg/m <sup>3</sup> (coal smoke) B[a]P: 0.15 µg/m <sup>3</sup> (control, clean air), 50.5 µg/m <sup>3</sup> (coal smoke)	Control, clean air Coal smoke 113 M + 58 F (control) 160 M + 50 F (coal smoke)	Lung cancer: 29/171 (all adenocarcinoma); 188/210* (119/210, adenocarcinoma; 45/210, adenosquamous carcinoma; 24/210, squamous cell carcinoma)	* $P < 0.001$	Age at start NR; weight, 21 g

**Table 3.1 (continued)**

Species, strain (sex) Duration Reference	Test materials/ Type of samples	Dosing regimen Animals/group at start	Incidence of tumours	Statistical significance	Comments
Rat, Wistar (M, F) 19 mo <a href="#">Liang et al. (1988)</a> , <a href="#">IARC (2010b, 2012a)</a>	Room with indoor air pollution and a round, shallow pit in the centre where bituminous coal was incompletely combusted to simulate indoor air in Xuanwei County, China Total suspended particles: 0.91 mg/m <sup>3</sup> (control, clean air), 14.38 mg/m <sup>3</sup> (coal smoke) B[a]P: 0.15 µg/m <sup>3</sup> (control, clean air), 50.5 µg/m <sup>3</sup> (coal smoke)	Control, clean air Coal smoke 59 M + 51 F (control) 62 M + 63 F (coal smoke)	Lung cancer: 1/110 (adenocarcinoma), 84/125* (all squamous cell carcinoma)	* <i>P</i> < 0.001	Age at start NR; weight, 105 g
<i>Wood smoke</i>					
Mouse, Kunming (M, F) Mouse, Beijing (M) 15 mo <a href="#">Liang et al. (1988)</a> , <a href="#">IARC (2010b)</a>	Incompletely combusted wood smoke from a fire pit in the centre of a room: PM, 14.99 mg/m <sup>3</sup> ; B[a]P: 43.1 µg/10 m <sup>3</sup> (control: PM, 0.91 mg/m <sup>3</sup> ; B[a]P, 1.47 µg/10 m <sup>3</sup> ) Simulation of indoor air in Xuanwei County, China	12 h/d, 15 mo Kunming: 58 M, 59 F Beijing: 60 M Similar number of controls	Lung tumours: Exposed groups: 81/177 (45.8%) (sex and strain combined) Control groups: 29/171 (17.0%) Highest tumour incidence with F Kunming mice in exposed group (49.3%) and control group (26.9%), followed by M Kunming mice (exposed, 37.9%; controls, 13.2%)	NR [ <i>P</i> < 0.05]	Age at start NR; weight, 21 g Tumours were mainly adenocarcinomas
Mouse, A/J (M, F) 12 mo <a href="#">Reed et al. (2006)</a> , <a href="#">IARC (2010b)</a>	Whole hardwood smoke emissions	6 h/d, 7 d/wk, 6 mo; 6 mo follow-up PM: 0, 30, 100, 300, or 1000 µg/m <sup>3</sup> 20 M + 20 F	Lung tumours: 47–59% (both sexes combined)	NS (no differences in lung tumour incidence or multiplicity between exposure groups and control group)	Age at start: 6 wk No exposure-related mortality

**Table 3.1 (continued)**

Species, strain (sex) Duration Reference	Test materials/ Type of samples	Dosing regimen Animals/group at start	Incidence of tumours	Statistical significance	Comments
Rat, Wistar (M, F) 19 mo <a href="#">Liang et al. (1988)</a> , <a href="#">IARC (2010b)</a>	Incompletely combusted wood smoke from a fire pit in the centre of a room: PM, 14.99 mg/m <sup>3</sup> ; B[a]P, 43.1 µg/10 m <sup>3</sup> (control: PM, 0.91 mg/m <sup>3</sup> ; B[a]P, 1.47 µg/10 m <sup>3</sup> ) Simulation of indoor air in Xuanwei County, China	12 h/d, 19 mo 55 M, 55 F Similar number of controls	1 pulmonary tumour in controls; 0 in wood smoke-exposed rats	NS	Age at start NR; weight, 105 g
<i>Diesel engine exhaust</i>					
Mouse, NMRI (F) Lifetime (up to 120 wk) <a href="#">IARC (2013)</a> , <a href="#">Heinrich et al. (1986a, b)</a>	Filtered or unfiltered exhaust from a 1.6 L diesel engine (diluted 1 : 17; particles, 4.24 mg/m <sup>3</sup> ); control: clean air	19 h/d, 5 d/wk, lifetime 96 animals/group	Lung adenocarcinoma: Unfiltered exhaust: 18/93 (19%)* Filtered exhaust: 13/76 (17%)* Control: 2/84 (2%)	* <i>P</i> < 0.05	Age at start: 8–10 wk Incidence of lung tumours in “historical controls” in this laboratory reported to reach 32% in untreated controls and 12.5% in inhalation controls
Mouse, NMRI (F) 23 mo Mouse, C57BL/6N (F) 30 mo <a href="#">IARC (2013)</a> , <a href="#">Heinrich et al. (1995)</a>	Exhaust from a 1.6 L diesel engine operated according to US-72 FTP driving cycle or under constant load conditions (diluted 1 : 9; particles, 7.0 mg/m <sup>3</sup> ); exhaust from a 1.6 L diesel engine (diluted 1 : 15; particles, 4.5 mg/m <sup>3</sup> ); particle-free exhaust; control: clean air	18 h/d, 5 d/wk, 13.5 mo; then kept untreated for 9.5 mo 18 h/d, 5 d/wk; 24 mo; then kept untreated for 6 mo 80 animals/group	No increase in the number of animals with lung tumours in groups of particle-exposed animals	NS	Age at start: 7 wk
Mouse, CD1 (M, F) 24 mo <a href="#">IARC (2013)</a> , <a href="#">Mauderly et al. (1996)</a>	Exhaust from a 1980 model 5.7 L V8 diesel engine; different levels of NO <sub>2</sub> and soot concentrations	6 h/d, 5 d/wk, 24 mo 0.35, 3.5, or 7.0 mg soot/m <sup>3</sup> (0.1 ± 0.1, 0.3 ± 0.2, or 0.7 ± 0.5 ppm NO <sub>2</sub> ) Number of animals NR	No increase in the incidence of lung tumours in exposed animals	NS	Age at start: 17 wk No effects on survival or bw

**Table 3.1 (continued)**

Species, strain (sex) Duration Reference	Test materials/ Type of samples	Dosing regimen Animals/group at start	Incidence of tumours	Statistical significance	Comments
Mouse, C57BL/N (newborn) (M, F) Mouse, ICR (M, F) 24 mo <a href="#">IARC (2013)</a> , <a href="#">Takemoto et al. (1986)</a>	Exhaust from a 269 cm <sup>3</sup> small diesel engine at idling speed (diluted 1 : 2 or 1 : 4 with clean air; PM, 1–4 mg/m <sup>3</sup> ; NO <sub>2</sub> , 2–4 ppm) Control group: clean air	4 h/d, 4 d/wk, 24 mo Number of exposed and control animals (combined): 225 M, 225 F (C57BL/N); 205 M, 205 F (ICR)	Low incidence of lung adenoma and/or adenocarcinoma in exposed and control animals of both strains	NS	
Rat, Wistar (M) 20 mo <a href="#">IARC (2013)</a> , <a href="#">Karagianes et al. (1981)</a>	Soot from exhaust from a 3-cylinder, 43 hp diesel engine driving a 15 kW electric generator (diluted 1 : 35); soot from diesel exhaust plus coal dust	6 h/d, 20 mo Clean air (control) 8.3 ± 2.0 mg/m <sup>3</sup> soot 8.3 ± 2.0 mg/m <sup>3</sup> soot + 5.8 ± 3.5 mg/m <sup>3</sup> coal dust 8.3 ± 2.0 mg/m <sup>3</sup> soot + 6.6 ± 1.9 mg/m <sup>3</sup> coal dust 8.3 ± 2.0 mg/m <sup>3</sup> soot + 14.9 ± 6.2 mg/m <sup>3</sup> coal dust 14.9 ± 6.2 mg/m <sup>3</sup> coal dust only 24 animals/group	One bronchioloalveolar adenoma in diesel-only exposed group and one in diesel + coal dust-exposed group No lung tumours in control group and coal dust-only exposed group	NS	Age at start: 18 wk Increased incidence of non-neoplastic lesions of the respiratory tract, with severity related to duration of exposure
Rat, Wistar (F) Lifetime <a href="#">IARC (2013)</a> , <a href="#">Heinrich et al. (1986a)</a>	Filtered or unfiltered exhaust from a 1.6 L diesel engine (diluted 1 : 17; particles, 4.24 mg/m <sup>3</sup> ) Control group: clean air	19 h/d, 5 d/wk, lifetime 96 animals/group	Bronchioloalveolar adenomas and lung squamous cell tumours (combined): Unfiltered diesel exhaust: 17/95 (18%)* Filtered diesel exhaust: 0/96 Control group: 0/96	*[P < 0.0001]	Age at start: 8–10 wk

**Table 3.1 (continued)**

Species, strain (sex) Duration Reference	Test materials/ Type of samples	Dosing regimen Animals/group at start	Incidence of tumours	Statistical significance	Comments
Rat, F344 (M, F) 30 mo <a href="#">IARC (2013)</a> , <a href="#">Ishinishi et al. (1986)</a>	Exhaust with particles from a light-duty 4-cylinder, 1.8 L diesel engine or a heavy-duty 6-cylinder, 11 L diesel engine (different particle concentrations and NO <sub>x</sub> concentrations tested) Control group: clean air	16 h/d, 6 d/wk, 30 mo 64 M, 61 F	Lung carcinoma: Heavy-duty engine exhaust: 5/64* (M, highest-dose group; particles, 2.32 mg/m <sup>3</sup> ) 3/60* (F, highest-dose group; particles, 2.32 mg/m <sup>3</sup> ) Control group: 0/64 (M) 1/59 (F)	* <i>P</i> < 0.05 (M + F combined)	Age at start: 5 wk No significant increase in incidence of lung tumours in groups exposed to light-duty diesel engine exhaust Lung carcinomas were adenocarcinomas, squamous cell carcinomas, or adenosquamous cell carcinomas
Rat, F344 (F) 30 mo <a href="#">IARC (2013)</a> , <a href="#">Iwai et al. (1986)</a>	Diluted exhaust and diluted filtered exhaust from a 2.4 L diesel truck engine (particles, 4.9 ± 1.6 mg/m <sup>3</sup> ) Control group: clean air	8 h/d, 7 d/wk, 24 mo; 6 mo follow-up 24 animals/group	Lung tumours: Unfiltered diesel: 8/19* (5 malignant tumours) Filtered diesel: 0/16 Control group: 1/22	* <i>P</i> < 0.01	Age at start: 7 wk
Rat, F344 (F) 24 mo <a href="#">IARC (2013)</a> , <a href="#">Takemoto et al. (1986)</a>	Exhaust from a 269 cm <sup>3</sup> small diesel engine (diluted 1 : 2 to 1 : 4 with clean air; PM, 2–4 mg/m <sup>3</sup> ) Control group: clean air	4 h/d, 4 d/wk, 24 mo 15 (exposed animals) 12 (control animals)	No lung tumours	NS	Age at start: 5 wk
Rat, F344 (M, F) 30 mo <a href="#">IARC (2013)</a> , <a href="#">Mauderly et al. (1986, 1987)</a>	3 concentrations of exhaust from a 1980 model 5.7 L V8 diesel engine	7 h/d, 5 d/wk, 30 mo 0.35, 3.5, 7.0 mg/m <sup>3</sup> (measured as soot) Controls: filtered air 221–230 M + F	Lung tumours: 1.3%, 3.6%*, 12.8%*, 0.9% (control) Lung adenocarcinoma or squamous cell carcinoma (combined): 1.3%, 0.5%, 7.5%*, 0.9% (control)	* <i>P</i> < 0.05	Age at start: 17 wk Lung tumours were bronchioloalveolar adenoma, adenocarcinoma, squamous cyst, or squamous cell carcinoma

**Table 3.1 (continued)**

Species, strain (sex) Duration Reference	Test materials/ Type of samples	Dosing regimen Animals/group at start	Incidence of tumours	Statistical significance	Comments
Rat, F344 (M, F) 24 mo <a href="#">IARC (2013)</a> , <a href="#">Lewis et al. (1986, 1989)</a>	Exhaust from a 7.0 L, 4-cycle Caterpillar model 3304 diesel engine (diluted 1 : 27) with specific limits on gaseous/vapour constituents, coal dust, coal dust + diesel exhaust, control (clean air)	7 h/d, 5 d/wk, 24 mo Controls 2 mg/m <sup>3</sup> coal dust 2 mg/m <sup>3</sup> diesel exhaust particles 1 mg/m <sup>3</sup> coal dust + 1 mg/m <sup>3</sup> diesel exhaust particles 72 M, 72 F	No statistically significant differences in incidence of lung tumours	NS	Age at start: 8–10 wk
Rat, F344 (M, F) 30 mo <a href="#">IARC (2013)</a> , <a href="#">Brightwell et al. (1989)</a>	3 concentrations of exhaust from a 1.5 L VW Rabbit diesel engine, filtered or unfiltered; diluted with a constant volume of 800 m <sup>3</sup> of air; further dilutions: 1 : 3 and 1 : 9 Control groups: clean air	16 h/d, 5 d/wk, 2 yr; 6 mo follow-up Particle concentration of unfiltered fraction: 0.9, 2.7, or 8.2 mg/m <sup>3</sup> 72 M, 72 F Control groups: 144 F, 144 M	Lung tumours (all) in controls: M, 2/134; F, 1/126 Lung tumours in high-dose group: M, 16/71*; F, 39/72* Lung tumours in high-dose group animals that survived 24 mo: M, 12/27 (10/27 malignant); F, 24/25 (19/25 malignant)	NR * $[P < 0.001]$ No increase in the incidence of respiratory tract tumours in groups exposed to filtered exhaust	Age at start: 6–8 wk
Rat, F344 (M, F) Duration NR <a href="#">IARC (2013)</a> , <a href="#">Takaki et al. (1989)</a>	Exhaust from a 1.8 L light-duty diesel engine or from a 11 L heavy-duty diesel engine	16 h/d, 5 d/wk, duration NR Particle concentrations from 1.8 L engine: 0, 0.1, 0.4, 1.1, or 2.3 mg/m <sup>3</sup> Particle concentrations from 11 L engine: 0, 0.5, 1.0, 1.8, or 3.7 mg/m <sup>3</sup> 64 M, 59 F	No significant increase in the incidence of lung adenoma or lung carcinoma in exposed groups	NS	Age at start: 5 wk
Rat, F344/N (M, F) 24 mo <a href="#">IARC (2013)</a> , <a href="#">Mauderly et al. (1994)</a>	Exhaust from a light-duty diesel engine	16 h/d, 5 d/wk, 24 mo Particle concentration in engine exhaust: 0 (clean air), 2.44, or 6.33 mg/m <sup>3</sup> 140 F, 140 M	Bronchioloalveolar adenoma: M: 0.8%, 0.9%, 2.6% F: 0%, 2.6%, 17%* Bronchioloalveolar adenocarcinoma: M: 0.8%, 1.8%, 3.5% F: 0%, 4.4%, 16%*	* $[P < 0.0001]$	Age at start: 8 wk

