JOURNAL OF AEROSOL MEDICINE
Volume 3, Supplement 1, 1990
Mary Ann Liebert, Inc., Publishers

Interspecies Comparison of Lung Clearance of "Insoluble" Particles

W.G. KREYLING

Gesellschaft für Strahlen-und Umweltforschung mbH München, Projekt Inhalation Ingolstädter Landstr. 1, 8042 Neuherberg, FRG

ABSTRACT

Lung clearance studies after the inhalation of monodisperse, radiolabelled test particles including lung retention measurements and excretion analysis allow for estimates of the kinetics of long-term particle transport out of the thorax into the gastro-intestinal tract. Data of several interspecies comparisons using either radiolabelled fused aluminosilicate particles or $^{57}Co_3O_4$ particles were reviewed and compared. Species included were: man, baboon, beagle dog, guinea pig, HMT rat, F-344 rat, Long-Evans rat, hamster, mouse.

Particle transport M(t) after the first days after inhalation is a slow clearance mechanism which is independent of the particle material and size used (0.5 - 4 μm geom. diameter). M(t) was reproducible in the experimental species studied. In man, baboon, and dog the initial daily fraction M_0 of the contemporary lung burden transported out of the thorax is 0.001 d^{-1} which is an order of magnitude less than the initial rates in rodents. Particle transport rate decreases rapidly from its initial value in all species studied. The decay of particle transport varies considerably between the species and strains. The half-life of the decreasing transport rate is slower in man, dog, F-344 rat, hamster and mouse (100 - 200 days) than in baboon, HMT rat and Long-Evans rat (< 50 days). From these studies estimates of lung retention during chronic aerosol exposure showed no equilibrium value indicating that long-term particle transport is not a sufficiently effective clearance mechanism to keep the lung burden from continuousy increasing during chronic exposure.

INTRODUCTION

Interspecies comparisons of lung clearance including human studies provide a potential tool to estimate the relevance of results obtained from experimental animal species with respect to their extrapolation to the human lung. In this paper data obtained from a few interspecies comparisons and studies on lung clearance of inhaled test aerosol par-

Key words: alveolar clearance, particle transport, interspecies comparison, monodisperse radiolabelled aerosol particles

ticles are reviewed with a special emphasis on the removal of intact particles from the lungs.

Aerosol particles deposited in the lungs are subject to two major clearance mechanisms out of the thorax depending on physical and chemical properties of the particles: mechanical transport M(t) of entire particles and/or translocation S(t) of dissolved material from the particle. Both clearance mechanisms are believed to be time dependent and competitive and independent (Cuddihy, 1984,1988). This differentiation of particle clearance from the lung parenchyma applies to man and a variety of experimental animal species (Bailey et al., 1989). The total rate of clearance $\lambda(t)$ is a fraction of the contemporary lung retention L(t):

$$\lambda(t) = - \frac{dL(t)/dt}{L(t)} \qquad \lambda(t) = M(t) + S(t) \qquad (1)$$

Soluble particles or soluble compounds of particles are mainly cleared by absorptive mechanisms consisting of transepithelial permeation and subsequent elimination via the blood. However, the dissociated material might bind to distinctive constituents of the various lung and cell fluids which will be translocated or retained in the lungs at time constants specific for these constituents. As a result, water soluble aerosol particles will disintegrate readily in the epithelial lining fluid but the material may or may not be cleared from the lungs.

It is generally recognized that particles which are not dissolved in the epithelial lining fluid, are phagocytized by alveolar macrophages within a few hours (Brain, 1985). Hence, long-term clearance from this region must involve these cells. Particles may be moved by macrophage mobility to the ciliated conducting airways to be cleared by mucociliary transport. Macrophage migration is effected by chemotactic and other biochemical factors, as was reviewed recently by Oberdörster (1988). Particles also will be transported across the epithelial barrier to be stored in interstitial tissue or to be carried on for storage in tracheobronchial and bifurcational lymph nodes (TBLN).

Moreover, particles which are negligibly soluble in water might be cleared from the lungs by translocation of dissolved particle material (Kreyling et al., 1986,1988, Bailey et al., 1989). Since the retained particles are incorporated in phagolysosomal vacuoles of alveolar macrophages on the epithelium almost all the time, the solvent is the aqueous vacuolar sol which also contains oxygen radical species, lysosomal, proteolytic and other enzymes, mediators, chelators and protons at a pH of about 5. Therefore, this solvent is different from extracellular lung fluids which eventually results in a more effective dissolution of various particle compounds in the lungs. Clearance of long-term retained particles out of the thorax by particle transport and translocation of dissolved particle material is shown schematically in Figure 1.

MECHANICAL PARTICLE TRANSPORT

The kinetics of mechanical particle transport from the alveolar region via the mucociliary escalator to the larynx cannot be determined directly. From lung retention measurements, total lung clearance is determined which is the sum of the clearance mechanisms from the alveolar region and from the tracheobronchial tree and the extrathoracic airways which might be superimposed even after extended times of retention (Gore et al., 1982; Stahlhofen et al. 1980). However, for this investigation data of fast clearance during the first few days after inhalation were excluded. Thereafter, it was assumed that

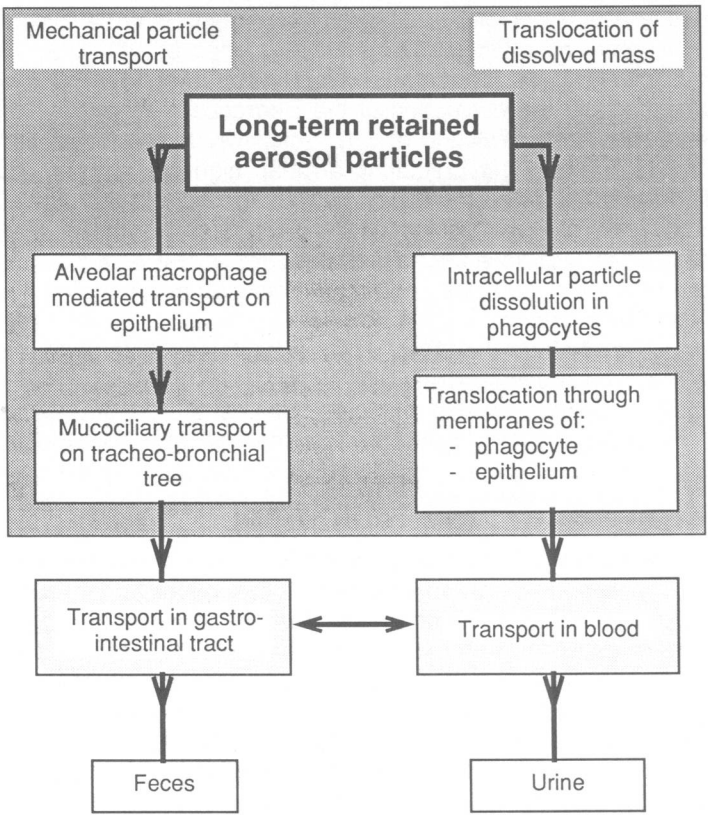

Figure 1. Lung clearance of long-term retained particles out of the thorax by particle transport and translocation of dissolved particle material.

long-term clearance from the tracheobronchial tree and the extrathoracic airways is a minor fraction of long-term clearance from the alveolar region.

