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## Pulmonary cystic keratinizing squamous cell lesions of rats after inhalation/instillation of different particles

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With 4 figures and 5 tables

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### Summary

Cystic keratinizing squamous cell lesions from three inhalation studies (Study A, B, C) and one intratracheal instillation study (Study D) in rats were reclassified and a certain number of lesions examined immunohistochemically for PCNA (proliferating cell nuclear antigen) as a marker of cellular proliferation. The following classification was used: squamous cell metaplasia with marked keratinization, keratinizing cyst, cystic keratinizing epithelioma, cystic keratinizing squamous cell carcinoma, keratinizing squamous cell carcinoma and non-keratinizing squamous cell carcinoma. In study A (inhalation of coal oven exhaust and subcutaneous injection of a high dose of DB (*ah*)A) 49.3 % of rats developed cystic keratinizing squamous cell carcinomas. Inhalation of coal oven exhaust gas together with intratracheal instillation of crocidolite or subcutaneous injection of a low dose DB(*ah*)A (dibenz(*ah*)anthracene) resulted in cystic keratinizing squamous cell carcinomas in 23 % to 24 % of the rats. High incidences of cystic squamous cell carcinomas in the range of 31.9 % to 76.4 % were observed in rats of Study B1 after a 10-months exposure to tar/pitch condensation aerosol (different B(*a*)P (benzo(*a*)pyrene) concentrations) with added carbon black in some groups. After a 20-months exposure period to the same inhalation atmospheres (Study B2) the incidence of squamous cell carcinomas was increased up to 95.8 %. Exposure of rats to va-

rious concentrations of unfiltered diesel exhaust (Study C) resulted in incidences of cystic keratinizing epitheliomas ranging from 2.5 % (2.5 mg/m<sup>3</sup>) to 10.7 % (7.5 mg/m<sup>3</sup>). Epitheliomas were also observed in 16.2 % of carbon black and 16.0 % of titanium dioxide exposed rats. Only a few cystic keratinizing squamous cell carcinomas occurred. In the intratracheal instillation study (Study D) increased incidences of cystic keratinizing epitheliomas occurred in rats exposed to native diesel exhaust particles (16.7 %), high dose of extracted diesel exhaust particles (14.6 %), extracted printex 90-carbon black particles (18.8 %), and extracted printex 90-carbon black particles + B(*a*)P (18.8 %). High incidences of cystic keratinizing squamous cell carcinomas were noted in rats that received 15 mg B(*a*)P (14.6 %) or 30 mg B(*a*)P (72.7 %) intratracheally. Immunohistochemical labeling of nuclei with PCNA demonstrated proliferative activity in one or two (and focally more than two) peripheral cell layers of cystic keratinizing epitheliomas and in more than three peripheral cell layers of cystic keratinizing squamous cell carcinomas and keratinizing squamous cell carcinomas. The wall of keratinizing cysts showed no or a weak reaction.

### Introduction

The threat of human health by airborne respirable particles has become a matter for increasing concern in the past twenty years. The health hazard arising from asbestos fibres in particular has been demonstrated by several epidemiological and animal studies with the result that

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asbestos was banned as building material. Several other particles (diesel exhaust particles, titanium dioxide, quartz dust, tar/pitch aerosols etc.) are suspected to be involved in the development of human lung cancer. However, results of epidemiological studies on these particles are unclear due to the complex exposure situation of human beings. Therefore chronic inhalation and instillation studies with laboratory rodents remain a helpful tool for the assessment of cancer risk in humans. Unfortunately, the interpretation of these studies sometimes becomes difficult if particle-induced lesions occur in the lungs of rodents but lack a known counterpart in the human pulmonary tissue.

Cystic keratinizing lesions have been observed in the lungs of rats after exposure to several different types of particles, including diesel engine exhaust particles (HEINRICH et al. 1986a; MOHR et al. 1986; MAUDERLY et al. 1986; MAUDERLY et al. 1994; POTT et al. 1994), titanium dioxide (LEE et al. 1985; LEE et al. 1986a; WARHEIT and HARTSKY 1994), chromium dioxide (LEE et al. 1988b; LEE et al. 1989), quartz dust (MUHLE et al. 1989; POTT et al. 1994), para-aramid fibrils (LEE et al. 1988a; WARHEIT 1995), carbon black (MAUDERLY et al. 1994; NIKULA et al. 1995; POTT et al. 1994), petroleum coke dust (KLONNE et al. 1987), talcum (HOBBS et al. 1994), coal dust (MARTIN et al. 1977), and nickel dioxide (POTT et al. 1994). The various terms used to describe cystic keratinizing lesions in the rat reflect the diverse opinions concerning the biological behavior of the lesions. In the past, terms such as squamous metaplasia (KUSCHNER and LASKIN 1970), keratinizing cyst (KLONNE et al. 1987), inverted papilloma (SHABAD and PYLEV 1970), benign cystic keratinizing tumour (MOHR and DUNGWORTH 1988), and cystic squamous cell carcinoma (LEE et al. 1988a) have been used.

Two recent attempts to clarify the histopathological nature of cystic keratinizing lesions of the rat lung were made at workshops in Newark, USA (1992) and Hannover, Germany (1995). At the Newark-workshop thirteen pathologists, experienced in lung pathology reviewed cystic keratinizing lesions from two inhalation studies. In these studies, rats were either exposed to titanium dioxide or para-aramid fibrils. Most of the slides were taken from a para-aramid fibril inhalation study. The majority of workshop participants agreed that the lesion should be considered as a non-neoplastic change and that the term "proliferative keratin cyst" should be applied for its appropriate description. Only a minority (3/13) considered the lesions as benign tumours. The keratinizing cysts were considered to have no significance for human health, since comparable lesions had not been observed in humans (CARLTON 1994; LEVY 1994; SCHULTZ 1995). Since the number of lesions reviewed at the Newark workshop was small and the presented cases were obtained from only two different rat inhalation studies, a second workshop on this topic (sponsored by the Deutsche Forschungsgemeinschaft) was initiated in Hannover. Based on a broad spectrum of particles (11 different test substances from 13 different inhalation studies) the biological behaviour

of cystic keratinizing lesions was discussed again by a panel of eleven pathologists. Sixty-one slides of cystic keratinizing lesions from 56 rats of different strains were reviewed. There was agreement by the participants that the cystic keratinizing lesions reviewed represented a family of lesions that contained many morphological similarities. It became apparent at the workshop that the lesions comprised a spectrum of related morphological changes that ranged from squamous metaplasia with marked keratinization through pulmonary keratinizing cysts to cystic keratinizing epithelioma and finally pulmonary squamous cell carcinoma (BOORMANN et al. 1996).

The present paper summarizes the results of three inhalation studies and one intratracheal instillation study on rats. Cystic keratinizing lesions occurring in the studies were reviewed and reclassified according to the criteria of the Hannover workshop published by BOORMANN et al. (1996). Special attention was paid to whether transitional stages between benign cystic keratinizing epitheliomas and malignant variants occur and whether an increased incidence of malignant cystic keratinizing tumours can be attributed to type, concentration and route of administration of particles or simultaneous treatment with co-carcinogens. For the detection of changes of proliferation behaviour of cells, immunohistochemical labeling of the marker of proliferative cell activity PCNA ("proliferating cell nuclear antigen") was considered a suitable supplementary method for the classification of tumours and the differentiation between neoplastic and non-neoplastic lesions.