**Table 3.1 (continued)**

Species, strain (sex) Duration Reference	Test materials/ Type of samples	Dosing regimen Animals/group at start	Incidence of tumours	Statistical significance	Comments
Rat, Wistar (F) 30 mo <a href="#">IARC (2013)</a> , <a href="#">Heinrich et al. (1995)</a>	Diluted exhaust soot from a 1.6 L VW diesel engine; dilution: 1 : 80, 1 : 27, or 1 : 9	18 h/d, 5 d/wk, 24 mo; 6 mo follow-up Control group: clean air Concentration of diesel soot particles: 0.8, 2.5, or 7.0 mg/m <sup>3</sup> 100–220 animals	Lung tumours (all): 1/217, 0/198, 11/200*, 22/100* Bronchioloalveolar adenoma: 0/217, 0/198, 2/200, 4/100** Lung adenocarcinoma: 1/217, 1/217, 0/198, 1/200, 5/100***	*[ <i>P</i> < 0.005] ** <i>P</i> < 0.01 *** <i>P</i> < 0.05	Age at start: 7 wk
Rat, F344 (M, F) 30 mo <a href="#">IARC (2013)</a> , <a href="#">Nikula et al. (1995)</a>	Exhaust soot from two 1988 model LH6 General Motors 6.2 L V8 diesel engines, diluted in filtered conditioned air	16 h/d, 5 d/wk, 24 mo; 6 mo follow-up 0 (control), 2.5, or 6.5 mg/m <sup>3</sup> 105–109 animals (M, F)	Bronchioloalveolar adenocarcinoma: M: 1/109, 1/105, 3/106 F: 0/105, 3/105, 19/106*	*[ <i>P</i> < 0.0001]	Age at start: 7–9 wk
Rat, F344 (F) 30 mo <a href="#">IARC (2013)</a> , <a href="#">Iwai et al. (1997)</a>	Diluted filtered or unfiltered exhaust from a 2.4 L diesel truck engine (particles, 9.4 mg/m <sup>3</sup> ) either directly or after particle exclusion through a HEPA filter	8 h/d, 7 d/wk, 24 mo; 6 mo follow-up Clean air (control group) Diluted filtered or unfiltered diesel engine exhaust 120 (control group or filtered exhaust) and 24 (unfiltered exhaust) animals	Lung tumours (all): Controls: 5/121 Unfiltered engine exhaust: 8/19* Filtered engine exhaust: 4/108	* <i>P</i> < 0.01	Age at start: 8 wk Tumours were mainly adenomas and adenocarcinomas
Rat, F344 (F) 30 mo <a href="#">IARC (2013)</a> , <a href="#">Iwai et al. (1997)</a>	Diluted filtered or unfiltered exhaust from a 2.4 L diesel truck engine (particles, 3.2 mg/m <sup>3</sup> ) either directly or after particle exclusion through a HEPA filter	8 h/d, 6 d/wk, 24 mo; 6 mo follow-up Clean air (control group) Diluted filtered or unfiltered diesel engine exhaust 120 (control group or filtered diesel exhaust) and 48 (unfiltered diesel exhaust) animals	Lung tumours (all): Controls: 5/121 Unfiltered engine exhaust: 5/43* Filtered engine exhaust: 4/108	* <i>P</i> < 0.01	Age at start: 8 wk Tumours were mainly adenomas and adenocarcinomas

**Table 3.1 (continued)**

Species, strain (sex) Duration Reference	Test materials/ Type of samples	Dosing regimen Animals/group at start	Incidence of tumours	Statistical significance	Comments
Rat, F344 (F) 30 mo <a href="#">IARC (2013)</a> , <a href="#">Iwai et al. (1997)</a>	Diluted filtered or unfiltered exhaust from a 2.4 L diesel truck engine (particles, 5.1 mg/m <sup>3</sup> ) either directly or after particle exclusion through a HEPA filter	18 h/d, 3 d/wk, 24 mo; 6 mo follow-up Clean air (control group) Diluted filtered or unfiltered diesel engine exhaust 120 (control group or filtered diesel exhaust) and 96 (unfiltered diesel exhaust) animals	Lung tumours (all): Controls: 5/121 Unfiltered engine exhaust: 40/96* Filtered engine exhaust group: 4/108	* <i>P</i> < 0.01	Age at start: 8 wk Tumours were mainly adenomas and adenocarcinomas
Rat, F344 (F) 30 mo <a href="#">IARC (2013)</a> , <a href="#">Iwai et al. (2000)</a>	Diluted filtered exhaust from a 2.4 L diesel truck engine (particles, 3.5 ± 1.4 mg/m <sup>3</sup> ) Controls: clean air	17 h/d, 3 d/wk, for 0 (control), 3, 6, 9, or 12 mo; follow-up to termination at 30 mo 48–50 animals/group	Lung tumours (all): 1/48, 0/48, 6/43, 19/47*, 10/44* Types of tumours: bronchioloalveolar adenoma (14 rats) or adenocarcinoma (22 rats), squamous cell carcinoma (3 rats), adenosquamous carcinoma (1 rat), and sarcoma (1 rat) Controls: 1/48	* <i>P</i> < 0.01	Age at start: 8 wk
Hamster, Syrian golden (F) Lifetime <a href="#">IARC (2013)</a> , <a href="#">Heinrich et al. (1982)</a>	Filtered or unfiltered exhaust from a 1.6 L Daimler-Benz diesel engine (dilution: 1 : 7; particles, 4.24 mg/m <sup>3</sup> )	7–8 h/d, 5 d/wk, lifetime Controls: clean air 48 animals/group	No lung tumours	NS	Age at start: 8 wk No effects on survival
Hamster, Syrian golden (M, F) Lifetime <a href="#">IARC (2013)</a> , <a href="#">Heinrich et al. (1986a)</a>	Filtered or unfiltered exhaust from a 1.6 L Daimler-Benz diesel engine (dilution: 1 : 17; particles, 4.24 mg/m <sup>3</sup> )	19 h/d, 5 d/wk, lifetime Controls: clean air 48 M, 48 F/group	No lung tumours	NS	Age at start: 8–10 wk No effects on survival

**Table 3.1 (continued)**

Species, strain (sex) Duration Reference	Test materials/ Type of samples	Dosing regimen Animals/group at start	Incidence of tumours	Statistical significance	Comments
Hamster, Syrian golden (M, F) 24 mo <a href="#">IARC (2013)</a> , <a href="#">Brightwell et al. (1989)</a>	Filtered or unfiltered exhaust from a VW Rabbit 1.5 L diesel engine (dilution to give a particle concentration of 0.7, 2.2, or 6.6 mg/m <sup>3</sup> )	16 h/d, 5 d/wk Controls: clean air 104 M, 104 F/exposure group 208 M, 208 F/control group	No lung tumours	NS	Age at start: 6–8 wk No effects on survival
Monkey, Cynomolgus (M) 24 mo <a href="#">IARC (2013)</a> , <a href="#">Lewis et al. (1989)</a>	Exhaust from a 7.0 L Caterpillar model 3304 diesel engine, diluted 1 : 27 (particle concentration, 4.98 ± 0.82 mg/m <sup>3</sup> ); coal dust at 2.00 ± 0.41 mg/m <sup>3</sup> ; or coal dust at 2.02 ± 0.30 mg/m <sup>3</sup> + diesel engine exhaust	7 h/d, 5 d/wk, 24 mo Controls: clean air 15 M/group	No differences in tumour incidence	NS	Age at start NR
<i>Gasoline engine exhaust</i>					
Mouse, NR (M, F) 25 mo <a href="#">IARC (2013)</a> , <a href="#">Campbell (1936)</a>	Exhaust from a 4-cylinder, 23 hp (unleaded) gasoline car engine or a 6-cylinder, 24 hp (leaded) gasoline car engine	7 h/d, 5 d/wk, 25 mo Controls: clean air 37–38 animals/group	Lung tumours (all) in M + F combined: Unleaded gasoline exhaust: 9/75 (12%) Controls: 8/74 (11%) Leaded gasoline exhaust: 12/75 (16%) Controls: 6/70 (9%)	NS	Age at start: 3 mo Study poorly reported; no details provided on survival and pathology
Mouse, ICR (F) 12 mo <a href="#">IARC (2013)</a> , <a href="#">Yoshimura (1983)</a>	Exhaust from a small gasoline engine diluted with clean air to give concentration of 0.1 mg/m <sup>3</sup>	2 h/d, 3 d/wk, 6–12 mo	Lung tumours: 2/15 (13%) (no malignant tumours)	—	Age NR Study poorly reported Lack of controls
Rat, Bor:WISW (F) 30 mo <a href="#">IARC (2013)</a> , <a href="#">Heinrich et al. (1986c)</a>	Exhaust from a 1.6 L (leaded) gasoline engine operated according to US-72 FTP driving cycle, diluted 1 : 27 or 1 : 61 with clean air Controls: clean air	18–19 h/d, 5 d/wk, 24 mo; 6 mo follow-up 80–83 animals/group	Lung tumours: 1 : 61 dilution: 1/83 (squamous cell carcinoma) 1 : 27 dilution: 3/78 (squamous cell carcinoma, adenoma) Controls: 1/78 (adenoma)	NS	Age at start: 10–12 wk

**Table 3.1 (continued)**

Species, strain (sex) Duration Reference	Test materials/ Type of samples	Dosing regimen Animals/group at start	Incidence of tumours	Statistical significance	Comments
Rat, F344 (M, F) 30 mo <a href="#">IARC (2013)</a> , <a href="#">Brightwell et al. (1989)</a>	Exhaust from a Renault R18 1.6 L (unleaded) gasoline engine, operated with or without a 3-way catalytic converter, diluted with a constant volume of 800 m <sup>3</sup> of air, or further diluted 1 : 3 Controls: clean air	16 h/d, 5 d/wk, 24 mo; additional 6 mo clean air 72 animals/group	No increase in lung tumours	NS	Age at start: 6–8 wk
Hamster, Syrian golden (F) 24 mo <a href="#">IARC (2013)</a> , <a href="#">Heinrich et al. (1986c)</a>	Exhaust from a 1.6 L (leaded) gasoline engine operated according to US-72 FTP driving cycle, diluted 1 : 27 or 1 : 61 with clean air Controls: clean air	18–19 h/d, 5 d/wk, 24 mo 80–83 animals/group	0/83 (control), 3/80 (1 : 61), 1/75 (1 : 27)	NS	Age at start: 10–12 wk No effects on survival
Hamster, Syrian golden (M, F) 24 mo <a href="#">IARC (2013)</a> , <a href="#">Brightwell et al. (1989)</a>	Exhaust from a Renault R18 1.6 L (unleaded) gasoline engine, operated with or without a 3-way catalytic converter, diluted with a constant volume of 800 m <sup>3</sup> of air, or further diluted 1 : 3 Controls: clean air	18–19 h/d, 5 d/wk, 24 mo 104 animals/group Control: 208 M and 208 F animals Half of the animals were treated with NDEA 3 d before start of exposure	No increase in lung tumours	NS	Age at start: 6–8 wk No effects on survival
Dog, Beagle (F) 104 mo <a href="#">IARC (2013)</a> , <a href="#">Stara et al. (1980)</a>	Exposure to exhaust from a 6-cylinder, 2.4 L (leaded) gasoline engine, operated to simulate urban driving, and/or to specific air pollutants Pb concentration: 14–26 µg/m <sup>3</sup>	16 h/d, 68 mo, 36 mo follow-up 12–20 animals/group Controls: 17 animals	No lung tumours observed in 41 surviving dogs from groups exposed to engine exhaust or in 17 surviving controls	[NS]	

B[a]P, benzo[a]pyrene; bw, body weight; d, day or days; F, female; h, hour or hours; HEPA, high-efficiency particulate air; hp, horse power; M, male; mo, month or months; NDEA, N-nitrosodiethylamine; NO<sub>2</sub>, nitrogen dioxide; NO<sub>x</sub>, nitrogen oxides; NR, not reported; NS, not significant; Pb, lead; PM, particulate matter; VW, Volkswagen; wk, week or weeks; yr, year or years

**Table 3.2 Studies of carcinogenicity in experimental animals of components of outdoor air pollution by intratracheal administration, intratracheal instillation, or intrapulmonary implantation**

Species, strain (sex) Duration Reference	Test materials/ Type of samples	Dosing regimen Animals/group at start	Incidence of tumours	Statistical significance	Comments
<i>Coal smoke and soot from household combustion of coal</i>					
Mouse, Kunming (M) 18 mo <a href="#">IARC (2010b, 2012a)</a> , <a href="#">Yin et al. (1984)</a>	Coal fume extracts from coal smoke collected from an area of Xuanwei County, China, in an aqueous solution of Tween 80 and 0.1 mL of vehicle solution (12.5 mg soot/mL)	Intratracheal instillation once/10 d for an average period of 100 d; follow-up to termination at 18 mo 43 controls and 72 exposed animals	Lung adenoma or adenocarcinoma (combined): 25.6% (vehicle control), 52.8%* Lung adenocarcinoma: 16.3% (vehicle control), 40.3%*	* $P < 0.01$	Age at start NR
<i>Diesel engine exhaust</i>					
Mouse, ICR (M) 12 mo <a href="#">IARC (2013)</a> , <a href="#">Ichinose et al. (1997)</a>	Exhaust emissions from a 1.5 L diesel engine (2740 cm <sup>3</sup> exhaust volume), collected on a glass filter, suspended in 50 mM phosphate-buffered 0.9% saline (pH 7.4) containing 0.05% Tween 80	Intratracheal instillation once/wk for 10 wk; follow-up to termination at 12 mo 0, 0.05, 0.1, or 0.2 mg/mouse 34 animals/group	No increase in incidence of lung adenoma, lung adenocarcinoma, or lymphoma	NS	Age at start: 4 wk
Rat, Osborne-Mendel (F) 24–140 wk <a href="#">IARC (2013)</a> , <a href="#">Grimmer et al. (1987)</a>	Vehicle alone (control); condensate from exhaust from a diesel car engine (3.0 L, Daimler-Benz 300D), separated into hydrophilic (6.7 mg) and hydrophobic (20 mg) fractions; hydrophobic fraction separated by column chromatography into several subfractions: (A) non-aromatic compounds plus PAHs with 2 or 3 rings (19.22 mg), (B) PAHs with 4–7 rings (0.21 mg), (C) polar PAHs (0.29 mg), and (D) nitro-PAHs (0.19 mg), or a hydrophobic fraction reconstituted from subfractions A–D (19.9 mg) in beeswax:trioctanoin (1 : 1)	Single intrapulmonary implantation of each fraction or subfraction 35 animals/group	Lung squamous cell carcinoma: 0/35 (control), 5/35 (hydrophobic)*, 6/35 (PAHs with 4–7 rings)*, 1/35 (nitro-PAHs), 7/35 (reconstituted hydrophobic)*, 0/35 (other fractions)	*[ $P < 0.05$ ]	Age at start: 3 mo Study poorly reported; details of results, including pathology and dosing regimen, not clear; results summarized in a general manner and difficult to interpret
Rat, F344 (M, F) 30 mo <a href="#">IARC (2013)</a> , <a href="#">Iwai et al. (1997)</a>	Diesel particulate suspension collected from the exhaust of a 2.4 L diesel truck engine, suspended in Tween 80 or DMSO phosphate buffer (pH 7.4)	Intratracheal instillation of 2, 4, 8, or 10 mg of diesel particulate suspension Once/wk for 2–10 wk; follow-up to termination at 30 mo 50 animals/group	Lung tumours (all): 6% (2% malignant), 20% (13% malignant), 43% (34% malignant), 74% (48% malignant)	NR [dose-related increase]	Age at start: 8 wk Results of study poorly reported; unclear whether there was a control group

Table 3.2 (continued)

Species, strain (sex) Duration Reference	Test materials/ Type of samples	Dosing regimen Animals/group at start	Incidence of tumours	Statistical significance	Comments
Hamster, Syrian golden (M, F) Lifetime <a href="#">IARC (2013)</a> , <a href="#">Kunitake et al. (1986)</a>	Suspension of tar from exhaust from a heavy-duty V6 11 L diesel engine, suspended in 0.1 mL of Tween 60, ethanol, and phosphate buffer solution	Intratracheal instillation of 0, 0.1, 0.5, or 1.0 mg of tar Control group: vehicle only 59–62 animals/group	No significant differences in the incidence of tumours of the lung, trachea, or larynx between treated groups and untreated controls	NS	Age at start: 8 wk Dose-related decrease in survival: 98% (control), 95%, 92%, 71%
<i>Gasoline engine exhaust</i>					
Rat, Osborne-Mendel (F) Lifetime <a href="#">IARC (2013)</a> , <a href="#">Grimmer et al. (1984)</a>	Condensate from exhaust emission from a 1.5 L gasoline car engine (operated on the European test cycle)	Single intrapulmonary implantation of 0 (control), 5.0, or 10.0 mg (A1, A2) condensate, or one of several fractions: 4.36, 8.73, or 17.45 mg PAH-free (B1, B2, B3); 0.50, 0.99, or 1.98 mg PAHs with 2 or 3 rings (C1, C2, C3); or 0.14, 0.28, or 0.56 mg PAHs with > 3 rings (D1, D2, D3) in beeswax:trioctanoin (1 : 1) into the left lobe of the lung 34–35 animals/group	Lung carcinoma: 0/34, 3/35 (9%; A1), 20/35 (57%; A2)**, 0/34 (B1), 3/34 (9%; B2), 1/34 (3%; B3), 0/35 (C1), 0/35 (C2), 3/35 (9%; C3), 3/35 (9%; D1), 15/34 (44%; D2)*, 24/35 (69%; D3)** Lung sarcoma: 0/34, 4/35 (11%; A1), 0/35 (A2), 0/34 (B1), 3/34 (9%; B2), 2/34 (6%; B3), 0/35 (C1), 0/35 (C2), 3/35 (9%; C3), 1/35 (3%; D1), 2/34 (6%; D2), 0/35 (D3)	[* <i>P</i> = 0.0002, ** <i>P</i> < 0.001]	Age at start: 3 mo Mean survival times: 80–11 wk No tumours in untreated or vehicle controls The authors reported that a lung tumour dose–response relationship was obtained with the total condensate and with the fraction of PAHs with > 3 rings
Hamster, Syrian golden (M) Lifetime <a href="#">IARC (2013)</a> , <a href="#">Mohr et al. (1976)</a> , <a href="#">Reznik-Schüller and Mohr (1977)</a>	Condensate from exhaust emission from a German gasoline car engine (operated on the European test cycle), containing 340 µg/g B[a]P, dissolved in 0.2 mL of Tris-HCl and EDTA	Intratracheal instillation of 0 (control), 2.5 or 5.0 mg; every other wk for life 6 animals/group	Pulmonary adenoma: 0/6, 6/6*, 6/6*	*[ <i>P</i> < 0.05]	Age at start: 12 wk Survival range: 30–60 wk Study poorly described
Hamster, Syrian golden (M) Lifetime <a href="#">IARC (2013)</a> , <a href="#">Künstler (1983)</a>	Condensate from exhaust emission from a VW 1500 Otto gasoline engine	Single intratracheal instillation of 0 (control), 0.5, 1.0, or 2.1 mg of exhaust condensate in Tris-buffer/saline 30 animals/group	No lung tumours observed	NS	Age at start: 16 wk Survival range: 68–87 wk

