Int Arch Occup Environ Health (2001) 74: 109–118

ORIGINAL ARTICLE

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Carcinogenicity assays of wood dust and wood additives in rats exposed by long-term inhalation

Received: 23 May 2000 / Accepted: 13 September 2000

Abstract ***Objective:*** This study evaluates whether wood dust and/or wood preservatives develop a carcinogenic potential against the tissues of the airways of rats. ***Methods:*** The formation of tumors of the respiratory tract after exposure to wood dust was studied in six groups of approximately 60 female Fischer 344 rats exposed by long-term inhalation to mean concentrations of (1) 18 mg/m^3 of untreated oak wood dust, (2) wood preservatives containing ca. 1 μg/m^3 lindane and 0.2 μg/m^3 of pentachlorophenol (PCP) in the exposure air, or lindane and 18 μg/m3 of PCP (group lindane/PCP vapors, and group oak wood treated with lindane/PCP), (3) 21 or 39 μg/m^3 of sodium dichromate (calculated as CrO_3, group chromate aerosol and group oak wood with chromate), and 72 μg/m^3 of *N*-nitrosodimethylamine vapors as positive control. The negative control group consisted of 115 animals (sham-exposed). ***Results:*** Tumors of the nasal cavity developed in two rats exposed to chromate aerosol or in combination with wood dust (2/102, 2%). Malignant tumors of the lower respiratory tract were induced only in exposed groups of rats (three adenocarcinomas of the lung and four bronchiolar lung carcinomas, 7/254, 2.8%). More respiratory tract tumors were observed in rats exposed to chromate or wood with chromate (5/102, 5%), also in groups exposed to oak wood dust (oak untreated, oak + chromate, oak + lindane/PCP; together 5/155, 3.2%). Analysis of 'unpreserved' oak wood dust revealed up to 5 μg/m^3 of chromate. When this exposure was taken into account, eight of nine animals with respiratory tract tumors (including nasal cavity) had exposure to chromate, while only one tumor occurred in the group lindane/PCP. Otherwise the incidence of systemic tumors was increased in animals exposed to lindane/PCP, due in particular to a significantly increased incidence of liver tumors (OR = 3.7; 1.24–11.3; $P = 0.019$). Fatal (mucoepidermoid) tumors were induced by *N*-nitrosodimethylamine (NDMA) in the positive control (14/46, 30%). No such tumors of the respiratory tract were observed in the negative control. ***Conclusions:*** Tumors in the respiratory tract were found only in exposed animals, predominantly in the groups which inhaled oak wood dust and chromate stain. Chromate may play a decisive role for the etiology of tumors of the nasal cavity in wood workers. This assumption should be supported by further dose-response studies.

Key words Wood dust · Wood preservatives · Inhalation · Rats · Carcinogenicity

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Introduction

In 1965, Macbeth reported on 20 patients with nasal adenocarcinomas in the area of High Wycombe in England [11], of whom 15 had worked in the furniture industry. Between 1967 and 1972, more detailed information was provided [1, 2], and in 1969, the tumors were recognized as an occupational disease [2]. Numerous epidemiological and toxicological studies of the carcinogenic effect of wood dusts were subsequently published, which basically confirmed the results ob-

tained in England. The carcinogenic mechanism, however, could not be elucidated, and the question remained whether it was the wood dusts themselves or wood preservatives that were responsible. Although the evidence was lacking, the carcinogenic activity was attributed by a working group convened by IARC to natural wood dusts, because increased incidences of nasal adenocarcinomas were observed in various occupational groups in various countries over different periods, and because single exposures to the preservative chemicals did not result in relative risks of the same order of magnitude as did exposure to wood dusts [6].

Consequently, classification and preventive measures were focused on the dusts. In 1985, oak and beech wood dusts were classified as carcinogenic to humans by a Senate commission of the German Society for the Advancement of Scientific Research (Deutsche Forschungsgemeinschaft) in Germany, and other wood dusts as suspected carcinogens [12]. In 1999, hardwood dusts were classified as carcinogenic agents by the Commission of the European Union [21], on the basis of the IARC classification of the carcinogenic potential of wood dusts in 1995 [6].

Early evidence showed that oak and beech wood dusts play a special role in carcinogenesis [1, 26, 30], and that fact is accepted in occupational medicine worldwide. As the disease has occurred predominantly among wood-working artisans and less frequently in the wood-working industry [30], wood dusts alone may not be the only cause of tumorigenesis. The tumor incidence differs not only between workers in industry and artisans but also regionally. Strongly increased risks were demonstrated in many European countries but not in the USA, where a lower risk was found [3]. In Germany, too, considerable regional differences in the incidence rates were seen [30]. Since such differences may be accounted for by differences in the wood preservatives used, we considered it indispensable to investigate the effects of wood preservatives in greater detail.

A research project was initiated to evaluate exposures occurring in the wood industry systematically, and to understand better the mode of formation of adenocarcinomas of the nose. Epidemiological studies were excluded, as the number of noxious agents was too great, and neither employers nor workers know exactly what type of agents were used, since although the type of wood and additives such as varnish, stains, glues and wood preservatives that are used are known, often the type of pretreatment of the wood is unknown [27].

