3. Studies of Cancer in Experimental Animals[1]

3.1 Inhalation

3.1.1 Rat

Sixteen female Sprague-Dawley rats, 11 weeks of age, were exposed to untreated beech wood dust (approximately 70% of the dust particles with a maximal diameter of about 10 μm and 10–20% of the particles with a diameter of about ≤ 5 μm) at 25 mg/m^3 for 6 h per day on five days per week for 104 weeks. Surviving animals were killed and autopsied; only the nasal cavities and respiratory tract were examined histologically. No respiratory tract tumour and no squamous metaplastic or dysplastic lesions were found among the 15 surviving animals. About 50% of the animals were reported to have tumours outside the respiratory tract, but the incidence was said not to differ from that in untreated controls (Holmström *et al.*, 1989a). [The Working Group noted the small number of animals and the inadequate reporting of the tumours outside the respiratory tract.]

Fifteen female Wistar rats, four weeks of age, were exposed to beech wood dust (mass median aerodynamic diameter, 7.2 μm; geometric standard deviation, 2.2 μm) at 15.3 ± 13.1 mg/m^3 for 6 h per day on five days per week for six months and were observed for up to 18 months, when survivors were killed. At autopsy, the animals were examined grossly, and

[1]The Working Group was aware of studies in progress in which rats are exposed to oak wood dust by inhalation (IARC, 1994b).

Table 30. Community-based case-control studies of cancer of the digestive tract

Reference	Country	Sex	Cases/ controls	Source of information on exposure	Exposure to which relative risk applies	OR/RR	95% CI or *p*	Comments
Stomach cancer								
(i) Exposure to wood dust								
Siemiatycki *et al.* (1986); Siemiatycki (1991)	Canada	M	156/1524	Interviews evaluated by panel of chemists and industrial hygienists	'Substantial' exposure			
					< 16 years	1.2	[0.6–2.6]	
					≥ 16 years	1.9	[1.0–3.7]	
			251/2397		Update	1.1	[0.7–1.7]	
(ii) Occupational group								
Dockerty *et al.* (1991)	New Zealand	M	1016/19 042	Tumour register	Foresters and loggers	1.8	1.0–3.3	Current or most recent occupation; adjusted for age, socioeconomic level and smoking
					Cabinet-makers	1.4	0.7–2.8	
					Wood preparation, pulp and paper workers	0.8	0.4–1.7	
					Carpenters	0.8	0.5–1.2	
Kawachi *et al.* (1989)	New Zealand	M	1014/18 890	Tumour register	All woodworkers	1.2	0.9–1.6	Same population and methods as Dockerty *et al.* (1991)
González *et al.* (1991)	Spain	MF	354/354	Interviews	Any wood dust-exposed job	1.0	0.4–2.3	Cases were adenocarcinomas; controls matched on age, sex and residence; adjusted for diet and socioeconomic status
					Forestry	1.0	0.3–3.6	
					Wood and paper production	0.5	0.2–1.7	
					Furniture/wood manufacture	1.8	0.5–6.9	
Colon cancer								
(i) Exposure to wood dust								
Spiegelman & Wegman (1985)	USA	MF	370/1861	Interviews Job–exposure matrix	Wood (men)	1.3	$p = 0.24$	Adjusted for age
					Wood (women)	1.5	$p = 0.07$	
Siemiatycki (1991)	Canada	M	497/2056	Interviews evaluated by panel of chemists and industrial hygienists	Any exposure to wood dust	1.0	[0.8–1.2]	
					'Substantial' exposure	0.9	[0.7–1.2]	

Table 30 (contd)

Reference	Country	Sex	Cases/ controls	Source of information on exposure	Exposure to which relative risk applies	OR/RR	95% CI or *p*	Comments
Colon cancer (contd)								
Exposure to wood dust (contd)								
Peters *et al.* (1989)	USA	M	106/106	Interviews	Wood dust	2.1 1.5 3.6	0.5–8.5 0.3–3.6 0.6–21	Right side of colon Transverse and descending colon Sigmoid colon Subjects aged 25–44; adjusted for age and education
(ii) Occupational group								
Brownson *et al.* (1989)	USA	M	1993/9965	Tumour register	Carpenters	0.9	0.6–1.4	Adjusted for age
Fredriksson *et al.* (1989)	Sweden	M	156/306	Questionnaire	Lumbermen Pulp workers Sawmill workers	0.7 0.7 0.5	0.4–1.0 0.3–1.6 0.3–0.9	Adjusted for age and physical activity. Cases were adenocarcinomas.
Kawachi *et al.* (1989)	New Zealand	M	2043/17 861	Tumour register	All woodworkers	0.7	0.5–0.9	Same population and methods as Dockerty *et al.* (1991)
Arbman *et al.* (1993)	Sweden	M	51/512	Questionnaire	Carpenters Forestry workers Sawmill workers	0.5 0.9 1.2	[0.1–2.7] [0.4–2.0] [0.4–3.3]	Cases were adenocarcinomas
Rectal cancer								
(i) Exposure to wood dust								
Spiegelman & Wegman (1985)	USA	MF	551/1861	Interviews and a job–exposure matrix	Wood (men) Wood (women)	1.1 1.5	$p = 0.69$ $p = 0.04$	Colon and rectum combined; adjusted for age