B[a]P, benzo[a]pyrene; d, day or days; DMSO, dimethyl sulfoxide; EDTA, ethylenediaminetetraacetic acid; F, female; M, male; mo, month or months; NR, not reported; NS, not significant; PAHs, polycyclic aromatic hydrocarbons; VW, Volkswagen; wk, week or weeks

**Table 3.3 Studies of carcinogenicity in experimental animals of components of outdoor air pollution by skin application**

Species, strain (sex) Duration Reference	Test materials/ Type of samples	Dosing regimen Animals/group at start	Incidence of tumours	Statistical significance	Comments
<i>Coal smoke and soot from household combustion of coal</i>					
Mouse, SENCAR (F) 77 wk <a href="#">IARC (2010b, 2012a)</a> , <a href="#">Mumford et al. (1990)</a>	Exposure to organic extracts of indoor air particles (< 10 µm) from burned smoky coal in Xuanwei County, China (B[a]P, 19.3 µg/m <sup>3</sup> air) Indoor air particles collected during cooking periods in Xuanwei homes. Smoky coal: 0.9% sulfur, high heating value (27.1 MJ/kg), 20% ash content	1 mg of smoky coal extract in 0.2 mL of acetone, twice/wk, 52 wk Acetone control 40 animals/ group	Skin carcinoma: Acetone control: no skin carcinoma at 52 wk (100% survival) or at 77 wk (78% survival) Smoky coal-exposed: 38%* (multiplicity, 1.3) at 52 wk (88% survival); 88%* (multiplicity, 1.1) at 77 wk (10% survival)	NR, *[significant]	Age at start: 7–9 wk
<i>Wood smoke</i>					
Mouse, NR (M, F) 2 yr <a href="#">IARC (2010b)</a> , <a href="#">Sulman &amp; Sulman (1946)</a>	Ethanol extract of wood (eucalyptus) soot	Daily application on the neck skin, 2 yr 10 exposed animals 20 controls	No skin tumours observed Two exposed mice with para-urinary bladder sarcoma, after 5 mo and 12 mo, and one exposed mouse with bladder sarcoma, after 21 mo	NS	Age at start NR, “adult” mice Dose NR
Mouse, SENCAR (F) 74 wk <a href="#">IARC (2010b)</a> , <a href="#">Mumford et al. (1990)</a>	Wood (pine) smoke extract in acetone (PM < 10 µm), collected from homes in Xuanwei County, China High-volume sampling onto fibreglass filters	Skin application of 1 mg/kg bw extract twice/wk, 52 wk; further observation for 25 wk Positive control: 50 mg of B[a]P Acetone control group 40 animals/group	Skin carcinoma: Wood smoke extract-treated mice: 5% Acetone control group: 0% Positive control group: 100%	NS	Age at start: 7–9 wk
Mouse, SENCAR (F) NR <a href="#">IARC (2010b)</a> , <a href="#">Lewtas (1993)</a>	Extracts of particle emissions of a mixture of softwoods (e.g. pine) and of a mixture of hardwoods (e.g. oak) burned in a wood stove	1, 2, 5, 10, or 20 mg of dichloromethane extracts in 0.2 mL of acetone 40 animals/group	Skin papilloma (slope of dose–response curve) Particles from softwoods (0.046 papillomas/mouse/mg) more tumorigenic than those from hardwoods (0.009 papillomas/ mouse/mg)	—	Age at start NR No controls

**Table 3.3 (continued)**

Species, strain (sex) Duration Reference	Test materials/ Type of samples	Dosing regimen Animals/group at start	Incidence of tumours	Statistical significance	Comments
Mouse, SENCAR (F) NR <a href="#">IARC (2010b)</a> , <a href="#">Lewtas (1993)</a> , <a href="#">Cupitt et al. (1994)</a>	Sample A: composite outdoor air sample from Boise, Idaho, USA, of a mixture of 78% wood smoke, 11% mobile sources, and 11% residual unidentified mass Sample B: composite outdoor air sample of a mixture of 51% wood smoke, 33% mobile sources, and 16% residual unidentified mass	Skin application of 1, 2, 5, 10, or 20 mg of dichloromethane extract in 0.2 mL of acetone 40 animals/group	Skin papilloma (slope of dose–response curve) Sample A: 0.095 papillomas/mouse/mg Sample B: 0.21 papillomas/mouse/mg	—	Age at start NR No controls
<i>Diesel engine exhaust</i>					
Mouse, CBA (M, F) C57BL/Gr (M, F) A/Grf (M, F) GFF (M, F) GFF <sub>r</sub> (F) 13.5 mo <a href="#">Clemon et al. (1955)</a>	Two fractions of diesel engine exhaust extracts in benzene; controls received benzene only	Dermal application, 3 ×/wk 1–6 mice/strain/group	Lung nodules [not further described]: Fraction A: 5/21 Fraction B: 0/17 Controls: 1/21	[NS]	M and F mice of all strains combined Small number of animals. Poor study design
Mouse, SENCAR (M, F) 50–52 wk <a href="#">IARC (2013)</a> , <a href="#">Nesnow et al. (1983)</a>	Dichloromethane extracts of particles from the emission of a Nissan Datsun 220C diesel engine, dissolved in acetone	0 (control), 0.1, 0.5, 1.0, 2.0, or 4.0 mg/mouse Application once/wk for 50–52 wk (4 mg dose given as 2 mg twice/wk) 40 animals/group	Skin carcinoma: 0–2.0 mg-treated groups: 0% (M), 0% (F) 4 mg-treated group: 3% (M), 5% (F)	NS	Age at start: 7–9 wk Pathology poorly described
<i>Gasoline engine exhaust</i>					
Mouse, C57BL (NR) NR [> 390 d] <a href="#">IARC (2013)</a> , <a href="#">Kotin et al. (1954a)</a>	Extract of filtered exhaust from an overhauled Ford V8 gasoline engine, in benzene	Dose [NR] in benzene of an oil residue of the benzene extract Application “at frequent but irregular intervals” to the skin Controls: 42 Treated: 86	Skin tumours (all): 0/42, 38/86* Skin squamous cell carcinoma: 0/42, 22/86*	*[ <i>P</i> < 0.0005]	Age at start NR Study poorly reported

Table 3.3 (continued)

Species, strain (sex) Duration Reference	Test materials/ Type of samples	Dosing regimen Animals/group at start	Incidence of tumours	Statistical significance	Comments
Mouse, Swiss (F) Up to 18 mo <a href="#">IARC (2013)</a> , <a href="#">Wynder &amp; Hoffmann (1962)</a>	Oil residue of benzene extract of condensed and filtered exhaust from a V8 gasoline engine in acetone	3 ×/wk, 15 mo; 3 mo follow-up Skin application of 0%, 5%, 10%, 25%, 33%, or 50% of an oil residue of benzene extract 30–50 animals/group	Skin papilloma: 0%, 4%, 50%, 60%, 60%, 70% Skin carcinoma: 0%, 4%, 32%, 48%, 54%, 4%	NR, [significant]	Age at start: 6 wk All animals in the highest-dose group had died by 10 mo Study poorly designed
Mouse, Swiss (F) 18 mo <a href="#">IARC (2013)</a> , <a href="#">Hoffmann et al. (1965)</a>	Tar (in acetone) from exhaust of a V8 gasoline engine, using 0.3 L engine oil/100 km (A) or 0.04 L engine oil/100 km (B)	[Frequency and method of skin application NR] 50 animals/group	Skin tumours (all): A: 60% (48% carcinoma) B: 84% (52% carcinoma)	—	Age at start NR Study poorly designed. No control group
Mouse, CFLP (F) Lifetime <a href="#">IARC (2013)</a> , <a href="#">Brune et al. (1978)</a>	Different doses of exhaust condensate from a 1.5 L VW Otto gasoline engine in DMSO:acetone (3 : 1)	Application twice/wk for life of 0, 0, 0.5, 1.6, or 4.7 µg of exhaust condensate to the shaved interscapular region 80 animals/group in Hamburg laboratory 40 animals/group in Heidelberg laboratory	Hamburg laboratory study: Skin squamous cell tumours (all): 0/76, 1/76 (1%), 3/77 (4%), 26/74 (35%)*, 60/78 (77%)* Skin squamous cell carcinoma: 0/76, 0/76, 1/77 (1%), 22/74 (30%)*, 56/78 (72%)* Heidelberg laboratory study: Skin squamous cell tumours (all): 0/30, 0/37, 1/31 (3%), 3/37 (8%), 19/38 (50%)** Skin squamous cell carcinoma: 0/30, 0/37, 1/31 (3%), 2/37 (5%), 18/38 (47%)* Lung tumours (all): 3/40 (7%), 3/40 (7%), 3/40 (7%), 8/40 (20%)*, 9/40 (22%)*	[* $P < 0.0001$ , ** $P = 0.0002$ , *** $P = 0.001$ ]	Age at start: 12 wk Two control groups

**Table 3.3 (continued)**

Species, strain (sex) Duration Reference	Test materials/ Type of samples	Dosing regimen Animals/group at start	Incidence of tumours	Statistical significance	Comments
Mouse, CFLP (F) Lifetime <a href="#">IARC (2013)</a> , <a href="#">Grimmer et al. (1983a, b)</a>	Exhaust condensate from a 1.5 L gasoline car engine (50 hp); PAH-free fraction and PAH-containing fraction; mixture of PAHs simulating those in exhaust from gasoline automobile engine. Solution in DMSO:acetone (3 : 1)	Twice/wk, 104 wk; lifetime follow-up Application of 0.1 mL of solution of: 0 (control), 0.29, 0.87, or 2.6 mg/animal of exhaust condensate; 0.97 or 2.9 mg/animal of PAH-free fraction (A); 0.152 or 0.455 mg/animal of PAH-containing fraction (2 or 3 rings) (B); 0.02 or 0.06 mg/animal of PAH-containing fraction (> 3 rings) (C); or mixture of 15 PAHs (0.003 or 0.009 mg/animal) 65 controls and 80 treated animals/group	Skin tumours (all): Exhaust condensate: 0/65 (control); 6/80 (7%)*; 34/80 (42%)**; 65/80 (81%)** Fraction A: 4/80 (5%), 11/80 (14%)** Fraction C: 7/80 (9%)*, 50/80 (62%)** Mixture of 15 PAHs: 1/80 (1%), 29/80 (36%)** Fraction B: no significant increase in skin tumours	[* $P < 0.05$ , ** $P < 0.0001$ , *** $P = 0.003$ ]	Age at start: 7 wk Tumours were mainly squamous cell carcinomas

B[a]P, benzo[a]pyrene; bw, body weight; DMSO, dimethyl sulfoxide; F, female; hp, horse power; M, male; mo, month or months; NR, not reported; NS, not significant; PAHs, polycyclic aromatic hydrocarbons; PM, particulate matter; VW, Volkswagen; wk, week or weeks; yr, year or years

**Table 3.4 Studies of carcinogenicity in experimental animals of components of outdoor air pollution by subcutaneous injection or implantation**

Species, strain (sex) Duration Reference	Test materials/ Type of samples	Dosing regimen Animals/group at start	Incidence of tumours	Statistical significance	Comments
<i>Coal smoke and soot from household combustion of coal</i>					
Mouse, Hybrid F1 (C57Blx/CBA) (M) 55 wk <a href="#">IARC (2010b, 2012a)</a> , <a href="#">Khesina et al. (1977)</a>	Olive oil containing coal soot extracts collected from individual houses that were heated with brown coal	5 subcutaneous injections of 2.5 mL of olive oil containing coal soot extracts over 8 wk (total of 0.2 mg of B[a]P/animal); follow-up to 55 wk Vehicle group (olive oil) 30 animals/group	Subcutaneous tumours: Coal soot extract-treated group: 5/30 (17%)*, first tumour at 15 wk Controls: 0/30, no mortality at 55 wk	*[P < 0.05]	Age at start: 1.5–2 mo Tumour type NR
Mouse, Kunming (M) 10 mo <a href="#">IARC (2010b, 2012a)</a> , <a href="#">Liang et al. (1983)</a>	Cyclohexane extracts of coal soot from Xuanwei County, China, dissolved in Tween 80 and saline solution	Injection of 0.1 mL into the back of the neck, once/wk, 10 wk; follow-up to 10 mo 0 mg (vehicle control) 500 mg of coal soot extract (total dose) 1000 mg of coal soot extract (total dose) 38–57 animals/group	Lung cancer: 1/38 (2.6%), 44/57 (77.2%)*, 36/56 (64.3%)*	*P < 0.001	Age at start NR; weight, 18–26 g Lung cancers were squamous cell carcinoma, adenosquamous carcinoma, or adenocarcinoma
Mouse, Kunming (M) 311 d <a href="#">IARC (2010b, 2012a)</a> , <a href="#">Liang et al. (1984)</a>	Extracts of coal soot from Xuanwei County, China, dissolved in Tween 80 and saline solution	Injection of 0.1 mL into the back of the neck, once/wk, 10 wk; follow-up to 311 d 0 mg (vehicle control) 119 mg of soot extract (total dose) containing 0.15 µg of B[a]P 400 mg of soot extract (total dose) containing 0.52 µg of B[a]P ~60 animals/group	Lung cancer: 6/60 (10%) (all adenocarcinomas), 52/58 (89.5%)*, 39/59 (66.1%)*	*P < 0.001	Age at start NR; weight, 18–22 g Lung cancers were mainly squamous cell carcinoma, adenosquamous carcinoma, or adenocarcinoma (one fibrosarcoma in the low-dose group)
<i>Wood smoke</i>					
Mouse, Hybrid F1 (C57Blx/CBA) (M) 55 wk <a href="#">IARC (2010b)</a> , <a href="#">Khesina et al. (1977)</a>	Olive oil containing soot extracts from a wood-fired wood-working atelier	5 subcutaneous injections of 2.5 mL of olive oil containing soot extract over 8 wk (total of 0.2 mg of B[a]P/animal); follow-up to 55 wk Vehicle control group (olive oil) 30 animals/group	Subcutaneous tumours: Wood soot extract-treated group: 5/30 (17%)*, first tumour at 15 wk Controls: 0/30, no mortality at 55 wk	*[P < 0.05]	Age at start: 1.5–2 mo Tumour type NR

Table 3.4 (continued)

Species, strain (sex) Duration Reference	Test materials/ Type of samples	Dosing regimen Animals/group at start	Incidence of tumours	Statistical significance	Comments
Mouse, Kunming (M) 311 d <a href="#">IARC (2010b)</a> , <a href="#">Liang et al. (1984)</a>	Extract of wood smoke generated from a fire pit in the centre of a room to mimic that of rural inhabitants in Xuanwei County, China, in Tween 80 and saline solution	Injection of 0.1 mL into the back of the neck, once/wk, 10 wk; follow-up to 311 d 0 mg (vehicle control) 148 mg of extract (total dose) containing 0.074 µg of B[a]P 296 mg of extract (total dose) containing 0.15 µg of B[a]P ~60 animals/group	Lung cancer (all adenocarcinomas): 6/60 (10%), 31/60 (51.7%)*, 36/58 (62.1%)*	* $P < 0.001$	Age at start NR; weight, 18–22 g
Rat, NR (M, F) 2.5 yr <a href="#">IARC (2010b)</a> , <a href="#">Sulman &amp; Sulman (1946)</a>	Fragments of wood (eucalyptus) soot from the smoking chamber of a sausage factory	Implantation of 5–20 mg fragments near the right axilla and in the scrotal sac 18 M, 18 F Controls (untreated): 18 M, 18 F	Local sarcomas: Exposed animals: M: 0/18; F: 3/18 Controls: M: 0/18; F: 0/18	[NS]	Age at start NR; weight, 120–150 g Small number of animals Inadequate control group The 3 sarcomas had latency periods of 12, 17, and 14 mo
<i>Diesel engine exhaust</i>					
Mouse, C57BL/6N (F) 18 mo <a href="#">IARC (2013)</a> , <a href="#">Kunitake et al. (1986)</a>	Residue from dichloromethane extraction of particles collected from a V6 11 L heavy-duty diesel engine	Injection into the interscapular region, once/wk for 5 wk, follow-up to 18 mo, of a total dose of 0 (control), 10, 25, 50, 100, 200, or 500 mg/kg bw of residue in olive oil containing DMSO 15–50 animals/group	Malignant fibrous histiocytomas: 0/38 (control), 0/15, 1/15, 2/14, 3/30, 1/15, 5/22*	* $P < 0.01$	Age at start: 6 wk
Mouse, ICR (newborn) (M, F) 24 mo <a href="#">IARC (2013)</a> , <a href="#">Kunitake et al. (1986)</a>	Residue from dichloromethane extraction of particles collected from a V6 11 L heavy-duty diesel engine	Single subcutaneous injection 24 h after birth of 0, 2.5, 5, or 10 mg/mouse of residue in olive oil containing DMSO 12–36 animals/group	Malignant lymphoma: 2/14 (control), 4/12 (10 mg/mouse) No significant increase in hepatoma, malignant lymphoma, lung, mammary gland, or other tumours	[NS]	Newborn C57BL mice were also injected with doses of 0 (control) or 5 mg/mouse, and no increase in the incidence of tumours was observed in treated vs control animals