## Material and methods

The rats examined belonged to three inhalation studies and one intratracheal instillation study. In the first study (Study A, table 1) 720 female Iva: WIWU Wistar rats in eight groups were exposed in steel wire cages to diluted coal oven exhaust gas or clean air (control) for an average of 16 h/day, 5 d/week over a maximum period of 22 months. In the first 9 months of the study the animals were subjected to an exposure atmosphere of 0.3 µg B(a)P/m<sup>3</sup>. During a following one month period the animals inhaled diluted coal oven exhaust gas without any addition and in the final 12 months the B(a)P concentration after the addition of pyrolyzed pitch effluent to the coal oven exhaust gas was about 90 µg/m<sup>3</sup>. Three of the groups exposed to coal oven flue gas as well as three control groups received additional treatments with crocidolite (20 x 0.5 mg, intratracheal) or two different concentrations of DB(ah)A (20 x 0.25 or 20 x 0.5 mg, subcutaneously). Out of the 720 rats 661 were examined histologically. For details of study design see HEINRICH et al. (1986b).

In the second study (Study B1 and B2, table 2 and table 3) 1296 female Crl:[WI] BR Wistar rats in 9 groups were exposed to PAH-rich coal tar/pitch condensation aerosol (adjusted to different B(a)P-contents) for 17 h/day and 5 d/week. The aerosol was generated by heating hard coal/tar pitch to 750 °C under nitrogen atmosphere and diluting the high temperature tar/pitch vapour with 12 °C clean air. In this

**Table 1.** Incidence of keratinizing cysts and neoplasms in Study A (Coal oven exhaust gas).

Exposure atmosphere	Incidence of lesions							
	Clean air				Coal oven exhaust gas			
Additional treatment	NaCl 0,9 % 20 x 0.3 ml	Croci- dolite 20 x 0.5 mg	Dibenz (a,h)-an- thracene 20 x 0.25 mg	Dibenz (a,h)-an- thracene 20 x 0.5 mg	NaCl 0,9 % 20 x 0.3 ml	Croci- dolite 20 x 0.5 mg	Dibenz (a,h)-an- thracene 20 x 0.25 mg	Dibenz (a,h)-an- thracene 20 x 0.5 mg
Number of rats examined histologically	120	68	69	69	121	71	72	71
Diagnosis								
Keratinizing cyst	0	0	1 (1.4%)	6** (8.7%)	1 (0.8%)	7*** (9.8%)	2 (2.8%)	4* (5.6%)
Cystic keratinizing epithelioma	0	0	2 (2.9%)	1 (1.4%)	3 (2.5%)	7*** (9.8%)	7*** (9.7%)	7*** (9.9%)
Cystic keratinizing epithelioma associated with squamous cell carcinoma	0	0	0	1 (1.4%)	0	2 (2.8%)	2 (2.8%)	1 (1.4%)
• Total number of cystic keratinizing epitheliomas	0	0	2 (2.9%)	2 (2.9%)	3 (2.5%)	9*** (12.7%)	9*** (12.5%)	8*** (11.3%)
Cystic keratinizing squamous cell carcinoma	0	0	2 (2.9%)	6** (8.7%)	3 (2.5%)	17*** (23.9%)	16*** (22.2%)	35*** (49.3%)
Keratinizing squamous cell carcinoma	0	2 (2.9%)	1 (1.4%)	7*** (10.1%)	12*** (9.9%)	8*** (11.3%)	6** (8.3%)	6** (8.5%)
• Total number of squamous cell carcinomas	0	2 (2.9%)	3 (4.3%)	13*** (18.8%)	15*** (12.4%)	25*** (35.5%)	22*** (30.6%)	41*** (57.7%)

Significance of difference between control and treated groups in Fisher test: \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001

**Table 2.** Incidence of neoplasms in Study B1 (Tar/pitch condensation aerosol, 10 months exposure).

Exposure atmosphere	Incidence of lesions								
	Clean air	Irritant gas	Carbon black 6 mg/m <sup>3</sup>	Tar/pitch aerosol					
				B(a)P 20µg/m <sup>3</sup>	B(a)P 50µg/m <sup>3</sup>	B(a)P 125µg/m <sup>3</sup>	B(a)P 50µg/m <sup>3</sup> +Carbon black 2 mg/m <sup>3</sup>	B(a)P 50µg/m <sup>3</sup> +Carbon black 6 mg/m <sup>3</sup>	B(a)P 50µg/m <sup>3</sup> +Irritant gas
Number of rats examined histologically	72	72	72	72	72	72	72	72	72
Diagnosis									
Cystic keratinizing epithelioma	0	0	1 (1.4%)	0	0	0	0	1 (1.4%)	0
Cystic keratinizing squamous cell carcinoma	0	0	5 (6.9%)	0	23*** (31.9%)	38*** (52.8%)	55*** (76.4%)	37*** (51.4%)	4 (5.6%)
Keratinizing squamous cell carcinoma	0	1 (1.4%)	2 (2.8%)	1 (1.4%)	4 (5.6%)	3 (4.2%)	9** (12.5%)	8** (11.1%)	2 (2.8%)
• Total number of squamous cell carcinomas	0	1 (1.4%)	7* (9.7%)	1 (1.4%)	27*** (37.5%)	41*** (56.9%)	64*** (88.9%)	45*** (62.5%)	6* (8.3%)

Significance of difference between control and treated groups in Fisher exact test: \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001

**Table 3.** Incidence of keratinizing cysts and neoplasms in Study B2 (Tar/pitch condensation aerosol, 20 months exposure).

Exposure atmosphere	Incidence of lesions								
	Clean air	Irritant gas	Carbon black 6 mg/m <sup>3</sup>	Tar/pitch aerosol					
				B(a)P 20µg/m <sup>3</sup>	B(a)P 50µg/m <sup>3</sup>	B(a)P 90µg/m <sup>3</sup>	B(a)P 50µg/m <sup>3</sup> +Carbon black 2 mg/m <sup>3</sup>	B(a)P 50µg/m <sup>3</sup> +Carbon black 6 mg/m <sup>3</sup>	B(a)P 50µg/m <sup>3</sup> +Irritant gas
Number of rats examined histologically	72	72	72	72	72	72	72	72	72
Diagnosis									
Keratinizing cyst	0	0	0	1 (1.4%)	0	0	0	0	3 (4.2%)
Cystic keratinizing epithelioma	0	0	1 (1.4%)	0	0	0	0	0	1 (1.4%)
Cystic keratinizing squamous cell carcinoma	0	0	3 (4.2%)	19*** (26.4%)	63*** (87.5%)	62*** (86.1%)	69*** (95.8%)	67*** (93.1%)	49*** (68.1%)
Keratinizing squamous cell carcinoma	0	0	1 (1.4%)	4 (5.6%)	5 (6.9%)	4 (5.6%)	0	1 (1.4%)	3 (4.2%)
• Total number of squamous cell carcinomas	0	0	4 (5.6%)	23*** (31.9%)	68*** (94.4%)	66*** (91.7%)	69*** (95.8%)	68*** (94.4%)	52*** (72.2%)

Significance of difference between control and treated groups in Fisher exact test: \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001