According to equation 1, M(t) can be evaluated from lung retention measurements as long as S(t) is negligible, i.e. the test particles are negligibly soluble in the lungs over the entire time of observation. It is emphasized, however, that in vivo dissolution might be different from in vitro dissolution in simulant solvents (Lundborg et al., 1984; Kreyling et al., 1986,1988,1990b). Moreover, translocation from the lungs might vary between the species (Bailey et al., 1989). Although it is not necessarily required to study clearance of monodisperse test particles, their use is desirable to test the size dependence of particle transport. Additionally, particle dissolution and hence, translocation, becomes more complicated since particle dissolution extracellularly and intracellularly is particle size dependent (Mercer, 1967; Moss and Kanapilly, 1980; Kreyling et al., 1990a,1990b) resulting in erroneous estimates of particle transport. Therefore, this review is restricted to interspecies comparisons of lung clearance using monodisperse test particles.

In the past several investigators have used Fe_2O_3 test particles labelled with various radio-isotopes in human or experimental animal lung clearance studies (Albert et al., 1967, Morrow et al., 1967a,1967b; Bellmann et al., 1983,1986; Muhle et al., 1988). Translocation S(t) of dissociated Fe from these particles was generally difficult to determine due to the metabolism of Fe. However, long-term retention of dissociated Fe in the lungs and in other organs could not be ruled out. Hence, neither exclusive particle retention in the lungs nor estimates of S(t) were given excluding a proper estimation of M(t).

Using magnetic Fe_3O_4 particles, retention in the lungs was determined by magnetopneumography in man and experimental animals (Cohen et al., 1979; Oberdörster et al., 1984; Kalliomäki et al., 1985; Freedman et al., 1988). From these data total particle clearance was calculated but the evaluation of the clearance mechanism of particle transport M(t) would have required knowledge of S(t). Sensitivity of the magnetometer was another limitation of this approach, still requiring a rather high dose of retained magnetic particles. Since high lung burdens of test particles and/or apparently toxic particles might effect phagocytic and migratory functions of alveolar macrophages, particle transport might also be altered (McClellan et al., 1982,1986; Vostal et al., 1982). Therefore, the deposited dose of the test particles should be as low as possible and the material should not be specified to be cytotoxic.

A better means to estimate the kinetics of mechanical particle transport was the combination of lung retention measurements and excretion analysis after the inhalation of more or less insoluble, radiolabelled test particles (Snipes et al. 1983; Bailey et al. 1985a, 1985b, 1989, Kreyling et al., 1986+1988). This method makes use of the fact that particles which had been transported to the larynx are subsequently swallowed into the gastro-intestinal (GI) tract and are eventually excreted in feces. The amount of excreted particles in the feces and the amount of retained particles in the lungs were determined by radioactivity measurements from which the particle transport rate was determined. However, the analysis was complicated by the fact that a fraction of the particle material and/or the radiolabel might have been absorbed during passage through the GI-tract which might have been redistributed systemically and not excreted in feces. Another complication arose from the other clearance mechanism of translocation, i.e. a fraction of the particle material which was dissociated and translocated from the lungs to blood might have entered the GI-tract and was excreted in the feces.

Recently, the importance of translocation of dissolved particle material from the lungs to blood was clearly demonstrated for ^{57}Co-labelled fused aluminosilicate particles (^{57}Co-FAP) which were considered to be almost insoluble (Kreyling et al., 1988).
During a three year clearance study with beagle dogs not only lung retention measurements and excretion analysis was carried but also a chemical procedure was involved which separated ^{57}Co-FAP from non-particulate ^{57}Co in fecal samples. From these measurements and a supplementary study for the absorption of the radiolabel from FAP during passage of the GI-tract after gavage, particle transport M(t) was evaluated. The initial particle transport rate was 0.0006 d^{-1} and decreased monotonically with a rate of 0.004 d^{-1} (half-life 170 d). Since S(t) was 0.0005 d^{-1} and constant over the entire period it became the predominant clearance mechanism from the lungs after one year. The fraction of non-particulate ^{57}Co in fecal samples increased from an initially minor fraction to a similar amount as the ^{57}Co-FAP fraction, since 5% of the translocated non-particulate ^{57}Co circulating in blood was not excreted in urine but in feces.

Bailey et al. (1989) proposed a simple model (Figure 2) taking these metabolic effects into account from which particle transport was evaluated:

$$U(t) = b_u\, S(t) + g_u\, M(t) \qquad F(t) = g_f\, M(t) + b_f\, S(t) \qquad (2)$$

where U(t) and F(t) were the urinary and fecal excretion rates, i.e. the amounts of radioactivity excreted per day as fractions of the contemporary lung content L(t); b_u and b_f were the fractions of the radiolabel in urine and feces, respectively, following translocation of the radiolabel from the lungs to blood; and g_u and g_f were the fractions of the radiolabel excreted in urine and feces respectively, after particles had entered the GI tract. The transfer coefficients b_u, b_f, g_u, g_f were determined by supplementary excretion analyses after the radiolabel was injected intravenously or the test particles were ingested. Thus:

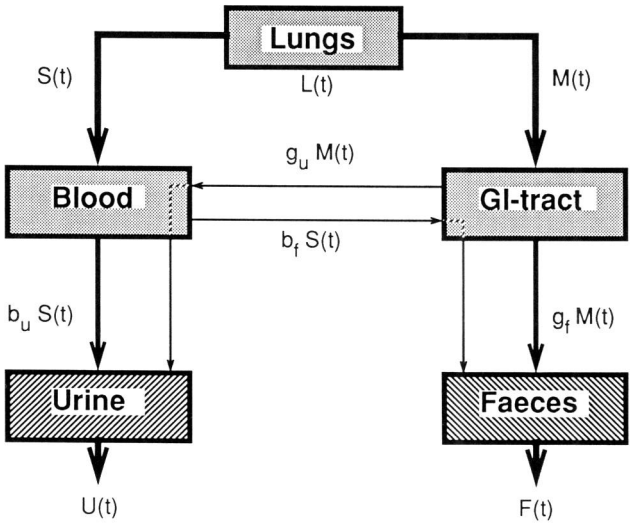

Figure 2. Transfer coefficients for the radiolabel following inhalation of test particles, showing the fractions of lung content L(t) cleared per day by mechanical particle transport M(t) and translocation of dissolved particle material S(t) at time t.

$$S(t) = \frac{g_f U(t) - g_u F(t)}{b_u g_f - b_f g_u} \qquad M(t) = \frac{b_u F(t) - b_f U(t)}{b_u g_f - b_f g_u} \qquad (3)$$

This model allowed systemic uptake of the radiolabel by other organs but it did not take into account clearance from these organs since it was assumed that rate constants for those organs would be negligibly small to effect the transfer coefficients b_u, b_f, g_u, g_f.