**Table 3.4 (continued)**

Species, strain (sex) Duration Reference	Test materials/ Type of samples	Dosing regimen Animals/group at start	Incidence of tumours	Statistical significance	Comments
<i>Gasoline engine exhaust</i>					
Mouse, NMRI (F) NR <a href="#">IARC (2013)</a> , <a href="#">Pott et al. (1977)</a>	Different doses of gasoline engine [type NR] exhaust condensate	Single subcutaneous injection of 0 (control), 20, or 60 mg of exhaust condensate in 0.5 mL of tricapylin 87–88 animals/group Fourth group: 3 injections of 60 mg dose 45 animals/group	Local fibrosarcomas: Control: 3/89 (3%) Condensate-treated groups: 10/87 (11%), 6/88 (7%), 5/45 (11%)	NR [NS]	Age at start NR Decrease of survival as a function of dose. Survival time in the low- and mid-dose group: 80–88 wk (in the range of the control group); in the high-dose group: 57 wk Study poorly reported; no details provided on histopathology

B[a]P, benzo[a]pyrene; bw, body weight; d, day or days; DMSO, dimethyl sulfoxide; F, female; h, hour or hours; M, male; mo, month or months; NR, not reported; NS, not significant; wk, week or weeks; yr, year or years

**Table 3.5 Studies of administration in experimental animals of components of outdoor air pollution with known carcinogens or modifying factors**

Species, strain (sex) Duration Reference	Test materials/ Type of samples	Dosing regimen Animals/group at start	Incidence of tumours	Statistical significance	Comments
<i>Diesel engine exhaust</i>					
Mouse, NMRI (F) Lifetime (up to 120 wk) <a href="#">IARC (2013)</a> , <a href="#">Heinrich et al. (1986a)</a>	B[a]P or DB[a,h]A, followed by exposure to filtered or unfiltered exhaust from a 1.6 L VW diesel engine, diluted (1 : 17) with air (particles, 4.24 mg/m <sup>3</sup> )	19 h/d, 5 d/wk, lifetime Initial intratracheal instillation with 50 or 100 µg of B[a]P for 20 or 10 wk, respectively, or 50 µg of DB[a,h]A for 10 wk, followed by exposure to clean air, or filtered or unfiltered exhaust Controls: DB[a,h]A + clean air or B[a]P + clean air 64–96 mice/group	Lung tumours: Inconsistent results for the various treatments B[a]P: 71% lung tumour rate B[a]P + diesel exhaust: only 41% lung tumour rate (not reproduced)	NS	Age at start: 8–10 wk
Mouse, NMRI (newborn) (F) 6 mo <a href="#">IARC (2013)</a> , <a href="#">Heinrich et al. (1986a)</a>	DB[a,h]A, followed by exposure to filtered or unfiltered exhaust from a 1.6 L VW diesel engine, diluted (1 : 17) with air (particles, 4.24 mg/m <sup>3</sup> )	19 h/d, 5 d/wk, 6 mo Initial subcutaneous injection of 5 or 10 µg of DB[a,h]A, followed by exposure to clean air, or filtered or unfiltered exhaust Control: DB[a,h]A + clean air 96 mice/group	Lung tumours: Incidence NR. The various treatments gave erratic and inconsistent results	NS	
Rat, F344 (F) Up to 24 mo <a href="#">IARC (2013)</a> , <a href="#">Takemoto et al. (1986)</a>	Exhaust from a 269 cm <sup>3</sup> small diesel engine (diluted 1 : 2 to 1 : 4 with clean air), followed by DIPN	4 h/d, 4 d/wk, 24 mo; after 1 mo, the exposure group was injected with 1 g/kg bw DIPN once/wk for 3 wk Control: DIPN + clean air 20–35 animals/group	Lung carcinoma: 4/21 (control), 7/18 Lung adenoma: 10/21 (control), 12/18	NS	Age at start: 5 wk Small number of animals
<i>Gasoline engine exhaust</i>					
Mouse, NMRI (F) 93 wk <a href="#">IARC (2013)</a> , <a href="#">Heinrich et al. (1986c)</a>	B[a]P or DB[a,h]A, followed by inhalation exposure to exhaust from a 1.6 L gasoline engine (leaded fuel) diluted 1 : 27 or 1 : 61 with air	18–19 h/d, 5 d/wk, 53 wk; 40 wk follow-up Initial treatment with 10 intratracheal instillations of 100 µg of B[a]P, 20 intratracheal instillations of 50 µg of B[a]P, or 10 intratracheal instillations of 50 µg of DB[a,h]A, followed by inhalation exposure to clean air (control) or dilutions of gasoline exhaust 60 animals/group	Similar lung tumour incidences in control groups and exhaust-exposed groups	NS	Age at start: 8–10 wk

Table 3.5 (continued)

Species, strain (sex) Duration Reference	Test materials/ Type of samples	Dosing regimen Animals/group at start	Incidence of tumours	Statistical significance	Comments
Mouse, NMRI (newborn) (M, F) 6 mo <a href="#">IARC (2013)</a> , <a href="#">Heinrich et al. (1986c)</a>	DB[a,h]A, followed by inhalation exposure of exhaust from a 1.6 L gasoline engine (leaded fuel) diluted 1 : 27 or 1 : 61 with air	Single injection of 4 µg or 10 µg of DB[a,h]A, followed by inhalation exposure to clean air (control) or dilutions of gasoline exhaust for 6 mo 61–83 animals/group	Number of lung tumours per animal was not significantly different from that in controls	NS	
Mouse, NMRI (F) NR <a href="#">IARC (2013)</a> , <a href="#">Pott et al. (1977)</a>	B[a]P alone or together with exhaust condensate from a gasoline engine [type NR] in tricaprylin	Single subcutaneous injection of 10, 30, 90, or 270 µg of B[a]P alone or together with 6.6, 20, or 60 mg of condensate in 0.5 mL of tricaprylin 87–88 animals/group	Significant reduction of the dose–response relationship for local fibrosarcoma incidence produced by B[a]P by addition of the condensate	—	Age at start NR Study poorly reported
Rat, Sprague-Dawley (F) 6–12 mo <a href="#">IARC (2013)</a> , <a href="#">Yoshimura (1983)</a>	DIPN alone or together with exhaust emissions from a small gasoline engine (diluted 1 : 250 with clean air)	2 h/d, 3 d/wk, 6 or 12 mo 0.01% DIPN in drinking-water, alone (control) or with 0.1 mg/m <sup>3</sup> exhaust emission Numbers NR	Lung tumours: DIPN control: 2/24 (8%) DIPN + exhaust: 11/37 (30%)* (10 undifferentiated carcinomas, squamous cell carcinomas, adenocarcinomas, or mixed tumours, and 1 adenoma)	*[P < 0.05]	Age at start NR
Rat, Bor:WISW (F) 30 mo <a href="#">IARC (2013)</a> , <a href="#">Heinrich et al. (1986c)</a>	NDPA, followed by inhalation exposure to clean air or exhaust from a 1.6 L gasoline engine (leaded fuel) operated according to US-72 FTP driving cycle, diluted 1 : 27 or 1 : 61 with clean air	18–19 h/d, 5 d/wk, 24 mo; 6 mo follow-up Subcutaneous injection of 0.25 or 0.5 g/kg bw NDPA, once/d for 25 d, followed by exposure to clean air (control) or exhaust 60 animals/group	Decrease in the incidence of benign or malignant (combined) lung tumours	—	Age at start: 10–12 wk
Hamster, Syrian golden (F) 24 mo <a href="#">IARC (2013)</a> , <a href="#">Heinrich et al. (1986c)</a>	NDEA or B[a]P, followed by inhalation exposure to clean air or exhaust from a 1.6 L gasoline engine (leaded fuel) operated according to US-72 FTP driving cycle, diluted 1 : 27 or 1 : 61 with air	18–19 h/d, 5 d/wk, 24 mo Single subcutaneous injection of 3 mg/kg bw NDEA or 20 intratracheal instillations of 0.25 mg of B[a]P, followed by exposure to clean air (control) or exhaust 80–81 animals/group	Basic rates of NDEA- or B[a]P-induced benign respiratory tract tumours were 12.8% and 6.5%, respectively; tumour rates in NDEA- and B[a]P-treated animals exposed to the 1 : 27 dilution were ~50% lower than those in treated animals exposed to the 1 : 61 dilution or clean air	—	Age at start: 10–12 wk

B[a]P, benzo[a]pyrene; bw, body weight; d, day or days; DB[a,h]A, dibenz[a,h]anthracene; DIPN, *N*-nitrosodiisopropanolamine; F, female; h, hour or hours; M, male; NDEA, *N*-nitrosodiethylamine; NDPA, *N*-nitrosodipentylamine; NR, not reported; NS, not significant; VW, Volkswagen

**Table 3.6 Initiation–promotion studies in experimental animals of components of outdoor air pollution**

Species, strain (sex) Duration Reference	Test materials/ Type of samples	Dosing regimen Animals/group at start	Incidence of tumours	Statistical significance	Comments
<i>Wood smoke</i>					
Mouse, Kunming (F) 32 wk <a href="#">IARC (2010b)</a> , <a href="#">Liang &amp; Wang (1987)</a>	Extracts of inhalable particles (< 10 µm) of indoor wood smoke collected from Xuanwei County, China	Skin application of 1, 5, 10, or 20 mg of inhalable particles of indoor wood smoke in acetone; promotion with TPA (application of 2 µg/mouse, twice/wk, 26 wk); further observation for 6 wk TPA control group 40 animals/group	Skin tumours (at 26 wk): TPA + wood smoke extract-treated group: 12.5–41%* TPA control group: 10%	* <i>P</i> < 0.01	Age at start NR; weight, ~28.7 g Time to first tumour incidence decreased with increasing dose of extract Histopathology of skin tumours NR
Mouse, SENCAR (F) Up to 52 wk <a href="#">IARC (2010b)</a> , <a href="#">Mumford et al. (1990)</a>	Wood (pine) smoke extract in acetone (PM < 10 µm); collected from homes in Xuanwei County, China High-volume sampling onto fibreglass filters	Skin application of two doses of 1, 2, 5, 10, or 20 mg of wood smoke extract/kg bw in 0.2 mL of acetone over 1–5 d and then 2 µg of TPA twice/wk for 26 wk TPA control group 40 animals/group	Skin papilloma (at 23 wk): TPA + wood smoke extract-treated groups: 40%*, 45%*, 70%*, 80%*, 90%* TPA control group: 10%	NR, *[significant]	Age at start: 7–9 wk
<i>Coal smoke and soot from household combustion of coal</i>					
Mouse, SENCAR (F) 27 wk <a href="#">IARC (2010b, 2012a)</a> , <a href="#">Mumford et al. (1990)</a>	Exposure to organic extracts of indoor air particles (< 10 µm) from burned smoky coal in Xuanwei County, China (B[a]P, 19.3 µg/m <sup>3</sup> air) Indoor particles collected during cooking periods in Xuanwei homes. Smoky coal: 0.9% sulfur, high heating value (27.1 MJ/kg), 20% ash content	Initiation with skin application of smoky coal extract, followed 1 wk later by promotion with TPA (application of 2 µg/mouse, twice/wk, 26 wk) Initiation dose: 0 (acetone control), 1, 2, 5, 10, or 20 mg in 0.2 mL of acetone 40 animals/group	Skin papilloma: 15%, 80%*, 90%*, > 90%*, > 90%*, 100%*	NR, *[significant]	Age at start: 7–9 wk Tumour incidences estimated from graphical presentation of data
Mouse, Kunming (M) 32 wk <a href="#">IARC (2010b, 2012a)</a> , <a href="#">Liang &amp; Wang (1987)</a>	Extracts of particles (< 10 µm) from smoky coal soot from Xuanwei County, China	Initiation with skin application of 1, 5, 10, or 20 mg of smoky coal soot extract in acetone; promotion with TPA (application of 2 µg/mouse, twice/wk, 26 wk); further observation for 6 wk Control group (TPA only) 40 animals/group	Skin tumours (at 26 wk): Smoky coal extract-treated groups: 25%, 54%*, 60%*, 40%* TPA control group: 10%	*[ <i>P</i> < 0.05]	Age at start NR; weight, ~28.7 g Histopathology of skin tumours NR

Table 3.6 (continued)

Species, strain (sex) Duration Reference	Test materials/ Type of samples	Dosing regimen Animals/group at start	Incidence of tumours	Statistical significance	Comments
<i>Diesel engine exhaust</i>					
Mouse, ICR (F) [29 wk] <a href="#">IARC (2013)</a> , <a href="#">Kunitake et al. (1986)</a>	Extracts of particles from a V6 11 L heavy-duty diesel engine, dissolved in acetone	Skin application every other d for 20 d of 0 (acetone control), 0.5, 1.5, or 4.5 mg/animal, followed by treatment with 2.5 µg of TPA in 0.1 mL of acetone 3 ×/wk for 25 wk 50 animals/group	Skin papilloma: 0/50, 0/49, 1/48, 4/50 No skin “cancers”	[NS]	Age at start: 8–9 wk Study poorly described
Mouse, SENCAR (M, F) 26 wk <a href="#">IARC (2013)</a> , <a href="#">Nesnow et al. (1982a, b)</a>	Extracts of diesel engine exhaust particles from emissions of (A) a 1973 Nissan Datsun 220C, (B) a 1978 Oldsmobile 350, (C) a prototype VW turbo-charged Rabbit, or (D) a 1972 heavy-duty Caterpillar 3304; collected on Teflon-coated fibreglass filters, extracted with dichloromethane, and dissolved in acetone	Application of 0 (control), 0.1, 0.5, 1.0, 2.0, or 10.0 mg of extract/mouse in 0.2 mL of acetone to shaved dorsal surface, followed 1 wk later by treatment with 2 µg of TPA in 0.2 mL of acetone, twice/wk for 25 wk Positive control: B[a]P 40 animals/group	Diesel engine A: Skin papillomas/mouse: M: 0.08 (control), 0, 0.34, 0.38, 1.1, 5.5 F: 0.05 (control), 0.03, 0.39, 0.53, 1.6, 5.7 Skin squamous cell carcinoma: M: 0/37 (control) vs 12/38* (31%), high dose F: 1/38 (control) vs 14/38* (36%), high dose Diesel engines B, C, and D: Skin papillomas/mouse: M + F: 0.1–0.5 vs 0.05–0.08 in TPA controls	*[P < 0.001]	Age at start: 7–9 wk
<i>Gasoline engine exhaust</i>					
Mouse, SENCAR (M, F) 25 wk <a href="#">IARC (2013)</a> , <a href="#">Nesnow et al. (1982a, b)</a> , <a href="#">1983)</a>	Dichloromethane extract of gasoline engine exhaust particles from the emission of a 1977 Ford Mustang II-302 V8 engine with catalyst, collected on Teflon-coated fibreglass filters and dissolved in acetone	Single dose on shaved dorsal surface of 0 (control), 0.1, 0.5, 1.0, 2.0, or 3.0 mg of extract in 0.2 mL of acetone, followed 1 wk later by skin application of 2.0 µg of TPA in 0.2 mL of acetone, twice/wk for 25 wk 40 animals/group	Skin papilloma: M: 0% (control), 5%, 13%, 18%, 22%, 18% F: 5% (control), 13%, 18%, 10%, 21%, 23% Skin carcinoma: F: 0% (control), 20% (3.0 mg)	NR The authors stated that the responses at the higher doses were significantly higher than those in controls	Age at start: 7–9 wk

B[a]P, benzo[a]pyrene; bw, body weight; d, day or days; F, female; M, male; NR, not reported; NS, not significant; PM, particulate matter; TPA, 12-O-tetradecanoylphorbol-13-acetate; VW, Volkswagen; wk, week or weeks

**Table 3.7 Veterinary epidemiology studies of components of outdoor air pollution**

Species, strain (sex) Duration Reference	Test materials/ Type of samples	Dosing regimen Animals/group at start	Incidence of tumours	Statistical significance	Comments
<i>Coal smoke and soot from household combustion of coal</i>					
Dog, pet dogs (sex NR) 5 yr <a href="#">IARC (2010b, 2012a)</a> , <a href="#">Bukowski et al. (1998)</a>	Coal smoke	Exposure to coal smoke resulting from indoor use of coal Case-control study between 1989 and 1993 129 cases and 176 controls	Sinonasal cancer Odds ratio, 4.24 (95% CI, 1.30–16.52)	Indoor use of coal is a risk factor for sinonasal cancer	Histopathology database at the University of Pennsylvania School of Veterinary Medicine Data on exposure, confounders, and behaviour were obtained by questionnaire and by telephone from veterinarians and owners
<i>Wood smoke</i>					
Dog, pet dogs (sex NR) 5 yr <a href="#">IARC (2010b)</a> , <a href="#">Bukowski et al. (1998)</a>	Wood fires	Exposure to wood fires within a residence Case-control study between 1989 and 1993 129 cases and 176 controls	Sinonasal cancer Odds ratio, 1.58 (95% CI, 0.81–3.09)	NS	Histopathology database at the University of Pennsylvania School of Veterinary Medicine Data on exposures, confounders, and behaviour were obtained by questionnaire and telephone from veterinarians and owners More than 220 cumulative occurrences of exposure to wood fires

CI, confidence interval; NR, not reported; NS, not significant; yr, year or years

## 3.2 Inhalation studies of exposure to outdoor air

See [Table 3.8](#).