Long-term experiments in which animals were exposed by inhalation seemed to be a suitable method, provided that the cancers observed in humans could be induced in laboratory animals under comparable conditions. As the number of agents was too large for systematic testing in such experiments, short-term studies of toxicity and genotoxicity were carried out [30] to serve as a basis for the design of our long-term experiments.

Material and methods

Selection of agents to be tested

On the basis of our previous results [30], the following hazardous agents were selected for long-term study:

- Oak wood dust, because it was the most genotoxic of the woods tested; the genotoxicity was not tested directly with wood dusts, but with cyclohexane extracts [28].
- Wood preservatives containing lindane and pentachlorophenol (PCP), because lindane was genotoxic in human nasal cells from the central turbinate [17, 18], the mucosal area in which cancer arises. These wood preservatives have been used in Europe for decades to pretreat wood, including oak and beech, without the knowledge of the processing industry [26].
- Chromate, which is genotoxic, induces prolonged nasal clearance and, when used in wood preservatives, might contribute to the formation of dysplasia. Chromate has frequently been used in the wood-processing industry, particularly as potassium dichromate in stains but also as a fixing agent in wood preservatives. Stained furniture is made largely from oak and beech as they contain enough tannic acid to allow chemical staining.

Groups of animals

For technical reasons (available cage size), female Fischer 344 rats were chosen for the experiments. As rats inhale virtually all their air through the nose, the major portion of inhaled dust is deposited within the nose. Seven groups were formed (Table 1), the sham-

Table 1 Study design (*PCP* pentachlorophenol)

Exposure	Number of applications	Number of rats exposed			Animal number under life-long risk		
		Total	25 Weeks	Killed	25 Weeks exposure	Continuous exposure	Total
Oak wood dust, untreated	573	60	24	9	15	36	51
Oak wood dust treated with lindane- and PCP-containing wood preservative	573	60	24	9	15	36	51
Oak wood dust treated with chromate stain	573	61	24	8	16	37	53
Chromate stain aerosol	557	58	24	9	15	34	49
Lindane and PCP aerosol and vapor from lindane and PCP-containing wood preserving solution	557	59	24	9	15	35	50
N-Nitrosodimethylamine vapor	539	58	23	12	11	35	46
Sham-exposed	573/539	115	48	19	29	67	96

exposed control group being twice the size of the others for statistical reasons. A group exposed to *N*-nitrosodimethylamine (NDMA) served as positive controls, because rats exposed to certain nitrosamines are known to develop tumors of the nasal cavity [8, 10]. Exposure and animal handling were performed according to the German Law on the Protection of Animals.

The initial maximal size of approximately 60 animals per group had to be reduced as their body weights increased owing to the cage size. Thus, after 25 weeks, the groups were randomly divided into two, consisting of ca. 36 animals that continued to be exposed and approximately 24 (for exact numbers, see Table 1) that were no longer exposed. Of the latter, two to five animals were selected randomly from each group and killed after 26, 34 or 45 weeks, except that those exposed to NDMA were killed after 33, 74 or 82 weeks because of the very low dose and the calculable cancer risk [9]. As examinations revealed no histological changes, the remaining animals exposed for only 25 weeks were allowed to live until their natural death, thus allowing the development of any exposure-related tumors. The animals that continued to be exposed were also allowed to die naturally until only three or fewer animals were left, when exposure was terminated. Given mean daily exposure times of 4–5 h, the total duration of exposure of the individual groups was 2,430–2,580 h.

Production of wood dust

Oak wood was provided by a plant associated with the Holz-Berufsgenossenschaft (statutory accident prevention and insurance institution in wood industry). In view of the known possible contamination of wood with lindane and PCP [27], the wood was analyzed for these agents at the Professional Associations Institute for Occupational Safety (BIA), according to documented methods (N. Lichtenstein, personal communication, 1999; Gas chromatographic/electron capture detection; Analytische Methoden zur Prüfung Gesundheitsschädlicher Arbeitsstoffe – Luftanalysen, Deutsche Forschungsgemeinschaft, VCH, Weinheim 1992). Lindane and PCP were not detected in the woods (detection limits, 0.2 μg/g, and 0.4 μg/g respectively).