INTERSPECIES COMPARISON OF PARTICLE TRANSPORT

Data Base

There were a few interspecies comparisons of lung clearance which were based on this clearance model and the appropriate measurements. In these studies monodisperse test particles of two different materials were used: FAP labelled with various radiolabels and ^{57}Co labelled Co_3O_4 particles. Lung retention L(t) and both clearance mechanisms M(t) and S(t) of FAP were estimated in the following species:

man:	Bailey et al. 1985b
beagle dogs, Fischer-344 rats, CD-1 mice:	Snipes et al. (1983)
Hartley guinea pigs:	McClellan et al. (1984)
beagle dogs :	Kreyling et al., 1988
HMT rats, Syrian golden hamsters (DSN):	Bailey et al. 1985a

In a joint European attempt (Bailey et al., 1989, summary) organized by the European Late Effects Project Group (EULEP) of the Commission of the European Communities lung retention L(t) and both clearance mechanisms M(t) and S(t) of two different sizes of monodisperse, porous $^{57}Co_3O_4$ particles were evaluated and compared between:

man:	Foster et al., 1989, Pearman et al., 1990;
baboon:	André et al., 1989;
beagle dog:	Kreyling et al., 1989a;
Harwell guinea pig, HMT rat, Syrian golden hamster (DSN):	Collier et al., 1989;
Fischer-344 rat (SPF):	Patrick et al., 1989;
Sprague-Dawley rat:	Drosselmeyer et al., 1989;
mouse (CBA/H):	Talbot et al., 1989).

Particles from the same two batches were also used to determine clearance in Long-Evans rats. Additionally, lung clearance of monodisperse solid $^{57}Co_3O_4$ particles was investigated in baboons, beagle dogs and HMT rats (Kreyling et al.,1988,1989b; Collier et al., submitted). The solid $^{57}Co_3O_4$ particles were chosen to confirm that translocation S(t) in each species was proportional to the specific surface area of the particles. It also was chosen to prove reproducibility of particle transport M(t). Particle parameters are summarized in Table 1. Also incorporated in this interspecies comparison is another hu-

Table 1.

Parameters of monodisperse test particles and number N of subjects (animals) of each species for the interspecies comparisons of lung clearance.

Species / strains	fused aluminosilicate particles N	radiolabel	d_{geom} (µm)	$^{57}Co_3O_4$ particles N	density	d_{geom} (µm)
Man	13	^{88}Yt	4.0	4	porous	1.7, 0.8
	13	^{85}Sr	1.0			
Baboon				4	porous	1.7, 0.8
				2	solid	0.9
Beagle dog	2	^{57}Co	1.5	4,(8)*	porous	1.7, 0.8
	120	^{134}Sr	0.5, 1.0, 1.9	4	solid	1.6, 0.9
Guinea pig	49	^{134}Sr	1.3	24	porous	1.7, 0.8
HMT rat	30	^{85}Sr	1.2	60,(24)*	porous	1.7, 0.8
				20	solid	0.9
F-344 rat	320	^{134}Sr	0.5, 1.0, 1.9	44	porous	1.7, 0.8
Long-Evans rat				8	porous	1.7, 0.8
Syrian hamster	30	^{85}Sr	1.2	60	porous	1.7, 0.8
Mouse CD-1	320	^{134}Sr	0.5, 1.0, 1.9			
Mouse CBA/H				60	porous	0.8

* repetitive studies

man study using 4 μm monodisperse, ^{51}Cr labelled Teflon particles (Philipson et al., 1985).

Evaluation of Particle Transport Rate

Common to all species studied, long-term particle transport rate M(t) from the lungs to the GI-tract decreased drastically with time. In those studies where M(t) was not explicitly tabulated or given as a function, M(t) was calculated according to equation 1 from the given retention function L(t) and the given estimate of S(t). In all species M(t) was approximated by a sum of two exponential terms:

$$M(t) = M_{01} \exp(-m_1 t) + M_{02} \exp(-m_2 t) \qquad M_0 = M_{01} + M_{02} \qquad (4)$$

In Table 2 the values of the parameters of M(t) and the number of subjects/animals studied are given for each particle material and each species / strain. It is emphasized that the evaluation of a second term clearly depended on the parameters of the first term and the period of observation, i.e. the entire period must have been long enough that the first term was negligible for a sufficient time to determine the second term. It is notable, however, that particle transport in dogs followed a single exponential term for almost 1000 days if the most rigid analysis of fecal particle excretion was applied. The measurements of Snipes et al. (1983) confirmed the monotonic decay of particle transport. But the function of M(t) they gave in the paper was one exponential term and a final constant rate. This approximation was used for all data sets obtained from three species and 4 particle sizes. In Table 3 data of the transport rate M(t) are given at various times t during the first 400 days after inhalation which show the effectiveness of the clearance mechanism of particle transport in the various species at those times.

The initial transport rate M_0=M(0) was extrapolated from data obtained during the first weeks after inhalation of the test particles. Fast particle clearance was not taken into account in these estimations. As indicated above the latter was predominantly ascribed to particle removal from the tracheobronchial tree. However, there was no means to determine at what time and to which extent clearance of particles deposited on the tracheobronchial tree had diminished. Therefore, the extrapolated value of M(0) might have represented a superposition of both the vanishing clearance from the tracheobronchial tree and the initial clearance from the alveolar region.

Species differences

Both the initial transport rate and the decay of the transport rate varied considerably between the species. In the upper panel of Figure 3 M(t) in man, baboon and dog is shown for both FAP and Co_3O_4 particles. In the lower panel M(t) is shown in guinea pigs, three strains of rats, hamster and mice. Generally, in man and large animals the initial transport rate M_0 was in the range of 0.001 d^{-1} of the contemporary lung content. This was about an order of magnitude less than in mice, hamsters and the various strains of rats, where M_0 was $\geq$0.01 d^{-1}. It was quite astonishing that for each species of the rodents M_0 was very similar even though different strains had been studied. The latter was most striking in the three strains of rats studied. Interestingly, M_0 in guinea pigs was closer to man and large animals than to the other rodents. M_0 in man and baboon was very similar but in dogs it was even less according to the most rigid data evaluation for ^{57}Co-FAP.

The rate at which the particles reached the distal end of the mucociliary escalator of the tracheo-bronchial tree depended on the velocity of migration of the laden macrophages and the distance they had to travel. As was reviewed recently (Oberdörster, 1988) the velocity was effected by chemotactic and other biochemical factors eventually resulting in different velocities in different species. Interestingly, there were fewer airway

Table 2.

Parameters of the exponential terms of the particle transport rate M(t) according to equation 4 for man and various experimental animal species and for two different particle materials, FAP and Co_3O_4. Additionally, human data are given for Teflon particles. The period of observation is also given.

Species	Particle	Days of Observ.	M_{01}	m_1	M_{02}	m_2
Man	^{85}Sr-FAP/					
	^{88}Yt-FAP	400	.0031	.013	.00031	.0001
	$^{57}Co_3O_4$	700	.0018	.0059	.00040	.0016
	^{51}Cr-Teflon	300	.0090	.026	.00041	<.0001
Baboon	FAP		--	--		
	$^{57}Co_3O_4$	200	.0017	.013		
Dog	^{57}Co-FAP	1000	.0006	.0042		
	^{134}Cs-FAP	800	.005	.03	.0001	<.0001
	$^{57}Co_3O_4$	800	.0019	.067	.0004	.0039
Guinea pig	^{134}Cs-FAP	200	.005	.03	.0001	<.0001
	$^{57}Co_3O_4$	400		.0036	.0016	
HMT-rat	^{85}Sr-FAP	400	.019	.018	.0045	.0039
	$^{57}Co_3O_4$	400	.019	.028	.0066	.0056
F-344-rat	^{134}Cs-FAP	200	.020	.007	.001	<.0001
	$^{57}Co_3O_4$	200	.024	.009		
Long-Evans rat	FAP		--	--		
	$^{57}Co_3O_4$	200	.021	.019		
Hamster	^{85}Sr-FAP	500	.010	.014	.004	.0031
	$^{57}Co_3O_4$	400	.0022	.033	.0082	.0031
Mouse	^{134}Cs-FAP	200	.02	.006	.0015	<.0001
	$^{57}Co_3O_4$	300	.018	.0042		

generations in the zone of alveoli to respiratory bronchioli in rodents than in dogs, monkeys and man (Phalen et al., 1983), i.e. the distance from an alveolus to the terminal bronchiolus is shorter. This structural difference might have contributed to the larger initial transport rates M_0 and the subsequent, more effective transport in rodents compared to man and the large animals.