A series of studies was conducted in São Paulo, Brazil, to determine the effect of inhaled outdoor air on the initiation and promotion of lung tumours in Swiss mice treated with or without the known carcinogen urethane. A second series of studies was conducted in Los Angeles, USA, on the induction of lung tumours in several strains of mice and one rat strain exposed to outdoor air. Descriptions of these studies are given below.

### 3.2.1 Mice exposed to outdoor air in São Paulo, Brazil

[Cury et al. \(2000\)](#) studied Swiss mice aged 15 days [sex was not reported] that were injected intraperitoneally twice within 48 hours with 3 g/kg body weight (bw) of the carcinogen urethane. The animals were then housed in cages in either a low-pollution area (in a house in the rural region of Atibaia) or a high-pollution area (a church tower in São Paulo) for 6 months before being killed. The outdoor air pollution in São Paulo is mainly due to vehicular traffic. [Methanol and ethanol, besides gasoline and diesel, are used as vehicle fuels in Brazil; see Section 1.] The authors stated that the mean levels of pollutants in São Paulo between 1994 and 1997 were as follows: 63.8  $\mu\text{g}/\text{m}^3$  ozone, 66.2  $\mu\text{g}/\text{m}^3$  PM with particles of aerodynamic diameter less than 10  $\mu\text{m}$  ( $\text{PM}_{10}$ ), 125  $\mu\text{g}/\text{m}^3$  nitrogen dioxide ( $\text{NO}_2$ ), 4.4 ppm carbon monoxide (CO), and 21  $\mu\text{g}/\text{m}^3$  sulfur dioxide ( $\text{SO}_2$ ). [It is unclear whether the study was performed between 1994 and 1997.] Pollutant levels in the rural area were not reported. There was an increase ( $P = 0.002$ ) in lung “atypical” adenoma multiplicity (number of tumours per tumour-bearing animal). [These lesions exhibited cytological and architectural atypia, but no metastasis or invasion was found.] There was no increase in the multiplicity of lung adenoma or lung hyperplasia. [The Working Group noted

that the study was limited by the fact that lung tumour incidence was not provided, that there was no clean air control group, and that numerical values of tumour multiplicity were not tabulated but were shown on a graph.]

In a similar study, the same group of investigators ([Reymão et al., 1997](#)) used the same exposure protocol, both for administration of urethane and for geographical sites of outdoor air pollution exposure (São Paulo and Atibaia), to conduct two experiments. The relative contributions of the different sources of PM in São Paulo were 40% from automotive, 10% from industrial, and 50% from other sources.

The first experiment was designed to determine whether outdoor air pollution acts as an initiator and/or a promoter of lung cancer. Eight groups of 25–50 Swiss mice (half male, half female) were studied. For one set of four groups of mice (50 per group), each group was exposed to outdoor air in either São Paulo or Atibaia for 2 months with or without prior intraperitoneal injections of urethane. For another set of four groups of mice (25 per group), each group was exposed to outdoor air in either São Paulo or Atibaia for 6 months and then returned to the laboratory “vivarium” [animal house at the University of São Paulo] for 2 months before being killed; two groups of these mice, exposed to either São Paulo air or Atibaia air, were injected with urethane at the beginning of the 2-month holding period. Animals exposed only to air pollution were considered as being tested for initiation of lung tumours; animals exposed to both urethane and air pollution were being tested for promotion of lung tumours initiated by urethane.

In the 2-month initiation studies, the incidences of lung adenoma (both sexes combined) were 6/50 for mice exposed to São Paulo air and 0/50 for mice exposed to Atibaia air. In the 6-month initiation studies, the incidences were 11/21 for São Paulo air and 0/20 for Atibaia air. [The Working Group found both results to be



Table 3.8 (continued)

Species, strain (sex) Duration References	Dosing regimen Animals/group at start	Results	Statistical significance	Comments
Mouse, Swiss (F) 2 mo <a href="#">Pereira et al. (2011)</a>	100 mice were divided into 4 groups of 25 animals and housed in one of two chambers in São Paulo for 2 mo; half were injected intraperitoneally with urethane (3 g/kg bw) São Paulo outdoor air: 24.5 µg/m <sup>3</sup> PM <sub>10</sub> , 1.93 ppm CO, 116.72 µg/m <sup>3</sup> NO <sub>2</sub> , 14.47 µg/m <sup>3</sup> SO <sub>2</sub> 67.5% of PM <sub>2.5</sub> due to traffic; 42–70% ratio of carbon black to organic carbon One of two chambers had HEPA-filtered outdoor air (3 filters) [The Working Group assumed that there was no PM <sub>10</sub> left in the exposure atmosphere] Mean concentration of PM <sub>2.5</sub> in chambers: 4.54 µg/m <sup>3</sup> (filtered outdoor air); 17.66 µg/m <sup>3</sup> (unfiltered outdoor air)	Mean number of lung adenomas/animal in urethane-treated mice was higher in the chamber with unfiltered air (4.0 ± 3.0) than in the chamber with filtered air (2.0 ± 2.0) No lung adenomas were observed in groups of animals not exposed to urethane	<i>P</i> = 0.02	There were no clean air-exposed groups
<i>Studies in mice exposed to outdoor air in Los Angeles, USA</i>				
Mouse, A, A/J, and C57 (M, F) Up to 15 mo <a href="#">Gardner (1966)</a> , <a href="#">Wayne &amp; Chambers (1968)</a>	4 exposure sites were used: University of Southern California Medical School, Burbank, and the Hollywood Freeway (all with high pollution levels) and Azusa (lower pollution level) Exposures from age 6 wk for 6–15 mo continuously A group of control mice was exposed to air that had passed through an activated charcoal filter, which removed O <sub>3</sub> , NO <sub>2</sub> , and PM > 0.3 µm Subsets of 30 animals/group were killed at monthly intervals at age 7–16 mo and examined for lung tumours	No differences between sexes were observed for incidences of pulmonary adenoma; consequently, data for both sexes were pooled In one experiment in A mice, there was a significant increase in the incidence of pulmonary adenoma in mice older than 12 mo if data from the Burbank and Azusa sites were combined*. Incidence rates: Burbank site, 55/124 vs 32/116 controls; Azusa site, 46/120 vs 34/121 controls. This response was not repeated in a second experiment. There was no effect at the medical school site (35/129 vs 41/131 controls). Data from the Hollywood Freeway site were incomplete A/J mice developed more pulmonary adenomas than A mice, but there was no difference in incidence between outdoor air-exposed and control A/J mice C57 mice had a pulmonary adenoma incidence of only 4/381, but all tumours were observed in mice exposed to outdoor air (4/194 vs 0/187 controls)	* <i>P</i> < 0.01	Study performed beginning in 1962 No precise information on PM was provided. Concentrations of CO, NO, NO <sub>2</sub> , O <sub>3</sub> , “particulates”, and hydrocarbons provided. Concentrations for all sites were not very different Limited reporting of the study and inconsistent results

**Table 3.8 (continued)**

Species, strain (sex) Duration References	Dosing regimen Animals/group at start	Results	Statistical significance	Comments
Rat, Sprague-Dawley (M, F) Up to 28 mo <a href="#">Gardner et al. (1969)</a>	Exposures were at the same sites as in the <a href="#">Gardner (1966)</a> study (see above) Exposures from age 6 wk for either 4–5 mo (35–36 M and 29–31 F/group) or 27–28 mo (4–11 M and 5 F/group)	No lung tumours were observed in 92 controls and 153 outdoor air-exposed rats	NS	Study performed beginning in 1962 No precise information on PM was provided. Concentrations of CO, NO, NO <sub>2</sub> , O <sub>3</sub> , “particulates”, and hydrocarbons provided. Concentrations for all sites were not very different Limited reporting of the study

bw, body weight; CO, carbon monoxide; d, day or days; F, female; h, hour or hours; HEPA, high-efficiency particulate air; M, male; mo, month or months; NO, nitrogen oxide; NO<sub>2</sub>, nitrogen dioxide; NR, not reported; NS, not significant; O<sub>3</sub>, ozone; PM, particulate matter; PM<sub>10</sub>, particulate matter with particles of aerodynamic diameter < 10 µm; PM<sub>2.5</sub>, particulate matter with particles of aerodynamic diameter < 2.5 µm; SO<sub>2</sub>, sulfur dioxide; wk, week or weeks

statistically significant ( $P < 0.05$ ), although the number of animals was small in the 6-month study and the duration of exposure was short.] In the 2-month promotion studies, the incidences in the urethane-injected mice were 43/50 for São Paulo air and 30/50 for Atibaia air. [The Working Group found this result to be statistically significant ( $P < 0.05$ ) but noted the short duration of exposure.] In the 6-month promotion studies, the incidences in the urethane-injected mice were 17/20 for São Paulo air and 14/20 for Atibaia air [not significant]. The authors stated that the promotion arm of the experiment was positive, based on an increase in lung tumour multiplicity that was statistically significant ( $P = 0.005$ ). [The Working Group noted that numerical values of tumour multiplicity were not given.]

The second experiment was designed to determine whether the effect of outdoor air pollution on urethane-induced lung adenomas was dose-dependent. A group of Swiss mice aged 15 days was divided into five groups of 25 male and 25 female mice, all treated with intraperitoneal injections of urethane as previously described. One group was then sent to Atibaia for the duration of the experiment (60 days). The other four groups were exposed to outdoor air in São Paulo for 15, 30, 45, or 60 days, respectively. At the end of the designated exposure period, the four groups of mice were shipped to Atibaia for the remainder of the 60-day study. Only 172 of the 250 mice that started the experiment survived, due to early deaths from urethane within 3 days of injection of the drug. The authors reported a dose-dependent increase [no statistics were given], based on increasing duration of exposure to São Paulo air related to increasing promotion of lung adenomas in mice exposed to urethane. The incidences of lung adenoma after 15, 30, 45, or 60 days of exposure to São Paulo air were 13/26, 15/27, 33/39, and 34/39, respectively [positive trend,  $P < 0.005$ ]. The incidence in the group of animals exposed for 60 days in Atibaia was 28/41.

In a later study by [Pereira et al. \(2011\)](#), 100 female Swiss mice were divided into four groups of 25 animals and housed for 2 months in one of two chambers in São Paulo.  $PM_{10}$  concentration in the outdoor air was  $24.5 \mu\text{g}/\text{m}^3$ . Half of the animals were treated with intraperitoneal injections of urethane as described above, and half were not. One of the chambers had filtered outdoor air containing a mean of  $4.54 \mu\text{g}/\text{m}^3$  PM with particles of aerodynamic diameter less than  $2.5 \mu\text{m}$  ( $PM_{2.5}$ ), and the other chamber had unfiltered outdoor air with  $17.66 \mu\text{g}/\text{m}^3$   $PM_{2.5}$ . The mean number of lung adenomas per animal in the urethane-treated mice was significantly higher ( $P = 0.02$ ) in the chamber with unfiltered air ( $4.0 \pm 3.0$ ) than in the chamber with filtered air ( $2.0 \pm 2.0$ ). [No lung tumour incidences were reported.]

### 3.2.2 Mice exposed to outdoor air in Los Angeles, USA

One study has been performed on the effect of outdoor air in Los Angeles, USA, in mice ([Gardner, 1966](#); [Wayne & Chambers, 1968](#)). [Gardner \(1966\)](#) investigated whether outdoor air could induce lung tumours in several strains of mice exposed to Los Angeles outdoor air for 6–15 months, beginning in 1962. [The Working Group noted the limited reporting of all the publications, and especially that no precise information on PM was provided.] In the report by [Gardner \(1966\)](#), the experimental design was described as using three strains of mice: the lung tumour-susceptible A and A/J strains and the lung tumour-resistant C57 strain. The authors stated that four exposure sites were used, three of which had high pollution levels (University of Southern California Medical School, Burbank, and the Hollywood Freeway) and one of which had lower pollution levels (Azusa). With respect to describing outdoor air pollution, only concentrations of CO, nitrogen oxide,  $\text{NO}_2$ , ozone, “particulates”, and hydrocarbons were given;

the concentrations for all sites were not very different. Exposures began at age 6 weeks and lasted for 6–15 months continuously. There were 10 animals per cage, segregated by sex. The exposed mice were housed in rooms with ventilation from the outdoor air; the unexposed (control) mice were held in rooms with air that had passed through an activated charcoal filter, which removed ozone,  $PM > 0.3 \mu m$ , and  $NO_2$ . The number of mice used varied by strain, with almost 2000 mice of the A strain and 600 of the C57 strain.

Subsets of 30 animals were randomly chosen from each atmosphere to be killed at monthly intervals beginning at age 7 months. Lung tissue was examined for tumours. No difference between sexes in tumour incidence was noted in either the outdoor air-exposed or control groups, and consequently data for both sexes were pooled. In a first experiment in strain A mice, there was a significant ( $P < 0.01$ ) increase in the incidence of pulmonary adenoma in the outdoor air-exposed groups of animals older than 12 months if data from the Burbank and Azusa sites were combined. However, there was no significant increase in the incidence of pulmonary adenoma in mice exposed to outdoor air at the medical school site. Pooled incidence data for animals observed after 12 months were as follows: medical school site, 35/129 (controls, 41/131); Azusa site, 46/120 (controls, 34/121); Burbank site, 55/124 (controls, 32/116). [Data from the Hollywood Freeway site were incompletely reported.] In an additional experiment conducted 2 years later, there was no significant difference between the exposed and control groups. Results with the A/J strain of mice indicated a higher incidence of pulmonary adenoma in both the outdoor air-exposed and control groups compared with the incidence in strain A mice, but there was no difference in the incidences between the outdoor air-exposed and control A/J mice. As expected, a low incidence of pulmonary adenoma was found in C57 mice, but all of the tumours were found in the mice

exposed to outdoor air (exposed, 4/194; controls, 0/187) ([Gardner, 1966](#); [Wayne & Chambers, 1968](#)). [The Working Group noted the limited reporting and the inconsistent results, and found this study difficult to interpret.]

### 3.2.3 Rats exposed to outdoor air in Los Angeles, USA

In a study designed similarly to the one described in Section 3.2.2, male and female Sprague-Dawley rats were exposed at the same sites to Los Angeles outdoor air for 6–15 months, beginning in 1962 ([Gardner et al., 1969](#)). Rats were exposed continuously beginning at age 6 weeks and were killed after either 4–5 months or 27–28 months of exposure. No differences were noted in lifespan, body weight at death, or histology of lung tissue between the outdoor air-exposed and control groups. The total number of rats exposed to outdoor air was 287, of which 153 were examined for lung tumours. Control rats were exposed to charcoal-filtered air. There were 161 controls, of which 92 were examined for lung tumours. No lung tumours were observed in either group of animals. [The Working Group noted that the sensitivity to PM-induced lung carcinogenesis in this strain is unknown.]

## 3.3 Non-inhalation studies of exposure to outdoor air

See [Table 3.9](#).