**Table 4.** Incidence of neoplasms in Study C (Diesel engine exhaust).

Exposure atmosphere	Incidence of lesions					
	Clean air	Diesel motor engine exhaust			Carbon black 11.3 mg/m <sup>3</sup>	Titanium dioxide 10.4 mg/m <sup>3</sup>
		0.8 mg/m <sup>3</sup>	2.5 mg/m <sup>3</sup>	7.5 mg/m <sup>3</sup>		
Number of rats examined histologically	221	200	200	103	105	100
Diagnosis						
Cystic keratinizing epithelioma	0	0	5* (2.5%)	11*** (10.7%)	17*** (16.2%)	16*** (16.0%)
Cystic keratinizing epithelioma associated with squamous cell carcinoma	0	0	0	1 (1.0%)	0	0
• Total number of cystic keratinizing epitheliomas	0	0	5* (2.5%)	12*** (11.7%)	17*** (16.2%)	16*** (16.0%)
Cystic keratinizing squamous cell carcinoma	0	0	1 (0.5%)	2 (1.9%)	3 (2.9%)	3* (3.0%)
Keratinizing squamous cell carcinoma	1 (0.5%)	0	0	4* (3.9%)	1 (1.0%)	0
Non-keratinizing squamous cell carcinoma	0	0	1 (0.5%)	0	1 (1.0%)	1 (1.0%)
• Total number of squamous cell carcinomas	0	0	2 (1.0%)	6** (5.8%)	5** (4.8%)	4** (4.0%)

Significance of difference between control and treated groups in Fisher exact test: \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001

**Table 5.** Incidence of keratinizing cysts and neoplasms in Study D (Intratracheal instillation of diesel exhaust particles, carbon black and B(a)P).

Intratracheal instillation	Incidence of lesions									
	Control NaCl 4.5 ml	Diesel exhaust particles 15 mg	Diesel exhaust particles (extracted) 30 mg	Diesel exhaust particles (extracted) 15 mg	Printex 90 (extracted) 15 mg	Lamp black (extracted) 15 mg	Benzo-(a)-pyrene 30 mg	Benzo-(a)-pyrene 15 mg	Diesel exhaust particles (extracted) + Benzo(a)-pyren 15 mg	Printex 90 (extracted) + Benzo(a)-pyren 15 mg
Number of rats examined histologically	48	48	48	48	48	48	48	48	48	48
Diagnosis										
Keratinizing cyst	0	0	0	0	0	0	4 (8.3%)	1 (2.1%)	0	0
Cystic keratinizing epithelioma associated with squamous cell carcinoma	0	0	0	0	0	0	1 (2.1%)	0	0	0
Cystic keratinizing epithelioma	0	8** (16.7%)	7** (14.6%)	1 (2.1%)	9** (18.8%)	3 (6.3%)	1 (2.1%)	0	2 (4.2%)	9** (18.8%)
• Total number of cystic keratinizing epitheliomas	0	8** (16.7%)	7** (14.6%)	1 (2.1%)	9** (18.8%)	3 (6.3%)	2 (4.2%)	0	2 (4.2%)	9** (18.8%)
Cystic keratinizing squamous cell carcinomas	0	0	1 (2.1%)	0	0	1 (2.1%)	35*** (72.9%)	7** (14.6%)	1 (2.1%)	3 (6.3%)
Keratinizing squamous cell carcinoma	0	0	0	0	0	0	3 (6.3%)	1 (2.1%)	0	0
Non-keratinizing squamous cell carcinoma	0	0	0	0	0	0	1 (2.1%)	0	0	0
• Total number of squamous cell carcinomas	0	0	1 (2.1%)	0	0	1 (2.1%)	38*** (79.2%)	8** (16.7%)	1 (2.1%)	1 (2.1%)

Significance of difference between control and treated groups in Fisher exact test: \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001

way, a PAH-rich condensation aerosol free of any carbon black carrier particles was produced. Three groups inhaled exclusively tar/pitch condensation aerosols of different B(a)P concentrations (20 µg/m<sup>3</sup>, 50 µg/m<sup>3</sup> and 125 µg/m<sup>3</sup>), three groups were exposed to an aerosol (50 µg/m<sup>3</sup> B(a)P supplemented with carbon black (2 mg/m<sup>3</sup> or 6 mg/m<sup>3</sup>) or an irritant gas mixture (sulphur dioxide, nitric dioxide, formaldehyde). Three groups exclusively received clean air or irritant gas or carbon black (6 mg/m<sup>3</sup>). One half of the rats of each of the nine groups was exposed for 10 months followed by a 20 months clean air recovery period (Study B1). The second half of each exposure group was exposed for 20 months followed by clean air for 10 months (Study B2). All rats were examined histologically. For details of study design see HEINRICH et al. (1994a) and HEINRICH et al. (1994b).

In the third study (Study C, table 4) 1780 female CrI:[WI] BR Wistar rats were exposed by inhalation (18 h/day, 5 d/

week for 24 months followed by an up to 6 months long recovery period). Five groups of rats received different concentrations of unfiltered diesel engine exhaust (0.8 mg/m<sup>3</sup>, 2.5 mg/m<sup>3</sup> and 7.5 mg/m<sup>3</sup>), carbon black (11.3 mg/m<sup>3</sup>) or titanium dioxide (10.4 mg/m<sup>3</sup>). The carbon black (Printex 90) group received 7.4 mg/m<sup>3</sup> for the first 4 months and 12.2 mg/m<sup>3</sup> from the 5. to the 24. months. One additional group received clean air and served as control. Histological examination was performed on 929 rats.

Various particles were given intratracheally to different groups of 520 female CrI:[WI] BR Wistar rats in the fourth study (Study D, table 5). Test substances were dissolved in 0.9 % NaCl-solution with 0.25 % Tween 80. Animals were dosed intratracheally 16–17 times under halothane narcosis. The particles used included: native diesel exhaust particles (15 mg total dose), extracted diesel exhaust particles (devoid of polycyclic aromatic hydrocarbons, 15 mg and 30 mg total dose), Printex 90 (15 mg total dose), lamp black (15 mg

total dose), extracted Printex 90 + B(a)P (15 mg total dose), and extracted diesel exhaust particles + B(a)P (15 mg total dose). Two additional groups received B(a)P (15 mg or 30 mg total dose) and one group NaCl-solution. A total of 480 rats was examined histopathologically.

In all four studies, the animals were housed in a barrier-type animal room in Macrolon® cages type III on soft wood bedding. Room temperature was  $22 \pm 2$  °C, relative humidity  $60 \pm 15$  %, air exchange rate was 15 times/h and light/dark sequence 12:12 h. All rats were fed an autoclaved cereal-based diet (Study A, B: RMH-TM, Hope Farms, Woerden, Netherlands; Study C, D: Altromin N1324, Altromin, Lage, Germany) *ad libitum*. Filtered tap water was also available *ad libitum*. The rats were known to harbour no viral infections (Corona virus, Reovirus 3, Pneumonia virus of mice, Sendai virus and Kilham rat virus), Mycoplasma spec. or other pathogenic bacteria or parasites.

From all animals (found dead, killed moribund or sacrificed at termination of the studies) a complete necropsy was performed. Lungs were fixed in 10 % formalin by immersion (Study A) or by intratracheal instillation with 10 % formalin (Study B, C, D). All tissue samples from the lungs were embedded in paraffin, sectioned at 4 µm, and stained with haematoxylin and eosin. Special stains were applied if deemed necessary by the histopathologist.

