
Lung Clearance in Long-Evans Rats after Inhalation of Porous, Monodisperse Cobalt Oxide Particles

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ABSTRACT: *Lung clearance of a well-defined uniform and respirable material was conducted to aid in the development of models used to relate inhalation of inorganic hazardous particles to organ doses and bioassay measurements, and in particular to aid in the extrapolation of animal data to humans. In the present study, lung clearance was investigated in Long-Evans rats using monodisperse, porous, 0.8- and 1.7- μm -diameter cobalt oxide (Co_3O_4) test particles. An advanced inhalation technique for rats using endotracheal intubation yielded exclusive particle deposition in the pulmonary region without external pelt contamination, thus allowing for clearance studies starting directly after inhalation. The kinetics of lung clearance was distinguished between the two dominant clearance mechanisms of mechanical particle transport to the larynx and translocation of dissolved particle material to blood. A particle fraction of about 40% was cleared by short-term particle transport to the larynx. both the long-term particle transport rate and the translocation rate of dissolved particle material given as fractional rates of the retained particle mass in the lungs were not constant with time. The former declined from 0.03 to 0.004 d^{-1} during 6 months after inhalation. The latter depended on the specific surface area of the porous particles and increased with time from 0.08 and 0.04 d^{-1} for 0.8- and 1.7- μm particles, respectively. The results obtained were compared to previously reported data obtained from Fischer-344 rats and HMT rats. These were part of a previously reported inter-species comparison of lung clearance followed in seven species, including humans, and using the same batches of Co_3O_4 test particles. Long-term lung retention was*

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similar in Long-Evans rats and HMT rats but decreased faster for both particle sizes than in Fischer-344 rats, as a result of a significantly faster translocation of dissolved material from the test particles to blood. Mechanical particle transport to the larynx was comparable in all three species.

INTRODUCTION

Assessment of the hazards to humans from inhalation exposures to aerosols containing toxic substances requires estimates of the rates at which materials are cleared from the various regions of the respiratory tract. These rates determine the retention in the respiratory tract itself as well as the amounts transferred to secondary sites. In general, it is not possible to obtain relevant data for hazardous materials directly from observations in humans. Data from accidental or occupational exposures are often difficult to interpret because of uncertainties about the conditions of exposure, and for many substances potential hazards have to be assessed in the absence of any human data. Extrapolation to humans of the results of animal lung clearance studies is not straightforward. This is due to large interspecies differences in lung retention based on differences in the effectiveness of the various clearance mechanisms [1–4].

A model of particle clearance from the lungs has been proposed by a task group of the International Commission on Radiological Protection (ICRP) [5]. This model proposes that three clearance mechanisms compete for many inhaled particulate compounds in a variety of species:

- (1) Mechanical clearance of particulate material from the lungs to the larynx and subsequently to the gastro-intestinal tract (GI tract)
- (2) The translocation of material dissolved from the particles and transferred to blood
- (3) Mechanical clearance of intact particles from the lungs to lung-associated lymph nodes (LALN), which relocates the material inside the thorax.

An interspecies comparison of long-term lung clearance was carried out recently to investigate the kinetics of the first two clearance mechanisms from the lungs for long-term retained particles. Both were evaluated after the inhalation of 0.8- and 1.7- μm monodisperse, porous cobalt oxide (Co_3O_4) test particles radioactively labeled with ^{57}Co in the following species: male caucasian man [6]; papio papio baboon [7]; beagle dog [8]; Harwell guinea pig, HMT rat, DSN-Syrian hamster [9]; Fischer-344 rat [10]; Sprague-Dawley rat [11]; and CBA/H-mouse [12]. The data were summarized and compared by the authors [1]. The two sizes were used to compare the behavior of two materials with different in vivo dissolution rates. The smaller size selected was nominally 0.8 μm mean geometric diameter (MGD), since porous Co_3O_4 test particles smaller than this were expected to dissolve too rapidly. The larger size selected was nominally

1.8 μm MGD, which corresponded to a mass median aerodynamic diameter (MMAD) of about 3 μm . It was considered that this was the largest size that could be effectively administered by inhalation to small rodents [13]. Within each species and strain both the kinetics of mechanical particle transport and the kinetics of translocation of dissolved Co from lungs to blood varied little interindividually. However, the kinetics of both clearance mechanisms showed large differences between different species. Even between the three strains of rats [9–11], profound differences of lung retention and both clearance mechanisms were observed, whereas their invariability within one strain was confirmed by a repeat clearance study in HMT rats after one year [9].