### 3.3.1 Intratracheal instillation

[Ito et al. \(1997\)](#) collected PM from urban outdoor air in Tokyo, Japan, and extracted the tar with dichloromethane. The tar (25 mg) was mixed with 4.25 mg of carbon [not further described] and suspended in saline. Four groups of 5 male Fischer 344 rats (age, 5 weeks) were administered the tar-carbon mixture (1 mg in 0.2 mL of saline) once a week for 4 consecutive

**Table 3.9 Non-inhalation carcinogenicity studies of outdoor air particulates and extracts in experimental animals**

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	For each target organ: incidence (%) and/or multiplicity of tumours	Statistical significance	Comments
<i>Intratracheal instillation</i>				
Rat, F344 (M) 18 mo <a href="#">Ito et al. (1997)</a>	Particulates were collected from urban outdoor air and tar was extracted with dichloromethane. 25 mg of tar extract was mixed with 4.25 mg of carbon and suspended in 0.8 mL of saline 4 groups of 5 rats (age, 5 wk) were administered the tar-carbon mixture (1 mg/0.2 mL) by intratracheal instillation once/wk for 4 wk and exposed to the following gases for 11 mo: group A, 6 ppm NO <sub>2</sub> + 4 ppm SO <sub>2</sub> ; group B, 6 ppm NO <sub>2</sub> ; group C, 4 ppm SO <sub>2</sub> ; group D, filtered air Group E (control) rats ( <i>n</i> = 5) were injected intratracheally with 1 mg of carbon suspended in 0.2 mL of saline once/wk for 4 wk and housed in filtered air for 18 mo Group F (untreated control) rats ( <i>n</i> = 5) were housed in filtered air for 18 mo	Mean incidence of PEC hyperplasia (number of lesions/lung volume): Group A: 378 ± 105/cm <sup>3</sup> * Group B: 372 ± 104/cm <sup>3</sup> * Group C: 349 ± 126/cm <sup>3</sup> * Group D: 376 ± 146/cm <sup>3</sup> * Group E: 200 ± 75/cm <sup>3</sup> Group F: 194 ± 105/cm <sup>3</sup> A few PEC papillomas were found in all groups of tar-treated rats and not in controls; however, the incidences (22–33/cm <sup>3</sup> ) were not statistically significant	* <i>P</i> < 0.05  — —	Small number of animals The relevance of PEC hyperplasia and papilloma to cancer is not known No detailed information on outdoor air pollutants was reported
<i>Subcutaneous injection</i>				
Mouse, C3H (M) 12 mo <a href="#">Leiter et al. (1942)</a>	Outdoor air particulates from 6 sites in the USA were suspended in saline. Mice received a single subcutaneous injection containing ~20 mg of dust 6 groups of 20 M C3H mice (age, 2–3 mo); 120 in total	For pooled exposed groups Pulmonary tumours: 5/60 (8%) Hepatoma: 8/60 (13%) No injection-site sarcomas	Tumour incidence in treated mice was not greater than the incidence in historical controls (see comments)	Study was poorly designed and reported. Old age of animals. Short duration Saline-injected controls were not included Tumour types were not further specified. Spontaneous tumour incidence NR No detailed information on outdoor air pollutants was reported

Table 3.9 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	For each target organ: incidence (%) and/or multiplicity of tumours	Statistical significance	Comments
Mouse, strain A (M, F) Mouse, C3H (M) 12 mo <a href="#">Leiter et al. (1942)</a>	Outdoor air particulates from 6 sites in the USA were extracted with benzene, the benzene was removed, and the tar was suspended in tricaprylin. There were 8 tar extracts. 8 groups of 20 M C3H mice and 10 M and 10 F strain A mice received a single subcutaneous injection of extract containing 21–71 mg of tar 20 M C3H controls were injected with tricaprylin 30 strain A mice (sex NR) were kept as untreated controls	For pooled exposed groups Injection-site sarcoma: C3H (M): 10/154 (7%) Strain A (M, F): 4/126 (3%) Control C3H (M): 0/16 (0%) Pulmonary tumours: C3H (M): 5/81 (6%) Control C3H (M): 0/16 (0%) Strain A (M, F): 51/78 (65%) Control strain A: 5/10 (50%) Hepatoma: C3H (M): 21/81 (26%) Control C3H (M): 2/16 (13%)	[Fisher exact test, 1-tailed] [NS] [NS] — [NS] — [NS] — [NS] —	Study was poorly designed and reported. Old age of animals. Short duration Tumour types were not further specified Hepatoma incidence in treated M C3H mice (26%) was not greater than the incidence in M C3H historical controls No detailed information on outdoor air pollutants was reported
Mouse, C57BL/6 (M, F) Mouse, C3H (M) 24 mo <a href="#">Hueper et al. (1962)</a>	Monthly subcutaneous injections of extracts of outdoor air dusts from 8 cities in the USA (Atlanta, Birmingham, Cincinnati, Detroit, Los Angeles, Philadelphia, New Orleans, San Francisco). C57 mice were injected with the crude benzene extract (4 mg) or the aromatic fraction (obtained from 4 mg); these doses were doubled after 11 mo. C57 and C3H mice received either oxygenated (0.5 mg) or aliphatic (1 mg) fractions Vehicle control (C57, 36/group/sex) Crude benzene extract (36/group/sex) Aromatic fraction (C57, 36/group/sex) Aliphatic fraction (C57 [sex NR] and C3H, 50/group) Oxygenated fraction (C57 [sex NR] and C3H, 50/group)	For pooled exposed groups Injection-site tumours: Vehicle control (pooled), C57: 0/31 (0%) Crude benzene, C57: 26/576 (4.5%)* Aromatic, C57: 12/576 (2.1%) Aliphatic, C57: 2/372 (0.5%) Aliphatic, C3H: 2/372 (0.5%) Oxygenated, C57: 5/392 (1.3%) Oxygenated, C3H: 7/392 (1.8%)	[Fisher exact test, 1-tailed] — *[P < 0.05] [NS] [NS] [NS]	Majority of tumours were sarcomas and fibrosarcomas Study poorly designed and reported No detailed information on outdoor air pollutants was reported No C3H mice controls

Table 3.9 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	For each target organ: incidence (%) and/or multiplicity of tumours	Statistical significance	Comments
Mouse, Swiss ICR/Ha (newborn) (M, F) 50–52 wk <a href="#">Epstein et al. (1966)</a>	PM from outdoor air in 6 cities in the USA (Chicago, Cincinnati, Los Angeles, Philadelphia, New Orleans, Washington DC) was extracted with benzene 6 groups were injected subcutaneously with the extracts on d 1, 7, and 14 after birth $n = 105\text{--}137/\text{group}$ (M + F) Tricaprylin vehicle control, $n = 190$ (M + F)	For pooled exposed groups Hepatoma:  Control (M): 3/67 (4%) Treated (M): 37/85 (44%) Control (F): 0/68 (0%) Treated (F): 1/143 (0.7%)  Pulmonary adenoma (multiple): Control (M): 0/67 (0%) Treated (M): 49/85 (58%) Control (F): 0/68 (0%) Treated (F): 55/143 (38%)	[Fisher exact test, 1-tailed] — [ $P < 0.0001$ ] — [NS] — [ $P < 0.0001$ ] — [ $P < 0.0001$ ]	High mortality (29–61%) before weaning, due to acute toxicity Higher incidence of deaths after weaning in treated M mice than in controls, due to obstructive uropathy Due to lack of material, some animals of 2 groups received 2 injections only (15 mg) Tumour types were not further specified No detailed information on outdoor air pollutants was reported Short duration of the experiment Controls were untreated ( $n = 90$ ) or injected with tricapyrylin ( $n = 100$ )
Mouse, Swiss ICR/Ha (M, F) (pre-weaned) 49–52 wk <a href="#">Epstein et al. (1979)</a>	Groups of mice were injected subcutaneously with 0.1 mL (10 mg), 0.1 mL (10 mg), and 0.2 mL (20 mg) of benzene extract of a composite of air particulates (from several cities in the USA, collected in 1962) on d 1, 7, and/or 14 after birth. Various dosing sequences were used. Total doses were 1.1–8.3 mg/g bw $n = 89\text{--}233/\text{group}$ Tricaprylin vehicle controls, $n = 100$ ; untreated controls, $n = 90$	For pooled groups Pulmonary adenoma (single):  Controls: M: 9/76 (12%) F: 4/73 (5%) Treated: M: 58/334 (17%) F: 39/304 (13%)  Pulmonary adenoma (multiple): Controls: M: 0/76 (0%) F: 0/73 (0%) Treated: M: 28/334 (8%) F: 43/304 (13%)	[Fisher exact test, 1-tailed] — — [NS] [NS] — — [ $P = 0.0026$ ] [ $P < 0.0001$ ]	Treated mice aged 1 d and 7 d were generally more prone than treated mice aged 14 d to the development of pulmonary adenomas High mortality (13–61%) before weaning, due to acute toxicity. Relatively higher mortality in M mice, due to non-treatment-related obstructive uropathy No detailed information on outdoor air pollutants was reported. Short duration of the experiment No hepatocellular tumours in F mice

Table 3.9 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	For each target organ: incidence (%) and/or multiplicity of tumours	Statistical significance	Comments
Mouse, Swiss ICR/Ha (M, F) (pre-weaned) 49–52 wk <a href="#">Epstein et al. (1979)</a> (cont.)		Pulmonary adenocarcinoma: Controls: M: 0/76 (0%) F: 0/73 (0%) Treated: M: 18/334 (5%) F: 14/304 (5%) Hepatocellular carcinomas and neoplastic nodules (combined): Controls (M): 3/76 (4%) Treated (M): 26/334 (8%)	— — [P = 0.0229] [P = 0.0463] — [NS]	
Mouse, CFW white Swiss (sex NR) Up to 1 yr <a href="#">Rigdon &amp; Neal (1971)</a>	Samples ( <i>n</i> = 15) of airborne PM were collected near a petrochemical industrial area in Texas City, Texas, USA, from 1965 to 1969. Samples ( <i>n</i> = 11) were also collected from non-industrial areas. Benzene-soluble components were extracted from the samples. Mice (4–69/group) were injected subcutaneously with 1–20 mg of extracts in cottonseed oil. Vehicle controls ( <i>n</i> = 47) received only cottonseed oil. Animals were killed when a tumour was observed	For pooled exposed groups Fibrosarcomas: Industrial-area samples (1965–1968): 4/269 (1.5%) Industrial-area samples (1969): 95/232 (41%) Non-industrial area samples (1966–1969): 4/359 (1.1%) Vehicle controls: 0/47 (0%)	[Fisher exact test, 1-tailed] [NS] [P < 0.0001] [NS] —	Short duration of the experiment Non-industrial areas were mainly located in the state of Texas, USA.
Mouse, Swiss ICR/Ha (newborn) (M, F) 49–51 wk <a href="#">Asahina et al. (1972)</a>	Airborne PM was collected on air-conditioner filters in New York City and extracted with benzene, and the crude extract was fractionated. Mice (44–73/group) were injected subcutaneously with the benzene extract or fractions suspended in tricapyrylin (25, 50, or 100 mg/mL) on d 1, 7, and 14 after birth, resulting in total doses of 10, 20, or 40 mg/animal. Controls received tricapyrylin ( <i>n</i> = 86) or were not injected ( <i>n</i> = 81). Surviving mice were necropsied at age 49–51 wk	For pooled exposed groups Any tumours: M mice: Untreated controls: 0/23 (0%) Vehicle controls: 5/31 (16.1%) Benzene extract: 11/39 (28.2%) Acidic fraction: 2/31 (6.5%) Basic fraction: 13/28 (46.4%) Neutral fraction: 13/68 (19.1%) Aliphatic fraction: 8/67 (11.9%)	— — NS NS P < 0.05 NS NS	Study was compromised by high mortality after weaning in all M mice, due to non-treatment-related obstructive uropathy. High incidence of hepatomas [mainly hepatocellular tumours] in M mice in the basic fraction-treated group and slightly fewer in the benzene extract-treated group. High incidence of pulmonary adenoma in the basic, neutral, aromatic, and oxyneutral fraction-treated groups

**Table 3.9 (continued)**

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	For each target organ: incidence (%) and/or multiplicity of tumours	Statistical significance	Comments
Mouse, Swiss ICR/Ha (newborn) (M, F) 49–51 wk <a href="#">Asahina et al. (1972)</a> (cont.)		Aromatic fraction: 17/76 (22.4%) Oxyneutral fraction 9%: 18/35 (32.7%) Oxyneutral fraction 12%: 17/72 (23.6%) Oxyneutral fraction 36%: 11/50 (22.0%) Insoluble fraction: 9/58 (15.5%) F mice: Untreated controls: 1/23 (4.3%) Vehicle controls: 3/35 (8.6%) Benzene extract: 6/48 (12.5%) Acidic fraction: 0/23 (0%) Basic fraction: 10/23 (43.5%) Neutral fraction: 11/53 (20.8%) Aliphatic fraction: 21/66 (31.8%) Aromatic fraction: 33/81 (40.7%) Oxyneutral fraction 9%: 15/59 (25.4%) Oxyneutral fraction 12%: 23/65 (35.4%) Oxyneutral fraction 36%: 10/41 (24.4%) Insoluble fraction: 10/58 (17.2%)	NS NS NS NS NS — — NS NS $P < 0.05$ NS $P < 0.05$ $P < 0.01$ NS $P < 0.01$ NS NS	High mortality before weaning in high-dose groups treated with benzene extract and acidic and basic fractions. Short duration of the experiment
Mouse, NMRI (F) Up to 24 mo <a href="#">Pott et al. (1980)</a> , <a href="#">Pott &amp; Stöber (1983)</a>	Benzene extracts of airborne PM from 3 urban sites (Duisburg-Neuenkamp, Duisburg-Hamborn, Düsseldorf) and one rural site (Krahm) in western Germany were injected subcutaneously once Doses were based on the B[a]P content of the extracts (0.16, 0.63, 2.5, and 10 µg/mouse). Controls were injected with tricapyrylin vehicle 76–80/group	Injection-site tumours [sarcomas]: Duisburg-Neuenkamp: 18.3%, 31.7%, 65.5%, 68.3%; Duisburg-Hamborn: 10.3%, 31.7%, 53.3%, 61.0%; Düsseldorf: 16.7%, 25.9%, 46.7%, 39.0%; Krahm: 10.3%, 20.0%, 20.7%, 20.0% Tricaprylin controls, 1.7%		Exact numbers of mice per group NR Tumour incidences NR, only percentages Sampling period: winter 1975–1976 In the same study, a second experiment with an almost identical design in 3 urban sites and 2 rural sites in western Germany gave similar results

Table 3.9 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	For each target organ: incidence (%) and/or multiplicity of tumours	Statistical significance	Comments
Mouse, Jcl:ICR (M, F) (newborn) Up to 12 mo <a href="#">Sasaki et al. (1987)</a>	Air particulates were collected from a central urban area of Tokyo and extracted with benzene–ethanol. Neutral, acidic, and basic fractions were obtained. The crude extract or the fractions (10 mg in olive oil) were injected subcutaneously into newborn mice. Controls were injected with olive oil. 90 M and 77 F mice were killed at age 3, 6, or 12 mo Initial number of mice per group NR	Pulmonary adenomas:  Crude extract: M: 3/20 (15%) F: 1/5 (20%) Neutral fraction: M: 5/9 (56%) F: 2/16 (13%)  Basic fraction: M: 1/6 (17%) F: 0/5 (0%) Acidic fraction: M: 0/10 (0%) F: 0/10 (0%) Vehicle controls: M: 1/22 (4.5%) F: 1/17 (6%)	[Fisher exact test, 1-tailed]  [NS] [NS]  [ $P = 0.0039$ ] $P < 0.01$ , $\chi^2$ test  [NS] [NS]  [NS] [NS]  — —	High mortality before weaning in all groups, especially mice injected with the acidic and basic fractions Low and variable numbers of surviving mice Short duration of the experiment No detailed information on outdoor air pollutants was reported
<i>Skin application</i>				
Mouse C57BL/6 (M, F) > 15 mo <a href="#">Kotin et al. (1954b)</a>	Benzene extracts of outdoor air particulates Skin application, 3 ×/wk ( $n = 76$ ) Benzene control ( $n = 69$ )	Skin papilloma:  0/37 (control); 13/31* (42%; 9/13 also had squamous carcinomas**)	[Fisher exact test, 1-tailed]  *[ $P < 0.0001$ ] **[ $P < 0.001$ ]	Outdoor air particulates obtained in an industrial area during the smoggy season and in a high-traffic area during the non-smoggy season in Los Angeles County, USA M and F mice were combined. Exposed groups were pooled An unspecified number of mice died early due to toxicity and intercurrent infection

Table 3.9 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	For each target organ: incidence (%) and/or multiplicity of tumours	Statistical significance	Comments
Mouse, CBA (M, F) Mouse, C57BL/Gr (M, F) Mouse, A/Gr <sub>f</sub> (M, F) Mouse, C57BL/How (M, F) Up to 13.5 mo <a href="#">Clemo et al. (1955)</a>	3 fractions (A, B, C) of city smoke extract diluted to 1% in benzene. Controls received benzene only Skin application 3 ×/wk for 13.5 mo, or until malignant skin tumour was observed or animal died 1–6 mice/strain/group	For pooled groups Lung nodules [not further described]: Fraction A: 5/14 (36%) Fraction B: 6/15 (40%) Fraction C: 1/16 (6%) Benzene controls: 1/21 (5%) Skin papillomas: Fraction A: 0/14 (0%) Fraction B: 9/15 (60%) Fraction C: 5/16 (33%) Benzene controls: 0/21 (0%) Skin epitheliomas: Fraction A: 0/14 (0%) Fraction B: 5/15 (33%) Fraction C: 6/16 (38%) Benzene controls: 0/21 (0%)	[Fisher exact test, 1-tailed] [ <i>P</i> = 0.0278] [ <i>P</i> = 0.013] [NS] — [ <i>P</i> = 0.0008] [ <i>P</i> = 0.01] — [ <i>P</i> = 0.008] [ <i>P</i> = 0.0034] —	M and F treated mice of all strains were combined M and F control mice of all strains were combined Small number of animals and poor study design. Use of old animals (aged 2.5–9.5 mo)