The decay of particle transport was even more variable between the various species than its initial value (Figure 3). In man and dog M(t) decreased in parallel but in baboons it vanished faster. In the latter species, the period of observation was to short to deter-

Table 3.

Data of the particle transport rate M(t) at various times t for man and various experimental animal species and for two different particle materials, FAP and Co_3O_4. Additionally, human data are given for Teflon particles. The period of observation is also given.

Species	Particle	Days of Observ.	M(0)	M(100)	M(200)	M(400)
Man	^{85}Sr-FAP/					
	^{88}Yt-FAP	400	.0034	.0012	.00053	.00031
	$^{57}Co_3O_4$	200	.0022	.0014	.00086	.00034
	^{51}Cr-Teflon	300	.0094	.0011	.00048	.00041
Baboon	FAP		--	--		
	$^{57}Co_3O_4$	200	.0017	.00046	.00013	
Dog	^{57}Co-FAP	1000	.0006	.00039	.00026	.00011
	^{134}Cs-FAP	800	.0051	.00035	.00011	.00010
	$^{57}Co_3O_4$	800	.0023	.00027	.00018	.00008
Guinea pig	^{134}Cs-FAP	200	.0051	.00035	.00011	
	$^{57}Co_3O_4$	400	.0036	.0031	.0026	.0019
HMT-rat	^{85}Sr-FAP	400	.024	.0062	.0026	.00096
	$^{57}Co_3O_4$	400	.026	.0049	.0022	.00070
F-344-rat	^{134}Cs-FAP	200	.021	.0109	.0059	.0022
	$^{57}Co_3O_4$	200	.024	.0098	.0040	
Long-Evans rat	FAP		--	--		
	$^{57}Co_3O_4$	200	.021	.0031	.00047	
Hamster	^{85}Sr-FAP	500	.014	.0054	.0028	.0012
	$^{57}Co_3O_4$	400	.010	.0061	.0044	.0024
Mouse	^{134}Cs-FAP	200	.022	.013	.0075	.0033
	$^{57}Co_3O_4$	300	.018	.012	.0078	

mine a second term of M(t) (equation 4) indicating a slower decay or a final, more constant transport rate. The longest studies including excretion analysis up to 700 days in man (Foster et al. 1989; Pearman et al., 1990) and 850 days in dogs (Kreyling et al. 1988) suggested that there was no final constant transport rate but M(t) continued to decrease.

In guinea pigs, different decays of M(t) were observed for the two particle materials of FAP and Co_3O_4. In the 250 days study, M(t) of FAP decreased parallel to that of dogs to a final constant rate of 0.0001 d^{-1}, while M(t) of Co_3O_4 remained almost constant above 0.001 d^{-1} throughout 360 days, resulting in a more efficient particle transport during time.

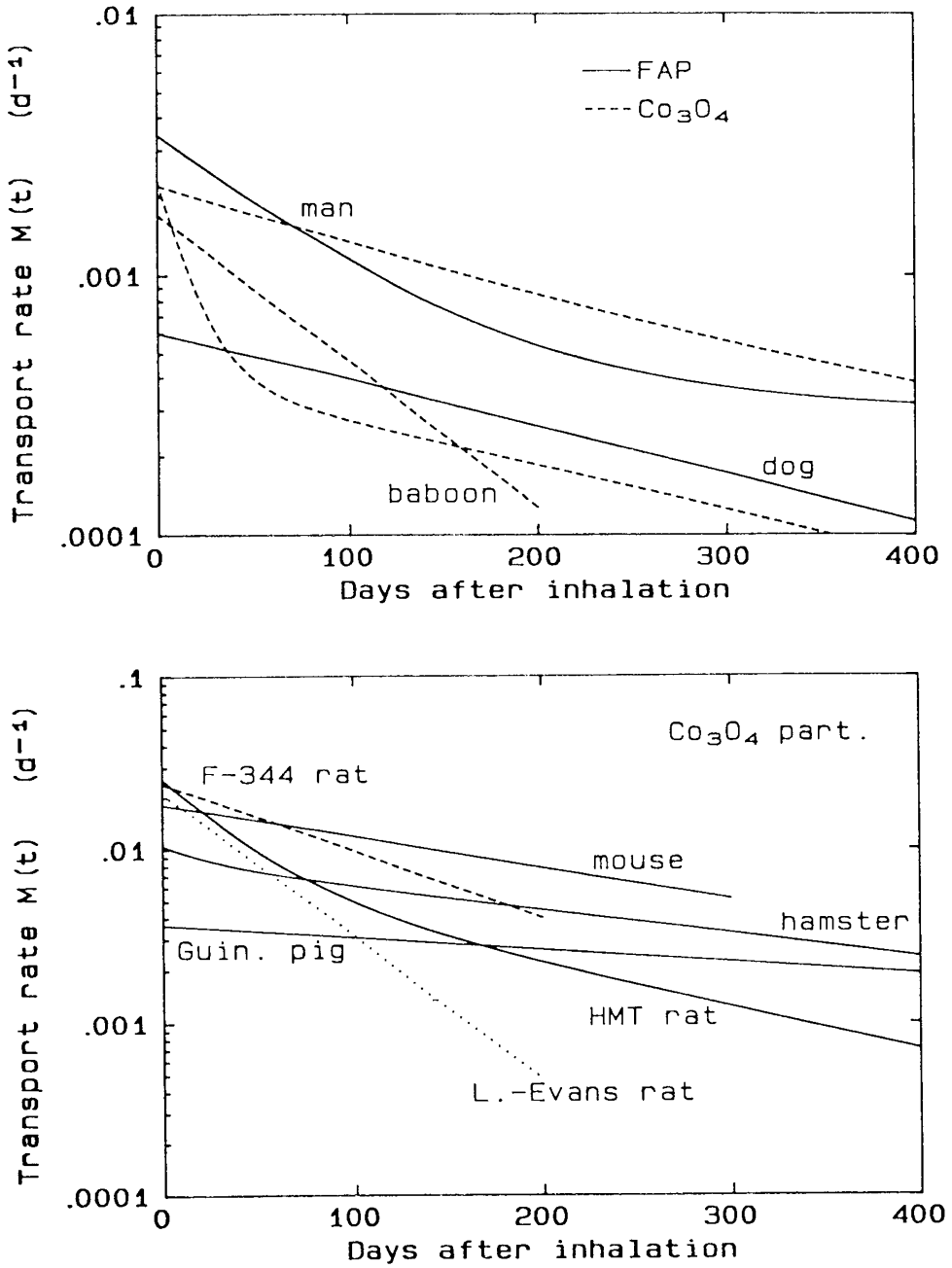

Figure 3. Particle transport M(t) of FAP and Co_3O_4 particles in man, baboon and dog (upper panel). Particle transport M(t) of Co_3O_4 particles in guinea pigs, three strains of rats (F-344 rat, HMT rat, Long-Evans rat), hamster and mice (lower panel).