The dust was generated by rotating sanding disks (diameter, 125 mm, type Siawat FC 1727, P 400, Kaindl-Schleiftechnik or diameter, 125 mm, type PS 22K, article No. 2215100, Wolfcraft; abrasive agent: corundum). The abrasive agents were free of quartz and chromium. The sanding disks were driven by standard angle sanders (Stayer SA 115, max 450 W; diameter, 115 mm; max. 10,000 min^{-1}). The number of revolutions of the three dust generators was adjusted to provide 5,000–6,000 min^{-1} by a circular core transformer and series resistance. The particle size of the dust samples collected on a seven-step impactor (Andersen) and filters from the exposure chambers ranged between 0.4 and 10 μm, the majority being in the range 2–7 μm. A cylindrical PVC housing was mounted around the sanding disk on top of the angle sander, which was fixed in a horizontal position, with tangential pipes for the air supply and waste air and a removable lid containing four vertical boreholes with sockets for fixation of untreated or pretreated pieces of wood (6 × 6 × 170 mm). Tightly sealed glass tubes placed over the wood rodlets allowed control of the sanding process. The mean daily amount of wood required for the 4–5-h exposure was 5–10 g per generator. The dust concentration to which the animals were exposed was kept constant throughout exposure by controlling the air supply and waste air exhaust measurements at various sites and intervals during exposure and daily cyclic rearrangement of the wire-grating cages within the boxes. Dust samples were collected daily from fiberglass filters in all boxes. The air supply was determined by measuring the flow in mainstream and sidestream air with bar gas meters (Elster, GA, 0.04–6 m^3/h). Control measurements and calculations were carried out by CO_2 dilution before exposure. Air and dust were supplied to the inhalation chambers through short glass pipes of a diameter of 25 mm with spherical cuts for flexible connections. Thus, the deposition of coarse-grained dust particles could be observed directly. An equilibrium was reached between deposition and transport of wood dust by the rapid flow.

Chromate stain

The stain (Arti-Paracidol-Nachbeize No. 265, Arti Holzlacke & Beizen, Wuppertal-Barmen), provided on 27 May 1993 and analyzed by BIA [13], was found to contain 9.7% sodium chromate (calculated as CrO_3). In order to produce a chromate stain aerosol, a 0.01% solution was prepared without ammonia in an ultrasound vaporizer (Devilbiss Ultraneb 99, 1.63 MHz, with additional digital flow meter for reproducible adjustment of the generator). A scavenging air flow of 25 l/h (Fischer & Porter, glass flow meter tube 1/8–16 G5/81, 0–60 l/h) drove the aerosol towards the mixing tube of the main air pipe. The mean daily requirement of chromate stain was 10–15 ml solution (13.3 ml; standard sampling deviation, SD = 4.6).

The Department of Biophysics and Medical Radiation Physics (German Cancer Research Center, DKFZ) analyzed wood dust samples collected daily and chromium aerosol collected from fiberglass filters after 8–14 days, for chromium detection by neutron activation analysis [23]. The measurements yielded 2,186 μg Cr as CrO_3 calculated per gram of treated wood (SD = 790), resulting in a concentration of 39.4 μg Cr as CrO_3 per m^3 of exhaust air at a dust concentration of 18.8 mg/m^3.

Lindane- and PCP-containing wood preservative and NDMA

The lindane- and PCP-containing wood preservative was a solvent-containing preparation (Fertighaus-Avenarol 8214, colorless, IfBt mark of conformity PA V-733; manufacturer R. Avenarius KG, Gau-Algesheim) produced between 1982 and 1987, and collected at a sawmill in Rhineland-Palatinate. The preparation contained 0.3% lindane and 3% PCP in addition to hydrocarbons used as solvent. The volatile compounds were vaporized in cylindrical glass vessels with fitted glass lids equipped with inlet and outlet pipes with spherical fitting and Teflon shut-off valves. An adjustable scavenging air flow (Fischer & Porter, glass precision flow rotor tube equipped with ruby floating balls; 1/16–12 G5/81 for 0–14 l/h NDMA vapors and 1/8–12 G5/81 for the lindane/PCP-containing Fertighaus-Avenarol wood preservative) carried the volatilized vapor from a weighing bottle to the main air flow. No additional heating above ambient temperature (20–23 °C) was needed for NDMA (maximum saturation concentration, 8,500 mg/m^3), whereas the hydrocarbon-containing wood preservative had to be heated to 30–50 °C in the evaporating vessel. Samples of PCP and lindane were collected in sampling tubes (ORBO 615, Supelco) and analyzed in a Hewlett Packard gas chromatograph 5890, series II with halogen-specific ECD. The wood preservative was applied to wood according to the manufacturer's instructions: freshly cut oak wood rods (6 × 6 × 17 mm) were dipped into the liquid solution for 1 h and then removed from the glass cylinders with fitted lids, and excess solution was allowed to drain off. The impregnated rods were kept in a partly closed glass vessel for 1 week, and two to four rods were then mounted in the dust generator. The wood particles were weighed before and after treatment, and the mean absorption of wood preservative per gram of oak wood was calculated by comparison. The chromate stain was prepared as a 2% solution containing aqueous ammonia according to the manufacturer's instructions. The wood rods were impregnated by dipping them into a cold solution, and were then allowed to drain and dry in a glass container with a loosely fitting lid that was placed in a closed vessel for 1 week. These rods were also weighed several times before and after treatment. Figure 1 shows a block diagram of an exposure chamber for two of eight units.