**Table 3.9 (continued)**

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	For each target organ: incidence (%) and/or multiplicity of tumours	Statistical significance	Comments
Mouse, Kunming (F) 28 wk <a href="#">Guan et al. (1990)</a>	Particle samples collected from outdoor air in Beijing, Taiyuan, and Xuanwei (China) in winter were separated into two fractions: $\geq 3.3 \mu\text{m}$ and $< 3.3 \mu\text{m}$ . The particulates were extracted with dichloromethane. Mice were treated with 5 mg of particle extracts (in acetone) by skin application once (5 mg group) or twice (10 mg group: 5 mg applied on d 1 and 2). From the second wk, mice were treated with 2.0 $\mu\text{g}$ of TPA twice/wk for 26 wk and observed for skin papilloma development until experimental wk 28	Skin papillomas: Beijing samples: Samples with particles $\geq 3.3 \mu\text{m}$ : 5 mg group: 4/19 (21.1%) Samples with particles $< 3.3 \mu\text{m}$ : 5 mg group: 16/40 (40.0%) 10 mg group: 20/36 (55.6%) Control group, acetone: 4/38 (10.5%) Taiyuan samples: Samples with particles $\geq 3.3 \mu\text{m}$ : 5 mg group: 9/30 (30.0%) Samples with particles $< 3.3 \mu\text{m}$ : 5 mg group: 25/39 (64.1%) 10 mg group: 22/39 (56.4%) Control group, acetone: 4/38 (10.5%) Xuanwei samples: Samples with particles $\geq 3.3 \mu\text{m}$ : 5 mg group: 17/36 (47.2%) 10 mg group: 24/39 (61.5%) Samples with particles $< 3.3 \mu\text{m}$ : 5 mg group: 25/39 (64.1%) 10 mg group: 26/39 (66.7%) Control group, acetone: 4/38 (10.5%)	[Fisher exact test, 1-tailed] [NS] [ $P = 0.015$ ] [ $P < 0.0001$ ] — [ $P = 0.0431$ ] [ $P < 0.0001$ ] [ $P < 0.0001$ ] — [ $P = 0.0005$ ] [ $P < 0.0001$ ] [ $P < 0.0001$ ] [ $P < 0.0001$ ] —	For all 3 locations, the first papilloma was observed at 10 wk for particles $< 3.3 \mu\text{m}$

Table 3.9 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	For each target organ: incidence (%) and/or multiplicity of tumours	Statistical significance	Comments
Mouse, BALB/c (M, F) 35 wk <a href="#">Zhao et al. (2003)</a>	Skin application of dichloromethane extracts of outdoor air particulates from 4 sites in Shanghai 5 mg (single application) or 10 mg (2 mg/d for 5 d) Vehicle control: 0.2 mL of acetone (single application) From 1 wk after initiation, TPA (2 µg in 0.2 mL of acetone) was applied dermally twice/wk for 30 wk 20/group/sex/extract	Skin papilloma (M): Controls, acetone: 0/40 (0%) 5 mg dose: 1/79 (1.3%) 10 mg dose: 9/75 (12%) Positive controls: 11/18 (61%) Skin papilloma (F): Controls, acetone: 0/40 (0%) 5 mg dose: 1/79 (1.3%) 10 mg dose: 1/80 (1.2%)	[Fisher exact test, 1-tailed] — [NS] [ <i>P</i> = 0.0179] [Fisher exact test, 1-tailed] — [NS] [NS]	Incidences were combined for the 4 extracts
Mouse, [Sv/129] AhR <sup>+/+</sup> or AhR <sup>-/-</sup> (F) 58 wk <a href="#">Matsumoto et al. (2007)</a>	PM from outdoor air (from the city of Sapporo, Japan) by skin application once/wk; 6.4 mg of particulate extract in 200 µL of acetone AhR <sup>+/+</sup> ( <i>n</i> = 17), AhR <sup>-/-</sup> ( <i>n</i> = 15)	Skin squamous cell carcinoma: AhR <sup>-/-</sup> : 0/15 (0%) AhR <sup>+/+</sup> : 8/17 (47%)	— <i>P</i> < 0.01	The samples were stored from 1988 to 2007. No clean air-exposed control groups. Extracts contained B[a]P and other PAHs
<i>Intravenous injection</i>				
Mouse, strain A (sex NR) 4 mo <a href="#">Leiter et al. (1942)</a>	Groups of mice received a single intravenous injection (tail vein) with outdoor air particulates from 6 sites in the USA. Dusts were suspended in saline (2.5 mg/0.25 mL). Controls were untreated Treated: 20–30/group Untreated controls: 20	Pulmonary tumours: Controls: 3/20 (15%) Treated: 15/138 (11%)	NS	The methods and results of this older study were poorly reported. Study duration was short. No detailed information on outdoor air pollutants was reported. Groups of treated mice were combined

**Table 3.9 (continued)**

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	For each target organ: incidence (%) and/or multiplicity of tumours	Statistical significance	Comments
<i>Intraperitoneal injection</i>				
Mouse, NMRI (M) (newborn) 52 wk <a href="#">Heussen et al. (1996)</a>	Outdoor air particulates from a non-industrial site (Wageningen, The Netherlands) were extracted with benzene, benzene was removed, and the extract was resuspended in propylene glycol. Mice were injected intraperitoneally on d 1, 8, and 15 after birth with 1.95 mg or 3.9 mg of extract. Controls were injected with propylene glycol Controls, <i>n</i> = 52 Extract (1.95 mg), <i>n</i> = 49 Extract (3.9 mg), <i>n</i> = 47	Bronchioloalveolar tumours: Controls: 16/52 (30.8%) 1.95 mg dose: 10/49 (20.4%) 3.9 mg dose: 17/47 (36.2%)	NS NS	No deaths occurred before weaning No significant difference in mortality between groups Short duration of the study. No detailed information on outdoor air pollutants was reported

AhR, aryl hydrocarbon receptor; B[a]P, benzo[a]pyrene; bw, body weight; d, day or days; F, female; h, hour or hours; M, male; mo, month or months; NO<sub>2</sub>, nitrogen dioxide; NR, not reported; NS, not significant; PAHs, polycyclic aromatic hydrocarbons; PEC, pulmonary endocrine cell; PM, particulate matter; SO<sub>2</sub>, sulfur dioxide; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; wk, week or weeks; yr, year or years

weeks by intratracheal injection [instillation]. Groups of instilled rats were also exposed by inhalation for 11 months to (group A) a mixture of 6 ppm NO<sub>2</sub> + 4 ppm SO<sub>2</sub>, (group B) 6 ppm NO<sub>2</sub>, (group C) 4 ppm SO<sub>2</sub>, or (group D) filtered air. A control group ( $n = 5$ ) received an intratracheal instillation of carbon suspension (1 mg in saline) once a week for 4 weeks and was housed in filtered air for 18 months. Untreated control rats ( $n = 5$ ) received no treatments and were housed in filtered air for 18 months. At 18 months, rats were killed and the incidence of pulmonary endocrine cell (PEC) hyperplasia and the incidence of PEC papilloma were estimated as the number of observed lesions per lung volume. The mean incidences of PEC hyperplasia were significantly greater ( $P < 0.05$ ) in animals treated with tar (groups A–D) relative to both control groups. Exposure to NO<sub>2</sub> and/or SO<sub>2</sub> did not promote hyperplasia or papilloma formation. A few PEC papillomas were found in animals treated with the tar extract (groups A–D) (not statistically significant); no papillomas were detected in controls. [The relevance of PEC hyperplasia and papilloma to cancer is not known. The Working Group noted the small number of animals. The study was judged inadequate for the evaluation.]

### 3.3.2 Subcutaneous injection

PM from outdoor air was collected by various methods at six sites in the USA: in Chelsea, Massachusetts, in Pittsburgh, Pennsylvania, and at four sites in the Holland Tunnel, which connects New York City and Jersey City (Leiter et al., 1942). In six groups of 20 male C3H mice (age, 2–3 months), each mouse received a single subcutaneous injection in the right axilla with one of six dust samples (~20 mg) suspended in 0.9% saline containing a small amount of emulsifying agent (dioctyl ester of sodium sulfosuccinate). The study was terminated 12 months after injection. Of the initial 120 treated mice, 60 were alive at 12 months. Of the 60 treated mice, 5 (8%) had

pulmonary tumours and 8 (13%) had hepatomas. [Tumour types were not further specified.] The authors stated that incidences of pulmonary tumours in treated mice were not higher than the incidence of spontaneous pulmonary tumours in untreated mice of a subline of C3H mice [spontaneous tumour incidence was not reported]. [No detailed information on outdoor air pollutants was reported. No saline-injected control group was included. The Working Group noted the old age of the animals at the start of the experiment and the short duration of the experiment. The study was judged inadequate for the evaluation.]

Leiter et al. (1942) also evaluated the carcinogenicity of eight tar fractions collected from the six outdoor air PM samples. The samples were extracted with benzene followed by ethyl ether. The solvents were removed by evaporation and the tar extract collected. Groups of 20 male C3H mice and 10 male and 10 female strain A mice (all aged 2–3 months) received a single subcutaneous injection of the tar extracts at doses ranging from 21 mg to 71 mg suspended in tricapyrylin vehicle. [No attempt was made to use equal doses.] A vehicle control group of 20 male C3H mice received a single subcutaneous injection of tricapyrylin. A group of 30 untreated strain A mice was kept for determining the spontaneous tumour incidence. The study was terminated 12 months after injection. Over the 12 months after injection of tars, 10 sarcomas were found in an effective total of 154 treated male C3H mice (7%), in contrast to 4 sarcomas in 126 treated strain A mice (3%). All sarcomas in strain A mice occurred in male mice. There were no injection-site sarcomas in vehicle controls. [Incidences of sarcomas in male C3H and strain A mice were not significantly different from the incidence in controls.] The incidence of pulmonary tumours [tumour type was not further specified] was 5/81 (6%) [not statistically significant] in treated C3H mice compared with 0/16 in C3H controls. Treated C3H mice (21/81 [26%]) also developed hepatomas [tumour type was not

further specified]; however, the incidence was not significantly different from that in vehicle control C3H mice (2/16 [13%]). The authors stated that the incidence of hepatomas in treated male C3H mice was not different from the incidence of spontaneous hepatomas in untreated male C3H mice [the incidence of spontaneous hepatomas was not reported]. Pulmonary tumours [tumour type was not further specified] were present in 51/78 (65%) strain A mice injected with tar extracts and in 5/10 (50%) untreated control strain A mice [not significantly different]. [This older study was not adequately designed and reported. No detailed information on outdoor air pollutants was reported. The Working Group noted the old age of the animals at the start of the experiment and the short duration of the experiment. The study was judged inadequate for the evaluation.]

[Hueper et al. \(1962\)](#) collected atmospheric dusts from eight cities in the USA and extracted the PM with benzol (benzene). The crude benzene extract was further fractionated to obtain aromatic, oxygenated, and aliphatic fractions. Groups of 36 male and 36 female C57BL/6 mice (age, 2 months) were injected subcutaneously (nape of the neck) once a month with either the crude extract (4 mg in 0.1 mL of tricapyrylin) or the aromatic fraction obtained from 4 mg of crude extract (in 0.1 mL of tricapyrylin vehicle). [The actual dose (mg) of the aromatic fraction was not reported.] These doses were doubled after 11 months. The oxygenated (0.5 mg in 0.1 mL of tricapyrylin) and aliphatic (1.0 mg in 0.1 mL of ethyl laurate) fractions were also administered to groups of 50 C57BL/6 mice [sex was not reported] and 50 C3H mice [male] once a month by subcutaneous injection. Groups of 36 male and 36 female control C57BL/6 mice were injected subcutaneously with 0.1 mL of tricapyrylin or ethyl laurate once a month. After 24 months, all the particulate extracts except one caused injection-site tumours (primarily sarcomas and fibrosarcomas). Compared with

C57BL/6 controls, the tumour incidence was higher in C57BL/6 mice receiving the crude benzene extract (26/576 [4.5%]) [ $P < 0.05$ ] and the aromatic fraction (12/576 [2.1%]) [not significant]. Tumour incidences in male and female C57BL/6 mice (combined) injected with the oxygenated or aliphatic fractions were 5/392 (1.3%) and 2/372 (0.5%), respectively [not significant]. In male and female C3H mice (combined), the tumour incidences were 7/392 (1.8%) with the oxygenated fraction and 2/372 (0.5%) with the aliphatic fraction. None of the C57BL/6 control mice (0/31) had tumours after 12 months. [Tumour incidences in mice treated with the oxygenated and aliphatic fractions were not statistically different from that in controls.] [The experiments were not well designed and reported. No detailed information on outdoor air pollutants was reported. There were no C3H control mice.]

Male and female newborn Swiss ICR/Ha mice (105–137 per group) were injected subcutaneously (nape of the neck) with benzene-soluble extracts of organic atmospheric particulates collected from six cities in the USA ([Epstein et al., 1966](#)). Mice received 5 mg of extract in 0.05 mL of tricapyrylin on the day of birth, 10 mg in 0.1 mL on day 7, and 10 mg in 0.1 mL on day 14 after birth. Control mice ( $n = 190$ ) were untreated ( $n = 90$ ) or injected with the tricapyrylin vehicle only ( $n = 100$ ). The final injection was omitted for some animals of two groups because of a lack of material; these mice received only 15 mg. Mortality before weaning was high (29–61%) in all treated groups (16% in controls), and deaths after weaning were higher in treated males than in controls, due to obstructive uropathy. At age 50–52 weeks, the overall incidence of hepatomas [tumour type was not further specified] in treated male mice (37/85 [44%]) was significantly greater [ $P < 0.0001$ ] than that in controls (3/67 [4%]), but the difference in incidence was not statistically significant in female mice. A significantly increased overall incidence of pulmonary adenoma (multiple) was observed in treated male

(49/85 [58%]) [ $P < 0.0001$ ] and female (55/143 [38%]) [ $P < 0.0001$ ] mice relative to controls (male, 0/67; female, 0/68). In addition, there was a small incidence of lymphomas in groups of treated mice (up to 18.5%), compared with 0–1.5% in controls. [The Working Group noted the short duration of the experiment and the high mortality. No detailed information on outdoor air pollutants was reported.]

In a subsequent study ([Epstein et al., 1979](#)), atmospheric particulates collected in 1962 from several cities in the USA were combined and extracted with benzene, and the extracts were suspended in tricaprilyn (100 mg/mL). Groups of pre-weaned male and female Swiss ICR/Ha mice were injected subcutaneously (nape of the neck) with 10 mg, 10 mg, and 20 mg of extract suspension on day 1, 7, and/or 14 after birth, respectively. Two control groups were either untreated ( $n = 90$ ) or were injected subcutaneously with tricaprilyn vehicle on days 1, 7, and 14 ( $n = 100$ ). The animals were injected using various dosing sequences. Total doses were 1.1–8.3 mg/g bw. The study was terminated at 49–52 weeks. Mortality ranged from 13% to 61% in the various test groups. Groups injected on day 1 had the highest mortality. In addition, a relatively high mortality was observed in male mice, due to non-treatment-related obstructive uropathy. The tumour incidences in both sexes were dose-related in all test groups. The incidences of pulmonary adenoma (single) in all exposed groups combined were 58/334 (17%) in male mice [not statistically significant] and 39/304 (13%) in female mice [not statistically significant]; corresponding values for control groups were 9/76 (12%) in male mice and 4/73 (5%) in female mice. There was a significantly increased incidence of pulmonary adenoma (multiple) in treated male (28/334 [8%]) [ $P = 0.0026$ ] and female (43/304 [13%]) [ $P < 0.0001$ ] mice relative to controls (male, 0/76; female, 0/73). A significantly increased incidence of pulmonary adenocarcinoma was observed in treated male

(18/334 [5%]) [ $P = 0.0229$ ] and female (14/304 [5%]) [ $P = 0.0463$ ] mice relative to controls (male, 0/76; female, 0/73). Hepatocellular tumours were observed only in male mice and were not dose-related. The incidence of hepatocellular carcinomas and neoplastic nodules (combined) in male mice of all exposed groups combined was 26/334 (8%) [not statistically significant] and in control male mice was 3/76 (4%). [The Working Group noted that the study duration was short. The study was compromised by the high mortality, due to acute toxicity in treated groups and non-treatment-related uropathy in male mice.]