A total of 122 cystic keratinizing lesions was examined immunohistochemically for the endogenous marker of cell proliferation PCNA (proliferating cell nuclear antigen) by the standard biotin peroxidase complex method. The details of the method used for the immunostaining were as follows: 4 µm sections were deparaffinized in xylene and antigens unmasked in a pressure-cooker. Endogenous peroxidase was blocked with H<sub>2</sub>O<sub>2</sub> at room temperature. The slides were then incubated with normal serum and with the primary antibody (mouse anti-PCNA, clone PC10, 1:300) for 30 minutes. Afterwards the slides were washed in citric acid-phosphate buffer and incubated with the secondary antibody (horse-anti-mouse-Ig G 1:200) for 30 minutes. Again after washing, the slides were incubated with ABC peroxidase. Peroxidase activity was demonstrated by treating the slides with diaminobenzidine. The slides were counterstained with Mayers haemalaun. The degree of immunoreactivity of cystic keratinizing lesions was determined semi-quantitatively by counting the cell layers with positive reacting nuclei in the periphery in ten representative areas of the lesions.

Incidences of cystic keratinizing lesions of the different treatment groups in the four studies were compared by a pairwise Fischer's test.

## Results

### Histopathological features and immunohistochemistry

**Squamous metaplasia:** Severe keratinizing squamous metaplasia was occasionally observed in small airways and pulmonary parenchyma and this had to be distinguished from cystic keratinizing epithelioma. In severely keratinized squamous metaplasia a gradual transition of alveolar cells into squamous metaplastic cells almost always could be recognised and a discrete cyst was not formed. Mitoses were not usually detectable in foci of squamous metaplasia and only few nuclei were stained by the anti-PCNA antibody.

**Keratinizing cysts:** All keratinizing cysts revealed a large keratin filled central cavity. In the great majority of cysts the keratin was surrounded by connective tissue infiltrated by inflammatory cells or by a thin rim (one to three layers) of epithelial cells. In some areas of the epithelial wall goblet cells were occasionally found. In contrast to cystic keratinizing epitheliomas, the epithelial parts of the wall were always thin and lacked any nests of epithelial cells expanding into adjacent alveolar spaces. Therefore the cysts had a smooth border and were clearly separated from the surrounding lung tissue. Epithelial parts of cyst walls only showed a weak or no reaction to PCNA in the peripheral cell layer.

**Cystic keratinizing epithelioma:** Cystic keratinizing epithelioma were comprised of a highly keratinized wall of squamous epithelium and a central lumen that was filled with keratin. The squamous wall consisted of several layers of epithelial cells (three to ten) and peripheral cell nests that projected into the adjacent alveoli, resulting in an irregular border of the tumours (fig. 1). The squamous epithelium was well differentiated and showed a distinct stratum basale and stratum spinosum in the thicker parts of the tumour wall. Epithelial cells in the periphery of the tumour wall and of the cell nests were basal cell-like and had little cytoplasm. In the more central layers of the wall, cells had more cytoplasm and nuclei were round to

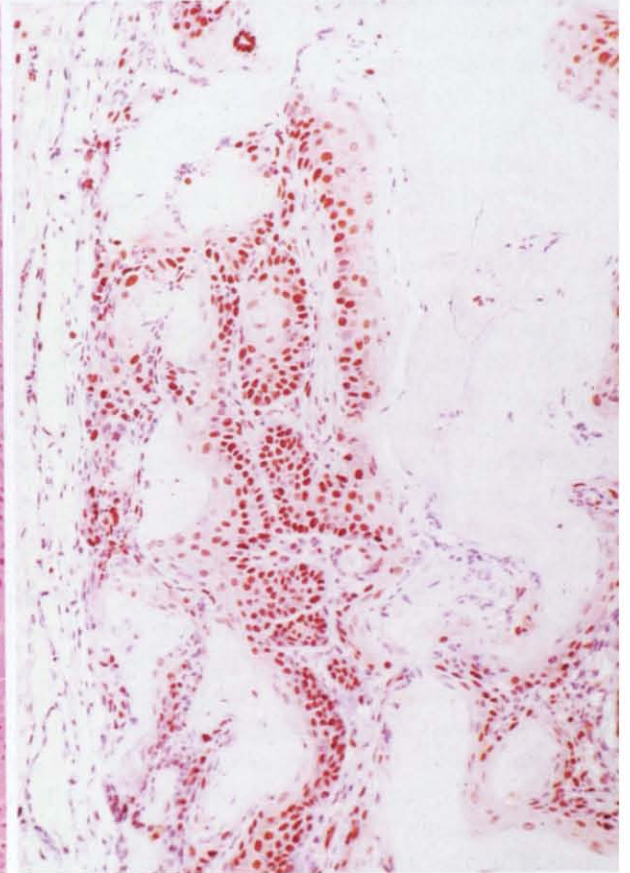
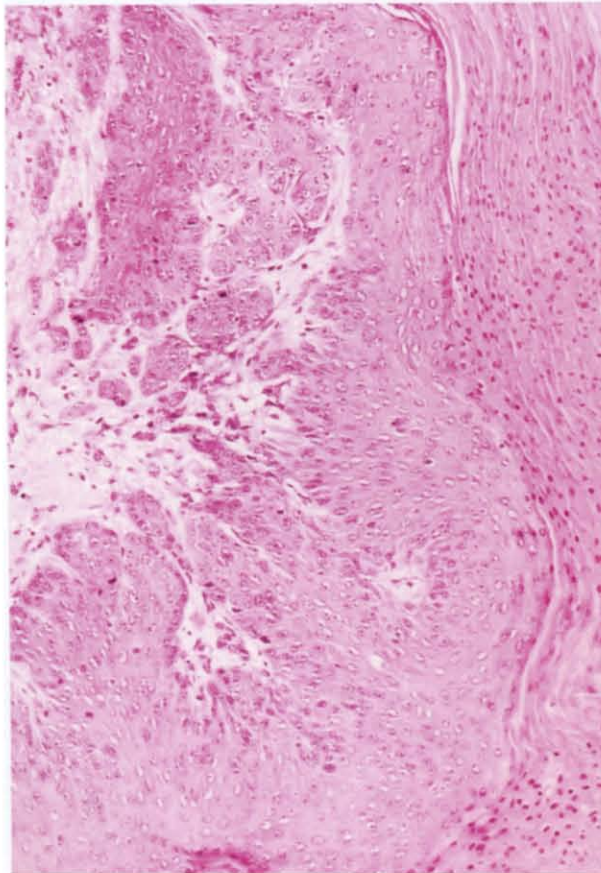
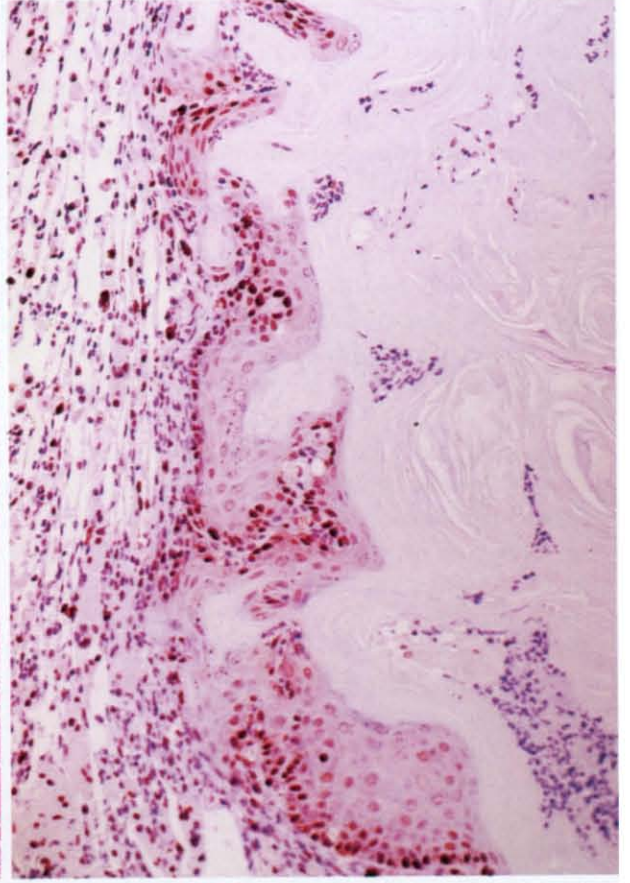
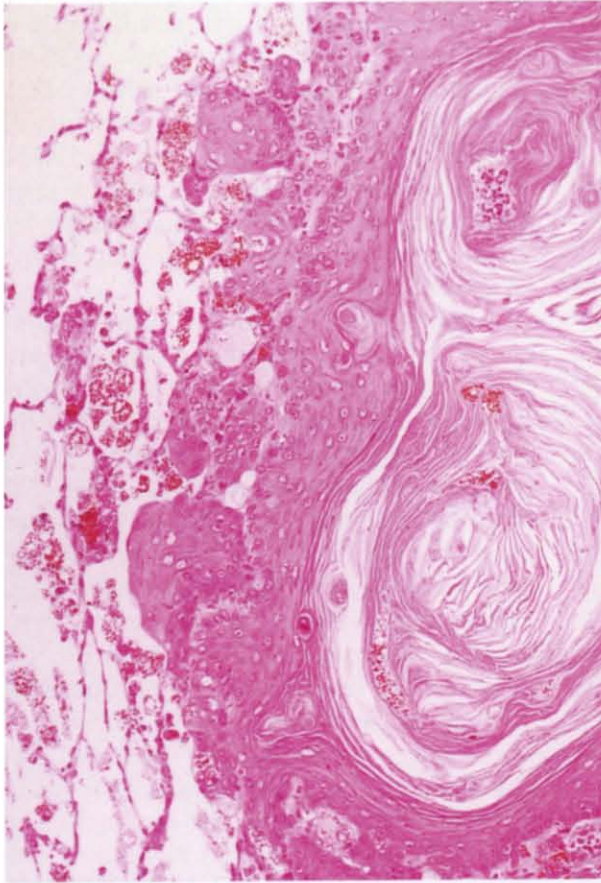
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**Fig. 1.** Cystic keratinizing epithelioma. Note the epithelial nests expanding into the adjacent alveolar spaces in the periphery of the tumor. H & E, x 125.