Independent control of exposures

To validate the measured parameters during inhalation, we determined exposure to wood dust and chromate as mass concentration in ambient air after each experimental series. Additionally, after the end of the exposure period the empty cages were sampled with a portable pump used for human sampling (Ametak, type Alpha-1

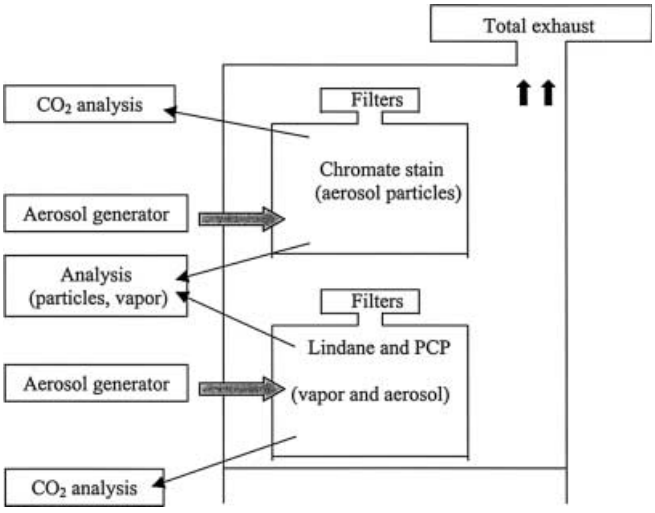

Fig. 1 Scheme of apparatus for exposure of rats to wood dust and preserving agents: examples of groups exposed to chromate stain aerosol and lindane/pentachlorophenol (PCP) vapors

with sampling system GSP). The procedures for sampling and calculation of wood dust corresponded to BGI No. 505–41 [13] and that for detection of chromate aerosol to BIA No. 6665 [14].

Exposure

The cages of all seven experimental groups were rotated daily within their exposure boxes, according to a scheme documented for each group and day, in order to balance flow-related local differences in the concentration within each box. During inhalation, the flow rate of fresh air, the CO_2 concentration in each box and the concentrations of dusts, vapors and aerosols were determined regularly, and the doses of dust and aerosol kept within the desired range by measuring deposits on the filters. The activity of animals during inhalation could be observed through inspection glasses in each box. Each inhalation phase (mean, 4.5 h) was followed by a 30–60-min flow of fresh air and subsequent transfer of all cages by group into boxes with an electronically regulated day-night cycle, where food and water were available up to the next inhalation phase. This schedule was repeated on 5 days a week. The technical staff wore independently ventilated protective clothes during cage transfer.

Histopathological examination

Histopathological examinations were performed in the Division of Histodiagnostics and Pathomorphological Documentation (German Cancer Research Center, DKFZ). Slides were examined without prior knowledge of the exposure group, and the results documented and discussed, if necessary, with a second pathologist (Professor D. Komitowski or Professor H.J. Gröne), who was also unaware of the exposure of the animal. Animals that died or were killed were dissected, and the individual organs were examined macroscopically and fixed in 10% formalin for histopathological diagnosis. All data were documented on dissection protocol forms and the findings subdivided as follows:

a Benign and malignant tumors: Mucoepidermoid tumors in the nasal cavity played a special role. Due to their expanding growth they were not only fatal but also represented a conspicuous tumor response to NDMA exposure in the positive control. In similar experimental settings of a dose-response study, rats developed adenocarcinomas of the nasal cavity at higher NDMA exposure [8]. Therefore, mucoepidermoid tumors of the nasal cavity were evaluated together with relevant malignant tumors of the respiratory tract. Malignant and mucoepidermoid tumors of the airways were subsumed as 'fatal respiratory tract tumors'. Tumors of the respiratory tract: These tumors were listed separately as a local effect apart from nasal cavity tumors. Tumors of the palate and gingiva which were close to the respiratory tract, i.e., the contaminated area of the mucociliary pathway, were designated nonspecific tumors of the respiratory tract to distinguish them from 'genuine' respiratory tract tumors, in lung, trachea or nasal cavity. A malignant small-cell mediastinal tumor that broke through into the trachea was not considered a respiratory tract tumor.
b Tumors outside the respiratory tract: These were recorded to assess systemic activity. As exposure to lindane and PCP might induce liver tumors in rats [17, 18], malignant liver tumors were documented separately.
c Other tumors, such as of the skin and eyelid, and carcinomas of the auditory canal, that did not belong to any of the above groups were designated as other tumors.
d Strain-specific tumors in F344 rats such as mammary tumors and leukemias.

Documentation and statistical evaluation

Survival times and body weight development were recorded continuously. Animals bearing one or more tumors at the same site were registered as '1' and those with no tumors at the same site as '0'. For example, one positive control had a second respiratory tract tumor (adenocarcinoma) in the trachea in addition to a primary nasal tumor (mucoepidermoid tumor), but only the latter was documented. Animals exposed for only 25 weeks were evaluated separately from animals with long-term exposure. Animals exposed for 25 weeks that were killed were excluded from the evaluation, because no tumors had developed, which would have falsified the results.

Odds ratios (ORs) and all statistical parameters of variation (95% confidence interval, level of significance) were calculated by specific software [19].

Results

Scope of exposure during inhalation

The results of continuous exposure measurements and independent controls are shown in Table 2.

Survival

The mean survival times in the individual groups were not significantly affected by treatment. The first natural deaths occurred after 400 days and were equally distributed among all groups until almost the end of the study, when some variation in the increased frequency was seen. The mean survival time was approximately 850 days, which is a good rate in rats of this strain, although strain-specific spontaneous tumors such as mammary tumors (mostly fibroadenomas of the mammary gland) and in particular the characteristic leukemias occurred more frequently with age.

