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Editorial/Editoriale

Carcinogenicity of the dietary sweetener aspartame: thirty years later

Cancerogenicità del dolcificante dietetico aspartame: trenta anni dopo

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Thirty-one years ago, the US FDA[1] approved as safe a new sweetener, aspartame (APM), for limited use in commerce. Today, APM is used in over 6,000 products by an estimated several hundred million consumers.

In support of the safety of APM, three chronic studies were submitted to the US FDA. These studies were judged to be negative, even though small numbers of animals were used per dose. The studies were carried out for a period of two years, which was standard at that time. Studies as designed could not adequately answer the question of the possible carcinogenicity of APM.

Prior to the initial approval of APM, Olney[2] raised a number of questions about the safety of APM and believed that APM might cause brain tumours. Olney's studies[3] have also raised serious questions regarding adverse effects of glutamate and aspartate, where it was shown that APM at 0.5 g/kg produced lesions in 50% of animals tested. The safety of APM was also questioned by a number of authors between 1970 and 1980.

In 2005, Soffritti and associates[4] were the first to report in this journal that APM caused leukaemias and lymphomas in rats. Soffritti's final findings were presented at the international scientific conference "Framing the future in light of the past: living in a chemical world", promoted by the Collegium Ramazzini and held in Bologna in September this year. The full data of the experiment have been reported in Environmental Health Perspectives[5], an independent publication of the National Institute of Environmental Health Sciences (NIEHS), a division of National Institutes of Health (NIH). Soffritti and associates have shown that APM is a multipotential carcinogen when administered to Sprague-Dawley rats in feed. Experiments were carried out in the Cesare Maltoni Cancer Research Centre, European Ramazzini Foundation of Oncology and Environmental Sciences in Bologna, Italy. The protocol of carrying out the experiment until the spontaneous death of the animal, pioneered by Maltoni, rather than for the usual two years was followed.

This important study demonstrated increased incidence of malignant tumours in animals and showed a statistically significant trend in male ($p \leq 0.05$) and in female ($p \leq 0.01$) Sprague-Dawley rats. Statistically significant increases were found in lymphomas, leukaemias, transitional cell carcinomas of the renal pelvis and ureter, and an increased incidence of malignant schwannomas of peripheral nerves.

From a public health standpoint, these studies necessitate the re-examination of the safety of APM as an additive in foods and beverages and of the use of 2-year-duration-chronic studies for carcinogenicity.

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Commentary/Commentario

Particle toxicology: past work and future implications

Tossicologia delle particelle: conoscenze acquisite nel passato ed implicazioni per il futuro

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The issue of dusts and their effects upon man has been around for centuries and our knowledge about such problems is long-standing. The ancient Romans knew of the hazards to miners of asbestos, and this was written about contemporaneously. In India the matter of mica mining showed how that material could lead to disease. The work in the collieries of Great Britain pointed to the hazards of coal dust, and silicosis was the biggest occupational health issue of the first part of the 20th Century.

Unfortunately each of these problems remains with us. The issue of dust and disease is both one of the past and one we can expect to see in the future. We have added to our knowledge base over time by recognizing that materials that have been known to have one hazard in reality have more. The matter of understanding that silica can also cause lung cancer is a relatively new appreciation, for example. What the work of Professor Pott does for us is point out that other materials not always thought of as carcinogenic may in fact be so. This is a new way of appreciating this issue, and his work considers many dimensions of dust-related problems. If we are thoughtful about what his work has to offer us, we can be protective of health in the future as workers not only work with traditional dust exposures, but with materials yet to be designed.

Pott and his colleagues have for many years been producing high quality research on the hazards of dusts. He, and many others, have studied the hazards of those dusts long recognized as having special risks, such as asbestos and silica. With the new studies presented in the extensive paper in this volume, the insights about dusts, their ability to cause a range of diseases, and some thought provoking issues are presented.

The paper is fairly straightforward, and represents extensive animal studies, and is appropriate regarding assumptions that the authors make regarding their work. While others might have chosen a different set of assumptions regarding several issues, there is little to quarrel with those chosen, and they are clearly stated.

The findings in some ways are not surprising, though the range of tumours, both benign and malignant, are beyond what might have been thought of as usual, and there were certain surprises, especially regarding the severe acute toxicity of materials. Hydrophobic titanium dioxide was found to be especially toxic.

Pott is also especially thoughtful as he raises issues regarding how to judge toxicity, should it be by weight, surface area, or particulate size. Most papers regarding dust never address this fairly fundamental question. There are significant implications regarding this matter. The ongoing debate regarding the relative toxicity of the amphiboles versus chrysotile might be seen differently if such issues were further considered.

The range of issues discussed is broad, with many implications. There are data regarding diesel particles and how this data fits with other particles. The issue of phagocytosis is also thoughtfully discussed.

Also, there are significant future implications of this work as it refers to small particles. As nano-particles are further considered, there will be some significant implications of the potential hazards of these small particles, based upon the findings of this research. Given these findings, attention should be paid to the special hazards that may result from these smaller particles.

It would be good if more researchers on the matter of particulates and cancer followed as thoughtful an approach as does Professor Pott.

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Carcinogenicity study with nineteen granular dusts in rats

Saggio di cancerogenicità con diciannove polveri granulari nei ratti

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Summary

The primary aims of the study were to test the carcinogenicity of granular dusts on the rat lung after intratracheal instillation and to find an optimal metric for the carcinogenic potency of non-specifically toxic dust types which constitute a particular dust category. Nineteen dusts were chosen which differed significantly in at least one of the following properties: chemical composition, density, specific surface area (BET), mean particle size. Quartz and amorphous SiO_2 were included in the test, for comparison's sake, because of their well-known specific toxicity. In addition, coated hydrophobic titanium dioxide (TiO_2), not tested before, was instilled and was found to be acutely toxic. Sixteen of the 19 dusts formed a group for which no specific toxicity was detected as the cause of their carcinogenicity. These dusts were thus defined as respirable granular bio-durable particles without known significant specific toxicity (GBP) in the framework of this carcinogenicity experiment. Regarding their particle size, 4 of the 16 GBP were classified as ultrafine (GBP-UF, mean diameters 0.01-0.03 μm), 4 as fine small (GBP-F-small, mean diameters 0.09-0.2 μm), and 8 as fine large (GBP-F-large, mean diameters 1.8-4.0 μm). All 16 GBP produced lung tumours, many more than expected. The microscopically diagnosed lung tumour incidences were used for non-linear regression analysis, which included especially instilled masses, volumes, dust surface areas, and particle sizes of the GBP. The GBP volume in connection

Riassunto

Gli scopi principali dello studio sono stati quelli di valutare la cancerogenicità delle polveri granulari per il polmone di ratto dopo instillazione intratracheale e di trovare un modo ottimale di misurare il potere cancerogeno di tipi di polveri non specificamente tossiche, che costituiscono una particolare categoria di polveri. Sono state scelte 19 polveri che differivano significativamente in almeno una delle seguenti proprietà: composizione chimica, densità, superficie specifica (BET), dimensione media delle particelle. Il quarzo e la SiO_2 amorfa sono stati inclusi nello studio come controlli positivi, per la loro ben nota tossicità specifica. Inoltre è stato instillato biossido di titanio (TiO_2) rivestito, idrofobo, non studiato prima, ed è stato trovato dotato di tossicità acuta. Sedici delle 19 polveri hanno formato un gruppo nel quale non è stata trovata una tossicità specifica che potesse essere causa della loro cancerogenicità. Nell'ambito di questo esperimento di cancerogenicità queste polveri sono state così definite particelle respirabili granulari bioresistenti senza una nota e significativa tossicità specifica (GBP). Per quanto riguarda la dimensione delle particelle, 4 delle 16 GBP sono state classificate ultrafini (GBP-UF, diametri medi 0,01-0,03 μm), 4 fini piccole (GBP-F-piccole, diametri medi 0,09-0,2 μm), ed 8 fini grandi (GBP-F-grandi, diametri medi 1,8-4,0 μm). Tutte le 16 GBP hanno causato tumori polmonari, molte in misura maggiore dell'atteso. Le incidenze dei tumori polmonari con diagnosi microscopica sono state usate per l'analisi di regres-

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with particle size turned out to be the most adequate dose metric for the carcinogenicity of GBP. The GBP-UF were twice as effective as the small GBP-F and 5½ times more effective than the large GBP-F. Their differing distribution pattern in epithelial and other cells, as well as the alveoli, interstitium, and lymph nodes was considered to be an important factor for the differing potencies. Eur. J. Oncol., 10 (4), 249-281, 2005

sione non lineare, che comprende soprattutto masse, volumi, superfici delle polveri e dimensione delle particelle di GBP. Il volume delle GBP, insieme alla dimensione delle particelle, si è rivelato la misura di dose più affidabile per valutare la cancerogenicità delle GBP. Le GBP-UF sono risultate 2 volte più potenti delle GBP-F-piccole e 5 volte e mezza più potenti delle GBP-F-grandi. Il loro diverso modo di distribuirsi nelle cellule epiteliali e nelle altre cellule, negli alveoli, nell'interstizio e nei linfonodi è stato considerato un fattore importante alla base del diverso potere cancerogeno. Eur. J. Oncol., 10 (4), 249-281, 2005

Key words: **dust, particles, rat, lung, cancer**

Parole chiave: **polvere, particelle, ratto, polmone, cancro**

Introduction

Several carcinogenicity studies on chronic inhalation exposure, especially to diesel engine exhaust, carbon black, and titanium dioxide (TiO_2), have resulted in benign and malignant lung tumours in rats[1-8]. Hamsters did not respond with tumours[2, 3, 9]. Tumours in mice were debatable or did not occur[3, 4, 7, 10, 11]. Repeated intratracheal instillation of diesel soot and other granular (=non-fibrous) bio-durable dusts of different chemical compositions without known significant specific toxicity also induced lung tumours in rats[12-15].

Regarding the analogy between diesel exhaust and other particles, the interpretation of the effect is not unequivocal. The common opinion is that there exists a significant difference between the carcinogenicity of diesel and that of other particles, because the organic substances with numerous well-known carcinogenic polycyclic aromatic hydrocarbons (PAH) are adsorbed at the elemental carbon core of diesel particles[16]. However, two inhalation experiments with rats can usefully be compared: pyrolysed tar pitch aerosol which contained about 90 μg benzo[a]pyrene (BaP) per m^3 as a reference substance for PAH and a minimal concentration of elemental carbon resulted in an 18% tumour response[17]; inhalation exposure to 4.2 mg diesel exhaust per m^3 with 12 ng BaP induced lung tumours in 16%[3]. The relationship between the BaP concentrations per m^3 amounts to about 7,500 to 1. Assuming that the PAH-composition with BaP as a reference substance was more potent by a factor of 10 in diesel exhaust than in tar pitch aerosol, and that the adsorption of PAH at the surface of elemental carbon core enhances the carcinogenic potency of PAH again by a factor of 10, then little more than 1% of the carcinogenicity of diesel exhaust in rat inhalation studies can be explained by PAH. Yet another inhalation experiment with pyrolysed pitch and carbon black showed that a very high PAH concentration (20 μg BaP/m^3, total aerosol 1 mg/m^3) resulted in 4% lung tumours, while 6 mg carbon black/m^3 (BaP < 0.1 ng/m^3) resulted in 18%[18]. The most reasonable explanation for the carcinogenicity of diesel soot is its content of respirable granular bio-durable particles[19]. However, they are up to now not classified as a carcinogen.

In hamsters, even after instillations of a high total dose of 60 mg carbon black, no lung tumours were detected[20]. However, some substances which are human carcinogens did not show a lung tumour response in hamsters either. These are asbestos fibres[21], PAH-rich pyrolysis exhaust[17], cadmium compounds[22], quartz[23], and nickel compounds[24].

At the time of the start of our experiment with 19 dusts in 1995, the state of knowledge on dust carcinogenicity had been described and interpreted by several authors in the proceedings of the conference "Particle overload in the rat lung and lung cancer"[25]. In general, it was concluded that rat lung tumours are rat-specific and only occur under so-called overload conditions of the lung. However, the rat lung has been used for decades as an important model for lung diseases in man. If one can really produce enhanced tumour incidences from granular dusts without significant specific toxicity by using doses which are in the range of dust burden incurred by human lungs, this would raise certain questions which could well be answered by an instillation study on 19 dusts in rats:

1) Which of the nineteen dusts can be described as granular dusts without significant specific toxicity (earlier termed *inert dusts*) although they are different in chemical composition and some physical properties?

2) Do such dusts induce tumours only when they are *ultrafine* (diameters smaller than 0.1 μm)? Or do so-called *fine particles* between 0.1-1 μm and 1-4 μm also produce statistically significantly higher tumour incidences (excess risks higher than about 10%) from a very high or moderate lung dust burden, like miners' exposure, if one were to use a more sensitive test model than the inhalation test?
3) Which characteristics describe the carcinogenic potency of these dusts best? Which is the appropriate physical dose metric? Mass, specific surface area, volume, particle sizes, and particle number should especially be considered.
4) Which method is adequate for assessing an ethically or socio-politically relevant but not statistically significant excess risk between 0.1 and 10%, sufficiently reliable from higher tumour incidences which are statistically significantly increased?
5) What is the difference between the carcinogenic potency of not specifically toxic dust and pure quartz dust type DQ12 which has been used in experimental studies for decades?
6) Does the original quartz content in coal mine dust increase carcinogenicity more than quartz-free coal dust?
7) Does the bio-soluble and generally well-known toxic amorphous silica (SiO_2) cause tumours and fibrosis by chronic inflammation, although the particles do not persist for a longer time?
8) Does the hydrophobisation of a dust surface change the effect?
9) What is the relation in terms of sensitivity between the inhalation and the instillation test models?
10) To what extent is it reasonable to extrapolate the carcinogenicity of GBP in rats to humans?

The 19 dusts tested differed from each other in at least one of the following properties: chemical composition, density, specific surface area, mean particle size, particle number per mass unit. At the beginning of the experiment, knowledge of the specific toxicity of some dusts and the relevance of this to their carcinogenic potency was equivocal or insufficient. Coal mine dusts were considered to have a specific toxicity because of their quartz content. Other dusts, such as zirconium dioxide (ZrO_2) and hydrophobic TiO_2 have never been tested before. After the end of the experiment and a prior evaluation of new data and previous information, we classified 16 of the 19 tested dusts as *respirable granular bio-durable particles without known significant specific toxicity*. The chosen abbreviation *GBP* means only a *special selection* of the uncounted number of *granular bio-durable dusts* which includes a lot of specifically toxic dusts like quartz or genotoxic particles like nickel oxide. So, the abbreviation *GBP* should be reserved strictly to the nine-word definition. In the literature, the abbreviation *PSP* is used for such particles. It has been defined as *non-fibrous poorly soluble particles of low acute toxicity, chemically distinct*[26]. However, the definition of GBP as given above is more precise.

Of the 19 dusts, three were clearly and significantly specific toxic. However, it has to be considered that they are active due to different toxic principles:

1) *quartz* (crystalline silica) has a strong inflammatory and fibrotic potency in the lungs of rats and man and is carcinogenic in both species due to one or more non-soluble toxic surface properties like silanol groups, which have been an object of research for decades[23, 27];
2) *Pyrogenic amorphous SiO_2* is not bio-persistent in the lung; it induces acute inflammation and at least a transient fibrosis in the lung. Acute mortality occurs from much lower doses than quartz doses used in intratracheal instillation[28, 29] and intraperitoneal injection[30];
3) *Hydrophobic TiO_2* was unknown to us before the study. Surprisingly, a high acute toxicity was observed[31]. This dust consists of two substances: the bio-durable core of ultrafine TiO_2, and an organic silicon compound which coats the hydrophilic TiO_2 surface and changes it from hydrophilic to hydrophobic. This latter path of particle toxicity, by dissolution of adsorbed toxic substances from the surface area of not specifically toxic particles, is supposed to contribute causally to the associations between particulate air pollution and daily mortality from respiratory and cardiovascular effects. These associations have been found in epidemiologic investigations since 1990[32-35]. Our study only touches on such effects in connection with hydrophobic TiO_2. That there is an appreciable effect on the general population can only be speculated.

Materials and methods

Dusts

The five coal-containing dusts and quartz applied in part 1 of the total experiment (so-called coal dust study) are characterized in Table 1. The further 13 dusts administered in part 2 ("non-mining dusts") are described in Table 2. Table 3 shows particle numbers and specific

Table 1 - Characteristics[a] of the 6 dusts in part 1 (mining dusts)

Substance	Received from	Particle size (µm)[b]			Specific particle number[c] (10^9/mg)	Density (g/ml)	Specific surface area (m^2/g)	SiO_2 (%)	Ash (%)
		10% <	50% <	90% <					
Lean coal, milled	Niederrhein. Bergwerks-AG[d]	1.6	4.0	7.6	0.27	1.4	4.1[e] 1.50[f]	<0.1	5.1
Lower rich coal, milled[g]	DMT 1994	1.0	1.8	3.4	1.7	1.4	9.9[e] 2.80[f]	<0.1	6
Rich coal, mine[h]	DMT 1994	1.5	3.4	6.1	0.54	1.8	6.4[e] 1.75[f]	1.3	~ 40
Steam coal, mine[i]	DMT 1994	1.0	2.4	4.7	1.5	2.2	10.9[e] 2.32[f]	9.0	~ 60
Rock, coal mine[j]	DMT 1994	0.8	2.3	4.6	1.2	2.4	17.6[e] 1.78[f]	16.7	86
Quartz, Dörentrup milling no. 12	Hauptstelle[k] 1966/67	0.6	1.1	2.3	3.6	2.6	8.8[e] 3.10[f]	99.1	

[a] We thank Dr. Armbruster, Deutsche Montan Technologie GmbH (DMT), Essen, for providing us with 4 of the 6 dusts and for the dust characteristics.

[b] Measurement with the Coulter Counter[36]. This method determines the equivalent diameter of the volume of spheres according to their mass or volume as compared with that of irregularly formed particles. The lower limit of the measurable range is given with an equivalent diameter of 0.6 mm. The geometric diameters of the 10-, 50-, and 90-percentiles measured by electron microscopy are expected to be significantly smaller than the given mass-equivalent diameters determined with the Coulter Counter.

[c] Data are not results of direct measurements, they are calculated from the particle size distribution.

[d] Received from Dr. Weller, ca. 1970; dust also applied in intraperitoneal tests in rats and in an inhalation experiment with rhesus monkeys[37].

[e] From Eickhoff[38]. This value was used for further calculations.

[f] Specific surface area, calculated from the particle surface which results from the particle size distribution, surface of spheres assumed (see footnote [b]).

[g] Tremonia II, cleaned and milled.

[h] Dust from the air underground in the *Bochumer Schichten* sampled with BAT II dust sampler[39]. The sample was also tested in other experiments[40, 41].

[i] Dust from the air underground in the *Essener Schichten*; sampled and tested as described in footnote [h].

[j] Dust from the air underground in a road leading into a coal mine; fine fraction of the dust of a filter unit. The dust is called stone dust instead of mine dust because of the high percentage of ash and the low content of coal.

[k] Hauptstelle für Staub- und Silikosebekämpfung des Steinkohlenbergbauvereins, Essen (Precursor of DMT, see[a]).

surface areas of "ideal" spherical dusts on the basis of assumed uniform diameters and, for comparison, the 19 tested dusts. Some additional information is given on the six dusts from coal, coal mines, and quartz in the following paragraphs.

Dusts from coal and coal mines. - All the coal-containing dusts come from materials of the Ruhr coal mining area. Two dusts are a fine fraction of purified and milled coal: lean coal (*Magerkohle*), from the former Niederrheinische Bergwerks AG, and lower rich coal (*untere Fettkohle*), sample Tremonia II. Two coal mine dusts were collected with a BAT II sampler[39] from the air in the mines, extracting rich coal (*Fettkohle*) from the lower Bochum strata (*untere Bochumer Schichten*) and extracting steam coal (*Gasflammkohle*) from the middle Essen strata (*Essener Schichten*); they contain much higher proportions of minerals (ash about 40% and 60%, respectively) and varying amounts of SiO_2 (1.3% and 9.0%, respectively). Samples of the same origin have been studied in other experiments[40, 41]. The fifth coal dust was collected from a road leading underground; the sample tested is the fine fraction of a dust from a filter unit. The mineral and quartz content are high (ash 86%; SiO_2 16.7%), therefore, the dust can be called coal *containing rock dust*.

Quartz dust DQ12. - It was administered as a positive carcinogenic and fibrogenic control substance. It is a tertiary quartz sand extracted from the Dörentrup deposit in North-East-Westphalia, milled and supplied by Dörentruper Sand- und Tonwerke GmbH under the name

Table 2 - Characteristics of the 13 dusts in part 2 (non-mining dusts)

Substance	Particle size, mean (μm)[a]	Density (g/ml)	Specific surface area (BET)[b] (m^2/g)	Supplier / producer	References
Carbon black, lamp black 101	0.095	1.8-1.9 1.85[c]	18.4	Degussa	Degussa[43, 44]
Carbon black, furnace black (Printex 90)	0.014		337		
Aluminium oxide C[d] > 99.6% Al_2O_3	0.013[c] 0.020	3.2[c, e] 2.9	124	Sigma-Aldrich / Degussa	Degussa[42, 45]
Aluminium silicate P 820, 9.6% Al_2O_3, 82% SiO_2, 8% Na_2O	0.015	2.1	62.9	Sigma-Aldrich / Degussa	Degussa[46]
Kaolin, ~$Al_2Si_2O_5(OH)_4$, K 7375 *[1332-58-7]*	~ 2[f]	2.5[c]	19	Sigma-Aldrich	
Diesel soot, lorry	0.2[c] (agglomerates + aggregates)	1.85[g]	native 12.9 extracted 34.5	Dr. Tomingas	Pott and Roller[47] UBA[19]
TiO_2 P 25, hydrophilic, majority anatase	0.030/0.021 0.025[c]	3.8	52	Degussa	Degussa[42, 45] Nolan *et al*[48]
TiO_2 P 805[h], AL 90,003-2, hydrophobic[i]	0.021 (data of T 805)	3.8	32.5	Sigma-Aldrich[h]	Degussa[45, 49] Pott *et al*[31]
Test Toner for copier[j], polymer with nucleus of carbon black	3.5 (MMAD 4.0; geom. standard deviation 1.5)	1.2	3.6	Xerox	Muhle *et al*[50, 51] Bellmann *et al*[52]
TiO_2 anatase AL 23,203-3 *[1317-70-0]*, (hydrophilic)	0.2[c]	3.9	9.9	Sigma-Aldrich	Nolan *et al*[53] Pott *et al*[14]
Zircon (IV)-oxide, 99% ZrO_2, AL 23,069-3	< 5 ~ 2[k]	5.85[m]	4.4	Sigma-Aldrich	
Lung dust, coal miner, silicosis degree III (336/1)	0.2[c]	~ 2[c]	12.2	Dr. Brockhaus	Pott and Roller[47]
Silica fumed, Si S5505[n] (pyrogenic amorphous SiO_2)	0.014	2.2[n]	210	Sigma-Aldrich	

[a] As far as dusts from Degussa are concerned, calculation of the arithmetical mean took place after measurement of the diameter of 3,000 – 5,000 primary particles by electron microscopy[42]; partly differing data of the mean diameter in different publications by Degussa, e.g. on titanium dioxide. Primary particles accumulate more or less to larger particles (bio-durable aggregates or not bio-durable agglomerates). So, the effective particle size in the lung is difficult to estimate.

[b] From Eickhoff[38]. This value was used for further calculations.

[c] There are no clearly measured values or more than one piece of information. On the basis of the data available, the value with footnote[c] was assumed to be close to the correct value. It was used for further calculations.

[d] Crystallographically, Al_2O_3 "C" belongs exclusively to the δ-group[45].

[e] Measured by pycnometer.

[f] Shapes are small plates, size not precisely detectable because of accumulated small primary particles (Rödelsperger, personal communication 2005).

[g] By analogy with technically produced carbon black, the density for the core of elementary carbon of diesel soot was assumed to be 1.85 g/ml. For the adsorbed organic substances see Table 4, footnote[r].

[h] Titanium dioxide T 805 from Degussa was ordered from Sigma-Aldrich, but the supplier only offered an amount of at least 40 kg P 805. Neither Sigma-Aldrich nor Degussa answered at all clearly when questioned insistently as to the difference between T 805 and P 805. So, it is not proven that P 805 is identical with T 805 from Degussa.

[i] In the case of T 805, ultrafine TiO_2 with the specification P 25 is coated with trimethoxyoctyl-silane to change the particle surface from hydrophilic to hydrophobic[49].

[j] The powder contains about 90% random copolymer and 10% high purity, medium colour furnace type carbon black. The polymer is composed of styrene and 1-butylmethacrylate in the ratio of 58:42, and has a molecular weight of approximately 70,000[51].