Therefore, we investigated lung clearance in a different rat strain, which is often used for inhalation toxicology studies, the Long-Evans rat, using the same aerosol material. We hypothesized that we would find again differences in the kinetics of both long-term clearance mechanisms between this rat strain and the others since it had been found in a previous study that titanium dioxide particles in Long-Evans rats were cleared faster than in Fischer-344 rats [14].

In the aforementioned interspecies comparison [9–11] only the noses of the rats were exposed. This resulted in extrathoracic particle deposition and possible pelt contamination. Therefore, the short-term phase of both clearance mechanisms during the first week after inhalation could not be analyzed. In the present study we applied an advanced inhalation technique for rats using endotracheal intubation to prevent extrathoracic particle deposition and pelt contamination. Thus, short- and long-term kinetics of both clearance mechanisms of particle transport to the larynx and initial translocation of dissolved particle material to blood could be analyzed in this small laboratory animal species, similar to the analysis carried out previously in large experimental animals [7, 8].

MATERIALS AND METHODS

Animals and Experimental Protocol

Eight-week-old, male Long-Evans rats with a mean body weight of 220 g were obtained from Charles River Laboratory. Twelve animals were used for the inhalation studies and two were used for the supplementary study. The experimental protocol of the clearance measurements was closely associated to those of the rodents studies within the interspecies comparison of lung clearance [1].

Preparation of Aerosol Particles

Monodisperse ^{57}Co -labeled Co_3O_4 particles were produced and characterized at the GSF using a spinning top aerosol generator as described

earlier [15–18]. Briefly, a solution of ^{57}Co -labeled $\text{Co}(\text{NO}_3)_2$ was dispersed into monodisperse droplets which were dried airborne. These $^{57}\text{Co}(\text{NO}_3)_2$ particles were preheated at 130°C to remove water of crystallization prior to thermal degradation in a tube furnace at 800°C . Particles were collected on PTFE membrane filters and stored dry. Two batches of monodisperse, porous particles with geometric diameters of 0.8 and $1.7\ \mu\text{m}$ were produced in February 1985 and were used also in the previous interspecies comparison of lung clearance [1].

Inhalation

Rats were anesthetized with halothane (3%) and intratracheally intubated using a flexible Teflon 14-gauge canula. A shortened pipet tip sliding along the canula was gently moved against the larynx to assure a tight seal so that breathing occurred only through the canula, thereby preventing inhalation of bypass air. After canulation, the rats received pentobarbital, 3.0 mg/kg body wt intraperitoneal, for continuing general anesthesia.

The $^{57}\text{Co}_3\text{O}_4$ particles were resuspended from the filter into distilled water containing 0.003% Tween 80 (polyoxyethylene-sorbitan monooleate) detergent by ultrasonic agitation. For inhalation the particle suspension was aerosolized using a jet nebulizer. The aerosol was diluted with dry air to obtain a final relative humidity of 50–60% to assure a dry $^{57}\text{Co}_3\text{O}_4$ aerosol. The generated aerosol was administered by an intratracheal inhalation technique. To achieve this, the rats were intubated and connected to the combined inhalation and breathing system described below.

For inhalation of each particle size, six rats were connected to the inhalation apparatus via their endotracheal canulas. This system consisted of a main metal tubing (inner diameter 10 mm) to which the endotracheal tubes were attached. The diluted aerosol entered this main tube. Downstream from the attached endotracheal tubes, the system divided into two branches: One branch was connected to a respiratory Rudolph valve with an opening pressure set at 1000 Pa; the other branch was connected to a solenoid which was operated by a timer so that it was closed for 2 s and open for 0.5 s. During the close time, the aerosol was pushed into the rats' lungs until the preset pressure of 1000 Pa was reached. At this point, achieved in less than 1 s, the respiratory valve opened and the air and aerosol were released while the lungs of the rats remained inflated at 1000 Pa, i.e., simulating a breathholding pause to increase particle deposition in the lungs. As soon as the solenoid opened, the animals exhaled passively since the pressure in the tubing was then equal to ambient pressure. The $^{57}\text{Co}_3\text{O}_4$ particles passing through the solenoid and the respiratory valve were collected on a filter connected to a pump via a loose coupling in the exhaust system.