Body weight development

The body weights of exposed animals developed normally according to age. No toxic effects of the dusts or aerosols were observed.

Table 2 Concentrations of agents in animal cages (*NDMA N*-nitrosodimethylamine, *PCP* pentachlorophenol)

Exposure	Oak wood dust (mg/m^3)		Chromate (μg/m^3)[a]		Lindane (μg/m^3)	PCP (μg/m^3)	NDMA (μg/m^3)
	Continuously[b]	Independent measurement	Continuously[b]	Independent measurement	Continuously[b]	Continuously[b]	Continuously[b]
Oak wood, untreated	18.0	75.36	–	5	–	–	–
Oak wood treated with lindane- and PCP-containing wood preservative	16.6	–	–	–	0.3–1	18	–
Oak wood treated with chromate stain	18.8	38.15	39.4	23 14	–	–	–
Chromate stain, aerosol	–	–	21.2 (SD 9.3)	29 39 26	–	–	–
Lindane and PCP aerosol and vapor from lindane- and PCP-containing wood preservative	–	–	–	–	0.07–1.13	0.15–0.26	–
NDMA vapor	–	–	–	–	–	–	72 (SD 25, 6)
Occupational exposure limit (air) in Germany [13]	2 mg/m^3	2 mg/m^3	50 μg/m^3	50 μg/m^3	500 μg/m^3	1 μg/m^2	1 μg/m^3

[a] Quantitation of chromate based on CrO_3
[b] Time-related mean

Tumor formation

The formation of tumors in the individual groups is summarized in Tables 3, 4 and 5. Respiratory tract tumors occurred less frequently in animals exposed for only 25 weeks than in those exposed for longer. Eight of 116 animals had malignant or mucoepidermoid tumors in the region of the respiratory tract (Table 3). Three were located in the palate, two of these in the group exposed to unstained oak wood dust and one in the sham-exposed negative control; and one was located in the oral cavity also in the sham-exposed control. Four fatal tumors, for an incidence of 3.4%, were found, one in an animal exposed to oak wood dust alone, one exposed to oak wood plus chromate, and in two positive control rats. The two nasal cavity tumors developed only in the positive control. Malignant tumors in other organs occurred at an incidence of 29%, with an incidence of 10% for liver tumors. The only significant finding in animals exposed for 25 weeks was an increased incidence over controls of benign tumors in 'other organs' in the group exposed to chromate aerosol.

The 280 animals exposed for life developed 26 respiratory tract tumors (14 respiratory tract including oral cavity and 12 mucoepidermoid tumors), including four in the palate (in animals exposed to chromate stain aerosol or lindane- and PCP-containing vapor, and in a positive and a negative control rat), two in the gingiva (in animals exposed to chromate-treated and untreated wood dust) and one in the oral cavity (exposed to chromate stain aerosol). The remaining 19 tumors of the respiratory tract (including mucoepidermoid tumors without oral cavity), for an incidence of 6.8%, occurred in all exposed groups but not in the negative controls (sham-exposed). Malignant tumors developed in other organs in 29% of the animals exposed for life (the same rate as in animals exposed for 25 weeks) and included malignant liver tumors at an incidence of 8.9%. The positive control group of 35 animals developed 12 nasal cavity tumors, and benign respiratory tract tumors occurred significantly more frequently in the positive controls than in the negative controls (OR = 26.4, 3.2–217; $P = 0.002$). Nasal cavity tumors also occurred in one animal exposed to wood dust treated with chromate stain and one exposed to chromate stain aerosol, but none were found in animals exposed only to wood dust, indicating that chromate should be studied in more detail.

Malignant tumors in organs other than the respiratory tract were observed more frequently in the groups exposed to lindane and PCP than the other groups (except the positive control), but this result was not significant. When the incidences of liver tumors in the different groups were compared analogously, a significant result was obtained (OR = 3.7; 1.24–11.3; $P = 0.019$). Comparison with the negative controls yielded OR = 2.9 (0.87–9.57; $P = 0.083$). Remarkably, no malignant mammary tumors occurred in the groups exposed to dust from wood treated with lindane and PCP or chromate stain aerosol, although this tumor

Table 3 Tumors in rats exposed for 25 weeks (numbers of prematurely killed animals in parentheses) (*PCP* pentachlorophenol, NDMA *N*-nitrosodimethylamine)