[k] Particle size < 5 μm described by the supplier. Rödelsperger (personal communication, 2005) found a heterogeneous material with 2 components containing ultrafine and large particles up to 10 μm by scanning electron microscopy. However, it was concluded from the relatively small specific surface area (4.4 m^2/g) that the mean diameter may be about 2 μm and that the dust is adequately sorted into the size class GBP-F-large.

[m] According to Weast *et al*[54].

[n] The producer of this sample is not stated, possibly it is Aerosil® from Degussa. The density given in the Table is that of Aerosil[55]. The specific surface area of silica fumed stated by Sigma-Aldrich is identical with that of Aerosil 200. The average size of primary particles of Aerosil 200 is 0.012 μm, particle surface smooth and without pores.

Table 3 - Comparison of five ideal dusts with the tested dusts. On the left: data of the ideal dusts (all particles with uniform size, spherical, smooth, density 1 g/ml), particle diameter descending from 2 to 0.02 μm, particle numbers and specific surface areas are calculated from these data. On the right: the data of the 19 tested dusts are arranged in the order of their mean diameter as it was given (not all were detected by the same method)

Ideal dusts [a]			The 19 tested dusts					
Particle diameter (μm)	Specific particle number (10^9/mg)	Specific surface area (m^2/g)	Dust specification	Specific part. no. (10^9/mg)	Mean diameter (μm)	Specific surf. area (BET) (m^2/g)	Density (g/ml)	Specific volume (μl/mg)
Fine dusts "large" (GBP-F-la)			[b] Lean coal	0.27	4.0	4.1	1.4	0.71
2	0.24	3.0	[b] Toner		3.5	3.6	1.2	0.83
			[b] Rich coal	0.54	3.4	6.4	1.8	0.56
			[b] Steam coal	1.5	2.4	10.9	2.2	0.45
			[b] Rock	1.2	2.3	17.6	2.4	0.42
			[b] ZrO_2		2.0	4.4	5.85	0.17
			[b] Kaolin		2.0	19	2.5	0.40
			[b] Lower rich coal	1.7	1.8	9.9	1.4	0.71
1	1.9	6.0	[c] Quartz	3.6	1.1	8.8	2.6	0.38
Fine dusts "small" (GBP-F-sm)			[b] TiO_2 fine		0.2	10	3.9	0.26
0.2	240	30	[b] Lung dust		0.2	12	2.0	0.50
			[b] Diesel soot, truck		0.2	13	1.85 [d]	0.54 [d]
0.1	1,900	60	[b] Carbon black 101		0.095	18	1.85	0.54
Ultrafine dusts (GBP-UF)			[b] TiO_2 hydrophilic		0.025	52	3.8	0.26
0.02	240,000	300	[e] TiO_2 hydrophobic		0.02	33	3.8	0.26
			[b] Aluminium silicate		0.015	63	2.1	0.48
			[b] Carbon black Pr. 90		0.014	337	1.85	0.54
			[f] Amorphous SiO_2		0.014	210	2.2	0.45
			[b] Aluminium oxide		0.013	124	3.2	0.34

[a] For uniform spheres in an ideal dust, the relationship between diameter, specific surface area and number of spheres per mass unit can generally be described as follows: when the diameter becomes smaller by a factor of 2, the specific surface area increases by a factor of 2, and the number increases by a factor of 2^3.

[b] Valued as *GBP*: *respirable granular bio-durable particles without known significant specific toxicity*.

[c] Specifically toxic by an unknown non-soluble toxic surface property. Independent of this specific toxicity, quartz dust additionally has the general toxicity of GBP, which remains and has to be considered when the specific toxic surface property is inactivated, e.g. by coating with polyvinyl-pyridine-N-oxide (PVNO).

[d] See Table 4, footnote [r].

[e] Specifically toxic by dissolution of a coating organic silicon compound which changes the surface from hydrophilic into hydrophobic[31].

[f] Soluble in the lung and specifically acutely toxic.

Dörentruper Quarz Mahlung Nr. 12 (in short: *DQ12*), with a size distribution of <60 μm. A <5 μm size fraction was prepared from this material by centrifugal separation in air with a separator from Walther-Staubtechnik, Cologne[56]. This standard quartz sample is officially designated as *DQ12* <5 μm, and has been used in a large number of *in vivo* and *in vitro* experiments in silicosis research. There were many bags of the primary coarse dust which had obviously been sized at different times. The size which we have used in nearly all of our experiments since 1967 is numbered as *no. 6 from bag no. 17*. Further data on DQ12 are given by Klosterkötter and Leiteritz[57], Robock[56] and Nolan *et al*[53].

Laboratory animals, experimental design and examinations of the rats

Female SPF Wistar rats (HsdCpb:WU) were delivered at the age of 7 weeks from Harlan Winkelmann, D-33176 Borchen, in three batches containing a similar number of rats, one batch for the coal dust study containing 642 rats at the start and two slightly larger batches for part 2 instilled with the 13 non-mining dusts. Rats were randomly distributed to the animal groups listed in Table 4. After adaptation, some animals with different body weights were exchanged between the groups in order to have similar body weight distributions per group. The

animals were marked by tattooing a group number on the left ear and an individual number on the right ear. The first intratracheal instillation was carried out at an age of 8 to 9 weeks. Part 1 was started on 4th July, part 2 with two series at 10-week intervals on 13th September and 21st November 1995. Body weight was registered weekly until week 20, once per month until week 39, and finally at intervals of 3 months. Rats were housed in plastic cages on wood granule bedding in groups of 8 or 6 at the start. Management reasons entailed changing the animal house with strict barrier conditions for another animal house for part 1 in the 92nd week. From that time onwards, rats were individually maintained. Regardless of this change, they were provided with a standard laboratory diet (sniff R/M-H, V 1536 Extruded) and water *ad libitum*, temperature bedded 23±2°C, 12 h light - dark cycle. They were inspected for clinical signs of morbidity and mortality twice per weekday and once on Saturday and Sunday and died either spontaneously, or were put to sleep by anaesthesia with CO_2 when in bad health conditions, or when suffering from a growing subcutaneous tumour, or in the 30th month when the experiment was finished.

The dusts were suspended by ultrasonification in 0.4 ml 0.9% phosphate buffered sodium chloride (NaCl) solution. Tween 80® (Polyoxyethylen(20)-sorbitan monooleate from Aldrich 27,436-4, Ch. 7804818) was added as a detergent to improve the homogeneity of the following dusts in part 1: coal dusts (except rock dust) and the control group received 0.5 % Tween 80. In part 2, 0.5% Tween 80 was added to the suspensions of diesel soot and commercial carbon blacks and 1.0% to hydrophobic TiO_2 P 805 and toner. The control groups of the two batches of part 2 which followed the first batch after 10 and 20 weeks were maintained untreated. The intratracheal instillations were administered under CO_2 anaesthesia at weekly intervals. The instilled doses are given in Table 4. The full report for the Federal Institute for Occupational Safety and Health (FIOSH)[47] contains more details on materials and methods, data on weight development, mortality and

Table 4 – Animal groups in the sequence of the experiment, size classes of the dusts according to the mean diameters, doses instilled (mass and volume dose), estimated dust volume retained in the lungs for a longer period, rats at risk, survival times, lung tumour incidences macroscopically and microscopically, and tumour incidence per µl dust burden in the lung. Original data on tumour types and incidences of part 1 in Pott *et al*[58], of part 2 in Bellmann *et al*[59]

Dust, size class (F-la = large fine F-sm = small fine UF = ultrafine)	Dose instilled		Dust volume / lung[b]	Rats at start / at risk[c]	Survival 50%[d]	Lungs with tumour(s) (%)					Lungs (%) with metastases of other tumours	Tum. / lung dust[h]
						Macroscopy		Microscopy (primary)[g]				
	Number of inst. x mg	Vol.[a] (µl)	(ml)		(wks)	Total[e]	Primary[f]	Ben.	Mal.	Total		(%/µl)
Part 1: mining dusts (coal dust study), start 4th July 1995												
Carrier fluid	20 x 0	–	–	48/47	110	6.4	2.1	0.0	0.0	0.0	17.0	–
Lean coal, F-la	11 x 6	47	31	48/47	109	48.9	34.0	8.5	48.9	57.4	17.0	1.8
<0.1% SiO_2	20 x 6	86	57	48/48	101	43.8	39.6	2.1	62.5	64.6	20.8	1.1
Lower rich coal, F-la	10 x 6	43	29	48/48	108	33.3	25.0	20.8	33.3	54.2	10.4	1.9
<0.1% SiO_2	20 x 6	86	57	48/44	106	70.5	61.4	4.5	72.7	77.3	13.6	1.4
Rich coal, F-la	10 x 6	33	22	48/48	106	45.8	43.8	10.4	45.8	56.3	14.6	2.5
1.3% SiO_2	20 x 6	67	44	48/45	99	57.8	44.4	22.2	57.8	80.0	24.4	1.8
Steam coal, F-la	10 x 6	27	18	48/43	108	39.5	32.6	11.6	60.5	72.1	14.0	4.0
9.0% SiO_2	20 x 6	55	36	48/45	95	62.2	53.3	17.8	66.7	84.4	8.9	2.3
Rock, F-la	10 x 6	25	17	48/47	102	27.7	19.1	6.4	27.7	34.0	17.0	2.0
16.7% SiO_2	20 x 6	50	33	48/45	105	44.4	37.8	11.1	46.7	57.8	15.6	1.7
Quartz DQ12, F-la	5 x 1	1.9	0.85[i]	38/35	103	60.0	51.4	17.1	48.6	65.7	22.9	*> 51*[i]
99.1% SiO_2	10 x 1	3.8	1.7[i]	38/35	106	65.7	54.3	14.3	57.1	71.4	11.4	*> 28*[i]
	10 x 2	7.7	3.4[i]	38/36[j]	100	67.6	64.9	22.2	55.6	77.8	5.6	*> 15*[i]

(footnotes next page)

(to be continued on the next page)

Table 4 – continued

Dust, size class (F-la = large fine F-sm = small fine UF = ultrafine)	Dose instilled		Dust volume / lung[b] (ml)	Rats at start / at risk[c]	Survival 50%[d] (wks)	Lungs with tumour(s) (%)					Lungs (%) with metastases of other tumours	Tum. / lung dust[h] (%/µl)
						Macroscopy		Microscopy (primary)[g]				
	Number of inst. x mg	Vol.[a] (µl)				Total[e]	Primary[f]	Ben.	Mal.	Total		
Part 2: non-mining dusts, series (a), start 13th September 1995												
Carbon black, F-sm	5 x 6[k]	18	11	48/45	106	44.4	35.6	33.3	26.7	60.0	15.6	5.5
lamp bl. 101	10 x 6[m]	34	22	48/46	104	50.0	45.7	26.1	37.0	63.0	10.9	2.9
	20 x 6[n]	68	44	48/47	108	70.2	53.2	No histology				
Carbon black, UF	5 x 1.5[o]	5	3	48/46	110	56.5	45.7	30.4	37.0	67.4	13.0	22.5
furnace black	*5 x 3*[p, q]	*10*	*6*	*21/18*	*112*	*83.3*	*83.3*	*22.2*	*66.7*	*88.9*	11.1	14.8
Printex 90	*5 x 3*[q]	*8*	*5*	*27/27*	*107*	*70.4*	*48.1*	*22.2*	*55.5*	*77.8*	22.2	15.6
	5 x 3	9	5.8	48/45				22.2	60.0	82.2	17.8	15.1
	5 x 6	16	11	48/48	108	75.0	62.5	14.6	68.6	83.3	10.4	7.6
	10 x 6	32	22	48/47	100	72.3	57.4	No histology				
Al-oxide, UF	5 x 6	9	6	48/44	111	75.0	63.6	15.9	65.9	81.8	15.9	13.2
	10 x 6	19	12	48/47	97	70.2	55.3	25.9	46.8	72.3	10.6	6.0
Al-silicate, UF	5 x 6	14	10	48/47	107	59.6	48.9	21.3	38.3	59.6	23.4	6.0
	10 x 6	29	19	48/45	108	73.3	48.9	33.3	42.2	75.6	22.2	4.0
Kaolin, UF	10 x 6	24	16	48/48	115	37.5	29.2	16.7	25.0	41.7	8.3	2.6
	20 x 6	48	32	48/47	121	59.6	51.1	14.9	59.6	74.5	4.3	2.3
No treatment (1)	–	–	–	48/46	124	0.0	0.0	2.2	0.0	2.2	4.3	–
Part 2: non-mining dusts, series (b), start 21st November 1995												
Diesel soot, F-sm	3 x 2.5	4.1	1.4[r]	48/45	117	8.9	4.4	2.2	2.2	4.4	20.0	3.1
	5 x 3	8.1	2.7[r]	48/47	115	23.4	12.8	14.9	10.6	25.5	21.3	9.4
	5 x 6	16.2	5.4[r]	48/45	108	48.9	31.1	26.7	13.3	40.0	22.2	7.4
TiO_2, UF	5 x 3	3.9	2.6	48/42	114	47.6	35.7	21.4	31.0	52.4	14.3	20.2
hydrophilic	5 x 6	7.9	5.3	48/46	114	52.2	47.8	17.4	50.0	67.4	15.2	12.7
	10 x 6	16	11	48/46	104	54.3	43.5	23.9	45.7	69.6	15.2	6.3
TiO_2, UF	15 x 0.5[s]	2.0	(toxic)	24/11	86	9.1	0.0	0.0	0.0	0.0	9.1	
hydrophobic	30 x 0.5[s]	3.9		48/15	114	20.0	20.0	6.7	0.0	6.7	6.7	
Toner, F-la	10 x 6	50	33	24/24	111	41.7	41.7	25.0	29.2	54.2	0.0	1.6
	20 x 6	100	67	24/24	101	66.7	62.5	29.2	58.3	87.5	16.7	1.3
TiO_2, anatase, F-sm	10 x 6	15	10	48/44	108	22.7	22.7	15.9	13.6	29.5	11.4	3.0
	20 x 6	31	21	48/44	113	36.4	36.4	38.6	25.0	63.6	2.3	3.0
ZrO_2, F-la	10 x 6	10	6.8	48/47	115	12.8	12.8	8.5	0.0	8.5	10.6	1.3
Lung dust, collier,	10 x 6	30	20	40/40	117	67.5	57.5	12.5	67.5	80.0	20.0	4.0
silicosis III, F-sm	20 x 6	60	40	40/34	107	67.6	47.1	No histology				
SiO_2, amorph., UF	5 x 3	6.8	(soluble	40/37	113	13.5	8.1	No histology				
(silica fumed)	10 x 3	14	+ toxic)	40/35	112	2.9	0.0	5.7	0.0	5.7	14.3	
No treatment (2)	–	–	–	48/46	113	6.5	0.0	0.0	0.0	0.0	13.0	–

(Footnotes next page)

[a] Total volume calculated from mass instilled and density. According to the *overload hypothesis*[60], the retardation of the macrophage-mediated alveolar lung clearance of particles is caused by the volume of GBP rather than by their mass. A threshold cannot be concluded from the data[61]. A standard for a "non-overload situation" in rats was set at a lung burden of 1 µl dust per g wet weight of control lungs deduced from experiments with Fischer rats. At this level, the halftime of lung clearance is about doubled. The lung wet weight of the control rats (Fischer strain) is given as 1.5 g[62].

[b] With the exception of quartz (see footnote [i]), hydrophobic TiO_2, amorphous silica, and diesel soot (see footnote [r]) the dust part retained in the lung in the long term is consistently assumed at a mean of 2/3 of the dose instilled according to published data which are in a relatively wide range; they were summarized and discussed by Driscoll e*t al* [63] and Pott and Roller[47].

[c] Number of sufficiently examined rats which survived at least 26 weeks after first instillation.

[d] Period after first instillation in which 50 % of the animals died excluding rats which died immediately after anaesthesia.

[e] Percentage of rats with any macroscopically diagnosed lung tumour regardless of existing tumours located at other sites which lead to the conclusion that the lung tumour detected might be a metastasis.

[f] Percentage of rats with lung tumour(s) which are probably not a metastasis of a tumour located at other sites; these lung tumours were classified as *macroscopically primary lung tumours*.

[g] Primary lung tumour types diagnosed: *benign:* adenoma, epithelioma; *malignant:* adenocarcinoma, squamous cell carcinoma. Lungs with one or more malignant tumours may additionally have benign tumours.

[h] Relation of percentage of rats with primary lung tumours to the dust volume dose in the lung (see footnotes [a] and [b]) as a measure of the carcinogenic potency in this experimental group.

[i] Due to the known tendency of quartz to migrate from the lung to the lymph nodes in inhalation experiments, the percentage of quartz retained in the lungs is expected to decrease more than the lung burden of GBP. In numerous earlier instillation experiments with DQ12, bronchial clearance and lymphotrophy reduced the SiO_2 content in the lung to about 1/3 of the instilled mass 10 months after instillation[47]. This corresponds roughly with retention data of the inhalation experiment of Bellmann *et al* [52]. Hence, an additional reduction factor of 2/3 was regarded for DQ12 (estimated retention = DQ12 instilled x 2/3 x 2/3; e.g. this means: 10 mg quartz instilled with a density of 2.6 g/ml result in 1.7 µl retained dust). However, the values given for % lung tumours per µl quartz per lung are calculated as if 2/3 of the instilled quartz dose persisted in the lung like GBP. The symbol > which precedes the figures written *in italics* indicates the existing uncertainties.

[j] 37 rats macroscopically examined, 1 rat thereof not histologically examined.

[k–q] One additional instillation by error. The dust volume of this instillation is included in the calculation of the totally instilled volume:

- [k] Additionally 1 x 2.5 mg diesel soot.
- [m] Additionally 1 x 3 mg diesel soot.
- [n] Additionally 1 x 6 mg diesel soot.
- [o] Additionally 1 x 3 mg TiO_2 UF hydrophilic.
- [p] Additionally 1 x 6 mg TiO_2 UF hydrophilic.
- [q] These two subgroups were combined for further statistical calculations. The large difference in the tumour response may be due to an inhomogeneous suspension administered in small numbers of rats per subgroup and not caused by the additional instillation of the relatively small volume of TiO_2 (about 20% of the dose of the first subgroup).

[r] The density of the elemental carbon core was estimated at 1.85 g/ml by analogy with carbon black (Table 1). According to the Umweltbundesamt (Federal Environment Agency) (UBA)[19], it was assumed that 50% of the mass of the native diesel soot of lorries are organic substances and that they will be dissolved in the lung. Hence, 7.5 mg total particle mass of diesel soot has a volume of 4.05 µl. After instillation, 2/3 of this volume (2.7 µl) would be retained in the lung for a longer period but is considered likely to be reduced by dissolution of the organic part at 50% (1.35 µl). This volume corresponds with the dust volume standard of 1 µl/g lung explained in footnote [a].

[s] The doses had to be reduced because of unexpected acute toxicity.

basic data sheets describing individual lifetime and macroscopical tumour diagnosis for each animal, and microscopic tumour diagnoses for part 1.

The initial plan of dosage with hydrophobic TiO_2 contained – as for most other dusts – repeated instillations of 6 mg, but the surprisingly acute mortality after the first instillation called for a drastic reduction of the single doses to 0.5 mg[31]. A comprehensive re-examination of the acute mortality was carried out in July 1996. The experiment and the results are described in Table 5.

After death of animals and before starting necropsy of the thoracic and abdominal cavity, lungs were insufflated via trachea *in situ* with 6% neutral buffered formalin at a pressure of about 50 mm H_2O for half an hour. Only a few lungs were removed and weighed before fixation. In particular, the surface of the lung was inspected and lesions were recorded. After fixation for at least one day, the lungs were embedded in paraffin *in toto*, and sections were stained with haematoxylin-eosin. Tumour-suspected tissues, especially of abdominal organs, were also taken for histological examination of primary tumours which metastasized into the lung. The histological sections of part 1 of the study were diagnosed by the histopathology group of the Fraunhofer Institute of Toxicology and Aerosol

Table 5 - Acute mortality after intratracheal instillation (i.t.r.) and intraperitoneal injection (i.p.) of female Wistar rats (age 8 weeks at the first treatment) with hydrophobic ultrafine TiO_2 P 805 and hydrophilic TiO_2 P 25. Second instillation after 8 days of survivors of group 1, 2, 5, 6, and 8. Second i.p. injection of group 4 and 7 the following day after first injection [a]

Group no	Substance	Dose [b] (1st instill., 1st + 2nd i.p. inj. [c])	Number of rats		Dose [b] of 2nd instill. 8 days after 1st instillation	Dead rats 24 h after 2nd instill.; / after further 5 days
			Dead in 24 h after treatment / treated	Survivors 8 d after 1st treatment		
1	TiO_2 P 805	0.5 mg i.tr.	3 / 12 = 25 %	9	0.5 mg i.tr.	4 / 0
2	TiO_2 P 805	1 mg i.tr.	7 / 12 = 58 %	5	1 mg i.tr.	4 [d] / 0
3	TiO_2 P 805	2 mg i.tr.	11 / 12 = 92 %	1	–	–
4	TiO_2 P 805	125 mg i.p.	1 / 12 = 8 %			
		+125 mg i.p. [c]	0 / 11 = 0 %	11	–	–
5	TiO_2 P 25	10 mg i.tr.	0 / 12	12	10 mg i.tr.	0 / 0
6	TiO_2 P 25	20 mg i.tr.	0 / 12 [e]	11	20 mg i.tr.	0 / 0
7	TiO_2 P 25	125 mg i.p.	0 / 12			
		+125 mg i.p. [c]	0 / 12	12	–	–
8	Control	0.4 ml i.tr.	0 / 12	12	0.4 ml i.tr.	0 / 0

[a] This experiment was carried out in July 1996. It should examine the unexpected acute toxicity of this dust sample, which was observed after instillation of 6 mg for comparison with the hydrophilic counter part (see Table 4, footnote [s]). A small part of the data has been published by Pott *et al* [31]

[b] Carrier fluid: 0.9% NaCl solution, phosphate buffered, with 1% Tween 80; 0.4 ml i.tr. (as in control group); 5 ml i.p. (no i.p. control with carrier fluid).

[c] 2nd i.p. injection of 125 mg 1 day after 1st injection.

[d] The lung of the only surviving rat was investigated with the REM by D. Höhr. Represent pieces of the lung show enlarged alveoli; the septa are substantially thickened.

[e] 1 rat died 3 days after the 1st i.tr. instillation.

Research, in Hannover, H. Ernst, S. Rittinghausen and U. Mohr (see Pott *et al* [58]); part 2 was diagnosed by U. Mohr [59].

Histopathological examination of tissue sections stained with haematoxylin-eosin should focus on the detection and the classification of lung tumours and their precursor lesions. As a rule, 10 to 15 slides per lung were available. However, the slides were not thin enough to evaluate tumour precursors systematically. Diagnostics of the pulmonary fibrosis degree and other non-neoplastic lesions could not be performed because specially stained sections were not available. Histopathological classification of the lung tumours was performed according to Boorman *et al* [64] and the IARC criteria [65]. All tissues which were taken from other sites were diagnosed for histopathological lesions, especially for tumours which might be primary tumours with lung metastases. The observations were recorded and tabulated with an on-line computer programme (P.L.A.C.E.S. 2000.1).

Statistical analysis

The dose-response relationships based on the histologically confirmed primary lung tumours were analyzed by non-linear regression. As a first step, the multistage model was fitted to the data of each single dust using the US EPA BenchMark Dose Software (BMDS) Version 1.3.2. For most dusts, a good fit was obtained (p-value >0.1 for chi^2-goodness-of-fit). The curves are nearly linear or slightly sublinear. In the case of quartz and of 3 GBP-dusts (carbon black UF, Al-oxide UF and TiO_2 UF), the deviation of the multistage model from the data was statistically significant. This was due to the tumour incidences of the highest dose groups, which are not higher or even lower than in the next lower dose group. This can be explained as a saturation effect in the range of tumour frequencies of about 80%. To avoid bias in the dose-response curves by these four dose groups, they were excluded from further analyses.

In the second step, as many dose groups as reasonably possible were included in one multivariate logistic regression analysis, i.e. the 4 groups mentioned above, the dose groups without histological diagnoses and the acutely toxic hydrophobic TiO_2 dust were excluded, so that the analysis comprised 35 dose groups. The control groups were combined, because there was no tumour in the vehicle control group and only one tumour among the 92

untreated rats. The model fitted to the data using the software package STATISTICA (Version 5.5) included the following continuous variables: log (natural logarithm) of the numerical value of the mass (in mg) of dust instilled intratracheally, density (g/cm^3), specific surface area (m^2/g), mean or *typical* particle diameter (in μm). Furthermore, the following dichotomous (indicator) variables were simultaneously included: *pure quartz*, *relatively good solubility, ultrafine particles, small fine particles (0.09-0.2 μm)*. For the combined control group a dose value of 1/100 of the smallest mass dose was chosen (i.e. 0.05 mg, to avoid an undefined log zero); for the other variables in the control group the programme option "mean substitution of missing data" was chosen.