**Fig. 2.** Cystic keratinizing epithelioma immunostained for PCNA using peroxidase/DAB. Note one to two (focally more) layers of intensely stained nuclei in the periphery of the tumour wall. Mayers haemalaun, x 80.

**Fig. 3.** Cystic keratinizing squamous cell carcinoma. Note the high number of mitotic figures and focal invasion of adjacent lung tissue. H & E, x 125.

**Fig. 4.** Cystic keratinizing squamous cell carcinoma immunostained for PCNA using peroxidase/DAB. Note that generally more than two layers of nuclei in peripheral cell layers are stained positively. Mayers haemalaun, x 80.



1	2
3	4



ovoid and occasionally polygonal. A few peripheral cell nests completely consisted of basal cells. With haematoxylin-eosin stain mitotic figures were visible only in the basal or adjacent cell layer. The number of mitoses was low and atypical mitotic figures were rare.

The central lumen of cystic keratinizing epitheliomas always was filled with laminated keratin masses. Keratin lamellae showed a parallel orientation or consisted of confluent keratin pearls. The latter often was accompanied by the presence of many macrophages and occasionally some shadow cells. Enlargement by centrifugal growth was revealed by the presence of particles throughout the keratin-filled cavity together with whorled profiles representing sequentially incorporated keratinized peripheral air-spaces. Rarely epithelial island or projections from the tumour wall were found within the central keratin. Keratin pearls were also present in some of the larger peripheral islands of cells.

In some of the cystic keratinizing epitheliomas the keratin masses were infiltrated by numerous polymorphonuclear granulocytes. Infiltration was most pronounced in tumours with marked inflammation and fibrosis of the adjacent pulmonary tissue. Inflammation and fibrosis of varying severity was observed in all rats exposed to particles and was most severe in rats exposed to crocidolite. Compression of adjacent lung tissue could only be seen in larger tumours of lungs fixed by instillation. Even small tumours in lungs fixed by immersion appeared to cause compression but this was an artifact of the fixation method. Regressive changes were seen in some subpleural areas of the tumours. In these areas the tumour wall was thinner and the number of cell layers reduced to one or two. In occasional parts of the tumour circumference, the epithelial wall was missing with connective tissue manifesting a foreign body granulomatous response including the presence of macrophages, giant cells, mast cells and a few lymphocytes.

One or two (focally, sometimes more than two) layers of the peripheral squamous epithelium as well as almost all epithelial islands projecting into the adjacent alveoli revealed the great majority of nuclei positively labeled with a monoclonal anti-PCNA antibody (fig. 2). Staining intensity decreased in the more centrally located layers of epithelial cells. Islands of epithelial cells entrapped in the central keratin mass showed only weak or no staining. The great majority of nuclei of epithelial cells in areas with regressive changes showed no reaction.

**Cystic keratinizing squamous cell carcinoma:** Cystic keratinizing squamous cell carcinomas had a pleomorphic appearance. Generally, all of them showed at least one cavity filled with keratin and surrounded by a highly keratinized squamous epithelium. One type of cystic keratinizing squamous cell carcinoma seemed to develop from cystic epithelioma. These neoplasms revealed focal atypia of tumour cells, resulting from disorganised cells with enlarged and sometimes irregularly-formed nuclei. An increased number of mitotic figures was usually observed.

The thickness of epithelium was increased to more than eight layers in these parts and in addition to the basal layers, mitotic figures were seen in other layers of the epithelium. Sometimes focal invasion accompanied by a scirrhous reaction could be seen (fig. 3). Mast cells were frequently seen in connective tissue adjacent to the tumours. Other regions of the wall of these cystic keratinizing squamous cell carcinomas resembled cystic keratinizing epitheliomas. In these regions, the tumour wall was three to eight layers thick, regularly organised and cellular atypia was absent or minimal. Mitoses were restricted to the basal layers and extension of the tumours by peripheral extension of epithelial cell nests into adjacent alveolar spaces could be observed.

Another type of cystic keratinizing squamous cell carcinoma obviously developed a central keratin filled cavity due to degeneration and necrosis of centrally located squamous epithelium and replacement of the epithelial structures by accumulating keratin. A mesh of necrotic remnants of cords of epithelial cells was visible in parts of the keratin filled lumen of these neoplasms. Some tumours of both variants of cystic keratinizing squamous cell carcinomas had parts with predominantly spindle-shaped cells which had an increased number of mitoses. In these spindle-cell parts little or no keratinization was observed. The central keratin of the carcinomas had a laminated appearance and was mixed with cell detritus, macrophages and occasionally shadow cells. Frequently entrapped islands of squamous cells or papillary projections were seen in the central keratin. In many carcinomas the central keratin also was mixed with polymorphonuclear granulocytes. This was the case in almost all B(a)P induced neoplasms and also frequently seen in lungs with severe inflammation and fibrosis. Focal regressive changes were occasionally seen in the tumour periphery. Beside pressure atrophy (marked reduction of the number of cell layers) of the epithelial wall in subpleural areas, loss of squamous epithelium was also observed in parts of the tumour distant from the pleura. The epithelial wall was only few layers thick or replaced by connective tissue infiltrated by macrophages, giant cells and few mast cells or lymphocytes. Invasion of the pleura and of bronchi or even blood vessels was frequently observed. Occasionally invasive growth into tissue adjacent to the lung could be seen. Tissues involved included mediastinum, periaortic tissue and diaphragm. A few metastases were noted in intrapulmonary blood or lymph vessels, mediastinum, lung-associated lymph nodes, heart and kidney. Some cystic keratinizing squamous cell carcinomas exhibited small parts with glandular structures. These glandular structures contained goblet-cells, but no signs of malignant growth were present. Therefore a diagnosis as adenosquamous carcinoma was declined.