Lung Retention, Excretion, and Organ Analysis

Lung retention and urinary and fecal excretion of ^{57}Co were determined for 6 months following the intratracheal inhalation. ^{57}Co activity in the lungs was determined with two collimated, horizontally arranged, lead-shielded NaI(Tl) detectors (2-in. diam $\times$ 2-in length). The animal was placed in a thin-walled plastic tube in between the detectors that were collimated to count the thoracic field. Thorax retention was determined by serial external counting of ^{57}Co activity of the animal's thorax. To estimate long-term lung retention, thorax counts were corrected for interpolated factors obtained from the lung fractions determined at sacrifice and assuming that only half of the whole body retention without the lungs would contribute to the thoracic counts.

For excretion analysis, the animals were kept singly in metabolic cages for separate collection of urine and feces during the first month after inhalation and subsequently for 5 days during each following month up to 150 days after inhalation or prior to sacrifice. Daily urinary and fecal excretion was measured by a 2 $\times$ 2-in. NaI(Tl) well detector.

Organ ^{57}Co analysis was performed after serial sacrifice at days 0, 3, 100, and 180 after inhalation. Animals were sacrificed by IP pentobarbital (100 mg/kg) and exsanguinated, and the activity present in the lungs, GI tract, liver, kidney, and samples of muscle and bone was determined by the gamma well counter.

All gamma counts were corrected for background and the physical decay of ^{57}Co (half-life 271.8 days). For comparison with the previous interspecies study [1, 6–12], lung retention was expressed as a fraction of the estimated lung content at 3 days after inhalation. This was a good estimate of the amount deposited in long-term lung retention sites.

Analysis of Clearance Mechanisms

Based on the assumption of the ICRP Task Group [5] that mechanical particle transport and translocation of dissolved particle mass to blood are independent clearance mechanisms, a clearance model was developed previously [1], which is shown schematically in Fig. 1. In both this study and the previous interspecies comparison, it was not possible to distinguish the particles associated with the tracheobronchial and the alveolar region, although it would be expected that after the first day most of the particles in the lungs would have been in the alveolar region. We therefore estimated the rate of translocation of ^{57}Co from the lungs as a whole to blood, $S(t)$ at time t , and the rate of mechanical transport of particles from the lungs to the GI tract, $M(t)$. Both $S(t)$ and $M(t)$ were expressed as fractions of the contemporary lung burden $L(t)$, cleared per day by each route, and were determined from the amount of ^{57}Co retained in the lungs