Retained dust volume and retained dust surface area are the dose metrics most intensively discussed in the literature[66-72]. This being so, a third step in the analysis was added, which should also provide an opportunity for a comprehensive graphical presentation of the results. The Weibull model and the quantal-linear model as a special case of the multistage model, respectively, were fitted to the data of the GBP dusts only (thus, quartz and amorphous silica, which are not GBP according to our definition, were not considered in this analysis). BMD software and the module *User-specified Regression* of STATISTICA were applied. To obtain maximum likelihood estimates the following loss function was used: -(X*log(PRED)+(N-X)*log(1-PRED)), where X is the number of rats with tumour per dose group, N is the number of rats at risk per dose group, and PRED are the predicted risk values.

Results

Table 4 contains the experimental groups, mass and volume doses instilled and the lung tumour incidences of each group. In Table 6, the few data on lung wet weights at the end of the coal dust study are given. Quartz was used for comparison with the effect of the three quartz-containing coal mine dusts. The middle and higher quartz dose are the same as in the two rock dust groups. Fig. 1 shows the survival time of rats with histologically confirmed lung tumours after instillations of coal or coal mine dust. Detailed data of body weight gain and survival times have previously been reported[47]. The survival time of the high dose group of aluminium oxide was significantly shortened and the lung surface seemed more altered than with the other dusts. The mean body weights of all groups treated with an aluminium compound (Al-oxide, Al-silicate, kaolin) were significantly lower than in the control group; the maximum decrease one year after instillation of 120 mg aluminium oxide was -6.9%.

The three graphs in fig. 2 a-c show the dose-response relationships of the coal dust study with three dose metrics *mass*, *dust volume* and *surface area*.

An acute maximum tolerated single dose of 3 mg amorphous SiO_2 particles was found after instillation in a pre-test. Five and 10 instillations did not reduce the survival time or body weight. Two rats were found with lung tumours among 35 rats at risk, the lower dose group was not histopathologically diagnosed.

Ultrafine TiO_2 with a hydrophobic particle surface produced by coating with an organic silicon compound was

Table 6 - Lung wet weights without trachea and lung volumes of some rats in the 29th month of part one of the study (coal dust study)

Intratracheal instillation	Lung weight (g), mean ± SD	No. of rats	Lung volume (ml), mean ± SD	No. of rats
Carrier fluid 0.4 ml	1.8 ± 0.3	5	2.3 ± 0.6	3
60 mg [a] coal or coal-containing dust	3.3 ± 1.1 3.0 ± 0.6	24 21 [b]	4.2 ± 0.8	11
120 mg [a] coal or coal-containing dust	4.6 ± 1.5 4.3 ± 1.5	26 18 [b]	5.7 ± 2.0	10
5 mg quartz	5.9 ± 0.1	2	7.0	1
10 mg quartz	6.2 ± 2.6 4.3	2 1 [b]	–	
20 mg quartz	6.9 ± 0.8 6.9 ± 1.1	3 2 [b]	7.9 ± 0.6	3

[a] Estimated lung burden 2/3 of the instilled dose.

[b] Lungs without tumour size larger than about 2 cm.

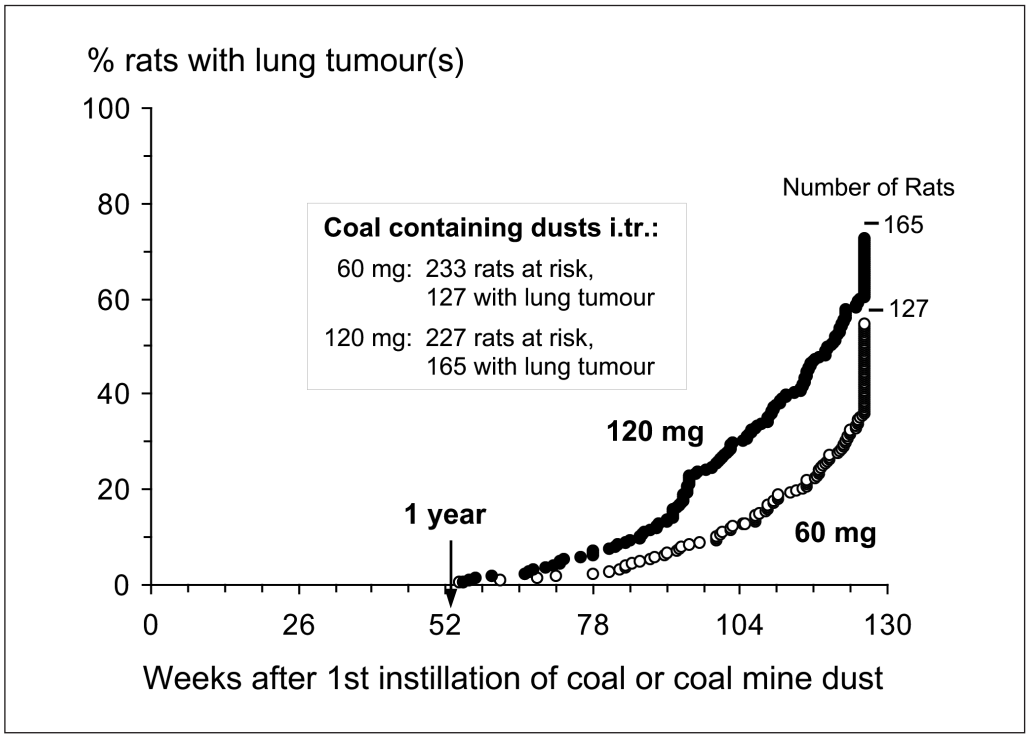

Fig. 1. Survival time of rats with one or more histopathologically confirmed primary lung tumours after instillation of 60 or 120 mg of one of the five coal-containing dusts. No animal with a lung tumour has survived for less than one year. Even in the high dose group, the majority of rats with lung tumours died in the third year of the experiment.

designed to be tested by weekly instillations of 6 mg in comparison with hydrophilic TiO_2. However, very surprisingly, the rats died within half an hour of instillation. After further tests, 0.5 mg was instilled 15 or 30 times respectively. In the higher dose group with a much reduced number of animals, 1 benign tumour was found in 15 rats. The acute mortality was re-examined in a separate experiment which is reported in Table 5.

Statistical analysis

Multivariate logistic regression analysis led to statistically highly significant results for most of the dose variables. The clearest significance ($p \leq 0.00001$) was obtained for the primary instilled-dose variable (log mass/mg), for density and for the variables representing the dust characteristics *pure quartz*, *relatively good solubility* and *ultrafine size*. It is hardly surprising that increasing the mass dose increases the tumour frequency, but obviously mass dose is not the only important parameter. The results may be expressed in terms of odds ratios. In the case of quartz, an odds ratio of 29.7 was obtained, which indicates that a dust consisting of pure DQ12 has a carcinogenic potency in the rat lung about 30 times higher than a GBP dust of the same density, particle size and specific surface area (note: the quartz type DQ12 has a substantially higher carcinogenic activity than other quartz types, e.g. Sikron F600 and Min-U-Sil 15[73]). The dust characteristic *relatively good solubility* only applies to the amorphous silica sample, which obviously has a much lower carcinogenic potency than the biodurable dusts (Table 4). The estimated odds ratio for the dust characteristic *ultrafine size* is 4.97, indicating an approximately 5-fold higher carcinogenic potency than a *non-ultrafine* dust. Density of the dusts is also highly significant. This points to the assumption that dust volume is a more suitable dose metric than dust mass, because of the simple relationship: *volume = mass / density*.

Logistic regression analysis has the advantage that a set of dust properties can be analyzed simultaneously and various different dusts like GBP, quartz and amorphous silica can be compared. However, there are some disadvantages. In a preliminary analysis, we used *dust mass* as a dose metric instead of the logarithm. The regression analysis led to an estimate of the background tumour frequency of 10%. Such a high lung tumour frequency in untreated Wistar rats is unrealistic, but obviously the data implied a hyperlinear curve shape that could not be modelled by the logistic function. Use of the logarithm for the numerical value of the dust mass led to realistic estimates of the tumour risk among controls. However, log zero is not defined and some numerical value for the dose of the controls has to be chosen. This is somewhat arbitrary and, in fact, the choice (e.g. 0.1 mg compared to 0.001 mg) influenced the results to some degree. Furthermore, it is not possible to present the dose response relationships for more than two variables graphically in any reasonable or illustrative way. Thus, an alternative analysis was made,

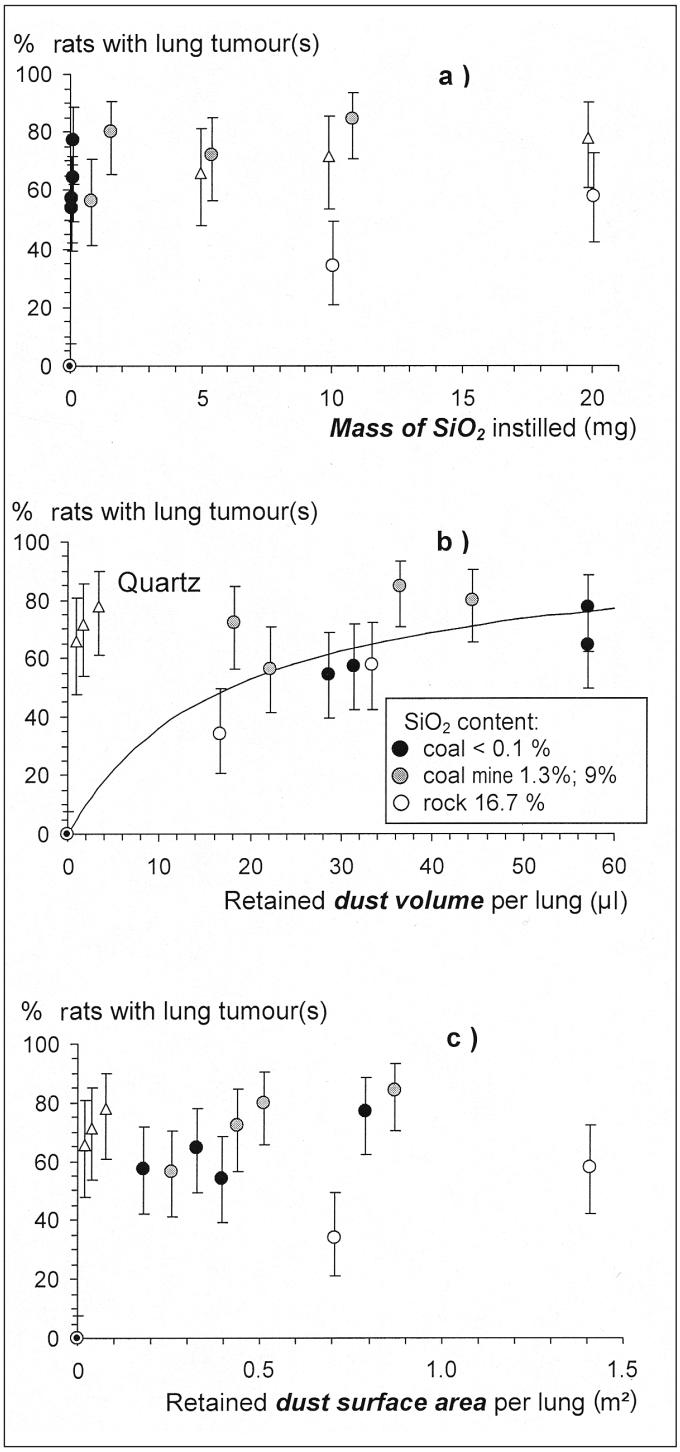

Fig. 2 a-c. Dose-response relationships of part 1 (coal dust study) with three dose metrics: a) *mass of silica* instilled, b) retained *dust volume* in the lung (curve: logistic regression model without quartz), c) *surface area* of retained dust.

directly comparing the dose metrics *dust mass combined with particle size*, *dust volume combined with particle size* and *dust surface area*.

The Weibull model is particularly suited to analysing the shape of dose-response curves in terms of *quantal-linear, sublinear, hyperlinear*. Hypotheses have been published in the literature purporting to explain the different carcinogenic potencies of different particle sizes, especially of ultrafine sizes, by dust surface area. This hypothesis is only reasonable if particle size does not matter when the surface area serves as a dose metric. We thus used a simple Weibull model to analyse the relationship between the retained surface area of GBP dust in the lungs of the rats (A_{ret}) and the proportion of rats with primary lung tumour (P). The Weibull model contains a parameter responsible for curve shape. If the value of the shape parameter is larger than 1, then the curve is sub- or hypolinear, if the value is smaller than 1, the curve is super- or hyperlinear; if it equals 1, the curve may be termed quantal-linear.

The maximum likelihood estimate of the shape parameter in our model was 0.42 in the case of retained surface area dose. This means that this curve is extremely hyperlinear with a very steeply rising curve in the lower range, flattening to a nearly horizontal line for the higher doses. Such a shape also means that the carcinogenic potency is dependent on dose, where smaller doses have a higher potency (tumour risk/dose ratio) than higher doses. A similar model was also applied to the dose metric retained dust volume in combination with particle size, and the regression again resulted in a hyperlinear shape, though not so pronounced as with the surface area (estimate 0.65). We have no biological explanation for an extremely hyperlinear dose response relationship for GBP. Further analysis was hence restricted to models with a constraint on the shape parameter (= 1). Such a model may be considered as a special case of the multistage model and is termed *quantal-linear* in the US EPA BMDS. If no further constraints are applied on this model, the hyperlinear curve will be compensated by a very high estimate of tumour risk for the control group. As a rule, the proportion of Wistar rats in our historical vehicle controls with primary lung tumours was zero. It is therefore unlikely that the true risk in the rats is higher than the combined tumour frequency of 0.72% in the 19 dust study. So we constrained the background tumour risk in the models that follow to 0.72%.

Fig. 3 - 5 show the dose-response relationships for the three different dose metrics, the data points are the "raw data" from Table 4, the curves are the results of non-linear regression (see Materials and methods) using quantal-linear models. The proportion of variance explained (R^2) may serve as a measure of how well the models fit the data. The three curves in fig. 3 and 4 provide an explained variance of 53% and 69% (R^2 = 0.53 and 0.69), respectively. The fit of the single curve in fig. 5 is extremely bad. The model accounts for only 2.8% of the variance in

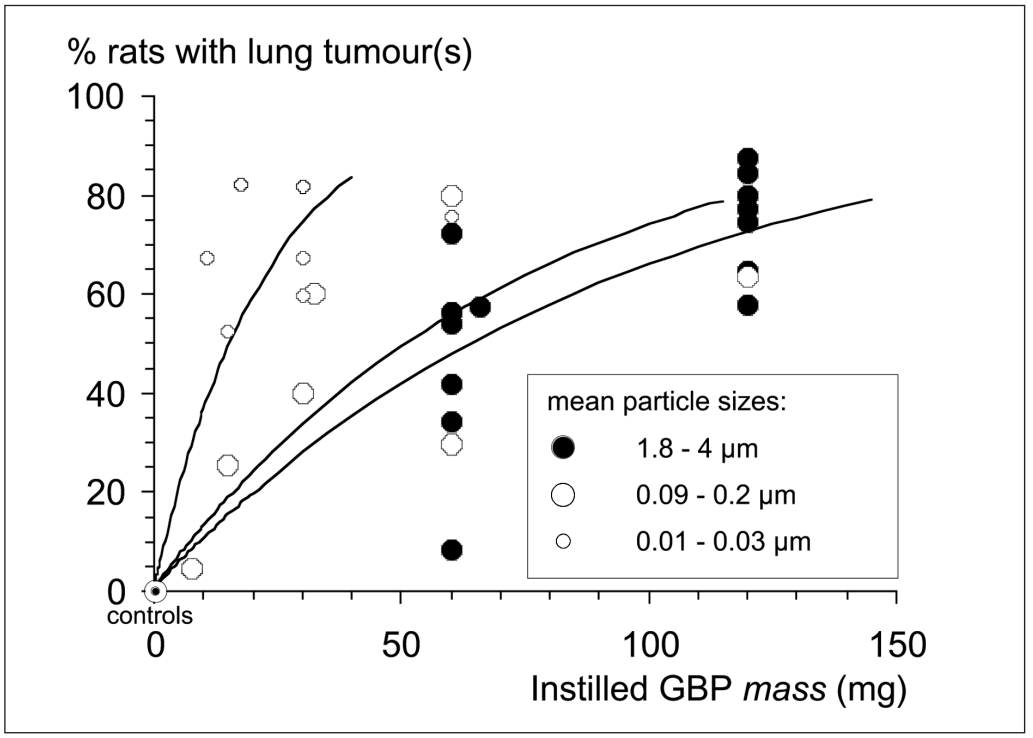

Fig. 3. Dose-response relationships after instillation of GBP in rats. Dose metric: instilled *dust mass*; non-linear regression for 3 particle size categories.

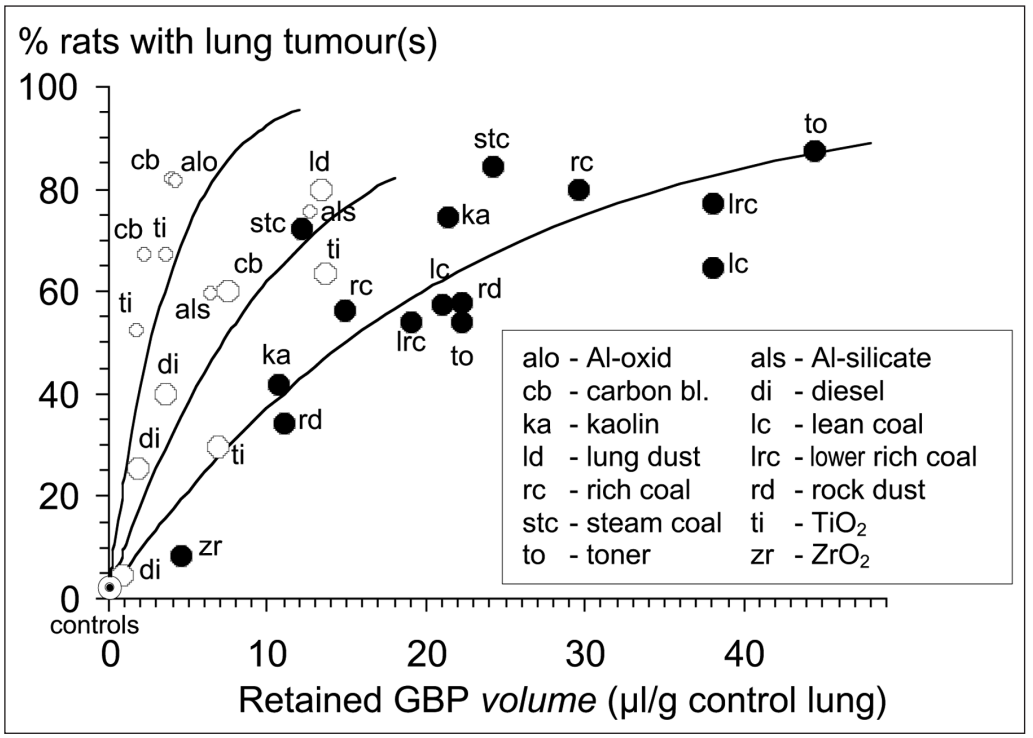

Fig. 4. Dose-response relationships after instillation of GBP in rats. Dose metric: retained *dust volume* (2/3 of the dose instilled is estimated to be retained in the lung for a long time); non-linear regression for 3 particle size categories (legend for dot symbols see fig. 3).

tumour frequencies ($R^2 = 0.028$). This may be surprising, but the large range of tumour frequencies associated with a retained surface area of 0.1 to 0.2 m^2/g in fig. 5 makes this plausible. The fit of a model with the surface area can be improved by allowing a hyperlinear shape (Weibull model see above, $R^2 = 0.47$) or by allowing 3 size classes ($R^2 = 0.25$), but the rationale for the use of particle surface area was to explain the carcinogenic potencies of different particle sizes, so the differentiation of size classes is in this case paradoxical. In any case, the fit for the surface area-models is substantially worse than for the volume-size-model.

Obviously, retained dust volume (V_{ret}) in combination with some parameters accounting for various particle sizes leads to the most plausible dose-response relationships. We therefore focussed on this dose metric. The three curves in fig. 4 are the graph of the following model, where the (rounded) maximum likelihood

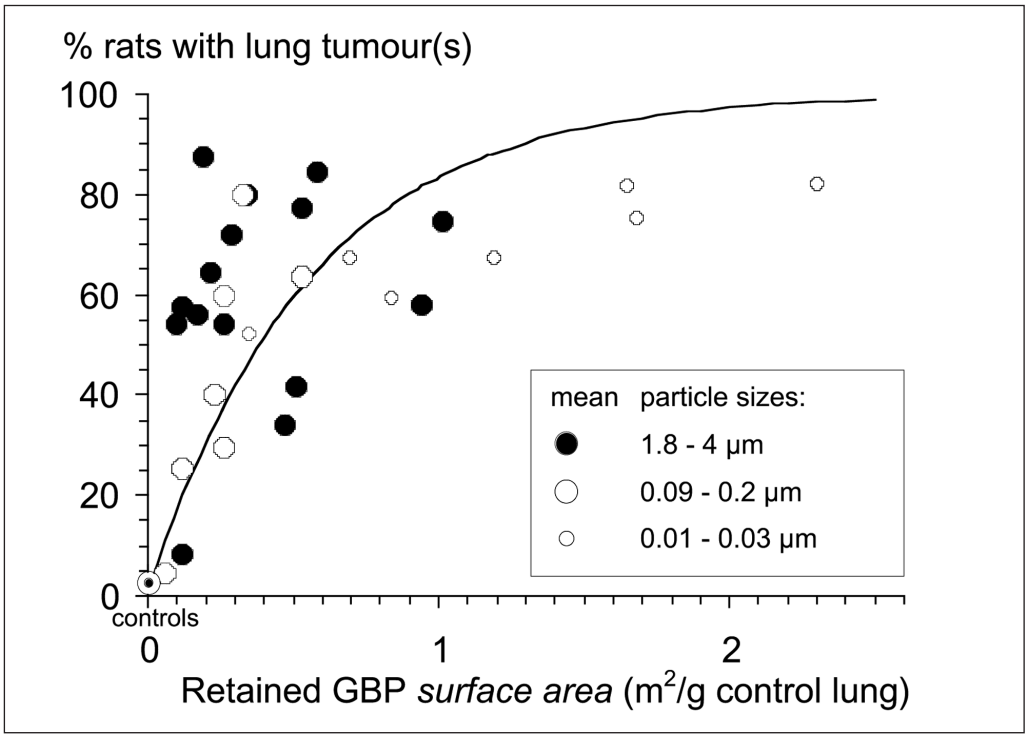

Fig. 5. Dose-response relationships after instillation of GBP in rats. Dose metric: retained *dust surface area*; non-linear regression for all dusts together (different dot symbols for illustrative purposes only).

estimates of the model parameters are directly written into the formula:

$$P = 1 - \exp\left(-0.0072 - \left(\frac{0.046 + 0.050\ S + 0.213\ U}{\mu l/g\ control\ lung}\right) V_{ret}\right) \quad \text{model (1)}$$

The dichotomous variables U and S indicate the size classes *ultrafine* and *small*; the rest contains sizes greater than 1 µm. The model directly contains measures of the carcinogenic potencies of the size classes by means of the terms associated with V_{ret}. The carcinogenic potency may be formulated as follows:

$$\text{potency}_{itr}\ (\text{size class}) = \frac{4.6\% + 5.0\% \cdot S + 21.3\% \cdot U}{\mu l/g\ control\ lung}$$

This means: for particle sizes that are not *ultrafine* and not *small fine* a dose of 1 µl retained dust volume per gram of control lung is associated with an additional tumour risk of 4.6%. The corresponding risk for *small fine* particles is 4.6% + 5.0% = 9.6%, while, for *ultrafine* particles it is 4.6% + 21.3% = 25.9% (*cf*. fig. 4).