Exposure	Numbers of rats	Malignant tumors					Fatal tumors	Benign tumors				Strain-specific tumors		
		Respiratory tract including oral cavity	Nasal cavity only	Other organs	Liver only	Others	Genuine respiratory tract without oral cavity	Respiratory tract including mucoepidermoid tumors without oral cavity	Mucoepidermoid nasal cavity	Respiratory tract without mucoepidermoid tumors	Nasal cavity without mucoepidermoid tumors	Other organs	Others	Leukemias	Malignant mammary	Benign mammary
Oak wood untreated	15 (9)	3	0	5	2	0	1	1	0	0	0	11	0	6	0	6
Oak wood treated with lindane- and PCP-containing wood preservative	15 (9)	0	0	3	1	0	0	0	0	0	0	11	0	7	1	2
Oak wood treated with chromate stain	16 (8)	1	0	4	1	0	1	1	0	2	2	10	0	9	0	4
Chromate stain, aerosol	15 (9)	0	0	4	1	0	0	0	0	0	0	14	1	10	0	2
Lindane and PCP aerosol and vapor from lindane- and PCP-containing wood preservative	15 (9)	0	0	6	4	0	0	0	0	1	0	9	0	8	2	3
NDMA vapor	11 (12)	0	0	4	1	0	0	2	2	2	2	7	0	2	1	3
Sham-exposed	29 (19)	2	0	8	2	3	0	0	0	1	0	10	1	11	0	6
All	116 (75)	6	0	34	12	3	2	4	2	6	4	72	2	53	4	26

type is strain-specific and would have been expected to occur in these groups as well.

When all exposed animals are considered together, the results become even more marked (Table 5). Fatal respiratory tract tumors occurred in five of 155 animals exposed to oak wood dust ($P = 0.08$, Fisher test, when compared with the negative control), in five of 102 animals exposed to chromate ($P = 0.03$) and in two of 101 animals exposed to lindane and PCP ($P = 0.17$), while such tumors were not seen in the 96 negative controls. Table 6 summarizes the histological types of these tumors. The large number of 14 nasal cavity tumors in animals in the positive control group confirms that the experimental design was appropriate to detect nasal cavity tumors induced by a suitable agent.

Discussion

We have succeeded in producing respiratory tract tumors in experimental animals which are comparable to the nasal cavity tumors diagnosed in workers in the wood-working industry. Our experimental design appears to have been adequate since, on the one hand, no acute toxic reaction was seen but, on the other, exposure-associated tumors occurred, i.e., two exposed animals developed nasal cavity tumors. These two tumors were not diagnosed in the group exposed to oak wood dust alone but in animals exposed to chromate either as an aerosol or in combination with oak wood dust. Additionally, nine genuine respiratory tract tumors developed in the exposed animals. The results justify our decision to investigate not only wood dust but also certain additives. It may be for this reason that the tumor yield in our study was markedly higher than that obtained by Wilhelmsson and Drettner [24, 25, 29], who found one nasal cavity tumor in one of 22 hamsters exposed for 40 weeks to beech wood dust and an observation time up to 40 weeks, but no other respiratory tract tumors. 50% of the rats in our study had a survival time with continued exposure of more than 122 weeks (range 115–131).

The concentrations of wood dust to which the animals were exposed was considerably higher than those measured nowadays in the wood industry [5, 14, 15, 21], amounting to a mean of 18 mg/m^3 over 3 years, with a maximum at 50 mg/m^3. Subsequent confirmation yielded concentrations up to 75.4 mg/m^3 (see Table 2). It should be borne in mind that human disease diagnosed today was initiated by the considerably higher exposures of 40 years ago [26]. Wilhelmsson and Drettner used concentrations of 15–30 mg/m^3, which are similar to those used in our experiments. The concentrations of chromate in breathing air in our study, 21 and 39 μg/m^3, were just below the German Occupational Exposure Limit of 50 μg/m^3 for CrO_3. In the solvent-containing wood preservatives tested, the mean concentration of lindane was 0.07–1.13 μg/m^3 and that of PCP was 0.15–0.26 μg/m^3, whereas the present air

Table 4 Tumors in animals exposed for life (*PCP* pentachlorophenol, *NDMA N*-nitrosodimethylamine)

Exposure	Number of rats	Malignant tumors						Fatal tumors	Benign tumors					Strain-specific tumors		
		Respiratory tract including oral cavity	Nasal cavity only	Other organs	Liver only	Other	Genuine respiratory tract without oral cavity	Respiratory tract including mucoepidermoid tumors	Mucoepidermoid nasal cavity	Respiratory tract without mucoepidermoid tumors	Nasal cavity without mucoepidermoid tumors	Other organs	Other	Leukemias	Malignant mammary	Benign mammary
Oak wood untreated	36	2	0	9	2	0	1	1	0	0	0	28	0	22	4	9
Oak wood treated with lindane- and PCP-containing wood preservative	36	1	0	12	6	2	1	1	0	0	0	29	0	17	0	11
Oak wood treated with chromate stain	37	1	0	7	1	3	0	1	1	2	1	27	3	23	2	11
Chromate stain, aerosol	34	5	1	8	2	1	3	3	0	1	0	26	0	11	0	11
Lindane and PCP aerosol and vapor from lindane- or PCP-containing wood preservative	35	2	0	11	5	3	1	1	0	0	0	27	1	22	3	8
NDMA vapor	35	2	1	14	5	3	1	12	11	10	10	30	0	18	4	7
Sham-exposed	67	1	0	21	4	6	0	0	0	1	0	45	0	28	8	10
All	280	14	2	82	25	18	7	19	12	14	11	212	4	141	21	67