More than three peripheral cell layers of the wall of cystic squamous cell carcinomas showed at least parts with positively stained nuclei for PCNA (fig. 4). Nuclei of cell layers adjacent to the central keratin were not labeled. Invasive growing tumour cells could be clearly identified

by the PCNA technique. A strong reaction was visible in the majority of nuclei in enlarged and pleomorphic cells. Papillary projections and islands of epithelial cells reacted positively as well as areas with spindle-shaped tumour cells.

**Keratinizing squamous cell carcinoma and non-keratinizing squamous cell carcinoma:** Beside cystic keratinizing squamous cell carcinomas numerous keratinizing carcinomas without large keratin filled cavities were observed. However, keratinization was also marked in these neoplasms. Generally, the tumours consisted of nests of squamous cells with distinct stratification and a low rate of mitosis. Some less differentiated squamous cell carcinomas had little keratinization, atypical cells and a tendency to a loss of cell orientation. Results of nuclear staining with the anti-PCNA antibody were comparable to cystic keratinizing squamous cell carcinomas. Four squamous cell tumours lacked keratinization. One of them was predominantly composed of cells with a basaloid appearance.

## Incidences of neoplasms

The incidences of the neoplastic lesions of the four different studies are presented in tables 1–5. After inhalation of coal oven exhaust (Study A) and additional treatment with different co-carcinogens (crocidolite, DB(*ah*)A) incidences of cystic keratinizing epitheliomas were similar in the different treatment groups (table 1). The majority of neoplasms observed were cystic keratinizing squamous cell carcinomas with an incidence of 23.9 % in rats additionally treated with crocidolite and 22.2 % after subcutaneous application of the low dose DB(*ah*)A. The highest incidence of cystic keratinizing squamous cell carcinomas (49.3 %) occurred in the group treated with the high dose of DB(*ah*)A.

Only single cystic keratinizing epitheliomas were seen in rats that inhaled tar/pitch condensation aerosol (containing different concentrations of benzo(*a*)pyrene) or aerosol supplemented with carbon black or irritant gas for 10 (Study B1) or 20 months (Study B2) (table 2 and table 3). However, in rats exposed for 10 months (followed by a 20 months clean air recovery period) high incidences of cystic keratinizing squamous cell carcinomas were found after inhalation of tar/pitch condensation aerosol with 50  $\mu\text{g}/\text{m}^3$  (31.9 %) or 125  $\mu\text{g}/\text{m}^3$  B(*a*)P (52.8 %), 50  $\mu\text{g}/\text{m}^3$  B(*a*)P + 2  $\text{mg}/\text{m}^3$  carbon black (76.4 %), and 50  $\mu\text{g}/\text{m}^3$  B(*a*)P + 6  $\text{mg}/\text{m}^3$  carbon black (51.4 %). Carbon black inhalation alone induced cystic keratinizing squamous cell carcinomas in 6.9 % of exposed rats. A 20-months exposure period followed by a 10-months recovery period even resulted in much higher incidences of cystic keratinizing carcinomas after exposure to tar/pitch condensation aerosol with 20  $\mu\text{g}/\text{m}^3$  (26.4 %), 50  $\mu\text{g}/\text{m}^3$  (87.5 %) or 90  $\mu\text{g}/\text{m}^3$  B(*a*)P (86.1 %), 50  $\mu\text{g}/\text{m}^3$  B(*a*)P + 2  $\text{mg}/\text{m}^3$  carbon black (95.8 %), and 50  $\mu\text{g}/\text{m}^3$  B(*a*)P +

6  $\text{mg}/\text{m}^3$  carbon black (93.1 %). In rats, exposed to a combination of aerosol (50  $\mu\text{m}^3$  B(*a*)P) and an irritant gas mixture the incidence of cystic keratinizing squamous cell carcinomas was 68.1 %.

Exposure of rats to different concentrations of unfiltered diesel exhaust (Study C) resulted in incidences of cystic keratinizing epitheliomas of 2.5 % (2.5  $\text{mg}/\text{m}^3$ ) and 10.7 % (7.5  $\text{mg}/\text{m}^3$ ). Epitheliomas were seen in 16.2 % of carbon black and 16.0 % of titanium dioxide exposed rats. Only a few cystic keratinizing squamous cell carcinomas occurred (table 4).

The tumour incidences in rats after intratracheal application of different particles (Study D) are given in table 5. Significantly increased incidences of cystic keratinizing epitheliomas occurred in rats exposed to native diesel exhaust particles (16.7 %), high dose of extracted diesel exhaust particles (14.6 %), extracted printex 90-carbon black particles (18.8 %), and extracted printex 90-carbon black particles + B(*a*)P (18.8 %). High incidences of cystic keratinizing squamous cell carcinomas were noted in rats that received 15  $\text{mg}$  B(*a*)P (14.6 %) or 30  $\text{mg}$  B(*a*)P (72.7 %) intratracheally.

## Discussion

Only a few chronic inhalation or intratracheal instillation studies are comparable to the four studies presented in this paper with respect to duration of exposure, number of animals and degree of histopathological examination. Only the studies of MAUDERLY et al. (1986) on 1147 F344 rats exposed to unfiltered diesel exhaust for 24 months, ISHINISHI et al. (1986) on 1256 F344 rats exposed to different concentrations of diesel exhaust for up to 30 months and of NIKULA et al. (1995) and MAUDERLY et al. (1994) on 1150 F344 rats that inhaled diesel exhaust atmospheres or carbon black for 24 months have a comparable study design. In the latter study, 9.7 % of the rats revealed keratinized “cysts” after inhalation of 6.5  $\text{mg}/\text{m}^3$  diesel exhaust, while 5.6 % were observed in rats exposed to 7  $\text{mg}/\text{m}^3$  diesel exhaust by MAUDERLY et al. (1986). Histologically comparable cystic keratinizing epitheliomas were seen in 11.0 % of high dose group rats (7.5  $\text{mg}/\text{m}^3$ ) in Study C on different concentrations of diesel exhaust. The incidence of cystic keratinizing epitheliomas in rats of Study C exposed to carbon black (11.3  $\text{mg}/\text{m}^3$ ) was slightly higher. They occurred in 15 % of rats. MAUDERLY et al. (1994) and NIKULA et al. (1995) found histologically identical keratinized “cysts” in 11.0 % of F344 rats (exposed to 6.5  $\text{mg}/\text{m}^3$  carbon black) of their study. The slightly higher incidence of epitheliomas in Study C might be the result of the higher carbon black concentrations given to aged rats in this study. In the study of ISHINISHI et al. (1986) a few adenomas and carcinomas of the lung were noted after exposure of rats to 4  $\text{mg}/\text{m}^3$  unfiltered diesel exhaust. Since no more detailed classification of the neoplasms was performed by the authors, comparison with our own findings is difficult.