Unfortunately in the FAP study no excretion data were given but only the function of M(t) which was derived from an entire lung clearance model (McClellan et al., 1984). The differences of M(t) observed in the two studies could reflect material dependence of particle transport but also differences between the two strains of guinea pigs.

Differences were observed between F-344 rats and the two other strains of rats independent of the particle material (Figure 3, lower panel). While M(t) diminished quickly in

HMT-rats and Long-Evans rats with a half-life of 40 days or less, the transport rate decreased more slowly in F-344 rats with 100 days half-life, i.e. particle transport in F-344 rats remained more effective than in the other two strains of rats. Unfortunately, M(t) was not determined in Sprague-Dawley rats, but lung retention decreased similar as in HMT rats and Long-Evans rats suggesting a similar, rapidly decreasing M(t) in these three strains. Hamsters and mice showed an even slower decrease of M(t) than F-344 rats (Figure 3, lower panel) with a half-life of 200 d and 140 d, respectively. Interestingly, no differences in the slow decrease of M(t) were found in the two strains of mice.

Particle transport to the hilar lymph nodes (TBLN) (Snipes et al., 1983; McClellan et al. 1984; Kreyling et al. 1986, 1988, 1989a; Harmsen et al., 1985) and observations of particles retained in the interstitium and subpleural spaces emphasized penetration of the particles through the epithelium. Significant fractions of particles had been found on the epithelium up to 500 days after inhalation by broncho-alveolar lavages (Kreyling et al. 1988, 1989a). From these findings, it is unclear how many of the particles remained on the epithelium. From those which penetrated through the membrane how many were carried to the TBLN? Also did particles penetrate reversibly through the epithelial barrier to appear again on the epithelium? In any event the decay of particle transport to the mucociliary escalator indicated an emptying pool of particles available for this transport mechanism which was macrophage mediated. Since the transport to TBLN was a minor and very slow mechanism, this means that an increasing fraction of particles was retained either on or beyond the epithelium in a state such that macrophages either could not phagocytize the particles or they phagocytized the particles but reached the mucociliary escalator increasingly more slowly.

Dependence on Particle Parameters

In each species except rats and guinea pigs, M(t) was independent of the two particle materials and the various particle sizes used, as shown in Table 3 by the data of M(t) at various time points. The three strains of rats showed clear differences in the decay of particle transport M(t). However, no material dependence of particle transport was found in F-344 rats and HMT rats in which both FAP or Co_3O_4 particles had been studied. In guinea pigs, different decays of M(t) were observed for the two particle materials of FAP and Co_3O_4 which might reflect differences in the two strains as discussed above. Interestingly, in man particle transport of another material, 4 μm ^{51}Cr labelled Teflon particles, was similar to those obtained for the other materials. Also no difference within a given species was found for porous and solid Co_3O_4 particles with a density of 2.5 - 3.5 and 6 g/cm3, respectively, (as long as M(t) was not too minute compared to S(t)). The particle size range varied in man from 1 to 4 μm geometric diameter (1.5 - 6 μm aerodynamic diameter) and in experimental animals from 0.5 to 2 μm (0.7 - 3.6 μm aerodynamic diameter). Administration of larger particles via inhalation would have been difficult since the particles would not have reached the peripheral lung due to their previous deposition in the upper airways.

Snipes et al. (1981,1984) found similar particle transport of 3 μm ^{141}Ce labelled polystyrene (PSL) particles in Fischer-344 rats and beagle dogs after administration via intratracheal instillation when compared to the inhalation data discussed here. However, in rats particle transport of 9 μm ^{85}Sr labelled PSL particles was much slower than that of the small PSL particles. Moreover, there was virtually no particle transport of 7.5 μm ^{85}Sr labelled PSL particles in dogs and no particle transport of 15 μm ^{46}Sc labelled PSL particles in either species during the entire four month period of observation. These results suggested that particle transport out of the peripheral lung of other species also might diminish for particles larger than 7-10 μm diameter. Since particle transport was macrophage mediated it is plausible to assume that the mobility of macrophages decreased with the increasing size of their load resulting in diminishing particle transport. The same ef-

fect of accumulated particle mass in macrophages had probably contributed to the vanishing test particle transport out of the lungs of various rodent species during chronic exposure of high concentrations of Diesel exhaust or other carbonaceous aerosols (Chan et al., 1984; Lee et al., 1987; Wolff et al., 1987; Muhle et al.; 1988, Bellmann et al., 1989).

Intersubject Variability and Reproducibility in Species

Intersubject variability of particle transport within a given species or strain was remarkably low for all rodents and dogs which were bred and maintained under controlled conditions of the various facilities. Moreover, the kinetics of particle transport in each of various species (F-344 rats, Syrian golden hamsters and the two strains of mice) were surprisingly reproducible in different investigations of different laboratories carried out more than three years apart from each other (Snipes et al., 1983; Collier et al., 1989; Talbot et al., 1989). Similarly, three studies on HMT rats at the National Radiological Protection Board, Chilton, UK, in 1985, 1986 and 1988 (Collier et al., 1989, submitted; Kreyling et al., 1989b) confirmed the invariance of the kinetics of particle transport within this strain. In an age-related investigation on the same species beginning at ages of 3, 13, 21 and 46 weeks Collier et al. (submitted) found no significant changes in particle transport M(t). As mentioned above the differences of M(t) found in guinea pigs for FAP and Co_3O_4 particles might be associated with the different strains.

Due to the predominant contribution of translocation S(t) to the clearance of porous Co_3O_4 particles in beagle dogs, M(t) could only be evaluated satisfactorily from studies using solid Co_3O_4 particles. Therefore, M(t) was obtained from only four animals, but intersubject variation was very low and matched excellently with the data for ^{57}Co-FAP for which the most rigid analysis was applied (Kreyling et al., 1988). These data are in good agreement with those obtained from 120 beagle dogs (Snipes et al., 1983). In baboons, wild-caught as young animals in West Africa, the intersubject variation of the kinetics of particle transport was slightly larger than in the other species discussed above. Yet, the mean pattern of the four animals studied in 1985 (André et al., 1989) was similar to those of the two animals studied in 1988 (Kreyling et al., 1989b). Largest intersubject variability was observed in man in each of the three studies (Bailey et al., 1985b; Philipson et al., 1985; Foster et al., 1989).