Biologically, a sharp borderline between the carcinogenic potencies of particle size classes is not plausible. We therefore looked for mathematical models suited for describing tumour probability dependent both on volume dose and on a continuous variable representing particle size (log d = natural logarithm of diameter/µm). We found that model (2) also fits the data quite well ($R^2 = 0.67$):

$$P = 1 - \exp\left(-0.0072 - \left(-0.068 + \frac{0.91}{\log d + 7}\right) \frac{V_{ret}}{\mu l/g\ control\ lung}\right) \quad \text{model (2)}$$

Fig. 6 shows the data and model (2) in a 3-dimensional plot. The dashed lines indicate the distance of the data points from the plane, which is the graph of the function model (2). The data points on the right hand side are the data of the GBP with mean particle sizes between 1.8 and 4 μm. A steeper increase in tumour frequencies can be seen for the data points of the ultrafine dusts plotted on the left-hand side. Within the plane, a curve (thick) next to the log-d-scale can be seen decreasing from left to right. This curve is a kind of graph of the term within model (2) that represents the dependency on particle size (log d). This term can also be used to describe carcinogenic potency dependent on particle size. This can be formulated as follows:

$$potency_{itr}\ (d) = \left(-6.8\% + \frac{91\%}{\log d + 7}\right) \frac{1}{\mu l/g\ control\ lung}$$

From this function, it can be calculated that - after instillation - for particles of 1 µm diameter a dose of 1 µl retained dust volume per gram of control lung is associated with an additional tumour risk of 6.2% (91% / 7-6.8%); the

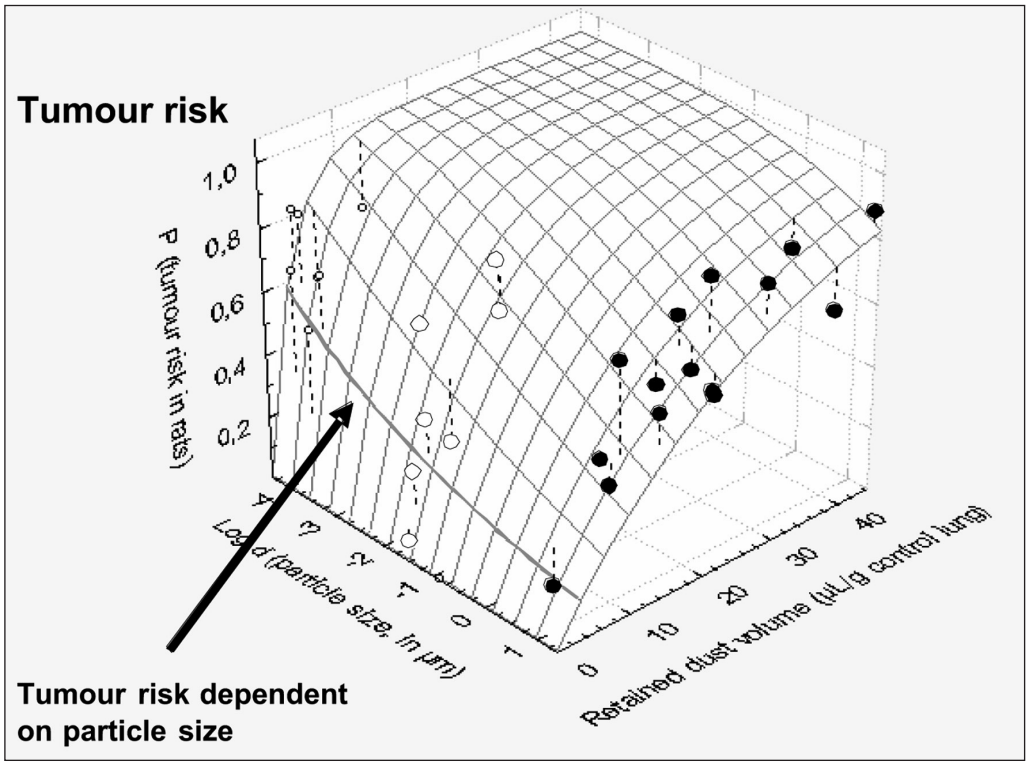

Fig. 6. Dose-response relationships after intratracheal instillation of GBP in rats. Dose metric: *retained dust volume together with the logarithm of the numerical value of particle size* (log *d*); non-linear regression with two continuous dose variables (see text; particle size is treated as a continuous variable, different dot symbols are only used for easier comparison with fig. 4).

corresponding risk for particle size 0.2 µm is 10%. By means of this function, the ratios of the carcinogenic potencies of any particle sizes can also be calculated. This may be formulated as:

$$relative\ potency_{itr}(d) = potency_{itr}(d) / potency_{itr}(d_0)$$

where d_0 is the "reference particle size". For fig. 7, a reference particle size of 2.5 µm is chosen (*cf PM2.5*). For particle sizes 0.015 µm and 0.2 µm, relative carcinogenic potencies of 5.5 and 2.2, respectively, are obtained. Of course, there are no data points in fig. 7: the curve is the result of the 3-dimensional model (2) plotted in fig. 6.

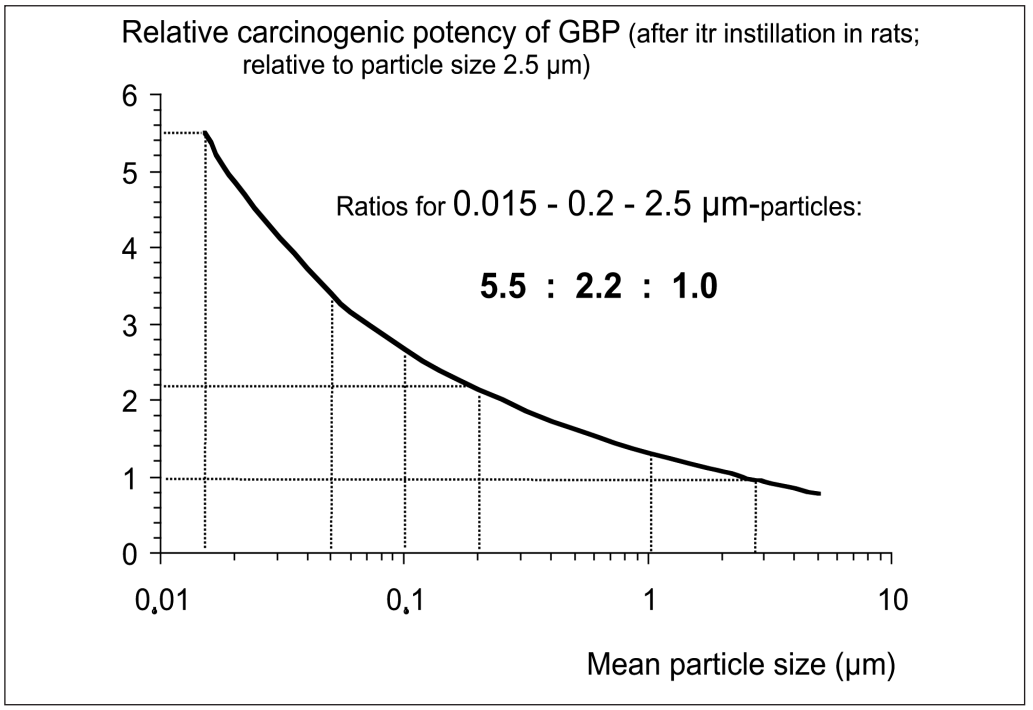

Fig. 7 Special result of the non-linear regression analysis presented in fig. 6: carcinogenic potency dependent on particle size (the curve corresponds to the thick curve within the plane of fig. 6).

From model (1) relative potencies of the three size classes can be calculated in a similar manner by using the term 0.046 + 0.050 *S* + 0.213 *U*. From this, ratios of 5.6 and 2.1 follow for ultrafine particles (U = 1) and particles F-small (S = 1), respectively, in relation to fine particles that are not *small*, so-called F-large.

Discussion

Dose selection background

From a glance at the high tumour responses in many groups, it can be concluded that lower doses of several dusts should frequently have been applied instead of 120 mg. However, this is a conclusion after the experiment prompted by the unexpected results. Dose selection was based on the following points: 1) the guidelines for testing chemical carcinogens by the US Interagency Staff Group on Carcinogens[74] and the Environmental Protection Agency guidelines for carcinogen risk assessment[75]; 2) the lung dust burden of coal workers of up to 100 g at maximum which needs to be reproduced by an equivalent lung burden in rats; 3) interpretation of the carcinogenicity studies by many authors, according to which carcinogenicity of GBP with metric diameters > 1 μm would be negligible if at all; and 4) our own experience from an earlier study which underestimated the carcinogenicity by a small number of lung sections[14].

The Guidelines[74] stated: *"The question of what maximum dose should be administered may be the most controversial issue concerning bioassays. …. The highest dose currently recommended is that which, when given for the duration of the chronic study, is just high enough to elicit signs of minimal toxicity without significantly altering the animal's normal life span. …. The MTD* (maximum tolerated dose) *should not produce morphologic evidence of toxicity of a severity that would interfere with the interpretation of the study. …. The purpose of using the MTD is to provide maximum opportunity for the detection of a neoplastic response"*.

Before we started the 19-dust study, an irrelevant or non-existent carcinogenicity of GBP for humans, at least from fine particles, was the interpretation given at a conference held in 1995[25] by several authors[67, 69, 76-80]. We intended to confirm or disprove this evaluation by a test model which was more sensitive than inhalation exposure and with a lung burden of relatively coarse coal fine dusts, equivalent to the lung burden of very highly exposed coal miners. More than 50 g dust and up to 100 g has been found in the lungs of coal miners[81-83]. Mauderly[84] calculated a weighted mean of 16.7 g total lung dust among 1,225 coal miners reported in eight publications. The lung dust burden of women without occupational dust exposure living in cities of the Ruhr area increased to nearly 3 g over their lifetime (fig. 8).

The wet lung mass of healthy men in their 20s amounts to about 1000 g[86] (of female Wistar rats at start of the experiments = nearly 1 g). A long-term lung burden of two thirds, or probably less, of the instilled dust mass can be assumed as a mean value among earlier data[14, 15, 63]. Subgroups in the 19-dust study which were instilled for detection of the lung dust burden after certain times could not be analysed at the end of the study. The range of the reported data on retained dust doses is relatively wide[63]. However at the end of our study, it still seemed reasonable to maintain the estimate that 2/3, as a mean of the nominally instilled total GBP dose, was retained at least for some months after the last instillation of max 6 mg single dose. The relatively low retained mean of 38.6 ± 12.1 mass % measured 6 months and 37.5 ± 10.9 mass % 12 months after a single instillation of 50 mg mine dusts might be due to the very high single dose[87, 88]. A substantial part of the dust may be deposited on the ciliated airways and cleared quickly into the oesophagus. According to the estimation that a mean of 2/3 of the nominally instilled dose in a rat group is retained for the first year of the experiment, the mean GBP burden in the lungs could be calculated (Table 4) and used as the value for considering the dose-response relationships. Hence the lung dust burden of about 40 mg or 80 mg after instillation of the total dose of 60 mg or 120 mg dust corresponds to that of heavily or very heavily exposed coal miners at the end of their lives. For comparison with the two rock dust doses of 60 and 120 mg which contain 16.7% quartz, the equivalent quartz doses (10 and 20 mg) were administered, in addition to the lower dose of 5 mg quartz.

A very low carcinogenic potency of TiO_2 with a mass median aerodynamic diameter (MMAD) of about 1.5-1.7 μm (equivalent to ~0.8 μm geometrical diameter) seemed to be documented by the inhalation study of Lee *et al*[1, 89]. Only the highest concentration of 250 mg/m^3 induced increased tumour incidence by 35% in female and 26% in male Sprague Dawley rats; the mean dust burden per lung amounted to 372 mg after 1 year and 665 mg after 2 years of exposure. However, the dust volume in the lung after one year of exposure may be the most relevant dose metric; it amounted to 32 μl/g lung; this is not extremely high in relation to the dust content in coal mine workers.

Similar to the Lee study, a previous instillation study with 15x3 mg and 20x3 mg of two TiO_2 samples resulted in tumour rates of only 3% and 5%[14, 73]. In that study, we did not systematically describe macroscopic lung tumours as we did in the 19-dust study, so they could not

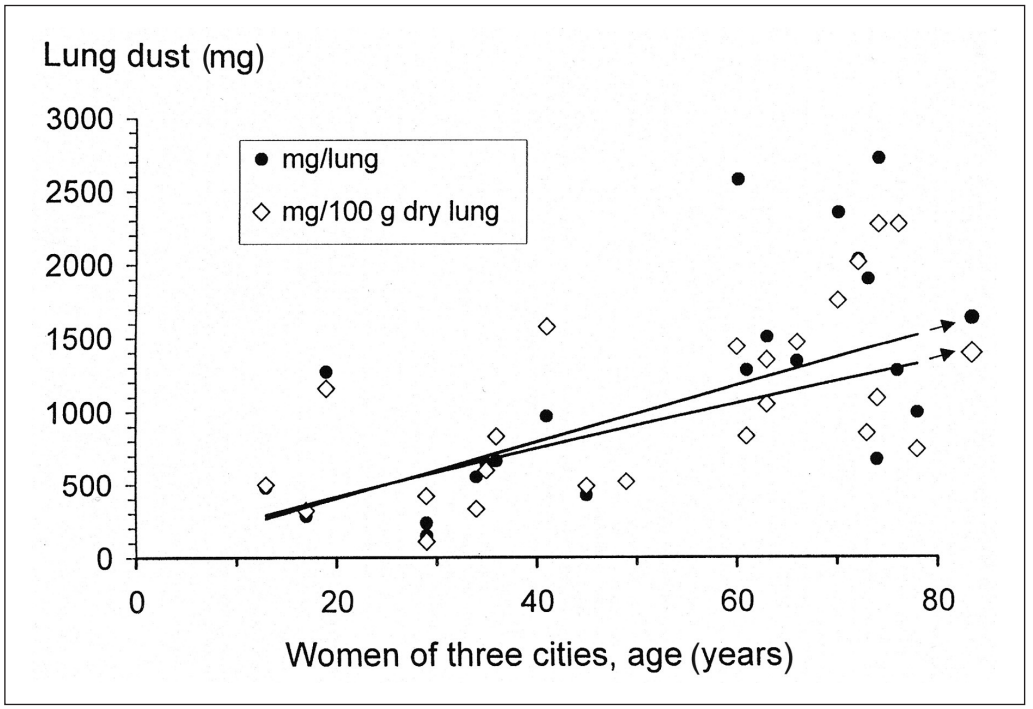

Fig. 8. Age-dependent increase in lung dust burden of women between 13 and 78 years without occupational dust exposure, who lived permanently in big towns of the industrial Ruhr area (Essen, Gelsenkirchen, Recklinghausen). Average dust burden of 11 lungs under 50 years 0.62 g/100 g dry lung; in 12 lungs of persons over 50 years 1.72 g dust/100 g dry lung (fat-free). Isolation of dusts from lungs with the formamide method; data from Einbrodt and Dohmes[85]; lung wet weight of women aged 20-21 years 793 g[86]. A steady state is not detectable.

be compared with the tumour incidences from microscopic diagnoses which based the examination on only three tissue sections per lung. Higher tumour incidences of up to 68% were found from 30-60 mg diesel soot, ultrafine carbon black, charcoal activated with a specific BET surface area of 860 m^2/g, iron oxides (50 or 150 mg), and three quartz specimens (DQ12, Min-U-Sil, Sikron F 600). We assumed that a substantial number of small tumours were missed by the three histological lung sections. Thus the number of sections per lung was increased in the present study to 10-15 as a rule; we tested fine TiO_2 from the same supply yet again; 60 mg resulted in a tumour rate of 29.5% (Table 4), as compared to 5.1% after instillation of 45 mg in the former study.

A further study which influenced our dosage system was the inhalation carcinogenicity study on toner by Muhle *et al*[51]. The so-called *negative results* were commented in the paper as follows: *"The comparable tumour frequencies observed for three different toner exposure groups and air-only and TiO_2 controls clearly demonstrate that toner inhaled over most of the life span of F-344 rats under the strictest international guidelines for chronic toxicity/carcinogenicity evaluation is not tumourigenic for the respiratory system. This outcome provides a clear demonstration of lack of carcinogenicity of the toner, an innocuous, benign dust, under conditions where both the MTD and maximum functionally tolerable dose (MFTD) criteria were satisfied*[52]*, and exposure conditions were not overtly excessive"*. The overwhelming majority of the dust experts agreed with this interpretation. We re-evaluated the study and came to quite different conclusions (see next chapter). However, at that time we assumed that the inhalation model was far from being sensitive enough to test the carcinogenicity of coarse particles, because the very effective nose filter of rats reduces the lung dust burden, but it can be bypassed with the instillation method. We concluded that respirable dust particles with geometrical diameters > 1 μm (toner: mean diameter 3.5 μm) either induce a statistically significantly increased tumour response only after instillation of more than 100 mg or that non-significantly increased tumour rates with this dose could be interpreted as a not positive result with a high dose in a sensitive test model. The dose-response relationships of inhalation and instillation experiments are compared by Roller and Pott[90].

Were the adequate high doses exceeded?

Figs. 3 and 5 show that quartz as well as GBP is associated with dose-dependent tumour responses. The tumour incidences are often substantially higher than expected. One wonders whether the recent Health Effects Test Guidelines Carcinogenicity, published after the end of the study[91], show that the *adequate high dose* was exceeded in any group or not (the *term maximum tolerated dose* is substituted in the guidelines by *adequate high dose*). In contrast to earlier guidelines, only very general guiding principles are given: *"A rationale for the doses selected must be provided"*, and *"the highest-dose*

level should elicit signs of toxicity without substantially altering the normal life span due to effects other than tumors". The dosing issues are described much as in the guidelines of 2005[92]. In our study, the normal life span was moderately reduced in only a few of the 36 groups treated with GBP and in one quartz group, irrespective of the acutely toxic hydrophobic TiO_2 groups. Retardation in the body weight gain by more than 6% as compared with controls was observed in three groups of the coal dust study (high dose quartz and two coal dusts between minus 6.6 and 7.1%) and the higher dose group of aluminium oxide (-6.9%)[47]. However although histopathology was not performed on fibrosis, it is argued in the chapter *tumour diagnosis* that much more than minimal inflammation and fibrosis in quartz-treated lungs are unequivocal signs. Does this finding show that the *adequate high dose* is exceeded? To answer this question, one should remember that severe lung fibrosis from coal dust, asbestos dust and many other bio-durable dust mixtures is a frequent occupational disease which has caused thousands of deaths among miners and other workers, partly combined with lung cancer. Quartz and asbestos are accepted by many institutions as inducing lung cancer in man and in rats. Consequently, an enhanced lung weight from silicosis which reduces the body weight and lifespan of rats by a mechanism which is analogous to that in the human lung cannot reasonably be claimed to exceed the adequate high dose for a carcinogenicity test. When this applies at least to the lowest and middle quartz dose group, then it does so all the more for the GBP groups which cause a much lower degree of fibrosis (Table 6).

In contrast to these arguments, the *overload hypothesis* with a *postulated no adverse effect level* could find an argument in the wording "*excessive toxicity or inducing inappropriate toxicokinetics*" by an "*overwhelming detoxification mechanism*" which were used in earlier guidelines. McConnell[78] stated "*It is becoming accepted that exposures that "overload" the lungs' ability to clear the particulate in a normal way would, by definition, represent a dose that exceeds the MTD*". If this is correct, adequate carcinogenicity studies with dusts become impossible, because a high excess risk of a few percent cannot be detected without extrapolation from higher significant tumour incidences. Morrow *et al*[60] have correctively concluded that the retardation of macrophage mediated lung clearance of inhaled particles is caused rather by lung volume clearance of GBP than by their mass, but a threshold cannot be concluded from the data[61]. Nevertheless, the German MAK commission set the dust standard at a lung burden of 1 µl dust per g wet weight of control lungs (equal to about 1.5 g per rat lung) deduced from experiments with Fischer rats[62]. According to that definition, a burden of up to 1.5 µl GBP/lung is not yet associated with an overload condition although the lung clearance is retarded by a factor of two and polymorphonuclear neutrophils (PMN) in the bronchoalveolar fluid were significantly increased at this level[61].

This convention was based essentially on inhalation studies with toner and TiO_2 in rats[50-52]. Muhle *et al*[50] suggested the use of a *maximum functionally tolerated dose* (MFTD) concept, in terms of a lung burden at which macrophage-mediated clearance half-time is increased by a factor of 2- 4. Oberdörster[93] called this a reasonable suggestion for defining the highest exposure concentration for a chronic particle inhalation study in rodents. Looking at the relationships between the retained dust doses and tumour responses, the introduction of such a test concept would prevent one from detecting a carcinogenic risk of some per cent from the equivalent workplace exposure to GBP in rats.

In connection with evaluating the *adequate high dose,* we again analysed the inhalation study using toner by Muhle *et al*[51] and Bellmann *et al*[52] and came to a quite different result than earlier supposed and quoted above. The critical points were:

1) The retained lung dust of women who lived in large cities without occupational dust exposure was in the range of approximately 2 mg ± 1 mg per gram of lung (fig. 8). When a mean dust burden in the lungs of coal workers is 16 g as quoted and maximum values of more than 50 g were detected, then it is inappropriate to conclude that the *adequate high dose* was reached in the highest exposure group of the toner inhalation study with 5.4 mg/m^3 for 30 h/week (time-weighed average - TWA - concentration ~4.5 mg/m^3). This exposure resulted in a lung burden of 13 mg in female and 18 mg in male rats after two years of exposure and is roughly equivalent to 16 g dust in the lung of workers.

2) The statement of *"comparable tumour frequencies observed for three different toner exposure groups and air-only and TiO_2 controls"* is a misleading description of the data. In the highest toner group, 5 out of 114 rats with lung tumours (4.4%) were diagnosed against 6 out of 450 rats (1.33%) which include controls, the low and medium toner groups and the lowly exposed TiO_2 group. The highest toner group is associated with a borderline statistically significantly increased tumour incidence as against the pooled 450 rats which were less or not exposed (p=0.0504). The increase in tumour frequency with increasing toner lung burden (after about 1 year and after 2 years of inhalation) is also associated with a borderline statistical significance ("exact" trend test, i.e. generalized Fisher exact test, p=0.049; Cochran-Armitage trend test, p=0.025).

3) The wording *"this outcome provides a clear demonstration of the lack of carcinogenicity of the toner"* is untenable. Even if the tumour incidence in the different groups did not indicate a cancer risk among the highest exposure group, *"a clear demonstration of the lack of carcinogenicity"* substantially overestimates the power of the test model, or a possible cancer risk of some percent is being viewed as a zero risk. The proposed EPA guidelines of 1996 stated: *"Also, it is recognized that animal studies (and epidemiologic studies as well) have a very low power to detect cancer effects. Detection of a 10% tumour incidence is generally the limit of power with currently conducted animal studies"*. This is a result of simple statistic calculations. This has to be considered in connection with a discussion of the boundaries of acceptable and unacceptable risk. The Occupational Safety and Health Administration (OSHA) commented on the view of the US Supreme Court with the words: *"So a risk of 1/1000 (10^{-3}) is clearly significant. It represents the uppermost end of the million-fold range suggested by the Court, somewhere below which the boundary of acceptable versus unacceptable must fall"*[94].

Acute toxicity

The acute lethal dosage of instilled quartz is very high. Two groups of 40 female Wistar rats (mean body weight 135 g) were intratracheally instilled with 50 mg DQ12 suspended in 0.5 or 1.0 ml 0.9 % NaCl solution[95]. After 4 hours, 1, 2, 4 or 6 days, 6-8 rats were sacrificed in order to analyse the quartz content in the lungs. It decreased to the same level, 77% and 74% in the two groups, after 6 days. Ten rats died in this period spontaneously. The administered quartz dose in relation to body weight was extremely high, yet the great majority of the rats survived. Instillation of 45 mg DQ12 in 40 female Wistar rats at the age of 15 weeks was tolerated by all animals; the first rat died after 32 weeks[14]. More examples could be cited which show that rats are very resistant to the acute and subacute toxicity of quartz compared with humans. In man, *acute silicosis* with cases of lethal outcome occurred after relatively short exposure to dusts with high quartz concentrations at certain workplaces like sand blasting or drilling in tunnel construction. The quartz content in the lungs of two miners who died from acute silicosis in their early twenties amounted to 1.5 g and 2.3 g; the total dust content was 4.2 g or 6.1 g respectively[96]. These quartz levels in human lungs correspond to about 2 mg quartz dust in the rat lung, which is much more than a factor of ten lower than a lethal dose. Short term experiments with dusts in rats are frequently used for investigating the details of mechanistic questions. However, it is not clear how to include the low acute sensitivity of rats in any adequate extrapolation of the results of short term experiments to mechanistic processes which occur in humans.

The acute lethality of soluble amorphous silica in rats is much higher than that of quartz. Subchronic and chronic effects of different types were investigated in several experiments[30, 97]. In a pre-test with intratracheal instillation, we found an acute maximum tolerated single dose of 3 mg which was instilled five or ten times in the chronic study without significant mortality. The maximum tolerated single doses published on other experiments and other exposure routes are different and depend on the product applied. Reuzel *et al* [28] exposed rats to three types of amorphous silica dusts for 13 weeks (30 mg/m^3, 6 hr/day, 5 days/wk). Although Aerosil 200 was quickly cleared from the lungs and regional lymph nodes, the changes in these organs were only partly reversed during the post-exposure period of 52 weeks. Warheit[29] reviewed further studies in which short term exposure to amorphous silica produced only transient inflammatory and fibrotic response. Recent results after 30 instillations at intervals of two weeks resulted in fibrosis by the end of the study in the 29th month[98].