Table 30 (contd)

Reference	Country	Sex	Cases/ controls	Source of information on exposure	Exposure to which relative risk applies	OR/RR	95% CI or *p*	Comments
Rectal cancer (contd)								
Exposure to wood dust (contd)								
Siemiatycki (1991)	Canada	M	257/1299	Interviews evaluated by panel of chemists and industrial hygienists	Any exposure to wood dust 'Substantial' exposure	1.0 1.3	[0.7–1.4] [0.8–2.0]	
Peters *et al.* (1989)	USA	M	41/41	Interviews	Wood dust	9.4	2.0–45	Subjects aged 25–44; adjusted for age and education
(ii) Occupational group								
Kawachi *et al.* (1989)	New Zealand	M	1376/18 528	Tumour register	All woodworkers	1.1	0.8–1.4	Same population and methods as Dockerty *et al.* (1991)
Arbman *et al.* (1993)	Sweden	M	48/512	Questionnaire	Carpenters Forestry workers Sawmill workers	0.9 0.5 0.4	[0.3-3.2] [0.2-1.5] [0.1-1.9]	Cases were adenocarcinomas

OR, odds ratio; RR, relative risk; CI, confidence interval; M, male; F, female

lungs, nasal cavities, livers, spleens and kidneys were examined histologically. No respiratory tract tumour was found, and the incidence of tumours outside the respiratory tract did not differ significantly from that in untreated controls (Tanaka *et al.*, 1991). [The Working Group noted the small number of animals in each group and the short exposure.]

3.1.2 Hamster

One group of 12 and one group of 24 male Syrian golden hamsters, 10 weeks of age, were exposed to beech wood dust (approximately 70% of the dust particles with a maximal diameter of about 10 μm and 10–20% of the particles with a diameter of about ≤ 5mm) at 15 and 30 mg/m^3 for 6 h per day on five days per week for 36 and 40 weeks, respectively. At these times, the survivors were killed and autopsied; nasal cavities, respiratory tracts, livers and kidneys were examined histologically. No respiratory tract tumour was reported in the 12 animals exposed to 15 mg/m^3, but 1/22 hamsters exposed to 30 mg/m^3 had an unclassifiable infiltrating malignant nasal tumour [not significant], and one other animal in this group had cuboidal metaplasia with mild dysplasia of the nasal epithelium (Wilhelmsson *et al.*, 1985a,b). [The Working Group noted the short duration of the experiment.]

3.2 Intraperitoneal injection

Rat: In a preliminary report on a study of the carcinogenic activity of various fibrous and granular dusts, one group of female Wistar rats [initial number unspecified], eight weeks of age, received three weekly intraperitoneal injections of beech wood dust [size of dust particles unspecified and total dose ambiguously reported as 250 or 300 mg/animal] suspended in 0.9% sodium chloride solution (50 mg wood dust/ml). The surviving animals were killed 140 weeks after the first treatment [survival time not clearly specified]. At post-mortem examination of the abdominal cavity, no mesothelioma or sarcoma was found in the 52 rats examined (Pott *et al.*, 1989). [The Working Group noted the limited reporting of the experimental details and that UICC chrysotile asbestos induced mesotheliomas when similarly administered in parallel groups.]

3.3 Administration with known carcinogens or other modifying factors

3.3.1 Rat

Four groups of 16 female Sprague-Dawley rats, 11 weeks old, were exposed by inhalation in chambers to (i) air (controls); (ii) about 25 mg/m^3 untreated beech wood dust (approximately 70% of the dust particles with a maximal diameter of about 10 mm and 10–20% of the particles with a diameter of about ≤ 5 mm); (iii) 12.4 ± 1.1 ppm [14.9 ± 1.3 mg/m^3] formaldehyde; or (iv) beech wood dust (as above) plus 12.7 ± 1.0 ppm [15.2 ± 1.2 mg/m^3] formaldehyde for 6 h per day on five days per week for 104 weeks. No difference in the mortality rates was reported between the groups at any time during the study [mortality rates and statistical test unspecified]. Surviving animals were killed and autopsied; only nasal cavities and respiratory tracts were examined histologically. One respiratory tract tumour, a nasal squamous-cell carcinoma, was

found in the group exposed to formaldehyde alone. Pronounced squamous metaplasia, with or without keratinization of the nasal epithelium at the level of the naso- and maxillary turbinates, was found in 9/16 rats exposed to formaldehyde and in 8/15 rats exposed to wood dust plus formaldehyde. In addition, pronounced squamous metaplasia accompanied by dysplasia of the nasal epithelium occurred in 1/16 rats exposed to formaldehyde and in 4/15 rats exposed to wood dust plus formaldehyde. No such metaplastic or dysplastic nasal cavity lesions were encountered in any of the controls or in rats exposed to wood dust alone. Tumours outside the respiratory tract were reported to affect about 50% of the animals, but this incidence was said not to differ from that in controls (Holmström *et al.*, 1989a). [The Working Group noted the small number of animals in each group and the inadequate reporting of tumours outside the respiratory tract.]