Table 5 Tumors in all animals (exposed for 25 weeks and for life) (*PCP* pentachlorophenol, *NDMA N*-nitrosodimethylamine)

Exposure	Number of rats	Malignant tumors						Fatal tumors	Benign tumors					Strain-specific tumors		
		Respiratory tract including oral cavity	Nasal cavity	Other organs	Liver only	Other	Genuine respiratory tract without oral cavity	Respiratory tract including mucoepidermoid tumors	Mucoepidermoid nasal cavity	Respiratory tract without mucoepidermoid tumors	Nasal cavity without mucoepidermoid tumors	Other organs	Other	Leukemias	Malignant mammary	Benign mammary
Oak wood untreated	51	5	0	14	4	0	2	2	0	0	0	39	0	28	4	15
Oak wood treated with lindane- and PCP-containing wood preservative	51	1	0	15	7	2	1	1	0	0	0	40	0	24	1	13
Oak wood treated with chromate stain	53	2	0	11	2	3	1	2	1	4	3	37	3	32	2	15
Chromate stain, aerosol	49	5	1	12	3	1	3	3	0	1	0	40	1	21	0	13
Lindane and PCP aerosol and vapor from lindane- and PCP-containing wood preservative	50	2	0	17	9	3	1	1	0	1	0	36	1	30	5	11
NDMA vapor	46	2	1	18	6	3	1	14	13	12	12	37	0	20	5	10
Sham-exposed	96	3	0	29	6	9	0	0	0	2	0	55	1	39	8	16
All	396	20	2	116	37	21	9	23	14	20	15	284	6	194	25	93

Table 6 Histological classification of genuine respiratory tract tumors found (*PCP* pentachlorophenol)

Exposure	Malignant and fatal tumors	Location	Proportion (%) of tumors (in relation to rats under risk)	Histology	Exposure time and life time in weeks
Oak wood dust, untreated	1	Lung	2/51	Undifferentiated adenocarcinoma	22/110
	1	Lung	3.9%	Bronchial carcinoma	176/180
Oak wood dust treated with lindane- and PCP-containing wood preservative	1	Lung	1/51 2.0%	Adenocarcinoma	94/98
Oak wood dust treated with chromate stain	1	Lung	2/53	Bronchial carcinoma	22/152
	1	Nasal cavity	3.8%	Mucoepidermoid tumor	65/169
Chromate stain aerosol	1	Lung	3/49	Bronchial carcinoma	155/159
	1	Lung	6.1%	Adenocarcinoma	136/140
	1	Nasal cavity		Expanded necrotizing tumor	127/131
Lindane and PCP aerosol and vapor from lindane and PCP-containing wood preserving solution	1	Lung	1/50 2%	Bronchial carcinoma (preliminary stage)	133/137
N-nitrosodimethylamine vapor	14	Nasal cavity	14/46	13 Mucoepidermoid tumors	From 78 to 167
			30%	[a]1 Adenocarcinoma	Mean 139
Sham-exposed	0	–	0/96 0%		

[a] One rat also developed a primary adenocarcinoma of the trachea as well as a nasal tumor

thresholds are 500 $\mu g/m^3$ for lindane and 1 $\mu g/m^3$ for PCP. The exposure of animals to these compounds from oak wood dust treated with a lindane- and PCP-containing preservative was calculated from the type of preservation (wood impregnation) and the concentration of wood dust. After impregnation and drying, the mean concentrations in 1 g of preservative per kilogram of wood, which were standardized in the past [26], were 1 $\mu g/m^3$ for lindane and 18 $\mu g/m^3$ for PCP in breathing air. Thus, the threshold value for PCP was greatly exceeded, while the concentration of lindane remained well below the threshold.

The incidences of liver tumors were 7/51 animals exposed to dust from wood treated with lindane- and PCP-containing preservative and 9/50 animals treated with an aerosol of and vapors from a wood-preserving solution containing these agents. This difference is random ($P = 0.56$). As the exposure to lindane was comparable in the two groups while that to PCP was 100 times higher in the group given the dust, a considerably increased rate of liver tumors would have been expected in the latter group if PCP were the causal agent. The observed effect, therefore, must be associated with lindane and/or other substances in the commercial wood preservative.

The fact that the two animals with nasal cavity tumors were exposed to chromate suggests that this is the causal agent. Since tumors of the palate, gingiva and oral cavity occurred in all groups, including the negative controls, they cannot be attributed to exposure. Inhalation of chromate aerosol by rats for 25 weeks induced an increased incidence over controls of benign tumors in 'other organs' (OR = 26.6 (3.04–232.7); $P = 0.003$). But this finding is not related to the question of whether there is a causal relationship between nasal tumors and inhalative uptake of exogenous hazardous agents. However, tumors in the lung and trachea were found exclusively in exposed animals, and predominantly in the groups exposed to oak wood dust and chromate stain. Our results do not permit us to conclude whether oak wood dust or chromate stain is the causal agent.