Tests with bronchoalveolar lavage fluid 2 to 28 days after instillation of 0.3 to 2 mg amorphous SiO_2 (Aerosil® 150) resulted in high concentrations of leukocytes and other parameters[99]. It was concluded that instillations of 0.5 mg at intervals of 2 weeks would maintain a chronic inflammation which would induce tumours, as with biodurable dusts, if a similar degree of chronic inflammation is the only reason for the carcinogenic effect of GBP. Intensive histopathological examinations are in progress[98].

The third specifically toxic dust was hydrophobic ultrafine TiO_2 which was supposed to be not more toxic than the ultrafine TiO_2 hydrophilic counterpart. These two dusts should be tested in parallel; however, the planned single dose of 6 mg proved lethal[31]. Hence, the single doses for the carcinogenicity study had to be reduced to 0.5 mg and the total doses were reduced to 7.5 and 15 mg. Because of the great discrepancy between the toxicological evaluation of the dust by the assumed producer and the mortality after instillation of a few mg, a large experiment for testing the acute mortality was performed. This secondary effect of the carcinogenicity study is shown in Table 5. The pathophysiological mechanisms of the acute lethal effect can be discussed as follows: 1) the lipophilic surface mediates a fast distribution of the ultrafine particles in the surfactant layer on the alveolar walls; 2) the organic silicon compound dissolves from the particle surface, damages the surfactant and the membranes of pneumocytes and capillaries; 3) the alveolo-capillary membranes swell, which results in a capillary block and haem-

orrhage in the alveoli: dark red spots were macroscopically detected on the lung surface by autopsy and erythrocytes were seen in the alveoli.

Relationship between carcinogenicity and non-neoplastic lung lesions from GBP and from quartz

Studies on the degree of inflammation and fibrosis have not yet been performed. The survival analysis of the 10 groups which received 60 or 120 mg of a coal-containing dust did not show any significant reduction in life span compared with controls; the mortality only increased faster in the highest quartz dose group and during in the last months of the experiment[47]. The lung weight can roughly be used as a measure of the degree of fibrosis (Table 6). Hence it can be concluded that fibrosis was substantially lower after instillation of 120 mg coal-containing dust than from instillation of 5 mg quartz, but the carcinogenic potency was similar. In other words: it is not possible to reduce the life span of rats by inducing severe fibrosis by a high GBP dose. Rats are more likely to die of suffocation from an extremely high GBP volume than from inducible fibrosis; nevertheless, tumour incidences from coal dusts were as high as 80% in our study. Looking at the so-called *not worth mentioning* but existing accumulation of macrophages and fibrosis in cases of simple anthracosis[100], it has to be asked: is this low reaction a strong indication that a cancer risk is lower than 1 in 1000 workers (see the section below, on differences and similarities between lung tumour risk for rats and humans)?

Using the volume metric and under the precondition that the percentage of dust retained after instillation of 5 mg quartz DQ12 and 120 mg coal-containing dust is the same, the relation between the lung burden of pure quartz and the mean of the five coal-containing dust volumes works out as 1 to 37. This relationship increases to <1 to 37 if the lung burden of quartz decreases because of its larger tendency to migrate into the lymph nodes. The logistic regression analysis gave an odds ratio of 30 for the carcinogenic potency of DQ12 compared to GBP after adjusting for particle characteristics like size and specific surface area. The ratio of about 30 to 1 roughly applies to the carcinogenic potencies of quartz and GBP-F-large but not to the fibrogenicity as explained above. This excess risk from pure quartz compared to GBP can be attributed to the *activity of the quartz dust surface* by an ultimately unknown mechanism; acid silanol groups (≡ Si-OH) may be the starting point, but especially the ionisized silanol groups (≡ Si-O-) which can be inhibited by cations.

It must be emphasized that the differing quartz contents of the five coal-containing dusts did not show any clear influence on the general carcinogenicity from GBP using the dose metric of dust volume per lung. This can be explained in analogy with the well known inhibition of fibrogenicity from quartz by accompanying minerals in mine dusts which has been extensively investigated for decades[101]. However, open questions exist on the fibrogenic surface property of quartz and the coating and masking of it by other mineral dust components. Tourmann[102] concludes from his particle analyses of mine dusts with quartz contents up to 26 mass %, using laser microprobe mass spectroscopy (LAMMA), that the particle composition does not change in human lungs in 40 to 50 years. Our results with the five coal containing dusts may be generalized as similar to mine dusts. They do at least justify the classification of the tested dusts as GBP instead of specifically toxic dusts, although three of them contain up to 16.7% quartz.

Tumour diagnosis

Regardless of the tumour incidence per group, the relationship between macroscopic and microscopic tumour incidence is interesting because the dose-response relationships for the total study have only been based so far on macroscopic diagnoses[103]. The macroscopic tumour diagnoses of rats with primary lung tumours in part 1 show a 92.5% correspondence with histologically confirmed tumours. On average among all groups of the first part, the histological tumour outcome was 53% higher than the macroscopically detected tumour incidence. This result was expected because tumours which do not reach the surface of the lung cannot be diagnosed macroscopically. Besides this, it is evident that small tumours have a reduced chance of becoming cut and found by microscopic examination when only three to five sections per lung are performed. In our recent study, 10 to 15 sections were diagnosed. This high number of sections is the best explanation for the high number of lungs with more than one detected tumour. In 296 out of 368 lungs in part 1, at least one malignant tumour was diagnosed, in 249 of them more than one, in 159 more than one of the same type, and in 90 at least two different malignant tumour types. As a consequence of these findings, more tissue sections per lung than 3-5 should routinely be performed. On the one hand, the high dust burdens of experimentally exposed lungs are criticised; on the other hand, the carcinogenic response, especially from low doses with a late development of tumours, is underestimated or a "no observed effect" is wrongly assumed because there is a large probability that incipient tumours will be missed where the number of sections is small. This has been proved by the findings of a carcinogenicity study which

indicates a tumour-inducing effect of amorphous SiO_2 that could only be detected by histological examination of 60 sections per lung and not with the routine histological method[98].

Rats with one or more primary lung tumours were evaluated as "positive" for the purposes of the test, regardless of tumour diagnosis according to the preamble of the IARC Monographs on the evaluation of carcinogenic risks to humans: *"When benign tumours occur together with and originate from the same cell type in an organ or tissue as malignant tumours in a particular study and appear to represent a stage in the progression of malignancy, it may be valid to combine them in assessing tumour incidence"*. The 653 primary lung tumours are distributed among different diagnoses. The distribution of primary lung tumour incidences (Table 7) shows that adenomas and epitheliomas were not diagnosed in humans in the studies cited, but that they amount to about 40% of the diagnosed rat tumours. In man, about the same percentage of lung tumour diagnoses are represented by small cell and large cell carcinomas which are not found in the rat lung. Regarding cystic keratinizing epitheliomas, Boorman *et al*[64] stated that they may not be relevant to human safety evaluation concerning a substance or particle, if this tumour type is the only evidence of tumourigenicity in a study. However, this clearly does not concern the tested coal-containing dusts and quartz.

A publication by Borm *et al*[72] reports on a minor part of our study; only three of the five rat lung lobes were histologically diagnosed. The groups selected and the results are not authentically described; their interpretations are substantially different from ours.

Dose metric

One of the important questions of the 19-dust study was "which physical dust characteristics determine carcinogenic potency?" or in other words "which is the appropriate dose metric?". The primary dose measure, of course, is the instilled dust mass. The retained dust mass and the density of the material determine the retained volume, the retained dust mass and the specific surface area of the dust determine the retained surface area. Particle size, density and specific surface area are interrelated (in the case of spherical particles, the relation is: *specific surface area* = 6 / [*density* x *diameter*]). Sometimes retained particle number has been proposed as an appropriate dose metric. But the particle number is interrelated with particle size and density, even more than specific surface area (for spherical particles, the specific particle content, i.e. particle number per mass unit, can be expressed as: $6 / [\pi \times density \times diameter^3]$). For real dusts with various particle shapes and sizes, the specific particle number is particularly difficult to measure. Against this background and due to the difficulties encountered with surface area, we did not see any opportunity for usefully conducting a dose-response analysis of the dose metric *particle number*.

The non-linear regression analysis of the dose-response relationships of our 16 GBP leads quite clearly to the conclusion that the retained dust volume in combination with some information about (mean) particle size is the best suitable dose metric to date. Goodness-of-fit was about the same when the information about particle size was expressed in terms of 3 size classes and when a continuous function was used. The continuous function

Table 7 - Frequencies of histological diagnoses of primary lung tumours in humans and rats

Origin of lung tissue	No.	Adenoma (%)	Adeno-carcinoma (%)	Epithelioma (%)	Squamous cell carcinoma (%)	Small cell carcinoma (%)	Large cell carcinoma (%)	Other carcinoma (%)
Biopsy	635 [a]		23		41	30	1	5
Operat.	163 [a]		40		45	10	5	< 1
Autopsy	107 [a]		18		31	36	7	8
Women[b]			36		25	23	9	16
Men[b]			15		46	25	13	14
Rats	368 [c]	12	32	27 [d]	27			1.7

[a] 1989 data from the Bergmannsheil clinic, Bochum, Germany[104].

[b] Cancer Registry of the German Democratic Republic 1978-1982[105]. About 6,870 new morbidity cases, of these about 70% histologically confirmed, about 10% women.

[c] Part 1 of the 19-dust study.

[d] These benign epithelial tumours were subdivided into three types: non-keratinizing 3.4%, cystic keratinizing 15%, keratinizing 8% according to the classification of Boorman *et al*[64].

describes an increase in carcinogenic potency with decreasing (mean) particle size - and a decrease in carcinogenic potency with increasing (mean) particle size, respectively. Using the surface area as a dose metric only makes sense when the different carcinogenic potencies of various dusts can be explained by their surface areas and particle sizes do not have to be considered separately - this is the rationale of the *surface area concept*: "smaller particles are more effective *because* of their larger surface area". However, no plausible dose-response relationship was obtained in our regression analysis when just the retained surface area was used as the dose metric - as can easily be seen in fig. 5 (and has already been described above). This contrasts with the reasonable volume - size - response curves in fig. 4 and fig. 6.

Surface area has been advocated in several publications as the most suitable dose metric. However, none of these analyses are as comprehensive as our analysis of the carcinogenicity study with 16 GBP. In the analysis of carcinogenicity data by Driscoll[69], for example, which has also been published by Miller[106] and Oberdörster[107], data of various origins were mixed, so that an interpretation is difficult[108]. Furthermore, these analyses were restricted to the dose metrics *mass* and *surface area* while the dose metric *volume* was not even considered. Some analyses of inflammatory symptoms (PMN) did not account for different particle sizes and they refer to relatively small data bases (e.g. only two dusts compared per publication)[70, 107]. Some of these aspects have already been discussed by Roller[61]. Finally, an important argument against the *surface area* hypothesis is that it is not plausible that size should not matter for the localization of particles within the lung. If only surface area, but not the different particle sizes, is considered, the varying site of particles is ignored. For this reason and given our empirical results, retained dust volume and some indicator of particle size should both be included in the characterization of dose.

Dose-response relationships and quantitative risk assessment

The dose-response relationships of the 19-dust study have already been described in detail within the sections *Materials and methods* and *Results*. It should be emphasized that the dose-response curves tended to a hyperlinear shape in most of the analyses. A slight indication of sublinear (sigmoidal) shapes was only found for a minority of single dusts. The dose-response relationships for each individual dust can be identified in fig. 4, but there is no clear indication of a threshold dose for carcinogenicity. The hyperlinear dose-response shape was extremely pronounced for the dose metric *retained dust surface area* when all GBP types were combined (fig. 5). We have no biological explanation for a hyperlinear curve shape for GBP and we think, in the case of the BET surface area, that it must be an "artefact" stemming from an inappropriate dose metric. Besides logistic regression, we selected the multistage-model, which is widely used and is implemented in the US EPA BMDS, as one of the basic mathematical regression functions. The multistage-model is not able to take on hyperlinear curve shapes. When there is no sublinearity the multistage-model of the BMDS is identical with the so-called quantal-linear model. We thus used multivariate versions of the quantal-linear model to analyze tumour frequencies dependent on retained dust volume and particle size, which led to reasonable dose-response graphs. Besides and apart from the model-based mathematical analyses, the position of the data points alone gives no indication of a threshold dose (figs. 4 and 6).

It is known that some data indicate slightly sublinear exposure-tumour response relationships after inhalation of diesel particles[2, 4, 7, 19]. However, other data indicate a virtually linear dependence of inflammatory effects on the retained lung burden. Furthermore, some epidemiological data speak of higher risks from diesel particles in humans than in rats[19, 109-112] and some epidemiological data have been interpreted as linear exposure-response relationships of health effects after environmental exposure to PM2.5[34]. Hence, as long as there is no clear proof that the tumour risk from equivalent GBP exposure is higher or smaller in humans than in experiments with rats, we recommend calculating the exposure-related risk in rats and taking these values as a measure for the evaluation of human exposures where a linear or quantal-linear calculation procedure appears appropriate in the range of workplace-like exposure concentrations.

On the differences and similarities between lung tumour risk for rats and for humans in the case of GBP

An important point should be stated clearly before proceeding with further considerations: there is no law of nature which predicts that the tumour response of rats and humans is the same or nearly the same after a so-called equivalent exposure to a carcinogen (*equivalent* means: same concentration per m^3 air, same hours per day or per week for the same percentage of the total life span). Surprisingly, some examples really exist which show an analogy between the experimental and epidemiologic results of the cancer risks of rats and humans. We conclude this against the backdrop of our experience with polycyclic aromatic hydrocarbons[113], and quartz[114, 115], but we could show that this does not apply at all in the case

of asbestos[116-119]. On the other hand, it is well known that humans are susceptible to arsenic carcinogenicity but rodents are not. Consequently, we think that a statement such as "*The tumour risk of rats and humans by a substance called xyz is very similar after equivalent exposure"* can only be given on the basis of sufficient carcinogenicity studies with rats and with humans. The example of asbestos fibres shows impressively that false conclusions will be drawn when the lung tumour and mesothelioma risk from inhalation studies in rats are extrapolated to humans, although our knowledge of the mechanistic details suggests a good analogy between the species. What are the reasons? We do not know them. Of course, the dosimetric comparisons of particle deposition and retention in rats and humans in the comprehensive study of Brown *et al*[120] are a meritorious work. However, the great problem regards the important assumption "*that comparable doses should cause comparable effects across species and that species respond similarly to a given dose at a target site, extrapolation only requiring that the dose be defined and the site characterized*". This simple assumption, which ought to cover an immense gap in science, is the reason for our conclusion that, at present, knowledge of mechanistic actions will not lead to reliable dose-response extrapolation from the carcinogenicity of an agent in rats to man without support by epidemiologic data. Does any rat carcinogen exist for which the mechanistic processes in rat and in man are comprehensively known so that a carcinogenic risk in a short life span of 2½ years of a minimal number of cell divisions compared with humans can be reliably predicted at a level of 0.1-1%? The molecular pathways leading to cancer are highly complex, involving many different biochemical mechanisms and differences between species. The clarification of some qualitative and quantitative differences between rats and humans is of basic scientific interest, but insufficient for reliable risk extrapolation.

There is no doubt that the cell number at mutation risk or cancer risk is much higher in a human lung, of about 1000 g, than in a rat lung of about 1 g, and that the number of cell generations is much higher in humans than in rats. The number of cell divisions at mutation risk in both species cannot be calculated. However, it *must* be very much higher in humans than in rats. Consequently, a similar cancer risk after an equivalent exposure to a carcinogen which occurs after 75 years in man and after 2½ years in rats requires at least quantitatively large differences between the cancer susceptibility or cancer risk per cell of both species[121]. A beam of light directed into the black box of carcinogenesis *must* show substantial differences between man and rat. It might be a great error to expect, for instance, the same degree of histopathologically detectable reactions to GBP in the lung of a rat as in a man or take efficiency of DNA repair as a precondition for a similar tumour risk in both species after equivalent exposure. Indeed the opposite should be expected. *Such non-analogies are inevitable preconditions for analogies between the tumour risks of rats and humans*. Fig. 9 illustrates the problem, ignored by the great majority of experts, who evidently think that detection of a few

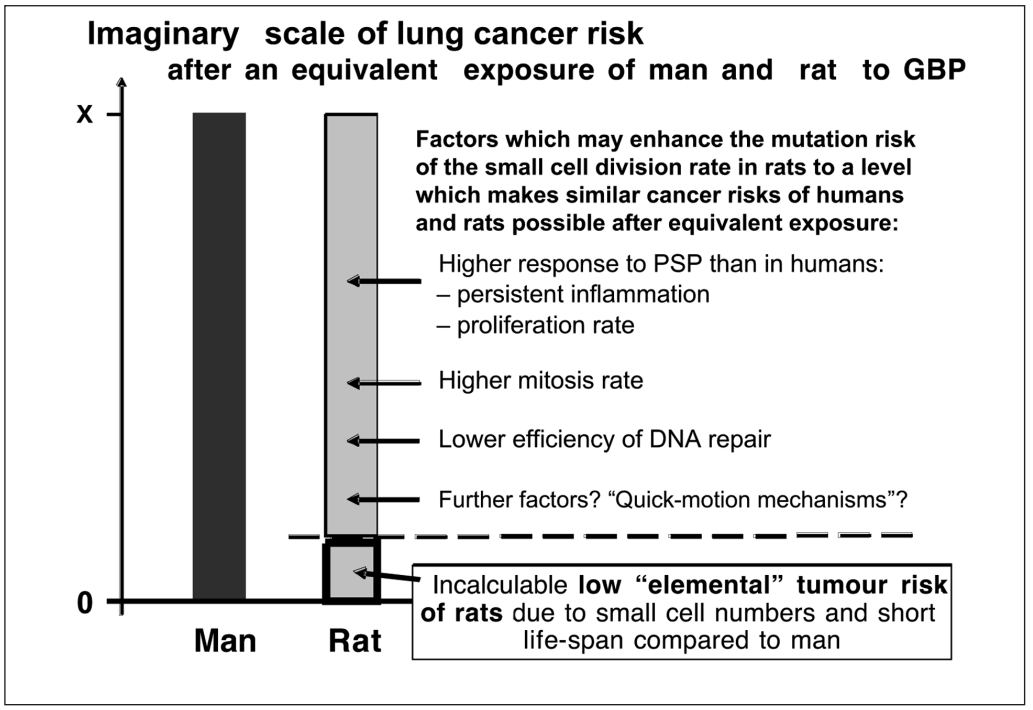

Fig. 9 - Illustration of the incalculably low basic lung tumour risk of rats from equivalent exposure to GBP, which has to be increased by species-specific differences of carcinogenesis to make any analogy to the human cancer risk possible.

significant differences will clear the way for reliable interspecies extrapolation.

Our conclusion that there may exist a relatively similar tumour risk from GBP among rats and humans is substantiated by epidemiologic carcinogenicity studies on workers exposed to quartz dust, coal dust and diesel particles. The good analogy with quartz dust has already been mentioned; the epidemiologic evidence for the carcinogenicity of coal dust is less convincing[122]. However, Morfeld *et al* [123] found that 10 SMR follow-up studies resulted in a statistically significantly lower relative risk and the 4 case-control studies in a statistically significantly increased risk. The contrast between the results of two different study types lends some weight to the conclusion that there is a systematic downward bias by SMR studies. The authors explained the bias as a healthy worker selection effect because workers with an occupational disease, mostly pneumoconiosis, had been weeded out of the cohort. In addition to this bias created by removing workers with an occupational disease from the cohort, it is plausible to assume that coal workers are a selected healthy population after some years in their profession, who probably tend less than the average of the general population to inflammatory reactions of the lung. Becklake[124] reports: *"There is evidence that changes to less dusty assignments, or job turnover rates early in a mining career are related to airway hyper responsiveness. For instance, recent studies in US coal miners have shown that those employed in dusty jobs are less likely than their unexposed coworkers to exhibit increased airway hyper-responsiveness"*. Mauderly[84] used coal dust as a reasonable objection to rebut the validity of positive diesel studies. He argued that 12 reports indicated that coal dust exposure does not cause lung cancer in humans, although lungs of heavily exposed coal workers accumulate specific lung burdens of dusts in the same dose range as in heavily exposed rats. At the other end of the total population, patients with idiopathic pulmonary fibrosis could represent a group which is especially sensitive to the carcinogenic effect of chronic inflammation[125, 126]. It remains to be seen whether the healthy worker selection effect was sufficiently regarded in the interpretation of the non-positive older coal worker studies which were the basis for Mauderly's conclusion in 1994.

Meanwhile, the carcinogenicity of diesel particles in humans is accepted by the majority of scientists. However, there is a contradiction regarding the cause of the assumed high cancer risk. The relative carcinogenic potency of diesel engine emissions seems to be much higher in humans than in rats, if the excess risk is attributed totally to diesel particles. Fig. 10 illustrates this contradiction. This has been explained by the adsorbed organic substances which contain carcinogenic PAH. The results of Dasenbrock *et al* [15] seem to support this conclusion at first sight, because the group of rats which received 15 mg extracted diesel particles per instillation produced lung tumours in only 4% compared to 17% exposed to 15 mg original diesel soot, which contained 43% organic material. However, the results of other groups, especially extracted diesel particles with adsorbed BaP show inconsistencies which cannot be reasonably explained. In inhalation experiments with tar pitch exhaust and diesel exhaust the same tumour incidences were found (16% and 18%)[3, 17], although the concentrations of BaP in the air were extremely different: 12 ng and 90,000 ng/m^3 are extremely different (relation 1 to 7500), but elemental carbon in diesel exhaust amounted to 85% of the 4.2 mg/m^3 total particle mass, and yet was very low in the tar pitch exhaust. Considering a much more than additional effect, the data lead to the conclusion that only about 1% of the carcinogenicity of diesel exhaust can be attributed to PAH.

The most reasonable explanation for the seemingly much higher potency of diesel particles in epidemiologic studies than in rat studies is that GBP were not considered as important confounders as explained in fig. 10. Up to now, GBP have not even been accepted as carcinogenic in experimental animals, on a par with diesel soot. A combined carcinogenic effect of diesel soot with other GBP was not taken into consideration to explain the discrepancy between humans and rats shown in the figure. However, it is reasonable to assume that GBP were often present in a substantial concentration of 1 mg/m^3 or higher in the workplace atmosphere of workers who were highly exposed to diesel soot. In a well reproduced study on the exposure conditions of potash mining workers, the mean value of the highest exposed group was 0.42 mg total carbon, which contained 63% elemental carbon[127, 128]. A re-evaluation of the diesel studies in the light of GBP as carcinogenic confounders is recommendable.