Two groups of 20 male Wistar rats, four weeks old, were exposed by inhalation in chambers to clean air (controls) or to beech wood dust (mass median aerodynamic diameter, 7.2 μm; geometric standard deviation, 2.2 μm) at 15.3 ± 13.1 mg/m^3 for 6 h per day on five days per week for six months (total exposure, 666 h). Immediately thereafter, five rats from each group were exposed to sidestream cigarette smoke (from 10 cigarettes per day) at 10.2 mg/m^3 [standard deviation unspecified] for 2 h per day on five days per week for one month (total exposure, 40 h). After clearance periods of 12 months for rats exposed only to wood dust and 11 months for rats exposed to wood dust plus cigarette smoke (i.e. 18 months after the start of the experiment), all rats, including the controls, were killed. At autopsy, animals were examined grossly, and lungs, nasal cavities, livers, spleens and kidneys were examined histologically. No intercurrent mortality occurred, and no tumours of the nose or of other segments of the respiratory tract were observed. The incidence of tumours outside the respiratory tract did not differ significantly from that in untreated controls (Tanaka *et al.*, 1991). [The Working Group noted the small number of animals in each group and the relatively short treatment and observation periods.]

3.3.2 Hamster

Two groups of 12 male Syrian golden hamsters, about 10 weeks old, were exposed by inhalation in chambers to air (controls) or to untreated beech wood dust (about 70% of the particles had a maximal diameter of about 10 μm, and 10–20% of the particles had a diameter of about ≤ 5 μm) at 15 mg/m^3 (range, 10–20 mg/m^3) for 6 h per day on five days per week for 36 weeks. A further two groups of hamsters were treated similarly but were also given *N*-nitrosodiethylamine (NDEA) at 1.5 mg/animal by subcutaneous injection, weekly for the first 12 consecutive weeks. All survivors were killed at week 36. No tumours of the nose were observed in 12 hamsters exposed to wood dust alone. Tracheal squamous-cell papillomas occurred in 1/7 controls, 0/8 hamsters exposed to wood dust alone, 3/8 hamsters treated with NDEA alone and 4/8 hamsters exposed to wood dust and NDEA (Wilhelmsson *et al.*, 1985a,b). [The Working Group noted the short duration of the experiment, the small numbers of animals in each group, the absence of data on mortality rates and the high losses of animals and tissues due to cannibalism.]

Two groups of 24 male Syrian golden hamsters, about 10 weeks old, were exposed by inhalation in chambers to air (controls) or to untreated beech wood dust (about 70% of the particles with a maximal diameter of about 10 μm, and 10–20% of the particles with a diameter of about ≤ 5 mm) at 30 mg/m^3 (range, 25–35 mg/m^3) for 6 h per day on five days per week for 40 weeks. A further two groups of hamsters were treated similarly but were also given 3.0 mg/animal NDEA by subcutaneous injection, weekly for the first 12 consecutive weeks. The survivors were killed at week 40. The death rate was very high in all groups and significantly higher in the two NDEA-treated groups than in the two other groups ($p < 0.05$; Fisher's exact test) [death rates not further specified]. One of the 22 hamsters exposed to wood dust alone had an unclassifiable, malignant, infiltrating nasal tumour, and another hamster in this group had focal cuboidal metaplasia with mild dysplasia of the nasal epithelium. No neoplastic, dysplastic or metaplastic changes occurred in the respiratory tracts of controls. The types and incidences of respiratory tract neoplasms and dysplasia in the groups exposed to NDEA and to wood dust plus NDEA were as follows: nasal tumours (papillomas and adenocarcinomas), 10/22 (46%) and 11/21 (52%); laryngeal and/or tracheal tumours (papillomas), 10/19 (53%) and 11/18 (61%); lung tumours (adenocarcinoma), 0/19 and 1/18 (6%); and nasal dysplasia, 8/18 (44%) and 4/17 (24%). The incidences of these respiratory tract lesions did not differ significantly between these two groups [Fisher exact test] (Wilhelmsson *et al.*, 1985a,b).