An intratracheal instillation study with female Wistar rats using diesel exhaust particles and carbon black was done by POTT et al. (1994). While in Study D cystic keratinizing epitheliomas occurred after intratracheal application of native diesel exhaust particles or Printex 90-carbon black particles in 8 of 48 rats of both groups, they were seen in the study of POTT et al. (1994) in 9 of 40 (diesel exhaust particles) and 4 of 37 (carbon black) rats. A few squamous cell carcinomas were additionally observed by POTT et al. (1994). In order to separate the effect of particles from the effect of polycyclic aromatic hydrocarbons (like B(a)P) adsorbed on their surface, one group of rats was exposed to titanium dioxide (10.4 mg/m<sup>3</sup>) in Study C. Cystic keratinizing epitheliomas were noted in 16 of 100 rats examined histologically. In a two-years study with SPRD rats on titanium dioxide, LEE et al. (1985, 1986a) found histological identical lesions in 1 of 75 males of the low-dose group (10 mg/m<sup>3</sup>) as well as in 1 of 77 males and 13 of 74 females of the high-dose group (250 mg/m<sup>3</sup>). The lesions were first classified as cystic keratinizing carcinomas, but later reclassified as "proliferative keratin cysts" (WARHEIT and HARTSKY 1994). Only the study of KLONNE et al. (1987) on PAH (polycyclic aromatic hydrocarbon)-rich particles generated by refineries during the coking process can be compared with the similar study on PAH-rich tar/pitch condensation aerosol (Study B) done by our group. Keratinized "cysts" histologically identical to cystic keratinizing epitheliomas were seen in 2 of 48 SPRD males and 10 of 44 SPRD females in the highest dose group (30.7 mg/m<sup>3</sup>, that reached an age between 18 and 24 months. A few benign cystic keratinizing epitheliomas but high numbers of cystic keratinizing squamous cell carcinomas occurred in Study B. This might be explained by longer duration of exposure (up to 30 months) in Study B or different sensitivity of Wistar and SPRD rats to squamous cell carcinoma induction by combinations of particles and PAHs.

Generally, comparison of the results of the chronic inhalation and instillation studies presented in this paper with former studies show that the cystic keratinizing epithelioma is a particle-induced benign neoplasm that can be expected to occur in inhalation or intratracheal instillation studies leading to high burden of particulates. Combined uptake of particles with other carcinogens like B(a)P, crocidolite or DB(ah)A regularly results in the occurrence of invasive cystic keratinizing squamous cell carcinomas. While the strong association of particle exposure and cystic keratinizing epitheliomas has been reported before in the literature (CARLTON 1994, LEVY 1994, BOORMAN et al. 1996) single epitheliomas also were observed after intratracheal instillation of B(a)P or subcutaneous application of DB(ah)A under clean air conditions in one of our studies. Although the great majority of cystic keratinizing squamous cell carcinomas were observed after combined exposure to particles and other carcinogenic substances, they also were noted in rats after intratracheal instillation of B(a)P in Study D.

Much of the discussion about the significance of cystic keratinizing lesions of the rat lung in chronic inhalation studies concentrates on the biological behaviour of that lesions. The majority of members of a workshop in Newark, U.S.A., denied the possibility of a malignant transformation of cystic keratinizing lesions and regarded them as non-neoplastic changes (CARLTON 1994, LEVY 1994). For the designation of the squamous lesions from two inhalation studies (titanium dioxide, para-aramid fibrils) the workshop members preferred the term "proliferative keratin cyst". Proliferative cysts are very rare in veterinary and human pathology. Variants described were derived from the epidermis or its associated structures. The term is only applied to a special variant of the isthmus-catagen (tricholemmal) cyst or some matrical cysts of dogs (WALDER and GROSS 1993). In both cases, proliferative cysts are looked upon as transitional stages of neoplastic development to cornifying keratoacanthoma (WEISS and FRESE 1974) or pilomatricoma and trichoepithelioma (WALDER and GROSS 1993). Also in human pathology proliferating tricholemmal cysts, classified according to the WHO-nomenclature of skin tumours, are regarded as transitional stages in the development of pilar tumours of the scalp (HEENAN et al. 1996; NOLTENIUS and COLMANT 1987). For that reasons the term proliferative cyst generally seems to be inappropriate for the description of what was believed to be a non-neoplastic lesion. As a result of the Hannover workshop, cystic keratinizing lesions were looked upon as a family of related changes, including squamous metaplasia with excessive keratinization, keratinizing cysts, benign cystic keratinizing epitheliomas and finally cystic keratinizing squamous cell carcinomas. The presence of focal cellular atypia, increased mitotic activity, loss of polarity of cells and invasive growth in the wall of cystic keratinizing tumours justified a classification of some of these tumours as carcinomas (BOORMAN et al. 1996). The findings in our own studies support this opinion. In two of four studies, cystic keratinizing tumours were observed which had a benign histological appearance in most parts of the tumour, but focally revealed invasive growth into adjacent connective tissue or bronchi, resulting in a classification as cystic squamous cell carcinomas. In a single case, a keratinizing squamous cell carcinoma focally arising from the wall of a cystic keratinizing epithelioma was observed in a rat exposed to titanium dioxide. Two similar cases were reported by MAUDERLY et al. (1994) in F344 rats exposed to diesel exhaust particles or carbon black by inhalation. All these findings indicate, that cystic keratinizing epitheliomas are not necessarily an endpoint of development, but may progress to (cystic keratinizing) squamous cell carcinoma.

Demonstration of PCNA in this study revealed that the cystic keratinizing lesions classified as cystic keratinizing epitheliomas have a distinct proliferative activity. PCNA is a 36-kDa nuclear protein, that is a co-factor of DNA-polymerase (BRAVO et al. 1987). This cell protein is considered as endogenous marker of proliferation (HALL et

al. 1990) that can be labeled immunohistochemically. In normal tissue, the monoclonal antibody PC10 directed against PCNA reveals positive reactions in all proliferating cells (HOFSTÄDTER et al. 1995). FRAME et al. (1996) reported the proliferative activity of cystic keratinizing lesions in the lung of rats after exposure to para-aramid fibrils in comparison to spontaneous keratoacanthomas. In the six examined cystic keratinizing lesions only a few nuclei were stained for PCNA in the basal cell layer or the outer layer of epithelial cell nests. In the present studies, over 100 cystic keratinizing squamous lesions were examined and it could be shown that numerous lesions classified as epitheliomas had positively labeled nuclei (anti-PCNA antibody, clone PC 10) in one or two, focally even in three or four peripheral cell layers, comparable to the proliferative activity of keratoacanthomas demonstrated by FRAME et al. (1996). Human squamous cell carcinomas of the lung were examined immunohistochemically with an anti-PCNA antibody (clone 19A2) by CAREY et al. (1992). Positive labeling of peripheral cell layers by the antibody was found in the majority of cases examined in primary squamous cell carcinomas and metastases. However, the authors indicated strong variations of positive reactions between different parts of one tumour. This was also confirmed by our own immunohistochemical examinations. Frequently, serial sections were necessary to detect strongly positive areas in the neoplasms. Generally, immunohistochemical demonstration of PCNA has proven to be an additional tool for evaluation of biological behaviour of cystic keratinizing lesions. Results of microscopic examination of haematoxylin and eosin stained slides were confirmed by this technique. Lesions classified as squamous metaplasia with abundant keratinization or keratinizing cysts revealed no or weakly positive reaction. Although neoplasms with distinct atypia or even invasive growth showed only staining reactions in two to four cell layers in some parts of the tumour, in other areas of the same tumour a much higher number of cell layers were positively labeled. The portion of PCNA-positive nuclei increased with increasing degree of cellular atypia. The estimation of the mean number of proliferating cell layers in the periphery of immunohistochemically stained tumours was the most reliable method for the distinction between keratinizing cyst, cystic keratinizing epithelioma and cystic keratinizing squamous cell carcinoma. Other classic criteria for malignant behavior like invasion or metastasis, cellular atypia, and number of mitosis however, are still the primary diagnostic aids.