ESTIMATED LUNG CONTENT DURING CHRONIC EXPOSURE

Particle transport M(t) of the test particles was determined while the human volunteers and the experimental animals were continuously exposed to the ambient aerosol which contained a certain fraction of nearly insoluble particles. Hence, M(t) was determined during chronic exposure of the insoluble particle fraction of the ambient aerosol. Using the functions of M(t) in Table 2 obtained from the interspecies comparisons, the accumulated lung burden in each species was estimated during chronic exposure to an aerosol of constant concentration. It was assumed that (1) in each species a unit dose D_0 of particle mass was deposited per day and (2) this material was exclusively cleared by particle transport, i.e. translocation S(t) = 0 during the entire period in all species. According to equation 1, the rate of change of retention becomes the result of the daily intake D_0 and clearance M(t) of the lung retention L(t):

$$\frac{dL(t)}{dt} = \{ D_0 - M(t) \} \; L(t) \qquad (5)$$

If $M(t) = M_0$ is constant, lung retention L(t) is:

$$L(t) = \frac{D_0}{M_0} \{1 - \exp(-M_0 t)\} \quad (6)$$

where $L(\infty) = D_0/M_0$ is the limiting value to which lung retention approximated during time. This value in units of the deposited dose D_0 and the time t_{95} at which L(t) reached 95% of $L(\infty)$ are given in Table 4 for each of the species. The equilibrium values for man, baboon, dog and guinea pig were up to an order of magnitude larger than those of rodents. Similarly, in the same species the time interval was much longer to reach 95% the of the equilibrium value.

Table 4.

The approximate limit $L(\infty)$ of lung retention during chronic aerosol exposure when $M(t) = M_0$ is constant according to equation 6. $L(\infty)$ is given in units of the daily deposited dose D_0; t_{95} is the time when lung retention L(t) has reached 95% of the value of $L(\infty)$.

Species / strain	$L(\infty)$	t_{95} (days)
man	290	860
baboon	590	1740
dog	440	1280
guinea pig	280	810
HMT rat	40	100
F-344 rat	36	90
Long-Evans rat	48	120
Hamster	71	190
Mouse	47	120

Assuming M(t) was a function of time as given in Table 2, equation 5 was numerically solved and the accumulating lung retention L(t) in units of the daily deposited dose D_0 is shown in Figure 4 for the various species. No equilibrium value L(t) was reached in any of the species since M(t) was a decreasing function with time although the increase was very minute in F-344 rats and mice. Since particle transport was less effective in man, baboon and dog, the accumulative retained dose was much higher than in rodents. The smaller the values of M(t) were, the higher was the accumulated, retained lung burden. Additionally, the accumulated retained dose remained lower in those species or strains in which M(t) decayed more slowly with time such as F-344 rats and mice compared to HMT rats and Long-Evans rats.

Figure 4 shows that after 1000 days of exposure about 70% of the deposited dose has been retained in the human lungs while only 30% has been removed by particle trans-

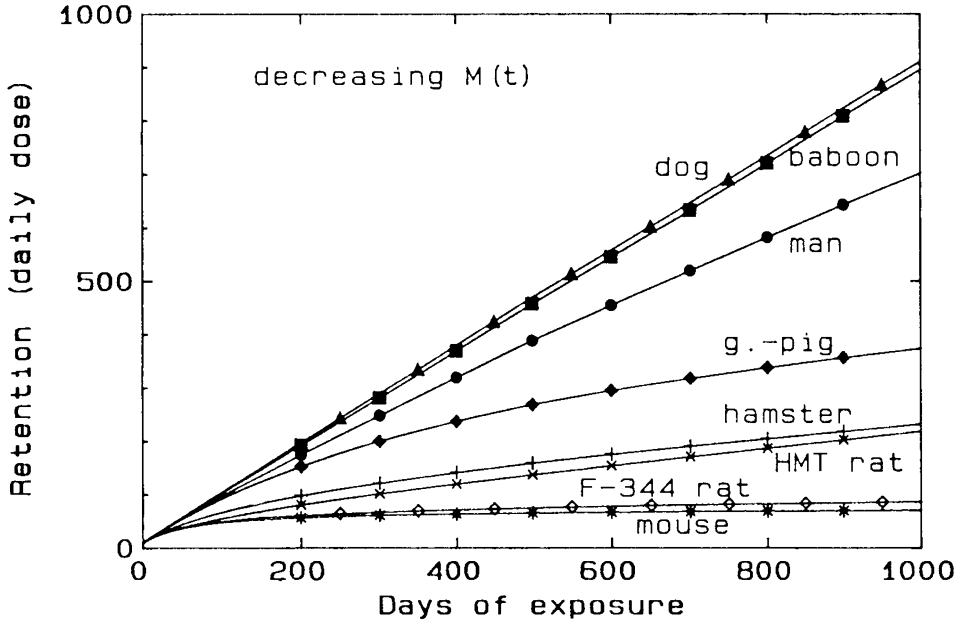

Figure 4. Estimates of the accumulated lung retention L(t) during chronic aerosol exposure to a daily deposited unity dose D_0.

port, whereas in F-344 rats and mice the accumulated retention would only account for about 10% of the deposited dose during a life time exposure. Applying this result to the chronic exposure of the ambient aerosol, it represents an estimated upper limit since particles are not completely insoluble and hence the translocation of dissolved material contributes to the total clearance of the particles from the lungs. The same is valid for other aerosols, for instance in specific occupational environments.

No limitations on the daily deposited dose were made in these calculations. It is well known if the deposited dose increases, recruitment of alveolar macrophages onto the epithelium also increases (Brain, 1985), eventually resulting in a more effective particle transport. However, the chronic exposure studies under "overload" conditions clearly indicate that beyond a certain administered dose, particle clearance even diminishes (Chan et al., 1984; Lee et al., 1987; Wolff et al., 1987; Muhle et al., 1988; Bellmann et al., 1989). Taking this and the less effective particle transport in man and large animal species into account, "overload" phenomena might occur at even lower concentrations during chronic aerosol exposure than was observed in rodents.

CONCLUSION

Particle transport from the alveolar epithelium to the beginning of the mucociliary escalator of the tracheo-bronchial tree was a slow, macrophage mediated clearance mechanism which decreased rapidly in all species studied. Both the initial transport rate M(0) and the kinetics of M(t) varied considerably between the species. As a result particle transport in man and large animal species was less effective by an order of magnitude than in rodents.

Particle transport was material independent in all species as long as the material was not cytotoxic to alveolar macrophages. It was also independent of the particle size within the range of 0.5 - 5 μm geometric diameter. Particle transport diminished for particles larger than 7 - 10 μm in rodents; but it is unknown in any species for fine and ultrafine

particles. Particle transport in rodents vanished during chronic aerosol exposure at high concentrations. The latter is unknown for man but it is plausible to assume a similar behavior. The estimated lung retention during chronic exposure which accumulated faster in man than in rodents suggested that "overloading" and decreasing particle transport might occur at even lower concentrations during chronic aerosol exposure than observed in rodents.

REFERENCES

ALBERT, R.E., LIPPMANN, M., SPIEGELMAN, J., STREHLOW, C., BRISCOE, W., WOLFSON, P. and NELSON, N. (1967) The clearance of radioactive particles from the human lung. Inhaled Particles and Vapours II (edited by C.N. Davies) Pergamon Press, Oxford, p. 361-378.

ANDRE, S, METIVIER, H. and MASSE R. (1989) An Interspecies Comparison of the Lung Clearance of Inhaled Monodisperse Cobalt Oxide Particles - Part III: Lung clearance of inhaled cobalt oxide particles in baboons. *J. Aerosol Sci.* 20, 205-218.

BAILEY, M.R., HODGSON, A.and SMITH, H. (1985a) Respiratory retention of relatively insoluble particles in rodents. *J. Aerosol Sci.* 16, 279-293.