3.4 Skin application of wood dust extracts

Mouse: Four groups of 70 young female NMRI mice [age unspecified], weighing 25–30 g, received skin applications of a mutagenic fraction of a methanol extract of dust from untreated, semi-dry beech wood in 30 μl acetone on a 1–2-cm^2 shaven area of the lower back twice a week for three months. The freshly prepared, weekly doses of the fraction were equivalent to 2.5, 5, 7.5 and 10 g wood dust per mouse. Five similar groups of mice served as controls: one was treated with acetone on the shaven skin, one was shaved only and one was neither shaved nor treated with acetone; two positive control groups were treated with 5 and 10 μg benzo[*a*]pyrene, respectively. All mice were observed until they died naturally or were killed to avoid severe suffering. The survival of treated mice was not significantly different from that of untreated mice ($p = 0.571$; Mann-Whitney U test). The positive controls and mice treated with the mutagenic wood dust extract developed precancerous skin lesions (epithelial hyperplasia and hyperkeratosis) and benign and malignant tumours of the skin and mammary glands just beneath the treated skin area (see Table 31). Comparison of the mice treated with wood dust with the negative controls was reported to show a significant overall carcinogenic effect ($p < 0.01$; χ^2 test) (Mohtashamipur *et al.*, 1989b). [The Working Group noted that a significant trend is observed for skin tumours, whether or not the analysis includes the keratoacanthoma and the papillary cystadenoma. The trend test for mammary tumours is significant when mammary gland adenocarcinomas, the adenoacanthoma and the mixed mammary tumours are grouped, and it is marginally significant when only the adenocarcinomas and the adenoacanthoma are considered.]

Table 31. Results of application to the skin of mice of mutagenic fractions of a methanol extract of dust from untreated, semi-dry beech wood, with negative and positive controls

Tumour	Negative controls			Extract (g)				Benzo[*a*]pyrene (μg)	
	Untreated ($n = 43$)	Shaven ($n = 44$)	Shaven, acetone-treated ($n = 42$)	2.5 ($n = 43$)	5.0 ($n = 50$)	7.5 ($n = 46$)	10.0 ($n = 49$)	5 ($n = 43$)	10 ($n = 42$)
Skin squamous-cell carcinoma	-	-	-	1	-	-	1[a]	1	15
Skin squamous-cell papilloma	-	-	-	1	1	6	5[a]	2	5
Skin keratoacanthoma	-	-	-	-	-	1	-	-	2
Skin papillary cystadenoma	-	-	-	-	1	-	-	-	-
Sebaceous gland adenoma	-	-	-	-	-	-	-	2	-
Mammary gland adenocarcinoma	-	-	-	-	4	3	2[b,c]	1	1
Mammary gland adenoacanthoma	-	-	-	-	-	-	1[b,c]	-	-
Mammary gland mixed tumours	-	-	-	-	-	-	2[b]	-	-
Fibrosarcoma	-	-	-	-	-	1	-	-	-
Haemangiosarcoma	-	-	-	-	1	-	-	-	-
Neurofibrosarcoma	-	-	-	-	1	-	-	-	-
Lymphoma	-	-	-	-	-	-	1	-	-
Anaplastic carcinoma	-	-	-	-	1	-	-	-	-
Precancerous skin lesions	-	1	2	2	4	8	6	13	18

Adapted from Mohtashamipur *et al.* (1989b), numbers of animals given are effective numbers

[a] [$p < 0.01$; Cochran-Armitage test for trend] where comparisons are made for 0 (acetone-treated controls), 2.5, 5.0, 7.5 and 10 g extract groups, including both squamous-cell carcinomas and papillomas, or papillomas alone

[b] [$p < 0.02$; Cochran-Armitage test for trend if included in the analysis] where comparisons are made for 0, 2.5, 5.0, 7.5 and 10 g extract groups, including mammary gland adenocarcinoma, adenoacanthoma and mixed mammary gland tumours

[c] [$p < 0.06$; Cochran-Armitage test for trend] where comparisons are made for 0, 2.5, 5.0, 7.5 and 10 g extract groups and only mammary gland adenocarcinoma and adenoacanthoma are considered

3.5 Experimental data on wood shavings

It has been suggested in several studies that cedar wood shavings, used as bedding for animals, are implicated in the prominent differences in the incidences of spontaneous liver and mammary tumours in mice, mainly of the C3H strain, maintained in different laboratories (Sabine *et al.*, 1973; Sabine, 1975). Others (Heston, 1975) have attributed these variations in incidence to different conditions of animal maintenance, such as food consumption, infestation with ectoparasites and general condition of health, rather than to use of cedar shavings as bedding. Additional attempts to demonstrate carcinogenic properties of cedar shavings used as bedding material for mice of the C3H (Vlahakis, 1977) and SWJ/Jac (Jacobs & Dieter, 1978) strains were not successful. In none of these studies were there control groups not exposed to cedar shavings.