In the studies, reported in this paper, exposure atmospheres of rats were characterised by high contents of particles. Exposure to particles and especially combinations of particles with B(a)P or other carcinogens resulted in the induction of a variety of squamous lung neoplasms including benign cystic keratinizing epitheliomas, cystic keratinizing squamous cell carcinomas and simple keratinizing squamous cell carcinomas. In contrast, there are only few epidemiological studies that report an increased risk of lung tumours in humans after exposure to the par-

ticles (diesel exhaust particles, titanium dioxide, carbon black, etc.) used in the rodent studies. Slightly increased cancer risk due to exposure to diesel motor exhaust was reported by GARSHICK et al. (1988) in railroad workers, by BOFFETTA et al. (1990) in railroad workers and miners and by DUBROW and WEGMAN (1983) in bus-, taxi- and truck drivers. In some rural areas of China, increased lung cancer risk seems to be associated with indoor air pollution by burning of smoky coal (MUMFORD et al. 1987) or sleeping on beds heated by coal-burning stoves (XU et al. 1989; WU-WILLIAMS et al. 1990). In contrast, several epidemiological studies on coal dust exposed coal miners (MAUDERLY 1994), workers of the carbon black industry (ROBERTSON and INGALLS 1980, 1989; HODGSON and JONES 1985) or workers of titanium dioxide producing factories (CHEN and FAYERWEATHER 1988) revealed no increased risk for lung cancer. The discrepancy between the results of chronic inhalation and instillation studies in rodents and the lack of epidemiological evidence for an increased risk of lung cancer in humans exposed to the same particles raises the question on the relevance of these studies for human beings. Since especially the rat (compared to mouse or hamster) seems to have a high sensibility for the lung tumour induction by particles (WATSON and VALBERG 1996) the occurrence of lung neoplasms in inhalation studies of this species is frequently interpreted as a species specific phenomenon and its relevance for humans is refused. In a number of studies it could indeed be demonstrated that the rat reveals some special characteristics concerning the lung reaction to inhaled particles and inhalative particle overload of the lung compared to other rodent species, monkeys and man. The location of particle deposition in the lung differs between monkeys (which lung is anatomically similar to the human lung) and rats. While intraalveolar deposition of particles is the most common feature in rats, NAU et al. (1962, 1976) observed only a few carbon black particles in the alveoli of monkeys exposed to carbon black by inhalation for years. Pigmented macrophages were rare. The great majority of carbon black particles was located in the peribronchial or perivascular interstitial tissue as free particles or phagocytized by macrophages. No neoplasms were found in the animals. In a 10-months inhalation study with manganese dioxide dust on rhesus monkeys, particles were also found in alveoli but were predominantly located in peribronchial and perivascular interstitium (SUZUKI et al. 1978). Low intraalveolar accumulation of particles in the lung of monkeys might result in a lower degree of direct injury of alveolar epithelium by particles in this species. Also the low degree of particle uptake by alveolar macrophages might result in decreased release of inflammatory mediators and reactive oxygen species by these cells. Both factors probably contribute to the chronic pulmonary inflammation observed in rats exposed to high burden of particles which is discussed as a cause of neoplastic transformation of lung cells in this species (DUNGWORTH et al. 1994). In contrast to other rodent species (mice and

hamsters) and man, particle deposition in the lung of rats is accompanied by a strong participation of polymorphonuclear leucocytes (which can release reactive oxygen species, with their mitogenic, cytotoxic and genotoxic effects) in the pulmonary response to particles (MUHLE et al. 1990; MUHLE et al. 1991; HEINRICH et al. 1986a; HENDERSON et al. 1988b; NATIONAL TOXICOLOGY PROGRAM 1993). In the study of MAUDERLY et al. (1994), cells in control rats harvested by lung lavage consisted predominantly of macrophages (97 %–99 %) with few polymorphonuclear leucocytes. In rats exposed to diesel exhaust or carbon black particles the proportion of macrophages decreased to 35 %–50 % and polymorphonuclear leucocytes increased to 50 %–65 % of the whole number of cells counted. In contrast, in humans that suffer chronic exposure to high concentrations of particles at their working place, only few polymorphonuclear leucocytes are involved in the lung reaction to particles. ROM et al. (1987) found in the lung lavage fluid of workers with asbestosis, pneumoconiosis or silicosis predominantly macrophages, while the portion of polymorphonuclear leucocytes was only 3 %–4 %. SABLONNIERE et al. (1983) found polymorphonuclear leucocytes only in the lung of six of 16 miners, but the portion of these cells was very low (0.68 %). The results of these studies reveal, that polymorphonuclear leucocytes not seem to be a significant part of the pulmonary response to particles in humans.

Differences between rats and other species also exist with regard to the release of mediators of inflammation after particle exposure. In a comparative study on B6C3F<sub>1</sub> mice and F344 rats, HENDERSON et al. (1988a) showed that rats have higher levels of leucotriene (LTB<sub>4</sub>), prostaglandin (PGE<sub>2</sub>) and thromboxane (TXB<sub>2</sub>) in the normal lung and that the increase of these substances in pulmonary lavage fluid is much higher in rats than in mice after inhalation of diesel exhaust particles (3.5 mg/m<sup>3</sup>). Additionally, it could be demonstrated that defence mechanisms against cell damage by reactive oxygen species released by macrophages and polymorphonuclear leucocytes in rats are not as effective as in other species (OBERDÖRSTER et al. 1994; Slade et al. 1985). In rats exposed to diesel exhaust, lower amounts of glutathione were found in lung and lung lavage fluid than in mice, and a cadmium inhalation induced increase of methallothionin seen in mice was missing in rats (OBERDÖRSTER et al. 1994). In a comparative study on the concentration of the antioxidants ascorbic acid,  $\alpha$ -tocopherole and glutathione in the lung, rats had the lowest levels of  $\alpha$ -tocopherole and glutathione of all laboratory animal species examined (SLADE et al. 1985).

Since particle-induced chronic inflammation of lung tissue, accompanied by hyperplastic, metaplastic and eventually neoplastic changes are thought to be fundamental sequential events in lung tumour induction in rats (DUNGWORTH et al. 1994) and rats are different to man and other species in so many respects of their particle-induced inflammatory response, direct extrapolation of rat studies to humans is not possible. Whether modified

extrapolation is appropriate is unclear because the extent of differences in particle response of rats and humans has not been yet fully explored. Further studies along these lines are therefore necessary to prove the relevance of rat studies for human risk assessment. Several types of neoplasms occurring in carcinogenicity studies in rats have not been accorded significance for risk assessment in humans because of their rat specific mechanisms of induction. Examples of these kind of tumours include renal tumours in male rats with  $\alpha_{2u}$ -globulin nephropathy following exposure to a variety of chemicals like isophorone, paradi-chlorobenzene, and pentachloroethane, mesovarian leiomyomas induced by  $\beta_2$  receptor stimulants and carcinoid tumours in the stomach induced by prolonged suppression of acid secretion with H<sub>2</sub> antagonists and proton pump inhibitors (ALISON et al. 1994). For cystic keratinizing neoplasms further studies on the mechanisms of tumour induction are necessary to determine whether these tumours truly belong to the group of rat neoplasms with no significance to man.

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