BAILEY, M.R., FRY, F. and JAMES, A.C. (1985b) Long-term retention of particles in the human respiratory tract. *J. Aerosol Sci.* 16, 295-305.

BAILEY, M.R., KREYLING, W.G., ANDRE, S. BATCHELOR, A. BLACK, A. COLLIER, C.G. DROSSELMEYER, E. FERRON, G.A., FOSTER, P. HAIDER, B., HODGSON, A., METIVIER, H. MOORES, S.R. MORGAN, A. MÜLLER, H.L. PATRICK, G. PEARMAN, I. PICKERING, S. RAMSDEN, D. STIRLING and C. TALBOT, R.J. (1989) An Interspecies Comparison of the Lung Clearance of Inhaled Monodisperse Cobalt Oxide Particles - Part I: Objectives and Summary of Results. *J. Aerosol Sci.* 20, 169-188.

BELLMANN, B., MUHLE, H. and HEINRICH, U. (1983) Lung clearance after long time exposure of rats to airborne pollutants. *J. Aerosol Sci.* 14, 194-196.

BELLMANN, B., MUHLE, H. and CREUTZENBERG, O. (1986) The effect of a "nuisance" dust inhalation on lung clearance. *Aerosols: Formation and Reactivity* (edited by W. Schikarski, H.J. Fissan, S. Friedlander) Pergamon Press, Oxford; p. 209-211.

BELLMANN, B., MUHLE, H., CREUTZENBERG, O., KILPPER, R., MORROW, P.E. and MERMELSTEIN, R. (1989) Reversibility of clearance impairment after subchronic test toner inhalation. *Exp. Path.* 37, 234-238.

BRAIN, J.D. (1985) Macrophages in the respiratory tract. *Handbook of Physiology - The Respiratory System I, Chapter 14.* (Edited by Fishman, A.P. and Fisher, A.B.), pp. 447-471. American Physiological Society, Bethesda.

CHAN, T.L., LEE, P.S. and HERING, W.E. (1984) Pulmonary retention of inhaled Diesel particles after prolonged exposures to Diesel exhaust. *Fund. Appl. Toxicol.* 4, 624-631.

COHEN, D, ARAI, S.F. and BRAIN, J.D. (1979) Smoking impairs long-term dust clearance from the lung. *Sci.* 204, 514-517.

COLLIER, C.G., BAILEY, M.R. and HODGSON, A. (1989) An Interspecies Comparison

of the Lung Clearance of Inhaled Monodisperse Cobalt Oxide Particles - Part V: Lung clearance of inhaled cobalt oxide particles in hamsters, rats and guinea pigs. *J. Aerosol Sci.* 20, 233-248.

COLLIER, C.G., HODGSON, A., GRAY, S., MOODY, J. and BALL, A. The lung kinetics of $^{57}Co_3O_4$ in rats of various ages. *J. Aerosol Sci.* (submitted).

CUDDIHY, R.G. (1984) Mathematical model for predicting clearance of inhaled radioactive materials. In: *Lung modeling for inhalation of radioactive materials.* (edited by H. Smith, G. Gerber) EUR 9384 EN. Brussels: Commission of the European Communities; 167-180.

CUDDIHY, R.G. and YEH, H.C. (1988) Respiratory tract clearance of particles and substances dissociated from particles. *Inhalation Toxicology.* (edited by U. Mohr) Springer, New York, p. 169-193.

DROSSELMEYER, E, MÜLLER, H.-L., and PICKERING, S. (1989) An Interspecies Comparison of the Lung Clearance of Inhaled Monodisperse Cobalt Oxide Particles - Part VII: Lung clearance of inhaled cobalt oxide particles in Sprague-Dawley rats. *J. Aerosol Sci.* 20, 257-260.

FOSTER, P.P., PEARMAN, I. and RAMSDEN, D. (1989) An Interspecies Comparison of the Lung Clearance of Inhaled Monodisperse Cobalt Oxide Particles - Part II: Lung clearance of inhaled cobalt oxide particles in man. *J. Aerosol Sci.* 20, 289-204.

FREEDMAN, P.A., ROBINSON, S.E. and STREET, M.R. (1988) Magnetopneumographic study of human alveolar clearance in health and disease. *Ann. Occup. Hyg. 32 (Inhaled Particles VI)*, p.809-820.

HARMSEN, A.G., MUGGENBURG, B.A., SNIPES, M.B. and BICE, D.E. (1985) The role of macrophages in particle translocation from lungs of lymph nodes. *Sci.* 230, 1277-1280.

GORE, D.J. and PATRICK, G. (1982) A quantitative study of the penetration of insoluble particles into the tissue of conducting airways. *Ann. Occup. Hyg.* 26, 149-159.

KALLIOMÄKI, P.L., KALLIOMÄKI, K., RAHKONEN, E.and JUNTILLA, M.L. (1985) Magnetopneumography - lung retention and clearance of manual metal arc welding fumes based on experimental and human data. *Proceedings of the Fifth World Conference on Biomagnetism* (edited by H. Weinberg, G. Stroink, T. Katila) Pergamon Press, New York, p. 416-421.

KREYLING W.G., FERRON G.A. and HAIDER B. (1986a) Metabolic fate of inhaled Co aerosols in beagle dogs. *Hlth. Phys.* 51, 773-795.

KREYLING, W.G., FERRON, G.A., GODLESKI, J.J., HAIDER, B.and KARIYA, S.T. (1986b) The dissolution of monodisperse, porous cobaltosic oxide particles in the dog's lungs and in its alveolar macrophages. *Aerosols: Formation and Reactivity* (edited by W. Schikarski, H.J. Fissan, S. Friedlander) Pergamon Press, Oxford; 232-236.

KREYLING, W.G., SCHUMANN, G., ORTMAIER, A., FERRON, G.A., and KARG E. (1988) Particle transport from the lower respiratory tract. *J. Aerosol Medicine* 1, 351-370.

KREYLING, W.G., FERRON G.A. and HAIDER B. (1989a) An Interspecies Comparison of the Lung Clearance of Inhaled Monodisperse Cobalt Oxide Particles - Part IV: Lung clearance of inhaled cobalt oxide particles in beagle dogs. *J. Aerosol Sci.* 20, 219-232.

KREYLING, W.G., ANDRE, S., COLLIER, C.G., FERRON, G.A., METIVIER, H. and SCHUMANN, G. (1989b) Interspecies comparison of lung clearance after inhalation of monodisperse, solid cobalt oxide aerosol particles. (A full paper has been submitted to J. Aerosol Sci.) *J. Aerosol Sci.* 20, 1317-1320.

KREYLING, W.G., GODLESKI, J.J., KARIYA, S.T., ROSE, R.M. and BRAIN, J.D. (1990a) In vitro dissolution of uniform cobalt oxide particles by human and canine alveolar macrophages. *Am. J. Resp. Cell & Molec. Biol.* 2, 413-422.

KREYLING, W.G. (1990b) Aerosol particle parameters maintaining lung clearance by intracellular dissolution and translocation. *J. Aerosol Sci.* 21, 371-374.

LEE,P.S., GORSKI, R.A., HERING W.E. and CHAN, T.L. (1987) Lung clearance of inhaled particles after exposure to carbon black generated from a resuspension system. *Environ. Res.* 43, 364-373.