Conclusions

The 10 questions asked in the introduction can be answered as follows:

1) According to the results, sixteen out of the nineteen tested dusts may be classified as *granular bio-durable particles without known significant specific toxicity* (GBP) regarding their carcinogenicity, although their physical and chemical properties are more or less different. This leads to the general conclusion that all respirable granular bio-durable dust

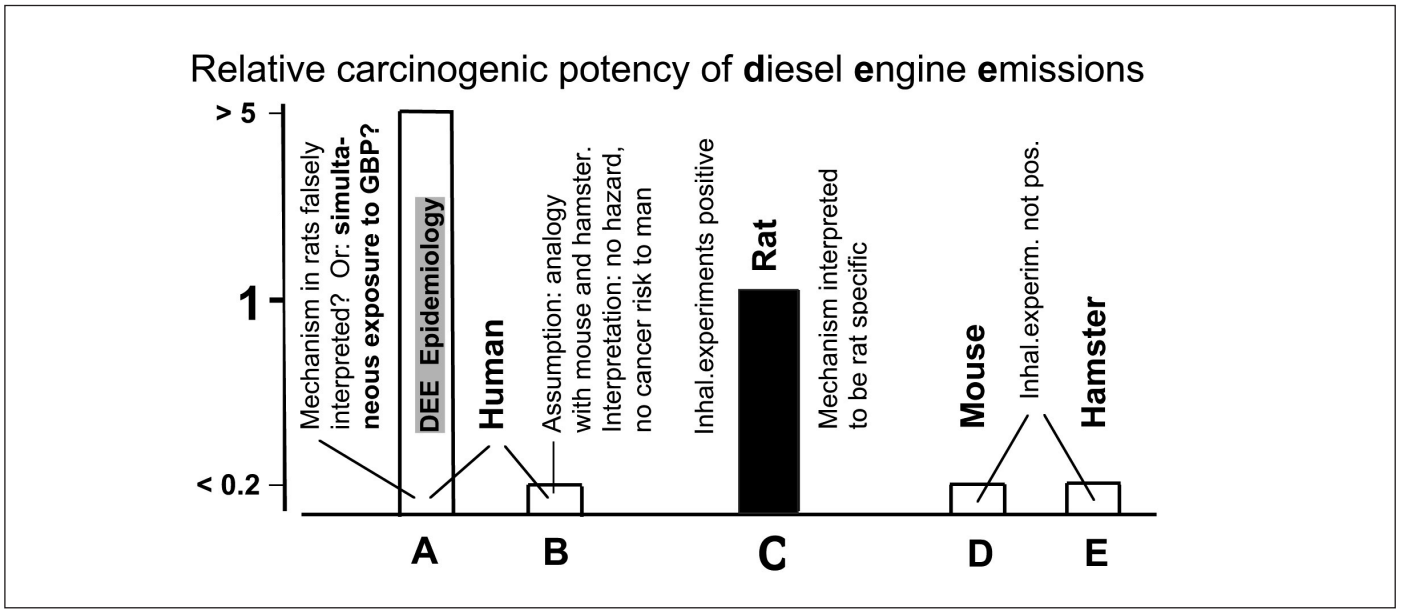

Fig. 10. Illustration of the contradiction between epidemiologic positive carcinogenicity studies with diesel engine emissions (DEE) (column A in the figure) and the common assumption of an analogy between human, mouse and hamster in the carcinogenic reaction to DEE (equal columns B, D, and E). Central point of the figure is the carcinogenicity of diesel soot detected in the rat lung; this potency is set at 1 (column C). If the enhanced lung cancer risk which was found in workers heavily exposed to DEE was caused by these emissions, the carcinogenic potency of diesel soot is either higher in man than in rats by the same mechanism (unit risk > 5 x 10^{-4} in man, 1 x 10^{-4} in rats) or DEE induce cancer in man by a mechanism different to that in rats. If the assumption is correct that the cancer risk in man is similar to that of mice and hamsters, the positive epidemiologic findings must be false positive. These contrary interpretations lead to the question: Is the rat model too sensitive (relation column C to B) to predict the cancer risk from DEE in man or is it not sensitive enough (relation C to A)? Conclusion: the epidemiologic results should be re-evaluated bearing in mind that GBP are carcinogenic confounders.

types have a carcinogenic potential in the rat lung. Diesel particles also belong among GBP because only 1% of their carcinogenic potency can be explained by organic substances with a minimal concentration of adsorbed PAH.

2) The carcinogenic potency of GBP depends on the particle size. The slopes for dose-response relationships have been calculated with the dose metric *particle volume* for the three size classes examined (mean diameters 0.01-0.03 μm; 0.09-0.2 μm; 1.8-4 μm) giving the relationship of 5.6**:**2.1**:**1.0. The risk ratio for the size classes *ultrafine* to *small fine* is 2.7**:**1.0. These results lead to the conclusion that the common formalistic division of respirable dusts into fine dust (>0.1 μm) and ultra-fine dust (<0.1 μm) is scientifically not justified. Instead of a large jump at the size of 0.1 μm, a continuous transition from a higher to lower carcinogenic potency is biologically much more plausible and is supported by the data.

3) Non-linear regression analyses show that the dose metric *dust volume combined with the particle size* correlates best with the carcinogenicity. There is no proof that the volume is the true carcinogenic agent, but it represents it best of all the checked criteria. In any case, the particle size influences the distribution in epithelial and other lung cells, alveoli, lymph nodes and other organs. In combination with the particle volume, the particle size is an important factor in determining the potency; the differing spectrum of particle sizes in the dust samples explains the differing potencies best. By contrast, the dose metric BET surface area assumes that the higher specific surface of smaller particles explains *per se* their higher effect, but this assumption neglects the fact that particle location in the lung depends on particle size and not on surface area. Nevertheless, surface properties may be involved in the mode of action. However, at least one property should be identified and quantitatively measurable, comparable with silanol groups on the surface of quartz.

4) Generally, major health risks which are induced by an agent should be quantified, especially if there is a cancer risk. We assessed the cancer risk from GBP in rats by a method based on the multistage model

using maximum-likelihood estimates (not the 95% confidence limit). The multistage model is well established. The assumption of linear dose-response relationships in the low dose range (<10%) probably does not underestimate the effect but even this cannot be stated with any certainty. This method for risk assessment is clearly transparent in comparison with the postulate or statement that no observed adverse effect levels of sensitive indicators offer proof for a threshold at this dose or, at least, exclude a risk of >1:1000. Such assumptions depend on the limit of effect detection, which may be associated with higher risks than 1:1000. The contrary should be established in every case.

There are indications that the uptake of particles by lung epithelial cells induces direct or indirect epithelial cell cytotoxicity[129-132] in addition to or independent from inflammatory cell response. The mechanisms are not sufficiently understood.

5) The odds ratio for the carcinogenic potency of pure quartz type DQ12 in comparison to GBP was estimated in this experiment by logistic regression as 30:1. However, it should be born in mind that other quartz dust types showed a substantially lower carcinogenic potency than DQ12.

6) The results with the 5 coal-containing dusts may be generalized to similar mine dusts. At least, they justify the classification of the tested dusts as GBP in this experiment, instead of "specifically toxic dusts", although three of them contain quartz up to 16.7%. The active quartz surfaces in coal mine dusts are covered with other minerals at least to a certain extent[101]. This bonding seems to be stable for decades[102]. This does not exclude toxic effects of metals, emitted in the air by combustion exhausts.

7) The question, whether an inflammation from 10 weekly instillations of 3 mg amorphous SiO_2 induces tumours could not be answered clearly. The low tumour rate of 5.7% led to a second study with longer exposure by 30 instillations of relatively low single doses of 0.5 mg[98]. Again, the tumour incidence found in 17 rats which survived more than 2 years was not significantly increased by normal histological examination, but by histological diagnostics of 60 sections per lung.

8) The hydrophobisation of so-called "inert" dusts can induce substantial toxic effects. There are many products with differently coated dusts on the market. Adequate toxicological test programmes should be carried out.

9) A comparison between lung tumour response in inhalation and instillation experiments was performed for carbon black, TiO_2 and diesel particles[90]. The results show that, on average, a five- to six-fold higher dust burden after one year of inhalation exposure corresponds with the effect of a few weekly instillations in terms of lung burden (estimated to be retained for a long time). The much higher tumour incidences after instillation are due to high early-life exposure which holds the high dust level in the lung for a much longer time for tumour induction than in inhalation experiments with slow increase of retained dust. A misleading bolus effect which can be expected with acutely toxic substances could not be substantiated.

10) We cannot assess to what extent a one-to-one extrapolation from the GBP carcinogenicity in rats to humans corresponds with the true relationships. Divergences to the one as well as to the other side are possible. We conclude that the mode of action which leads to lung tumours from GBP in workers is in principle similar in rats and in humans. This assumption is based on epidemiologic results and mechanistic data with three agents:

1. Quartz dust. The risk after equivalent exposure is approximately in the same range; as far as is known, quartz and GBP work qualitatively with a similar mechanism in both species and, of course, quantitatively on a much higher level than GBP.
2. Coal dust. Epidemiologic studies are partly not positive, but a general healthy worker selection effect among long-term workers who are not very sensitive to a dusty workplace and a bias by epidemiologic cohort mortality studies, in that they weed out workers with an occupational disease from the cohort, lends some weight to the conclusion that there is a systematic downward bias with an underestimation of the excess risk.
3. Diesel particles. As explained above, the generally much higher excess risk in epidemiologic studies than in animal studies is not due to the adsorbed organic substances. But at least a part of this difference may be explained by the fact that highly exposed diesel workers (mean ~ 0.25 mg elemental carbon/m^3 in the highest exposure group) tend to be simultaneously exposed to GBP, even in much higher concentrations than those of diesel particles. This exposure has not been

considered as a confounding factor up to now, because GBP are not classified as carcinogenic by IARC or any other institution which classifies substances regarding their carcinogenicity.

Mechanistic investigations have found few differences between rats and humans, but such as they are, they have been interpreted as a substantial impediment to risk extrapolation. However, similar tumour risks from equivalent exposure in rats and workers (e.g. 3 mg GBP/m^3, 40 h/week) require quantitatively large differences in parts of the complex mechanism of carcinogenesis between both species and a very high sensitivity of rat cells, in comparison to human cells, because of the very different cell numbers, life times, and – consequently – mutation risks per cell division. *Such non-analogies are fundamental preconditions for any analogies assumed between in tumour risks of rats and humans*.

On the basis of experimental results in rats and assuming that equivalent inhalation exposure of workers results in a similar lung tumour risk after 40 years to that in rats, the following excess risks (ER) after occupational exposure for 40 years, 40 h/week can be extrapolated for the three size classes of GBP (density of the material 2-2.5 g/ml) and an exposure concentration of 3.0 mg/m^3 (similar to one of the dust standards in Germany):

– GBP *fine large* (mean size 1-4 μm): 1% ER,
– GBP *fine small* (mean size 0.09-0.2 μm): 2% ER,
– GBP *ultra-fine* (mean size 0.01-0.03 μm): 5% ER.

The risk estimates are well above 1 : 1.000 and considerably higher than *minimal* risks, therefore linear interpolation to realistic lower occupational dust exposure limits is justified; e.g. for 0.3 mg/m^3 ultra-fine GBP (density 2-2.5 g/ml) an estimated excess lifetime lung cancer risk of 0.5% is obtained.

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Colecistectomia videolaparoscopica e carcinoma "incidentale" della colecisti: strategia terapeutica

Laparoscopic cholecystectomy and unsuspected gallbladder carcinoma: therapeutic strategy

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Riassunto

Il riscontro di un cancro della colecisti evidenziato "incidentalmente" dopo colecistectomia videolaparoscopica (CVL) pone il chirurgo di fronte a due problematiche di non facile risoluzione: 1) l'approccio laparoscopico favorisce la precoce disseminazione della neoplasia e, quindi, ne peggiora la prognosi? 2) è necessario radicalizzare la terapia con interventi più aggressivi? Gli Autori sottolineano che, mentre non vi sono certezze sull'influsso negativo della tecnica laparoscopica sulla prognosi, è opinione comune che la decisione sull'eventuale trattamento successivo dovrà basarsi sull'accurato studio istologico dell'intera colecisti asportata, per valutare esattamente la profondità di invasione del tumore. Analizzando le varie possibili strategie terapeutiche e confrontando i dati desunti dalla letteratura con quelli ricavati dalla loro esperienza, gli Autori concludono affermando che i pazienti con invasione limitata alla mucosa (pTis e pT1) non necessitano di alcun ulteriore trattamento, mentre quelli con tumori pT1b e pT2 possono trarre vantaggio da una ulteriore terapia resettiva. Nei tumori allo stadio pT3 o pT4, invece, i risultati della letteratura non mettono in evidenza una differenza statisticamente significativa, in termini di sopravvivenza, tra pazienti sottoposti ad ulteriore successiva terapia resettiva e pazienti non trattati. Eur. J. Oncol., 10 (4), 283-286, 2005

Parole chiave: **colecistectomia, laparoscopia, carcinoma**

Summary

The detection of an unsuspected gallbladder cancer, discovered at videolaparoscopic cholecystectomy (VLC), presents the surgeon with two not easily solvable problems: 1) does the laparoscopic approach favour early dissemination of the neoplasm, thus worsening the prognosis? 2) is it necessary to make the therapy more radical, by performing more aggressive surgery? The authors point out that, while there is no clear evidence of a negative influence on prognosis of the laparoscopic techniques, it is widely accepted that the choice for further treatment should be based on the accurate histological examination of the entire gallbladder removed, in order to exactly estimate the depth of neoplastic invasion. Analysing the different therapeutical strategies and comparing the data gleaned from the literature with those from their own experience, the authors conclude that patients with tumour invasion limited to the mucosal layer (pTis and pT1) do not need any further treatment, while those with pT1b and pT2 tumours may benefit from a more aggressive surgical therapy. In pT3 or pT4 stage tumours, instead, there is no evidence in the literature of a statistically significant difference, in terms of survival, between patients submitted to further surgical therapy and non-treated patients. Eur. J. Oncol., 10 (4), 283-286, 2005

Key words: **cholecystectomy, laparoscopy, carcinoma**

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Introduzione

Nell'ultimo decennio, la tecnica videolaparoscopica, inizialmente soprannominata, non senza una punta di disprezzo, *"key-hole surgery"*, ha sovvertito molti dei concetti della chirurgia tradizionale, e la "rivoluzione laparoscopica" si è diffusa con una velocità sbalorditiva.

Con il progredire e l'affinarsi della tecnica, la disponibilità di strumenti sempre più sofisticati e l'accrescersi dell'esperienza dei vari "*teams* laparoscopici", anche le colecistiti croniche, che inizialmente rappresentavano una controindicazione pressoché assoluta, vengono ormai routinariamente trattate per via laparoscopica con risultati soddisfacenti.

Il sempre maggiore consenso acquisito da questa metodica sia da parte dei chirurghi che dei pazienti e l'ampliamento delle sue indicazioni rispetto a quelle iniziali hanno portato, in ultima analisi, ad un incremento progressivo del numero di colecistectomie effettuate con tale accesso.

Conseguentemente, nelle casistiche più importanti, ha cominciato ad emergere una patologia che, già conosciuta in chirurgia laparotomica, costituiva per la laparoscopia una realtà ancora sconosciuta: il carcinoma "incidentale" della colecisti[1-5].

Il riscontro di un cancro della colecisti evidenziato "incidentalmente" dopo colecistectomia videolaparoscopica (CVL) pone il chirurgo di fronte a due problematiche di non facile risoluzione, e cioè:

– l'approccio laparoscopico favorisce la precoce disseminazione della neoplasia e, quindi, ne peggiora la prognosi?
– è necessario radicalizzare l'intervento già effettuato mettendo in atto un trattamento più aggressivo?

Lo scopo del presente studio è di valutare, alla luce dei dati desunti dalla letteratura e dall'esperienza personale, se ed in quale modo la via di approccio videolaparoscopica influenzi la prognosi di un cancro "incidentale" della colecisti, e se ed in quali casi esista la necessità di una radicalizzazione dell'intervento di colecistectomia già effettuato.

Pazienti e metodi

Nel periodo compreso tra il 1994 ed il 2004, presso la Cattedra di Chirurgia Generale dell'Università di Messina, sono stati osservati 6 pazienti con carcinoma "incidentale" della colecisti diagnosticato successivamente ad una CVL effettuata per litiasi, con un'incidenza pari allo 0,43%.

In tre casi l'indicazione all'intervento era stata una colecistite cronica litiasica; quattro pazienti erano di sesso femminile e due di sesso maschile; l'età media era di 63 anni, con un *range* tra 49 e 78 anni.

L'esame istologico postoperatorio ha messo in evidenza due tumori pTis, due pT1 (uno pT1a ed uno pT1b), un pT2 ed infine un tumore pT3. In nessun caso vi era interessamento del dotto cistico.

In una paziente si è verificata la perforazione della colecisti con fuoriuscita di bile durante le manovre di scollamento, ed è stato utilizzato un *endo-bag* per estrarre la colecisti.

Per quanto riguarda la strategia terapeutica adottata successivamente, nei tumori pTis e pT1, sulla guida dei dati desunti dalla letteratura, abbiamo ritenuto che la colecistectomia già effettuata potesse ritenersi di per sé curativa (il tumore pT1b era localizzato sul versante sieroso della parete della colecisti). Nel paziente con il tumore pT2, la successiva valutazione strumentale ha evidenziato un interessamento dei linfonodi peripancreatici, per cui la malattia è stata reputata ormai non più suscettibile di trattamento chirurgico. Il paziente allo stadio pT3, reso edotto sulla sua patologia, ha rifiutato il reintervento propostogli.

Risultati

Nella paziente pT1a, in cui vi era stato lo spandimento biliare intraoperatorio, il successivo *follow-up* ha evidenziato, 6 mesi dopo l'intervento, una recidiva nella sede del trocar ombelicale attraverso cui era stata estratta la colecisti, complicata successivamente da una carcinosi peritoneale.

La paziente pT1b, 7 mesi dopo l'intervento, ha sviluppato metastasi linfoghiandolari ilari e peripancreatiche.

Le due pazienti con la neoplasia pTis sono vive *disease-free* ad 8 e 6 anni. Negli altri pazienti, la sopravvivenza è stata rispettivamente di 10 mesi per la paziente pT1a in cui si era verificato lo spandimento biliare, di 11 mesi per la paziente pT1b, di 7 mesi per il paziente pT2, e di 5 mesi per il paziente pT3 (Tabella 1).

Discussione

Per quanto riguarda la problematica relativa alla strategia terapeutica da adottare nei confronti dei carcinomi della colecisti diagnosticati inaspettatamente, riscontrati cioè soltanto nel corso dell'esame istologico postoperatorio, la decisione sul successivo trattamento dovrà fondarsi sull'accurato studio dell'intera colecisti asportata che, sulla base della esatta valutazione della profondità di invasione del tumore, consentirà una stadiazione della neo-

Tabella 1. Sopravvivenza a distanza in relazione allo stadio

Stadio	Sesso	Sopravvivenza (mesi)	Osservazioni
pTis	F	98	Vivente "*disease-free*"
pTis	F	76	Vivente "*disease-free*"
pT1a	F	10	Spandimento biliare intraoperatorio
pT1b	F	11	Metastasi linfonodali 7 mesi dopo l'intervento
pT2	M	7	N+ alla stadiazione postoperatoria
pT3	M	5	Rifiuta il reintervento proposto

plasia e guiderà il chirurgo nella scelta della eventuale "radicalizzazione" della terapia mediante l'impiego di strategie terapeutiche più "aggressive"[6, 7].

Nei carcinomi *in situ* (pTis) o nei tumori che invadono solo la lamina propria (pT1), la maggioranza degli Autori ritiene che la colecistectomia da sola rappresenti un trattamento adeguato e definitivo. In questi pazienti, infatti, se non vi è stata la perforazione intraoperatoria della colecisti con perdita di bile ed il margine di sezione sul cistico non presenta invasione neoplastica, la percentuale di sopravvivenza a 5 anni oscilla tra il 79 ed il 100%[8-11].

Argomento di discussione ancora aperto è invece rappresentato dai tumori con invasione della tonaca muscolare (pT1b), situati sul versante epatico della colecisti: in questi casi, infatti, alcuni Autori ritengono opportuno procedere alla radicalizzazione dell'intervento. Le modalità con le quali ciò si può attuare variano dalla resezione del letto della colecisti con 2 cm di parenchima epatico, alla resezione epatica con asportazione dei segmenti IV e V.

Considerando che lo stato linfonodale rappresenta l'elemento predittivo di maggiore importanza per la prognosi di questi pazienti, l'intervento dovrà essere completato da una adeguata linfoadenectomia. Si dovrà pertanto associare alla resezione epatica la linfoadenectomia *en bloc* dei linfonodi dell'asse celiaco, dell'ilo epatico, del legamento epato-duodenale e della regione peripancreatica. Alcuni Autori[12, 13] procedono anche alla resezione del coledoco. Inoltre, malgrado non sia stata ancora definitivamente accertata la tendenza del cancro della colecisti a recidivare maggiormente nella sede dei trocars piuttosto che sulla breccia laparotomica[14], in questi casi è anche consigliabile effettuare la resezione di tali sedi[15-18].

Dalla valutazione dei risultati riportati in letteratura (complicanze 5-11%, mortalità 0-8,5%) noi, d'accordo con numerosi altri Autori[4, 6, 8, 13], riteniamo che non trovi indicazione l'esecuzione "di principio" di un intervento allargato, in quanto non è in grado di migliorare la prognosi di questi pazienti, generalmente favorevole già dopo semplice colecistectomia.

Nel caso in cui invece i margini di sezione sul dotto cistico presentino un interessamento neoplastico, allora si rende necessario il reintervento, allo scopo di evitare la recidiva precoce.

Se, infine, si è verificata la perforazione intraoperatoria della colecisti, evenienza documentata nel 26-33% dei casi di CVL[16, 19, 20], il problema diventa ormai quello della diffusione peritoneale e non più quello del controllo locale della neoplasia, e quindi la radicalizzazione dell'intervento non comporta un sensibile miglioramento della prognosi di questi pazienti.

Per quanto riguarda invece la terapia dei pazienti con un tumore pT2 (superamento della tonaca muscolare ed invasione del tessuto connettivo perimuscolare), i risultati non sono altrettanto incoraggianti. In questi casi, infatti, la sopravvivenza a 5 anni scende a valori anche inferiori al 35% se si effettua la sola colecistectomia, mentre può notevolmente migliorare fino a valori prossimi al 70% se si reinterviene radicalizzando il primitivo intervento[8, 21] con le modalità sopra riportate.

Infine, per i tumori più avanzati, pT3 (perforazione della sierosa o invasione di strutture anatomiche adiacenti) o pT4 (invasione della vena porta, dell'arteria epatica o 2 o più strutture extraepatiche), l'unica possibilità di migliorare la sopravvivenza è offerta, dopo un'accurata valutazione dell'estensione locale della neoplasia e della presenza di metastasi linfonodali o a distanza mediante ecotomografia, TC ed RM, dall'eventuale reintervento[4, 7, 11]. In ogni caso, in questi pazienti, anche quando è possibile effettuare un intervento ampiamente demolitivo (resezione epatica maggiore associata a linfoadenectomia allargata), l'aumento della sopravvivenza è minimo, e non mostra significative differenze rispetto ai pazienti non sottoposti ad alcuna terapia[10, 11, 22, 23].

Conclusioni

Malgrado i notevoli progressi nell'ambito delle tecniche diagnostiche strumentali, il reperto istologico di un carcinoma non evidenziato preoperatoriamente è una evenienza riscontrabile nello 0,3-1% di tutte le colecistectomie effettuate per litiasi.

I dati della letteratura sono d'accordo nell'affermare che la decisione sul trattamento da effettuare successivamente alla CVL debba essere basata sull'accurata stadiazione postoperatoria del tumore. Mentre i pazienti con invasione limitata alla mucosa (pTis e pT1a) non necessitano di ulteriore trattamento, quelli con tumori pT1b e pT2

possono trarre vantaggio da una ulteriore terapia resettiva[21].

Per i pazienti con tumori pT3 o pT4, pur se i risultati della letteratura non mettono in evidenza una differenza statisticamente significativa tra i pazienti sottoposti ad ulteriore terapia e pazienti non trattati, alcuni Autori, in base alla considerazione che oggi anche gli interventi più aggressivi presentano una bassa incidenza di complicanze e mortalità, sono favorevoli ad un successivo trattamento chirurgico più demolitivo[10, 24].

Pertanto, sebbene l'ancora relativamente esiguo numero di casi trattati non consenta di trarre delle considerazioni conclusive, alla luce dei risultati di molti studi presenti in letteratura ci sembra di poter affermare che il carcinoma della colecisti "incidentale" è una malattia curabile se riscontrata in stadio precoce, e che la CVL non peggiora in maniera determinante la prognosi di questi pazienti[25-28].

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Mesotelioma pleurico da asbesto in lavoratori del porto di Trieste

Asbestos-related pleural mesothelioma among workers of the port of Trieste, Italy

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Riassunto

Vengono riportati 23 casi di mesotelioma pleurico diagnosticati a Trieste in lavoratori del porto nel periodo 1968-2004. La diagnosi era basata su reperti autoptici in 18 casi. I pazienti, tutti maschi di età compresa tra 39 e 80 anni (media 61 anni), avevano lavorato generalmente nel carico-scarico di merci varie, tra le quali asbesto. Su 18 persone, per le quali erano disponibili dati cronologici sufficienti, 12 avevano iniziato la loro attività dopo il 1950. La maggioranza dei pazienti aveva lavorato per più di 20 anni. I periodi di latenza, intercorsi tra inizio dell'esposizione e diagnosi del tumore, variavano da 25 a 60 anni (media 38 anni). Corpi dell'asbesto furono osservati nelle sezioni istologiche di polmone allestite con i metodi di routine in 15 su 17 casi. Al confronto con altre categorie professionali esaminate nell'area di Trieste, i lavoratori portuali presentavano periodi di latenza più brevi e una più elevata prevalenza di corpi dell'asbesto nelle sezioni routinarie di polmone. Ambedue questi elementi sono indicativi di un'esposizione ad amianto di intensità elevata. Eur. J. Oncol., 10 (4), 287-290, 2005

Parole chiave: **mesotelioma, pleura, asbesto, porto, autopsia**

Summary

Twenty-three cases of pleural mesothelioma, observed among dock workers in Trieste between 1968 and 2004, were reviewed. Necropsy findings were available in 18 cases. The patients, all males, aged between 39 and 80 years (mean 61 years), had been generally employed in loading-unloading of a variety of goods, including asbestos. Of the 18 people, for whom sufficient chronological data were available, 12 had begun their activity after 1950. Most patients had worked for more than 20 years. Latency periods, between the start of exposure and tumour diagnosis, ranged between 25 and 60 years (mean 38 years). Routine histological sections of lung tissue, of the 17 cases examined, showed asbestos bodies in 15. When compared with other occupational groups investigated in the Trieste area, port workers showed shorter latency periods and higher prevalence of asbestos bodies in routine lung sections. Both the above findings indicate heavy exposure to asbestos. Eur. J. Oncol., 10 (4), 287-290, 2005

Key words: **mesothelioma, pleura, asbestos, port, necropsy**

Introduzione

Numerosi porti sono stati o sono tuttora stazioni di partenza e/o di arrivo nel commercio dell'asbesto. Nel passato la movimentazione del minerale ha portato a situazioni di grave inquinamento[1], tuttavia il problema è stato generalmente sottovalutato. Nel presente studio sono stati rivisti 23 casi di mesotelioma maligno della pleura, os-

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servati in lavoratori del porto di Trieste. Attraverso questo porto sono passate, nel periodo 1960-1989, quantità di asbesto comprese tra 5.000 e 18.000 tonnellate/anno.