Norpoth [16] found chromate in beech wood at a concentration of 0.07 ± 0.04 $\mu g/g$, and Ruetze et al. showed that chromate is not, as previously assumed, reduced rapidly and completely to trivalent chromium but is detectable as chromium VI in wood for a long time [22]. We therefore investigated whether chromate was present in the oak wood used to generate dust. These analyses were carried out in conjunction with an independent check after termination of all experiments (for method, see chapters "Chromate stain" and "Independent control of exposure"; detection limit, 1 $\mu g/m^3$ air and 5 $\mu g/m^3$, respectively). We found that the group exposed to 'pure' oak wood dust was also exposed to chromate at a concentration of 5 $\mu g/m^3$ as CrO_3. The detection limit represents approximately 20% and 12–14% of the mean concentrations received by animals in the groups exposed to chromate aerosol and wood dust treated with chromate stain, respectively. It is equivalent to almost 10% of the present occupational exposure limit in Germany [5, 15, 21]. Thus, the animals

assumed to have been exposed to 'chromate-free wood dust were also exposed to considerable amounts of chromate. Eight of the nine genuine respiratory tract tumors diagnosed in exposed animals can hence be associated with chromate and one with lindane and PCP. The results of our experiments thus indicate that the respiratory tract tumors, in particular the nasal tumors, were probably caused in cooperation by chromate.

This conclusion is in accordance with our earlier finding that chromate reduces the clearance and consequently prolongs the contact time on the mucosa. It also corresponds to the known marked genotoxicity of chromate [30]. Furthermore, the tumor types found in the respiratory tract after chromate exposure are often adenocarcinomas [20]. The occurrence of dysplasia after exposure to preservative-containing wood dust also corresponds to the formation of respiratory tract tumors after exposure to chromate. Chromate has frequently been used as a fixing agent for water-soluble constituents of preservatives. The increased occurrence of tumors among joiners can thus be explained by the frequent use of chromate-containing stains, although Ruetze et al. [22] could not establish such an association with tumorigenesis. Although we found in previous experiments that dysplasia was associated not specifically with chromate but also with preservative constituents in general, the increased occurrence of dysplasia after exposure to preservative-containing oak and beech wood dust supports the validity of our conclusion about the causal role of chromate. The conclusion also corroborates our earlier finding of cellular changes after inhalation of exogenous hazardous agents [30]. Combined exposure to wood dust and chemicals increased the incidence of squamous-cell metaplasia, suggesting the induction of squamous-cell carcinomas, but this applied only to combinations of chemicals with softwood dusts and not to those with oak and beech wood dusts.

In 1999, ten oak wood samples were collected from different sawmills to determine whether the chromate content of the oak wood used in our experiments was typical. The samples were analyzed by the same method as that used in this study. The chromate content was above or at the detection limit of 0.1 $\mu g/m^3$ in four samples, the highest value being 0.3 $\mu g/m^3$ (for concentration of wood dust of 0.32 and 5.02 mg/m^3). These measurements do not permit a conclusion about the extent to which chromate accounts for the effects associated with exposure to oak and beech wood dust or for the epidemiologically observed regional variations in tumorigenesis. More wood types from different regions will have to be analyzed for chromate, and compared.

Potential mechanisms involved in carcinogenic effects of wood dust and additives may be due to a special carrier function of the dust particles, especially for chromate, since tannic compounds in oak wood may slowly release trapped Cr(VI) ions. Katz and Salem have reviewed Cr(III) and Cr(VI) toxicity, cytotoxicity, genotoxicity and carcinogenicity. Cr(VI) is more toxic, more cytotoxic and more genotoxic than Cr(III) [7]. Cohen and Costa [4] support the hypothesis that slightly insoluble hexavalent Cr compounds may be potent carcinogens. Wistar rats developed lung cancers after long-term inhalative exposure to relatively low levels of sodium chromate. We suspect that the high content of tannic acid in oak wood may present very small amounts of Cr ions continuously in a bioavailable way for cancer induction at the site of sensitive tissues in the respiratory tract. It is reported that the final cellular form of Cr, Cr(III), becomes trapped intracellularly because it has low cell membrane permeability. A concentration gradient will be established and will enhance intracellular Cr levels. The Cr(VI) ion is readily taken up into eukaryotic cells. Also oxygen radicals are generated in cells by reduction of Cr(VI) which can cause DNA damage. Cr ions can form DNA adducts. Cr-DNA adducts have been observed in rats after in vivo exposure. It was shown that Cr(VI) can induce DNA-protein crosslinks which are highly stable and can be mutagenic [4].

Acknowledgements The authors wish to thank the Professional Associations Institute for Occupational Safety (BIA) for the analyses of chromate, lindane and PCP, Professor H.J. Gröne and Professor D. Komitowski (Division of Histodiagnostics and Pathomorphological Documentation, DKFZ) for their help in histopathological examinations, and Dr H. Wesch and A. Bindl (Division of Biophysics and Medical Radiation Physics, DKFZ) for chromium analysis, Regina Merkel, P. Waas, R. Hermann, E. Künzer and W. Dähmel for help in animal experimentation and care. The authors are grateful to G. Bielefeldt and E. Heseltine for their help in translating and editing the manuscript. The project was financially supported in part by the EU.

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