[Rigdon & Neal \(1971\)](#) collected samples of airborne PM near a petrochemical industrial area in Texas City, Texas, USA, and from non-industrial areas at various times between 1965 and 1969. Benzene-soluble extracts were obtained from the samples. Groups of 4–69 CFW white Swiss mice (“usually 30 to 50 days old”) [sex was not reported] were injected subcutaneously with 1–20 mg of the various benzene-soluble extracts in 0.5 mL of cottonseed oil vehicle. Controls ( $n = 47$ ) were injected with 0.5 mL of cottonseed oil vehicle. Animals were kept for up to 1 year and were killed when a tumour was observed. After injection of industrial-area samples (1–10 mg) collected from 1965 to 1968, in mice of pooled exposed groups only 4/269 (1.5%) developed fibrosarcomas. The incidence was not significantly different from that in vehicle controls (0/47). Injection of industrial-area samples (2.5–10 mg) collected in 1969 resulted in a significantly increased [ $P < 0.0001$ ] incidence of fibrosarcomas (pooled exposed groups; 95/232 [41%]) relative to that in vehicle controls. Benzene-soluble extracts of PM collected from non-industrial areas did not cause a statistically significant increase in fibrosarcomas (pooled exposed groups; 4/359 [1.1%]). [The Working Group noted the short duration of the experiment.]

In the study of [Asahina et al. \(1972\)](#), groups of 44–73 male and female newborn Swiss ICR/Ha mice were injected subcutaneously with extracts

of particulates collected on air-conditioner filters in New York City. Crude benzene extracts of the particulates were fractionated into acidic, basic, neutral, aliphatic, aromatic, water-ether insoluble, and oxygenated (oxyneutral, pentane-9%, -12%, or -36% ether) fractions. The extracts and fractions were resuspended in tricapyrylin at concentrations of 25, 50, and 100 mg/mL. Mice aged 1, 7, and 14 days were injected with 0.1 mL (days 1 and 7) and 0.2 mL (day 14) of suspension, resulting in total doses of 10, 20, or 40 mg. Controls consisted of newborn mice injected with tricapyrylin (0.1 mL on days 1 and 7 and 0.2 mL on day 14) ( $n = 86$ ) and non-injected newborn mice ( $n = 81$ ). Mice were necropsied at age 49–51 weeks. Mortality before weaning was high in neonates receiving high doses (40 mg) of the benzene-soluble extract (86%), acidic fraction (96%), and basic fraction (100%), and this precluded determining carcinogenicity in these groups. A large number of male mice in all groups (25–56%) developed non-treatment-related obstructive uropathy. Because of the small number of mice at risk at weaning, the numbers of tumour-bearing mice (all organs) were combined for all doses in each treatment group for purposes of comparison. The number of tumour-bearing male mice was significantly increased ( $P < 0.05$ ) in groups injected with the basic fraction (13/28, 46.4%) relative to tricapyrylin controls (5/31, 16.1%). The number of tumour-bearing female mice was significantly increased ( $P < 0.05$ ) in groups injected with the basic (10/23, 43.5%), aliphatic (21/66, 31.8%), aromatic (33/81, 40.7%), and oxyneutral pentane-12% ether (23/65, 35.4%) fractions relative to tricapyrylin controls (3/35, 8.6%). Tumours were not observed in any of the untreated male control mice. The highest incidences of lymphomas in male mice were found in the groups treated with the aliphatic, aromatic, oxyneutral pentane-9% ether, and insoluble fractions (7–13%). In female mice, the highest incidences of lymphomas were observed in the groups treated with the basic, insoluble,

aliphatic, aromatic, and oxyneutral fractions (12–30%). Lymphomas were found in 1/23 (4%) untreated female controls and 2/35 (6%) female vehicle controls. A relatively high incidence of pulmonary adenoma (single) was found in male mice injected with oxyneutral pentane-9% ether (10/55, 18%) and female mice injected with the neutral fraction (7/53, 13%). Multiple pulmonary adenomas occurred most frequently in male and female mice injected with the aromatic (9% and 14%, respectively) and the oxyneutral pentane-12% ether (7% and 11%, respectively) fractions. No multiple adenomas were observed in either control group. The highest incidence of hepatomas [mainly hepatocellular tumours] occurred in male mice injected with the basic fraction (12/28, 43%) and the benzene extract (9/39, 23%). In controls, only 3/31 vehicle-treated male mice developed hepatomas. [The Working Group noted the short duration of the experiment and the high mortality.]

[Pott et al. \(1980\)](#) collected airborne PM from three urban sites and one rural site in western Germany during the winter of 1975–1976. The particulates were extracted with benzene and then partially fractionated and analysed for benzo[*a*]pyrene (B[*a*]P) and other PAHs. The extracts (in 0.5 mL of tricapyrylin) were injected subcutaneously once into groups of 76–80 female NMRI mice aged 9–12 weeks at doses containing 0.16, 0.63, 2.5, or 10 µg of B[*a*]P per mouse. Controls were injected with the tricapyrylin vehicle only. [The exact number of mice per group was not reported.] The animals were observed for up to 2 years. The percentage of mice with injection-site tumours [sarcomas] increased with increasing B[*a*]P content of the injected extract. [Tumour incidences were not reported, only percentages.] The tumour rate was 1.7% in tricapyrylin-injected controls and reached up to 68.3% in mice treated with particulate extracts from the rural or urban sites. The extracts from the three urban sites showed a dose-dependent effect, and they showed a higher potency compared with extracts

from the rural site on the basis of the same B[a]P content. In the same study, a second experiment with an almost identical design, using samples from three urban sites and two rural sites in western Germany, gave similar results ([Pott et al., 1980](#); [Pott & Stöber, 1983](#)).

[Sasaki et al. \(1987\)](#) collected outdoor air particulates from an urban site in Tokyo, Japan. The particulates were extracted with a benzene-ethanol mixture. A portion of the crude extract was fractionated into acidic, basic, and neutral fractions. Newborn male and female Jcl:ICR mice [initial number of mice per group was not reported] were injected subcutaneously (nape of the neck) with 10 mg of the crude extract or fraction suspended in 0.05 mL of olive oil. Control mice were injected subcutaneously with 0.05 mL of olive oil. Mortality was high in all groups before weaning, particularly in mice that received the acidic and basic fractions. Surviving mice (90 male, 77 female) were killed and necropsied 3, 6, or 12 months after treatment. Within 1 year of treatment with the crude extract, pulmonary adenomas were observed in male (3/20, 15%) [not statistically significant] and female (1/5, 20%) [not statistically significant] mice injected with the crude extract, and in male (5/9, 56%) [ $P = 0.0039$ ] and female (2/16, 13%) ( $P < 0.01$ ) mice injected with the neutral fraction. One male mouse and one female mouse that received the neutral fraction had multiple pulmonary adenomas. In mice injected with the basic fraction, one male mouse (1/6, 17%) [not significant] and no female mice (0/5) had pulmonary adenoma. No tumours were found in mice that received the acidic fraction. Pulmonary adenomas were found in one male mouse (1/22, 4.5%) and one female mouse (1/17, 6%) in the vehicle control groups. [The Working Group noted the low and variable numbers of surviving mice and the short duration of the experiment.]

### 3.3.3 Skin application

[Kotin et al. \(1954b\)](#) collected outdoor air particulates in Los Angeles County, USA, in an industrial area during the smoggy season, and also adjacent to an area of high traffic density during the non-smoggy season. Pyrene, B[a]P, and 1,12-benzoperylene were detected in both particulate samples, and both contained relatively low concentrations of B[a]P. The samples were extracted with benzene, and both extracts were resuspended in benzene. Groups of 76 C57BL/6 mice (age, 3 months) [the exact numbers of male and female mice were not reported] were treated by skin application (interscapular area) 3 times a week with the extracts [dose was not reported] in approximately 0.5 mL of benzene. A control group of 69 C57BL/6 mice received skin applications of benzene. At the time of appearance of the first tumour (~15 months after treatment), 31 treated (pooled exposed groups) and 37 control mice were alive. Of the 31 treated mice, 13 (42%) [ $P < 0.0001$ ] developed skin papillomas, 9 of which also bore squamous carcinomas [ $P < 0.001$ ]. No skin tumours were observed in controls. [The Working Group noted that the study was incomplete. This appears to be an interim report because the authors state that “this 42% figure of positive tumour production is subject to upward revision in view of the possibility of tumour demonstration in nine remaining mice.” An unspecified number of mice died early due to toxicity and intercurrent infection.]

Groups of 1–6 male and female CBA, C57BL/Gr, A/Gr<sub>p</sub>, or C57BL/How mice (age, 2.5–9.5 months) were treated by skin application with one of three fractions (A, B, C) of an extract of smoke from chimneys diluted to 1% in benzene ([Clemo et al., 1955](#)). [The Working Group assumed that particles were mainly from emissions from combustion of coal.] Fractions were applied to the interscapular region 3 times a week, using two strokes of a No. 4 brush, for up to 13.5 months, or until malignant skin tumours

appeared or the animal died. Control mice (21 of various strains, both sexes) received the benzene vehicle only. For each fraction, groups of male and female mice of all strains were pooled. A significantly increased incidence of lung nodules [not further described] was observed in mice treated with fraction A (5/14, 36%) [ $P = 0.0278$ ] and fraction B (6/15, 40%) [ $P = 0.013$ ] relative to controls (1/21, 5%). In mice treated with fraction C, lung nodules [not further described] were observed in only 1/16 (6%) mice [not significant]. Skin papillomas were observed in 9/15 (60%) [ $P = 0.0008$ ] mice treated with fraction B and 5/16 (33%) [ $P = 0.01$ ] mice treated with fraction C. Skin epitheliomas were observed in 5/15 (33%) [ $P = 0.008$ ] mice treated with fraction B and 6/16 (38%) [ $P = 0.0034$ ] mice treated with fraction C. Female A/Gr<sub>f</sub> mice treated with fraction C were too old when the treatment started, and they survived only to 6.5–7.5 months of treatment. Of the C57BL/Gr mice treated with fraction C, all (6/6) developed skin epitheliomas and 5/6 developed skin papillomas. Of the 14 mice treated with fraction A, none survived more than 9.5 months of treatment, and no skin tumours were observed. Control mice did not develop skin tumours (0/21). [The Working Group judged this study inadequate due to several major design flaws, including the use of old mice of mixed strains.]

PM from outdoor air was collected in Beijing, Taiyuan, and Xuanwei (China) in winter using a multistage Andersen air sampler. Particle samples were separated into two fractions ( $\geq 3.3 \mu\text{m}$  and  $< 3.3 \mu\text{m}$  in diameter). Dichloromethane extracts of these particles were tested for skin tumour-initiating ability in a two-stage carcinogenesis assay (Guan et al., 1990). Female Kunming mice were treated with 5 mg of particle extracts (in acetone) by skin application once (5 mg group) or twice (10 mg group; 5 mg applied also on the second day). From the second week, mice were treated with 2.0  $\mu\text{g}$  of the tumour promoter 12-*O*-tetradecanoylphorbol-13-acetate (TPA) twice a week for 26 weeks and observed for skin

tumour development until experimental week 28. After 28 weeks, the extracts of outdoor air samples from Beijing with particles  $\geq 3.3 \mu\text{m}$  induced skin papillomas with an incidence of 4/19 [not significant] in the 5 mg group, whereas the extracts with particles  $< 3.3 \mu\text{m}$  induced skin papillomas with an incidence of 16/40 [ $P = 0.015$ ] in the 5 mg group and 20/36 [ $P < 0.0001$ ] in the 10 mg group. Similarly, the extracts of outdoor air samples from Taiyuan with particles  $\geq 3.3 \mu\text{m}$  induced skin papillomas with an incidence of 9/30 [ $P < 0.0431$ ] in the 5 mg group, whereas the extracts with particles  $< 3.3 \mu\text{m}$  induced skin papillomas with an incidence of 25/39 [ $P < 0.0001$ ] in the 5 mg group and 22/39 [ $P < 0.0001$ ] in the 10 mg group. The extracts of outdoor air samples from Xuanwei with particles  $\geq 3.3 \mu\text{m}$  induced skin papillomas with an incidence of 17/36 [ $P = 0.0005$ ] in the 5 mg group and 24/39 [ $P < 0.0001$ ] in the 10 mg group, whereas the extracts with particles  $< 3.3 \mu\text{m}$  induced skin papillomas with an incidence of 25/39 [ $P < 0.0001$ ] in the 5 mg group and 26/39 [ $P < 0.0001$ ] in the 10 mg group. The incidence of skin papilloma in the control group treated with acetone plus TPA was 4/38. For all three locations, the first skin papilloma was observed at 10 weeks for particles  $< 3.3 \mu\text{m}$ .

In a 24-month study, Hueper et al. (1962) collected atmospheric dusts from eight cities in the USA and extracted the PM with benzene. The crude benzene extract was further fractionated to obtain oxygenated and aliphatic fractions. Groups of male and female C57BL/6 mice and C3H mice received skin applications of the oxygenated or aliphatic fractions. [The limited reporting and poor design of the study, the weak response, and the lack of vehicle controls rendered this study inadequate for the evaluation.]

Groups of 20 male and 20 female BALB/c mice (age, 7–9 weeks) received skin applications of dichloromethane extracts of outdoor air particulates in 0.2 mL of acetone from four sites in Shanghai (samples A, E, I, or K) at a single

dose of 5 mg or at a dose of 2 mg per day for 5 days (cumulative dose of 10 mg) ([Zhao et al., 2003](#)). Vehicle control mice received 0.2 mL of acetone. From 1 week after initiation, the tumour promoter TPA was applied topically at 2 µg in 0.2 mL of acetone per mouse, twice a week for 30 weeks. None of the extracts produced skin cancers; however, all extracts initiated skin papillomas in 5% (1/20) to 17% (3/18) of male mice at the cumulative dose of 10 mg. Sample E initiated papillomas in male and female mice at all doses. Only sample E induced skin papillomas in female mice (1/20). The incidence of skin papillomas in male mice at the cumulative dose of 10 mg was significantly increased [9/75,  $P = 0.0179$ ] compared with male controls (0/40). No skin papillomas were observed in female controls (0/40).

[Matsumoto et al. \(2007\)](#) investigated the role of aryl hydrocarbon receptor (AhR) signalling on carcinogenicity of airborne PM. Groups of 17 female [Sv/129] AhR<sup>+/+</sup> (wild-type) and 15 female [Sv/129] AhR<sup>-/-</sup> (knockout) mice (age, 6–8 weeks) were treated with extracts of outdoor air particulates (from the city of Sapporo, Japan) by skin application. Extracts were shown to contain B[a]P and other PAHs. [The Working Group noted that the samples were stored from 1988 to 2007.] Mice received skin applications of extract (6.4 mg in 200 µL of acetone) once a week until a skin tumour appeared, and mice were necropsied after experimental week 58. In AhR<sup>+/+</sup> mice, the first tumour appeared after 29 weeks of treatment, and after 58 weeks, 8/17 (47%) AhR<sup>+/+</sup> mice bore squamous cell carcinomas. No skin tumours developed in AhR<sup>-/-</sup> mice (0/15). The difference in skin tumour incidence between AhR<sup>+/+</sup> and AhR<sup>-/-</sup> mice was significant ( $P < 0.01$ ). [The Working Group noted the lack of clean air control groups.]

### 3.3.4 Intravenous injection

[Leiter et al. \(1942\)](#) collected PM from outdoor air at six sites in the USA: in Chelsea, Massachusetts, in Pittsburgh, Pennsylvania, and at four sites in a road tunnel that connects New York City and Jersey City. Groups of 20–30 strain A mice (age, 2–3 months) [sex was not reported] were injected intravenously (tail vein) with the unextracted dusts suspended in saline (2.5 mg in 0.25 mL). Control mice were untreated. Four months after treatment, the incidence of pulmonary tumours in all dust-treated mice (15/138, 11%) [groups of treated mice were combined] was not increased compared with controls (3/20, 15%). [The methods and results of this older study were poorly reported. The study duration was short. No detailed information on outdoor air pollutants was reported.]

### 3.3.5 Intraperitoneal injection

[Heussen et al. \(1996\)](#) evaluated the carcinogenicity of outdoor air particulates collected over 2 years at a non-industrial site (Wageningen, The Netherlands). The particulates were extracted with benzene, benzene was removed by evaporation, and the extract was suspended in propylene glycol. Male newborn NMRI mice were injected intraperitoneally on days 1 (5 µL), 8 (10 µL), and 15 (20 µL) after birth with propylene glycol vehicle ( $n = 52$ ), 1.95 mg of extract ( $n = 49$ ), or 3.9 mg of extract ( $n = 47$ ). No deaths occurred before weaning. [There was no significant difference in mortality between groups.] Mice were necropsied at experimental week 52, and lungs and liver were examined for tumours. Incidences of bronchioloalveolar tumours in mice treated with 3.9 mg of extract (17/47, 36.2%) and 1.95 mg of extract (10/49, 20.4%) were not significantly different from the incidence in vehicle controls (16/52, 30.8%). [The Working Group noted the short duration of the study. No detailed information on outdoor air pollutants was reported.]

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