LUNDBORG, M., LIND, B. and CAMNER, P. (1984) Ability of rabbit alveolar macrophages to dissolve metals. *Exp. Lung Res.* 7: 11-22.

McCLELLAN, R.O., BROOKS, A. L., CUDDIHY, R.G., JONES, R.K., MAUDERLY, J.L. and WOLFF, R.K. (1982) Inhalation toxicology of Diesel exhaust particles. *Toxicological Effects of Emissions from Diesel Engines.* (edited by J.-E. Lewtas) Elsevier, New York, p. 99-120.

McCLELLAN, R.O., SNIPES, M.B. and BOECKER, B.B. (1984) Respiratory tract clearance of inhaled particles in laboratory animals. In: *Aerosols: Science, Technology and Industrial Applications of Airborne Particles* (edited by B.Y.H. Liu, D.Y.H. Pui, H.J. Fissan), Elsevier, New York (1984) 1047-1051.

McCLELLAN, R.O. (1986) Health effects of Diesel exhaust: A case study in risk assessment. *Am. Ind. Hyg. Assoc. J.* 47, 1-13.

MERCER, T.T. (1967) On the role of particle size in the dissolution of lung burdens. *Hlth. Phys.* 13, 1211-1224.

MOSS, O.R. and KANAPILLY, G.M. (1980) Dissolution of inhaled aerosols. *Generation of Aerosols and Facilities for Exposure Experiments.* (edited by K. Willeke), Ann Arbor Science Publishers Inc., Ann Arbor, p. 105-124.

MORROW, P.E., GIBB, F.R., and GAZIOGLU.K. (1967a) The clearance of dust from the lower respiratory tract of man. An experimental study. *Inhaled Particles and Vapours II* (edited by C.N. Davies) Pergamon Press, Oxford, p.351-359.

MORROW, P.E., GIBB, F.R., and GAZIOGLU.K. (1967b) A study of particulate clearance from the human lungs. *Am. Rev. Resp. Dis.* 96, 1209-1221.

MUHLE, H., BELLMANN, B. and HEINRICH, U. (1988) Overloading of lung clearance during chronic exposure of experimental animals to particles. *Ann. Occup. Hyg. 32, (Inhaled Particles VI)* 141-147.

OBERDÖRSTER, G., GREEN, F.H.Y. and FREEDMAN A.P. (1984) Clearance of $^{59}Fe_3O_4$ particle from the lungs of rats during exposure to coal mine dust and Diesel exhaust. *J. Aerosol Sci.* 15, 235-237.

OBERDÖRSTER, G. (1988) Lung clearance of inhaled insoluble and soluble particles. *J. Aerosol Med.* 1, 289-330.

PATRICK, G., BATCHELOR, A.L. and STIRLING, S. (1989) An Interspecies Comparison of the Lung Clearance of Inhaled Monodisperse Cobalt Oxide Particles - Part VI: Lung clearance of inhaled cobalt oxide particles in SPF Fischer rats. *J. Aerosol Sci.* 20, 249-256.

PEARMAN, I., FOSTER, P.P., RAMSDEN, D. and BAINS, M.E.D. (1990) Lung clearance of inhaled cobalt oxide in man: radiation protection - theory and practice. *Proceedings of the Fourth International Symposium of the SRP* (edited by E.P. Goldfinch) IOP Publishing, Bristol & New York, p. 251-254.

PHALEN, R.F. and OLDHAM, M.J. (1983) Airway structures: tracheobronchial airway structure as revealed by casting techniques. *Am. Rev. Resp. Dis.* 128, S1-S4.

PHILIPSON, K., FALK, R. and CAMNER, P. (1985) Long-term lung clearance in humans studied with Teflon particles labelled with chromium-51. *Exp. Lung Res.* 9, 31-42.

SNIPES, M.B.and CLEM, M.F. (1981) Retention of microspheres in the rat lung after intratracheal instillation. *Environ. Res.* 24, 33-39.

SNIPES, M.B., BOECKER, B.B. and McCLELLAN, R.O. (1983) Retention of monodisperse or polydisperse aluminosilicate particles inhaled by dogs, rats and mice. *Toxicol. Appl. Pharmacol.* 69, 345-362.

SNIPES, M.B., CHAVEZ, G.T. and MUGGENBURG, B.A. (1984) Disposition of 3-, 7-, and 13-μm microspheres instilled into lungs of dogs. *Environ. Res.* 33, 333-342.

STAHLHOFEN, W., GEBHART, J. and HEYDER, J. (1980) Experimental determination of the regional deposition of aerosol particles in the human respiratory tract. *Am. Ind. Hyg. Ass. J.* 41, 385-398.

TALBOT, R.J. and MORGAN, A. (1989) An Interspecies Comparison of the Lung Clearance of Inhaled Monodisperse Cobalt Oxide Particles - Part VIII: Lung clearance of inhaled cobalt oxide particles in mice. *J. Aerosol Sci.* 20, 261-266.

VOSTAL, J.J., SCHRECK, R.M., LEE, P.S., CHAN, T.L and SODERHOLM, S.C. (1982) Deposition and clearance of Diesel particles from the lung. *Toxicological Effects of Emissions from Diesel Engines.* (edited by J.-E. Lewtas) Elsevier, New York, p. 143-159.

WOLFF, R.K., HENDERSON, R.F., SNIPES, M.B., GRIFFITH, W.C., MAUDERLY, J.L., CUDDIHY, R.G. and McCLELLAN, R.O. (1987) Alterations in particle accumulation and clearance in lungs of rats chronically exposed to Diesel exhaust. *Fund. Appl. Toxicol.* 9, 154-166.

Article received in final form October 10, 1990
Reviewed by:
Timothy R. Gerrity
Sidney C. Soderholm

Address reprint requests to:
W. G. Kreyling
Gesellschaft für Strahlen-und Umweltforschung mbH München
Projekt Inhalation Ingolstädter Landstr. 1, D-8042 Neuherberg,
F. R. Germany

This article has been cited by:

1. Cory J. Hitzman, Lee W. Wattenberg, Timothy S. Wiedmann. 2006. Pharmacokinetics of 5-fluorouracil in the hamster following inhalation delivery of lipid-coated nanoparticles. *Journal of Pharmaceutical Sciences* **95**:6, 1196-1211. [CrossRef]
2. David B. Warheit, Mark A. Hartsky. 1993. Role of alveolar macrophage chemotaxis and phagocytosis in pulmonary clearance responses to inhaled particles: Comparisons among rodent species. *Microscopy Research and Technique* **26**:5, 412-422. [CrossRef]
3. Anthony J. Hickey. 1993. Lung Deposition and Clearance of Pharmaceutical Aerosols: What Can Be Learned from Inhalation Toxicology and Industrial Hygiene?. *Aerosol Science and Technology* **18**:3, 290-304. [CrossRef]
4. W KREYLING, F DIETL, M NEUNER, M ROSENBRUCH, F KROMBACH. 1992. Retained particle burden in the lungs of monkeys after chronic quartz dust exposure. *Journal of Aerosol Science* **23**, 507-510. [CrossRef]
5. W.G Kreyling, S André, C.G Collier, G.A Ferron, H Métivier, G Schumann. 1991. Interspecies comparison of lung clearance after inhalation of monodisperse, solid cobalt oxide aerosol particles. *Journal of Aerosol Science* **22**:4, 509-535. [CrossRef]