Pazienti e metodi

I casi sono stati diagnosticati presso l'Ospedale di Trieste nel periodo 1968-2004. La storia lavorativa dei pazienti è stata ricostruita in base ai dati forniti dal paziente stesso o dai suoi familiari, attraverso colloqui personali o telefonici. In tutti i casi la diagnosi era basata sull'esame istologico della neoplasia. In 18 casi venne effettuata l'autopsia. In 17 casi autoptici fu ricercata la presenza di corpi dell'asbesto sulle sezioni istologiche di tessuto polmonare, allestite con i metodi di *routine*.

Risultati

I pazienti, tutti di sesso maschile, erano di età compresa tra 39 e 80 anni (media 61 anni). La distribuzione per gruppi di età è riportata nella fig. 1. Quasi tutti i pazienti avevano lavorato nel porto di Trieste come braccianti, addetti al carico/scarico di merci svariate. Un paziente aveva svolto servizio in porto come finanziere e un altro come meccanico ed elettricista. L'attività in porto era iniziata per lo più dopo il 1950 (fig. 2). La durata del lavoro superava i 20 anni in larga parte dei casi (fig. 3). Il periodo di latenza intercorso tra l'inizio dell'attività lavorativa nel porto e la diagnosi di mesotelioma variava tra 25 e 60 anni (media 38 anni) (fig. 4).

Discussione

Il problema della movimentazione di amianto ha assunto proporzioni sempre maggiori nella seconda metà del secolo scorso, con il crescere progressivo della produzione mondiale del minerale. Se il totale prodotto nel 1950 era di 1.290.000 tonnellate, la produzione raggiunse nel 1977 la cifra di 4.793.000 tonnellate[2]. Solo alla fine degli anni '70 si diffondeva l'uso di *containers*. Fino a quel momento l'amianto era spesso trasportato in sacchi di iuta e di carta, che nelle operazioni portuali finivano non di rado con il rompersi. L'inquinamento del luogo di lavoro che ne risultava è stato ripetutamente descritto sia dai lavoratori che dalle Autorità Sanitarie.

Come in altri settori riguardanti gli usi dell'amianto, la gravità del problema è stata riconosciuta con grande ritardo. Già nel 1965 un gruppo di lavoratori del porto di Londra sollevò la questione se le operazioni di scarico dell'amianto comportassero pericoli[3]. Un esperto di medicina del lavoro, interpellato in quell'occasione, affermò che in effetti la situazione era pericolosa. Le Autorità in-

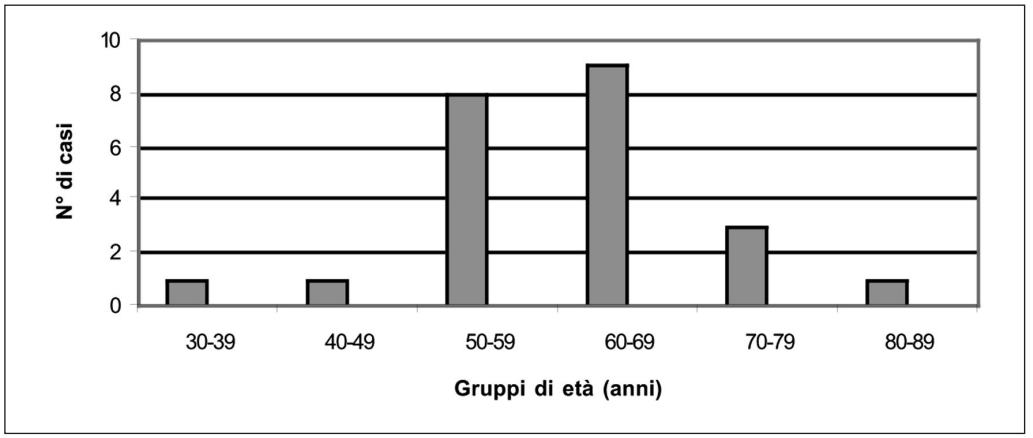

Fig. 1. Distribuzione per età in 23 casi

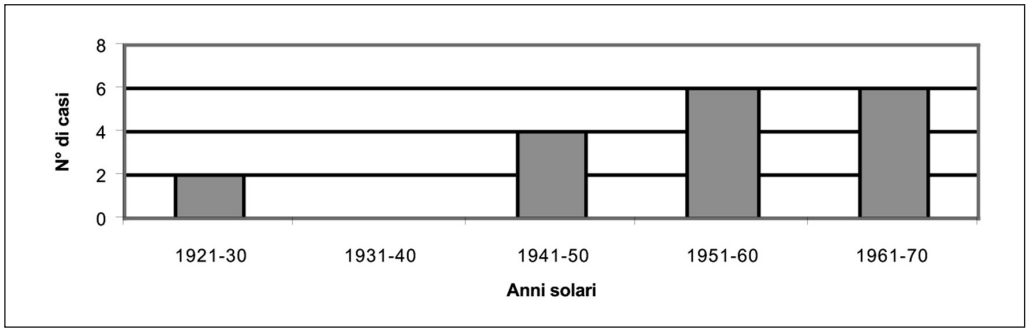

Fig. 2. Prima esposizione all'asbesto in 18 casi

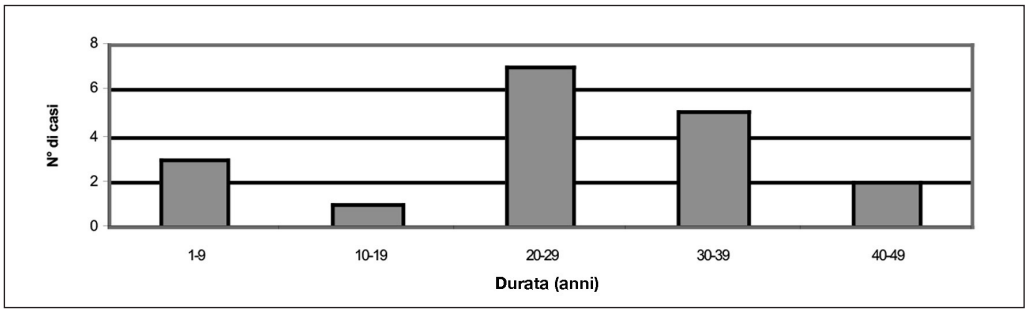

Fig. 3. Durata dell'esposizione all'asbesto in 18 casi

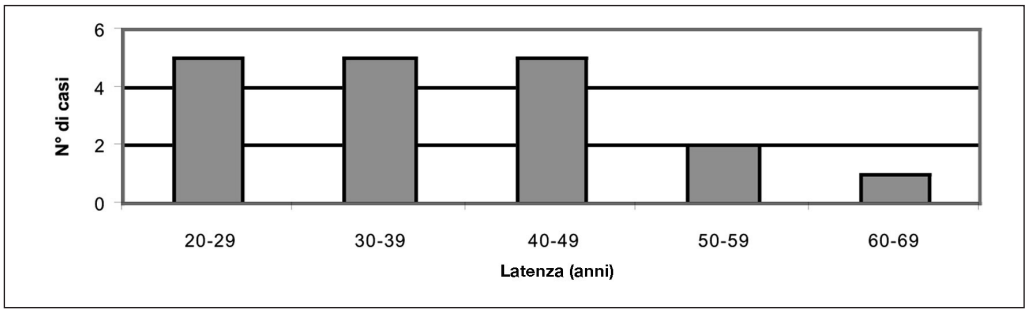

Fig. 4. Periodi di latenza in 18 casi

vece tranquillizzarono i lavoratori circa l'innocuità di quel lavoro. Nel far misconoscere la portata del rischio può aver giocato forse il fatto che le esposizioni in questione erano saltuarie. Tuttavia il carattere intermittente dell'esposizione era largamente compensato dall'elevata intensità.

Nelle casistiche di mesoteliomi pubblicate negli anni seguenti compaiono talora casi di mesotelioma in lavoratori portuali. Ad esempio un mesotelioma in un lavoratore del porto è citato in una serie di 38 mesoteliomi pleurici osservati nell'area di Marsiglia[4]. In una serie di 32 mesoteliomi pleurici osservati nel periodo 1976-1979 nell'area di Göteborg, Svezia, Märtensson *et al* menzionano un caso in un lavoratore portuale[5]. Nella serie di mesoteliomi osservati in Australia da Ferguson *et al*[6], figurano 19 casi.

Se ci si riferisce a dati più recenti, numerosi mesoteliomi in lavoratori portuali sono compresi in alcuni Registri Mesoteliomi. Così presso il Registro dei Mesoteliomi Australiano sono stati raccolti, nel periodo 1986-2001, 109 mesoteliomi in lavoratori portuali (comprendenti anche 13 casi con esposizioni miste)[7]. Nel Registro Mesoteliomi del Regno Unito sono stati registrati fino al 1999 266 decessi da mesotelioma in lavoratori portuali[3]. In Gran Bretagna l'analisi della mortalità da mesotelioma nelle varie categorie professionali ha mostrato un eccesso statisticamente significativo nella categoria "*stevedores, dockers*"[8]. Recentemente l'entità del rischio di mesotelioma connesso alle operazioni di carico-scarico è stata messa fortemente in risalto dai risultati di uno studio riguardante i portuali di Genova. Esaminando l'incidenza dei tumori in 2101 lavoratori, Puntoni *et al*[9] hanno osservato uno spiccato aumento di incidenza di mesotelioma pleurico (SIR=751; 95% CI = 302-1547).

Sul mancato riconoscimento del rischio in passato può aver influito il fatto che l'attività di lavoratore portuale non rappresenta per se stessa una certificazione automatica di esposizione all'amianto, come altre professioni. Non per tutti i porti è transitato amianto ed è quindi necessaria una ricostruzione storica delle condizioni del singolo porto. Nel caso del porto di Trieste sono disponibili dati sufficientemente dettagliati riguardanti il transito di amianto nel periodo 1960-1996. Non sono state finora raccolte notizie sulla quantità dei tipi di amianto in causa. È noto tuttavia che si trattava sia di crisotilo che di crocidolite. Uno studio di Biava *et al*[10] riporta che nel periodo 1971-1973 l'amianto giunto via mare a Trieste proveniva in prevalenza dal Sud Africa e da Cipro.

L'alto grado di polverosità che si raggiungeva nelle operazioni di carico-scarico, specialmente quando i sacchi si rompevano in ambienti chiusi come la stiva della nave o il vagone ferroviario, è stato descritto dettagliatamente dai lavoratori[1]. Ma la polverosità riguardava anche l'ambiente esterno. Un'indagine del Servizio di Medicina del Lavoro del Comune di Trieste, condotta nel novembre 1977, mostrava che le operazioni provocavano "sviluppo visibile di fibre di amianto nella zona di lavoro".

Le caratteristiche del mesotelioma rilevate nel presente gruppo di lavoratori portuali si differenziano da quelle osservate nei mesoteliomi dell'area di Trieste-Monfalcone in generale o da quelle osservate in altre categorie professionali[11]. I lavoratori del porto differiscono innanzitutto per distribuzione dell'età, che è spostata verso classi

più giovani (50-59, 60-69 anni). Parallelamente si nota anche una riduzione dei tempi di latenza, notevolmente più brevi che nelle altre categorie professionali, ad eccezione degli isolatori.

Benché la durata del periodo di latenza sia probabilmente condizionata da una serie di fattori, l'intensità dell'esposizione è certamente uno di questi. I periodi di latenza più brevi si osservano nelle categorie a più forte esposizione come gli isolatori e all'altro estremo i periodi più lunghi si riscontrano nei gruppi con esposizione poco intensa, come i marittimi[12-13]. Le latenze brevi dei lavoratori portuali sono quindi indicative, in linea di massima, di forti esposizioni, in armonia con quanto suggerito dalle descrizioni dell'ambiente di lavoro. I dati ottenuti dall'esame istologico delle sezioni di polmone confermano ulteriormente il fatto dell'esposizione intensa. La quasi totalità dei casi presentava corpi dell'asbesto nelle sezioni di *routine*, mentre la percentuale di reperti positivi in altre categorie è in genere minore[11].

In conclusione tutti gli elementi concordano nel far ritenere che i mesoteliomi dei lavoratori del porto di Trieste rappresentino l'effetto di esposizioni intense ad amianto. Il fatto che il trasporto sia continuato con mezzi convenzionali (sacchi di iuta e di carta) fino alla fine degli anni '70 del secolo scorso indica che le conseguenze di tale pratica si continueranno a verificare ancora per vari anni.

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Mioepitelioma della parotide: studio istopatologico ed ultrastrutturale di un caso

Myoepithelioma of the parotid gland: a histopathologic and ultrastructural study of one case

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Riassunto

***Finalità*. I mioepiteliomi, riconosciuti nel 1991 dall'Organizzazione Mondiale della Sanità come entità nosologica autonoma, sono tutt'ora motivo di interpretazioni contrastanti e di diagnosi differenziali delicate rispetto agli adenomi pleiomorfi. Una delle difficoltà è rappresentata dall'esistenza di aspetti strutturali e citologici eterogenei sul piano fenotipico, sebbene essi siano costituiti solo da elementi mioepiteliali. Per una diagnosi di certezza è indispensabile integrare l'istopatologia con l'immunoistochimica e la microscopia elettronica. La finalità di questo studio mira non solo a descrivere un caso di mioepitelioma, ma anche a mettere in evidenza la insostituibile validità dei risultati ultrastrutturali per una corretta diagnosi di tale neoplasia. *Pazienti e metodi*. Il reperto in esame si riferisce a un mioepitelioma della parotide sinistra in un uomo di 49 anni. Il campione chirurgico è stato trattato con metodiche di istologia e di microscopia elettronica a trasmissione. *Risultati*. I reperti ultrastrutturali hanno evidenziato in tutta la popolazione cellulare la presenza di strutture giunzionali, l'esistenza nel citoplasma di miofibrille e, lungo gli spazi intercellulari, di sottili strie riferibili a membrana basale. *Conclusioni*. I dati acquisiti mediante la microscopia elettronica documentano in modo diretto i caratteri mioepiteliali della popolazione cellulare. Pertanto l'Autore conclude sottolineando l'importanza di tale accertamento per lo studio e la diagnosi di tali neoplasie. Eur. J. Oncol., 10 (4), 291-296, 2005**

***Parole chiave:* mioepitelioma, miofibrille, desmosomi, membrana basale**

Summary

***Aim*. Recognized in 1991 by the World Health Organization as an independent nosological entity, myoepitheliomas are still the subject of conflicting interpretations and of difficult differential diagnoses as compared to pleomorphic adenomas. One of the difficulties is the existence of heterogeneous structural and cytological features from the point of view of phenotype, although they are made up only of myoepithelial elements. To obtain a sure diagnosis, the histopathological examination should be accompanied by an immunohistochemical examination and electron microscopy. The aim of this study was to describe a case of myoepithelioma, but also to underscore the importance of the ultrastructural tests for a correct diagnosis of this tumuor. *Patients and methods*. The study sample was taken from a myoepithelioma of the left parotid in a 49-year-old man. The surgical sample underwent histological examination and transmission electron microscopy. *Results*. The ultrastructural findings showed the presence of junctional structures in the whole cell population, the existence of myofibrilles in the cytoplasm and fine striations along the intercellular spaces relating to a basal lamina. *Conclusions*. The data acquired from electron microscopy clearly show the myoepithelial characteristics of the cell population. The author, therefore, underscores the importance of this examination for the study and diagnosis of this type of tumour. Eur. J. Oncol., 10 (4), 291-296, 2005**

***Key words:* myoepithelioma, myofibrilles, desmosomes, basal lamina**

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Introduzione

I mioepiteliomi sono tumori quasi sempre benigni, i quali sono costituiti da mioepiteli, sebbene possano avere sul piano fenotipico aspetti diversi[1].

Nel 1943 fu coniata l'etichetta di "mioepitelioma" delle ghiandole salivari quale riconoscimento di un istotipo neoplastico distinto dagli adenomi pleiomorfi[2].

Nel 1991, l'Organizzazione Mondiale della Sanità ha inserito nelle proprie classificazioni il mioepitelioma benigno e maligno quale entità neoplastica autonoma ed indipendente[3].

Intanto, il capitolo dedicato ai mioepiteliomi si è ampliato sia per una loro sempre maggiore incidenza, sia per la loro identificazione in organi diversi (cute, mammella, laringe, tessuti molli, etc), sia ancora per l'esistenza, accanto alle forme benigne, di forme con diversi gradi di aggressività biologica[4-7].

Probabilmente questa tardiva identificazione dei mioepiteliomi è conseguente alla loro eterogenea morfologia cellulare e stromale (forme solide, cistiche, ipervascolarizzate, laminari, trabecolari, compatte, a cellule fusate, plasmacitoidi, chiare)[3, 8, 9].

Questo polimorfismo con possibilità di manifestazioni intermedie è legato alle modalità proliferative delle cellule totipotenti progenitrici che hanno la capacità di differenziarsi sia in senso epiteliale sia in senso mioide[7].

Questa teoria istogenetica postula una derivazione globale e unitaria sia degli adenomi pleiomorfi sia dei mioepiteliomi[10]. Questi progressi diagnostici ed i suddetti approfondimenti istogenetici sono difficoltosi mediante la sola morfologia standard, mentre sono stati possibili mediante le metodiche di immunoistochimica e di microscopia elettronica.

Infatti le indagini di immunoistochimica hanno evidenziato la presenza nelle cellule mioepiteliali di un *pattern* antigenico non sovrapponibile a quello degli elementi epiteliali e a questa divaricazione del profilo antigenico è affidato il peso dell'identificazione delle neoplasie mioepiteliali, nonché delle complesse procedure di diagnosi differenziale. Purtroppo, un limite a tali possibilità è dato dall'esistenza della eterogeneità citologica, dai gradi di maturazione citoplasmatica, e dal rischio di processi di dedifferenziazione pre-maligna; questi vari livelli di maturazione cellulare possono essere causa di una espressività antigenica incerta o falsamente negativa[6].

Queste incertezze o erranze possono essere superate mediante le metodiche di microscopia elettronica; infatti, attraverso osservazioni ultrastrutturali, è possibile rilevare "in diretta" i caratteri citoplasmatici considerati specifici delle cellule mio-epiteliali (mio-filamenti, desmosomi, membrana basale pericellulare).

In particolare, il riscontro di miofilamenti, anche in quantità esigue, rappresenta il carattere specifico di tali popolazioni cellulari e ne consente la diagnosi anche nei casi in cui lo stato di differenziazione è quasi diffuso e/o molto basso.

Queste riflessioni hanno suggerito all'Autore di dare un contributo alla conoscenza del ruolo della microscopia elettronica nello studio e nella diagnostica dei mioepiteliomi delle ghiandole salivari attraverso la descrizione di un caso occorso alla sua attenzione.

Caso clinico

Il caso in esame riguarda un uomo di 49 anni, portatore di una neoformazione della parotide di sinistra; la neoformazione ha le dimensioni di 3x2cm, è delimitata da una sottile capsula, ha una forma ovalare ed evidenzia al taglio un'architettura plurinodulare. Essa ha un aspetto solido, colore bianco-lucente, consistenza molliccia.

Materiali e metodi

Il campione in esame è stato in buona parte processato per la microscopia elettronica e il rimanente è stato processato *in toto* per la microscopia luce.

I frammenti prelevati per la microscopia elettronica sono stati fissati in liquido di Karnowsky e, dopo opportuni lavaggi, sono stati post-fissati in soluzione acquosa di tetrossido di osmio al 2%. Dopo adeguata disidratazione sono stati inclusi in Epon. Da tutti i blocchetti così ottenuti sono state allestite sezioni semifini di 1 μm di spessore, che sono state colorate con blu di toluidina. Da tutti i blocchetti sono state allestite sezioni ultrasottili, che sono state contrastate con acetato di uranile e citrato di piombo.

La quota residua della neoplasia in esame è stata fissata, per la microscopia luce, in formalina tamponata al 10% e successivamente è stata inclusa in paraffina. Le sezioni allestite sono state colorate con i seguenti metodi: ematossilina-eosina (EE), ematossilina van Gieson, PAS, Alcian blu, tricromica di Mallory, impregnazione argentica di Gömori.

Esame istologico

Il campione in esame è stato sottoposto a tagli seriati. Esso è demarcato da una sottile capsula fibrosa che lo delimita da gruppi di acini ben strutturati di ghiandola salivare. La sua struttura d'insieme appare compatta e costituita da una componente cellulare e da una stromale. La prima è formata da aggregati solidi e da trabecole di cellule di media taglia tra loro coese; esse hanno forma ovoidale o fusata con discreta quota citoplasmatica e nucleo monocromatinico, isometrico ed isomorfo. Il citoplasma appare abitualmente acidofilo e compatto, tuttavia si ritrovano anche elementi con citoplasma vacuolizzato o chiaro.

La quota stromale è data da materiale addensato, compatto, tingibile con Alcian blu, positivo al PAS, di aspetto mucoide (fig. 1). Nel contesto di questo stroma si repertano rare ed isolate cellule con i caratteri simili alle precedenti. In nessun campo sono state ri-

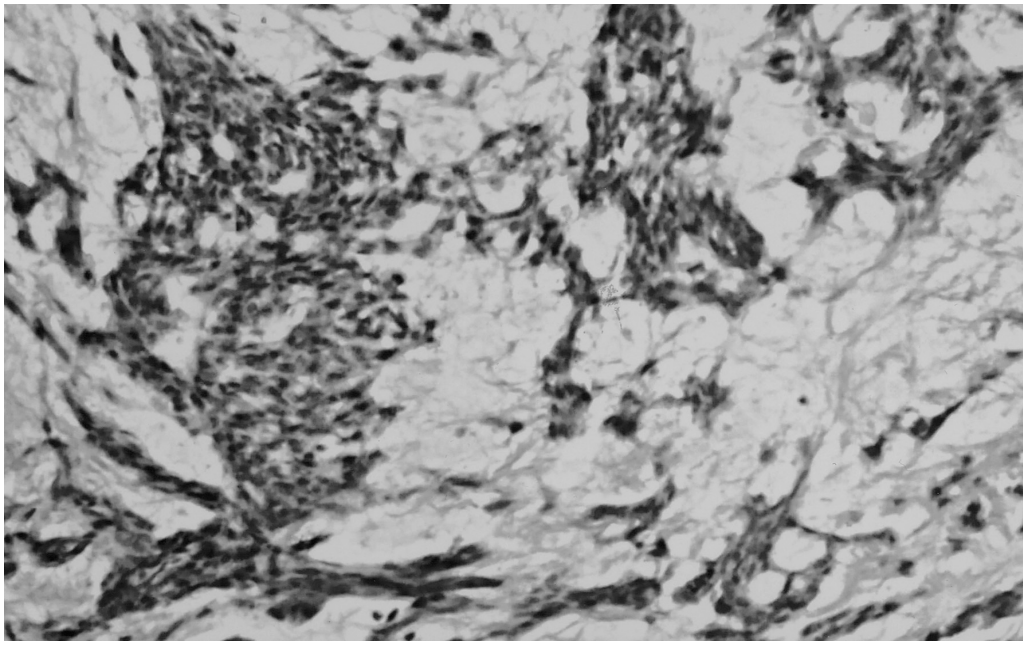

Fig. 1. Aggregati solidi, filiere e trabecole di cellule di media taglia tra loro coese; la quota stromale è fornita di materiale amorfo, con aspetto mucoide. Ematossilina-eosina (EE), 200x.

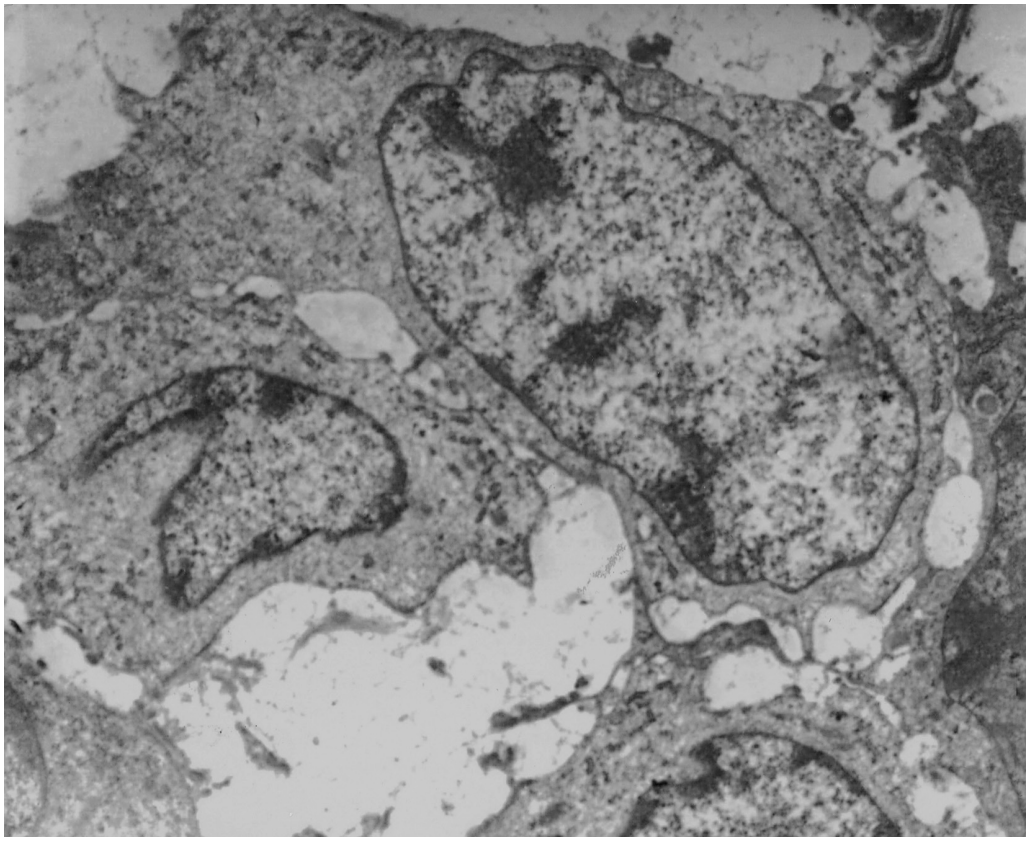

Fig. 2. Presenza di elementi cellulari fusati ed ovoidali, tra loro coesi, contenenti pochi organuli e sottili miofibrille. Microscopia elettronica a trasmissione (TEM), 4400x

levate strutture riferibili a dotti o duttuli ghiandolari; né sono stati repertati elementi in mitosi o con atipie nucleari.

Esame ultrastrutturale

I caratteri ultrastrutturali della popolazione cellulare presenti nella neoplasia in esame sono monomorfi e ripetitivi. Le cellule hanno un profilo abitualmente ovoidale o fusato, hanno un discreto alone citoplasmatico ben demarcato da una evidente membrana cellulare. Gli organuli citoplasmatici sono esigui e sono rappresentati da mitocondri e da corti segmenti di ergastoplasma; sono ben rappresentati sottili fasci di miofibrille disposte secondo l'asse maggiore delle cellule (figg. 2 e 3). I nuclei sono forniti di nucleolo evidente e mostrano la cromatina addensata a ridosso della membrana nucleare. La matrice citoplasmatica appare in alcuni elementi uniformemente e diffusamente compatta con presenza di

Fig. 3. Elementi mioepiteliali con citoplasma compatto contenente sottili miofibrille. Lo spazio intercellulare è demarcato da filiere di pseudovacuoli. TEM, 12000x

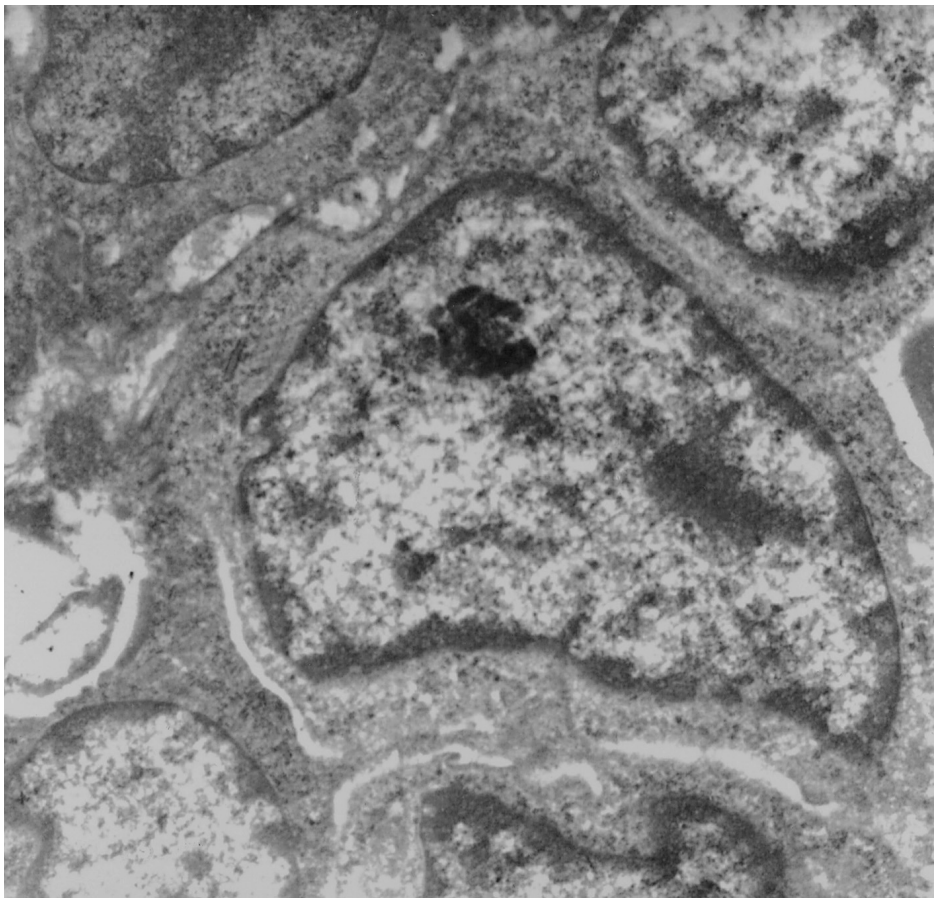

Fig. 4. Elementi mioepiteliali forniti di strutture desmosomiali. TEM, 8000x

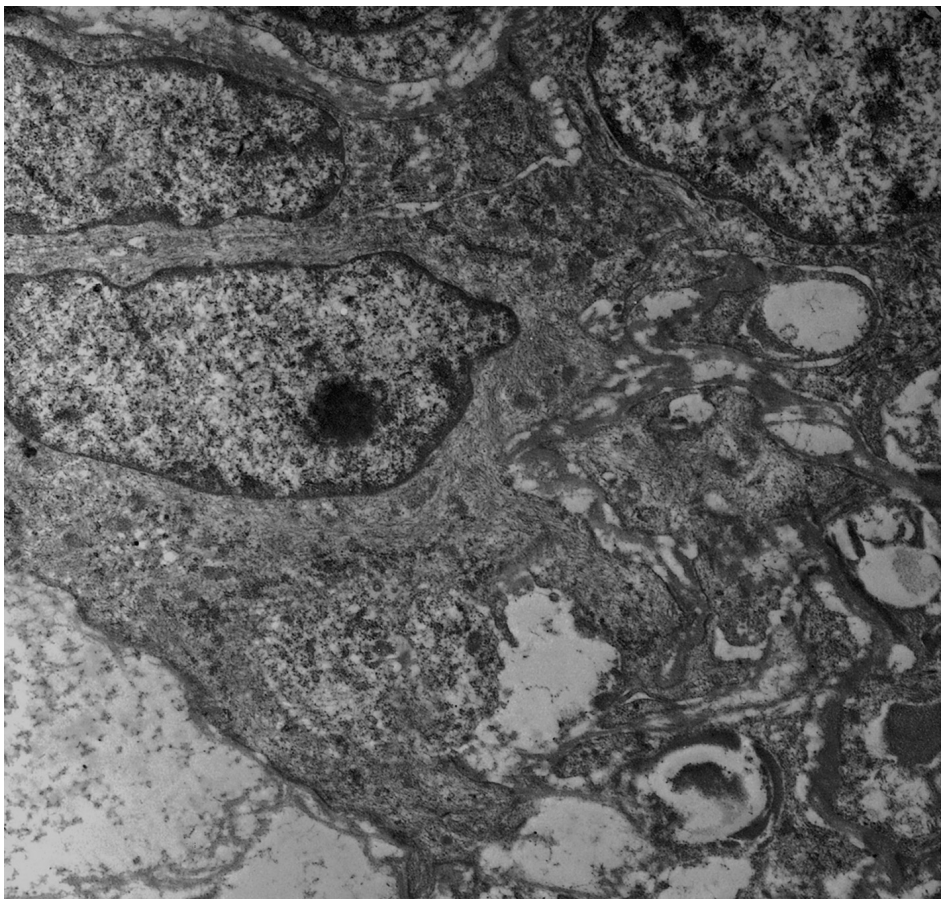

Fig. 5. Accanto a elementi mioepiteliali disposti a mutuo contatto sono presenti altri separati da una sottile membrana basale. TEM, 3000x

vacuoli distribuiti in filiera lungo gli spazi intercellulari (fig. 3). Non è raro repertare anche cellule sferoidali con citoplasma occupato da ampie lacune confluenti o trasformato in un unico ampio spazio privo di contenuto visibile agli elettroni.

I rapporti intercellulari sono variabili: oltre ad elementi isolati ed immersi in uno stroma amorfo, si ritrovano cellule raggruppate; alcune di esse sono coese mediante desmosomi, altre sono separate da una sottile quanto continua membrana basale (figg. 4 e 5).

Conclusioni

Il profilo morfologico dei mioepiteliomi può essere evidenziato e tracciato utilizzando in modo complementare ed integrato la microscopia luce e la microscopia elettronica. Mediante la prima è possibile fare l'inventario della popolazione cellulare esistente e stabilire i caratteri dello stroma, ricercando soprattutto gli aspetti differenziali nei confronti degli adenomi pleiomorfi.

Un ruolo decisivo e dirimente è affidato alla microscopia elettronica. Mediante questa strumentazione è possibile cogliere nei dettagli e nel modo più fine i caratteri subcitologici della popolazione cellulare, i rapporti intercellulari e la neosintesi di membrana basale.

Attraverso questa triade di reperti ultrastrutturali (miofilamenti intracitoplasmatici, apparato giunzionale, neosintesi di membrana basale) è possibile porre con certezza diagnosi, al di là delle eterogeneità delle manifestazioni fenotipiche, di mioepitelioma. Una loro valutazione semi-quantitativa contribuisce a fornire elementi per stabilire il grado di differenziazione e di conseguenza il loro grado di aggressività biologica.

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Eur. J. Oncol., vol. 10, n. 4, pp. 297-299, 2005

Special Award to Tushar K. Joshi
Premio Speciale a Tushar K. Joshi

Yasunosuke Suzuki, Tushar K. Joshi and Arthur L. Frank at the Tokyo conference

In the fall of 2004, participants from some 30 or more countries gathered in Tokyo, Japan to address the issue of a worldwide ban on asbestos and to share information regarding the science around asbestos disease. The Collegium Ramazzini has taken a clear stand regarding such a ban, and it was well represented at the meeting with several Fellows, from various countries in attendance; these included representation from Brazil, Sweden, the USA and India.

Collegium members made a significant number of presentations covering a wide variety of asbestos-related research issues ranging from pathology, the World Trade Center issues, research in China, and ban asbestos activities in India and Brazil. Two Collegium members were given major awards.

Fernanda Giannasi of Brazil was given a Japanese award, the Tajiri Muneaki Memorial Award, for her leadership in bringing the seriousness of asbestos hazards

in Brazil to the forefront, and for her efforts to limit exposure.

The Collegium Ramazzini decided to bestow a Special Award, in honour of Professor Irving J. Selikoff, to Dr. T.K. Joshi of India, also a Fellow. Dr. Joshi, almost single handedly, has begun high level professional training in occupational medicine for physicians in India, and in that country has led the campaign to discuss a ban on the use of asbestos. The Special Award was presented to him jointly by Dr. Yasunosuke Suzuki, himself a Ramazzini Award winner, and Dr. Arthur Frank, a member of the Executive Council of the Collegium and a frequent presenter on the topic of asbestos at Collegium meetings over the years. The full text of the Award was read, and the rôle of the Collegium in the area of asbestos disease was highlighted, and was clearly reflected in honouring Dr. Joshi in the name of Professor Selikoff and the Collegium. Doctors Suzuki and Frank had been professional colleagues of Professor Selikoff at Mount Sinai.

One highlight of the meeting was a session where Japanese family members who had lost loved ones to asbestos-related diseases presented their own special family stories. This, coupled with the high level of scientific presentations from around the world, made for an excellent conference, with many participants energized to continue to work for a worldwide ban on the use of asbestos.

Arthur L. Frank
Drexel University School of Public Health
Philadelphia, PA, USA

COLLEGIUM RAMAZZINI

The Collegium Ramazzini is proud to recognize the work of

Doctor

TUSHAR K. JOSHI

The Collegium Ramazzini presents this special award to Dr. T.K. Joshi in recognition of his heroic work in preventing the diseases caused by asbestos in India.

Dr. Joshi is a distinguished scientist and physician whose epidemiologic and clinical research has documented the enormous toll of disease, disability and death that asbestos has caused among Indian workers.

Dr. Joshi is also a courageous advocate who has translated the results of scientific research into prevention of disease. Despite having been assailed and reviled by the asbestos industry and its proponents, Dr. Joshi has persevered in his work of disease prevention and health promotion, and he has triumphed.

The Collegium Ramazzini takes enormous pleasure in recognizing the extraordinary contributions of Ramazzini Fellow, T.K. Joshi.

Tokyo, November 2004

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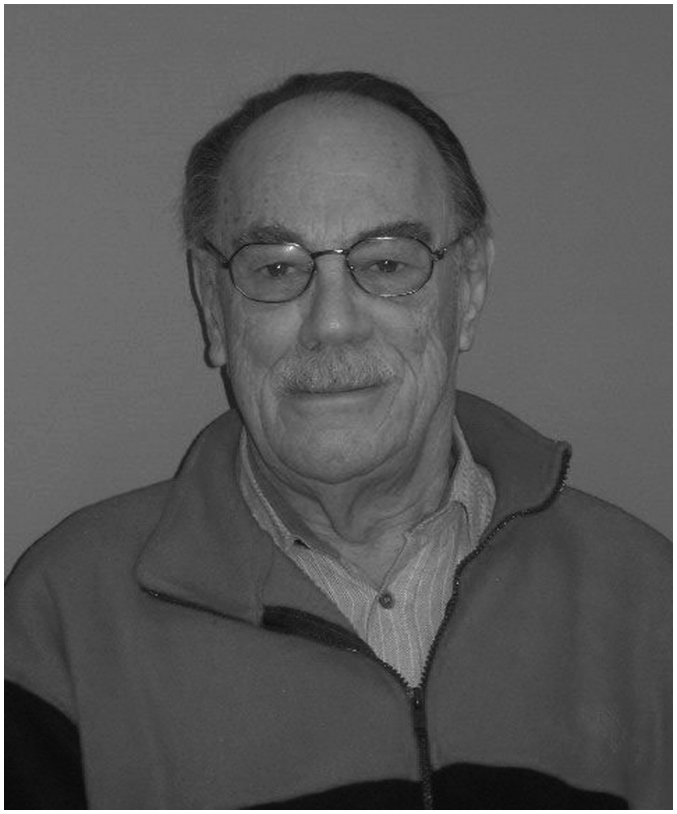
Professor Lars Ehrenberg
(1921-2005)



Lars Ehrenberg, a pioneer in risk assessment of genotoxic effects, died on 25th June, 2005 aged 84.

Lars Ehrenberg grew up in the town of Falun in the Swedish county of Dalecarlia. He received his first scientific education at Lund University in the south of Sweden. He graduated in genetics, botany and mathematics in 1943 and continued with studies in chemistry at Stockholm University, where, in 1955, he presented his doctoral thesis on the mechanism of action of ionising radiations in plant seeds. In 1962 Stockholm University created a personal Chair of Radiobiology for Lars Ehrenberg. He became instrumental in developing an Institute of Physical Biology funded by the Alice and Knut Wallenberg Foundation. From 1972 the Wallenberg Laboratory became the base for Lars Ehrenberg's research group.

Ehrenberg's broad scientific approach was established in his early studies in the fields of cytology, botany and biochemistry. Already during his studies in Lund, Ehrenberg came in contact with the group of plant breeders working with the geneticist Åke Gustafsson. Together with the plant breeders he introduced ionising radiation as a means of increasing hereditary variation in crop species. Different types of radiation were compared with regard to mutagenic effectiveness which, for instance, required that dosimetric methods were developed. Furthermore, action mechanisms were studied, and one of the achievements was the experimental demonstration (in collaboration with his brother, Anders Ehrenberg, and K.G. Zimmer) that free radicals are intermediates in the induction of biological radiation effects.

In 1954 mutagenic chemicals were introduced for the creation of genetic variability in the plant breeding work. Some alkylating agents were found to be far more efficient mutagens than ionising radiation. This finding led Ehrenberg to formulate a letter in 1959 with a warning about mutagens and carcinogens in the environment to the Swedish National Health Authorities. This was probably one of the first warnings of this kind. From then on he included the problem of risk assessment of environmental genotoxic agents in his research work.

The studies of biological effects of radiation and chemicals were carried out quantitatively, with definitions of concepts such as "mutagenic efficiency" and "mutagenic effectiveness", as well as a dose concept with regard to genotoxic chemicals, which formed a basis for the further work. The question about the true shape of the dose-response curve for mutation at very low doses was investigated in sensitive plant systems. In work aiming to find the properties that make certain alkylating agents to be highly efficient mutagens, it was shown that mutagenic effectiveness to a large extent followed reaction-kinetic laws and could be described by its reactivity towards certain centres (oxygens) in DNA.

The problems in cancer risk assessment for mutagens/carcinogens were gradually identified and could

then, in 1974, be formulated. His work continued to concentrate mainly on the development of improved methods for risk assessment for carcinogens which, to a large extent, was based on experience from radiation biology and radiation protection philosophy. One line in this research was Ehrenberg's suggestion to express the genotoxic potency of mutagenic chemicals, measured *in vitro*, as radiation-dose equivalents. This quantity could then be used in the developed model for cancer risk estimation, as an alternative to results from long-term animal tests.

Professor Ehrenberg realized that the reaction with blood proteins of reactive and short-lived genotoxic agents in humans and exposed animals offered possibilities to measure doses of genotoxic agents in blood. He suggested that such stable adducts, formed with haemoglobin, could be used to quantify exposure to genotoxic agents, as a basis for risk estimation. Along with the development of analytical methods, this approach has become an efficient tool in work aiming at cancer risk estimation, with a sensitivity exceeding that of epidemiological and animal studies by several orders of magnitude. The methods developed have also been applied for the detection of previously unknown exposure to carcinogens, shown to occur in the general population.

During the 1990s, Ehrenberg used his collective scientific experience for the evaluation of the applicability to chemically-induced cancer risk of the relative cancer risk model used for ionising radiation. This research, which is still ongoing, deeply engaged Ehrenberg, even when his former students had taken over the responsibility.

This summary of Ehrenberg's work is far from being complete; his unusual broad scientific knowledge and interest was reflected in his production of about 400 publications, ranging from mathematical-statistical and epidemiological papers to papers about, for instance, mechanisms of action of radioprotective agents. Furthermore, he was engaged as an expert by the Organization for Economic Co-operation and Development (OECD), the United Nations (UN), the Food and Agriculture Organization (FAO) and the World Health Organization (WHO), and worked in Yugoslavia, India and Bangladesh. He was honoured by several Swedish and international awards, among others a medal of the International Agency for Research on Cancer, and the Ramazzini Award in 1990. He was a selected member of the Swedish Royal Academy of Sciences and of the Collegium Ramazzini.

Lars Ehrenberg's pioneering work has received international recognition and given significant contributions. He was an unfailing source of knowledge to his students, and always showed interest in the work of junior scientists. Scientific problems were always the first priority for him.

As long as his health allowed, until the summer of 2004, he was at the laboratory every day, where he was appreciated for taking time for scientific discussions with younger colleagues and students, or just for telling some story, inspired above all from his six decades of experience in science. That was what he enjoyed very much, scientific discussion in small groups, much more than the larger and public contexts.

Through the years Lars Ehrenberg kept a keen interest in botany, and a walk together with him often became an exciting botanical excursion, with his impressive talent for identifying plant species. Lars Ehrenberg also had a great talent for languages. He had a good knowledge and appreciation for classical music, literature and art.

He was a genuine scholar.

He will be remembered as a charismatic scientist and person, and most fondly by the colleagues and students who experienced his generosity and good humour as a friend or mentor.

Maths Berlin
Professor Emeritus of Environmental Medicine, University of Lund, Sweden

Margareta Törnqvist
Assistant Professor,
Department of Environmental Chemistry, Stockholm University, Sweden

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Stile della bibliografia

Articolo in rivista, fino a 3 autori:

Sheibani K, Battifora H, Burke J. Antigenic phenotype of malignant mesotheliomas and pulmonary adenocarcinomas. Am J Pathol 1986; 123: 212-9.

Articolo in rivista, più di 3 Autori:

Fisher B, Costantino JP, Redmond CK, *et al*. Endometrial cancer in tamoxifen-treated breast cancer patients: findings from the National Sur-gical Adjuvant Breast and Bowel Project (NSABP) B-14. J Natl Cancer Inst 1994; 86: 527-37

Libro completo:

Selikoff IJ, Lee DHK. Asbestos and disease. New York: Academic Press, 1978.

Capitolo di libro:

Freedman AS, Nadler LM. Non-Hodgkin's lymphomas. In Holland JF, Breast RC J, Morton DL, *et al*. Cancer Medicine, IV Ed, 2. Baltimore: Williams and Wilkins, 1997, 2757-95.

Capitolo di libro che costituisce gli atti di un convegno:

Lipkin M. Current knowledge of the cancer latent period. Chemoprevention strategies during colonic cancer development. In Maltoni C, Soffritti M, Davis W. International Forum, The Scientific Bases of Cancer Chemoprevention, Amsterdam: Excerpta Medica, 1996, 61-71.

Abstract:

Abeloff MD, Gray R, Tarmey DC, *et al*. Randomized comparison of CMFPT versus CMFPT/VATHT and maintenance versus no maintenance tamoxifen in premenopausal, node positive breast cancer. An ECOG study. Proc Am Soc Clin Oncol 1991; 10, 43: abstr 47.

Supplemento:

Elison LO, Ekberg L. Ifosfamide, doxorubicin, vincristine, and etoposide in small cell lung cancer. Semin Oncol 1995; 22 suppl 2: 15-7.

Editoriale:

Morrow M. The natural history of ductal carcinoma in situ: implications for clinical decision making. Cancer 1995; 76: 1113-5 (editorial).

Lettera all'Editore:

Peat IM, Madden FJF. Neurological assessment of high grade astrocytomas following high dose radiotherapy as sole treatment. Clin Oncol 1995; 7: 273 (letter).

Resoconto scientifico o tecnico:

Akutsu T. Total heart replacement device - Bethesda (MD): National Institute of Health, National Heart and Lung Institute; 1974 Apr. Report No.: NIH-NHLI-69-2185-4

Articolo di giornale:

Rensberger B, Specter B. CFCs may be destroyed by natural process. The Washington Post 1989 Aug 7; Sect. A:2 (col. 5).

Tabelle. Le tabelle devono essere dattiloscritte o stampate, ciascuna in un foglio o più fogli separati, e vanno numerate progressivamente, in alto a sinistra, con numeri arabi (e come tali citate nel testo). Ogni tabella va illustrata con un titolo che deve comparire in testa a fianco del numero di identificazione. Eventuali legende, che devono comprendere anche la specificazione delle abbreviazioni, vanno posizionate ai piedi della tabella, e identificate con lettere minuscole. Le tabelle vanno inserite nel lavoro dopo il testo e la bibliografia.

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