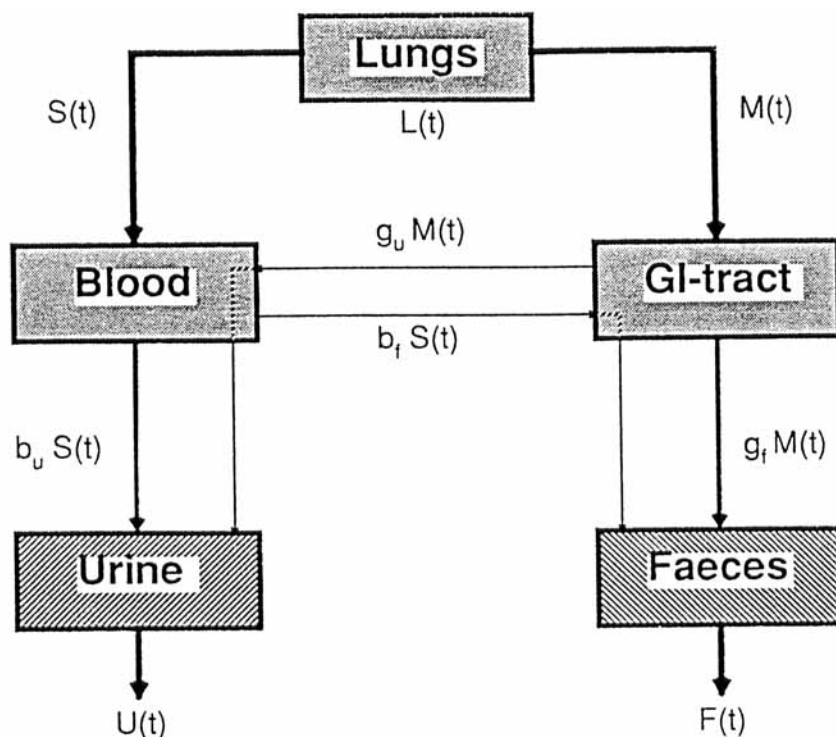


Figure 1 Lung clearance model and transfer factors for Co following inhalation of test particles, showing the fractions of the lung content $L(t)$ at time t cleared per day by each route. Transfer factors are explained in text and equations 1 and 2.

and the amounts excreted per day in urine and feces. Since most of the Co entering the blood or GI tract was rapidly excreted and there was little accumulation in any organ outside the lungs as shown before [1, 6–11], $S(t)$ and $M(t)$ were estimated by the simple model according to Fig. 1:

$$U(t) = b_u S(t) + g_u M(t) \quad F(t) = g_f M(t) + b_f S(t) \quad (1)$$

where $U(t)$ and $F(t)$ are the urinary and fecal excretion rates, i.e., the amounts of activity excreted per day as fractions of $L(t)$; b_u and b_f are the fractions of ^{57}Co in urine and feces, respectively, following translocation of dissolved ^{57}Co from the lungs to blood; and g_u and g_f are the fractions of ^{57}Co excreted in urine and feces respectively, after cobalt oxide particles enter the GI tract. Thus,

$$S(t) = \frac{g_f U(t) - g_u F(t)}{b_u g_f - b_f g_u} \quad M(t) = \frac{b_u F(t) - b_f U(t)}{b_u g_f - b_f g_u} \quad (2)$$

Since transfer of ^{57}Co to the blood from Co_3O_4 particles passing through the GI tract was minor between the two strains of rats in the previous interspecies comparison and varied very little, the means of the

transfer coefficients g_f , g_u were taken from the previous interspecies comparison of lung clearance. The transfer coefficients b_f , b_u for Co circulating in blood were determined by a supplementary experiment. Under Halothane anesthesia, two rats were intravenously injected with 0.25 mL of a 0.05% $^{57}\text{Co}(\text{NO}_3)_2 \times 6 \text{H}_2\text{O}$ solution in saline, through the tail. The mass of Co injected (30 μg) was comparable to the animals' daily dietary intake of Co (12 μg), which ensured that normal metabolism was maintained. Animals were kept singly in metabolism cages for 2 weeks. Urine and feces were continuously collected between whole body counts. Animals were then killed by an overdose of IP pentobarbital and the activity present in the lungs, GI tract, liver, kidney, and samples of muscle and bone was determined.

Statistical Analysis

Particle transport data were analyzed by fitting a two-compartment model. Group comparisons were performed using the approximate *F*-test from nonlinear regression. Translocation data were analyzed by analysis of covariance.

RESULTS

Aerosol Inhalation and Deposition

The aerosol parameters are summarized in Table 1. Under the scanning electron microscope (SEM), particles were spherically shaped with a rough surface (Fig. 2). Density of the porous particles was calculated from MGD and MMAD, yielding about half the density of 6.05 g/cm^3 for bulk Co_3O_4 . The total particle surface area was estimated to be about 10 times the spherical particle surface [16].

Table 1 Characterization of the $^{57}\text{Co}_3\text{O}_4$ Aerosol

Parameter	Large	Small
Mean geometric diameter (SEM) (μm)	1.73	0.81
Mass median aerodynamic diameter (SAC) (μm)	2.69	1.38
Geometric standard deviation (SAC)	1.10	1.08
Density (g/cm^3)	2.3	2.7
Particle mass (10^{-12} g)	6.2	0.75
Specific ^{57}Co activity of $^{57}\text{Co}_3\text{O}_4$ (GBq/g)	10	90
Specific ^{57}Co activity of one particle (Bq)	0.07	0.07

Note. SEM, scanning electron microscope; SAC, Stöber aerosol centrifuge.

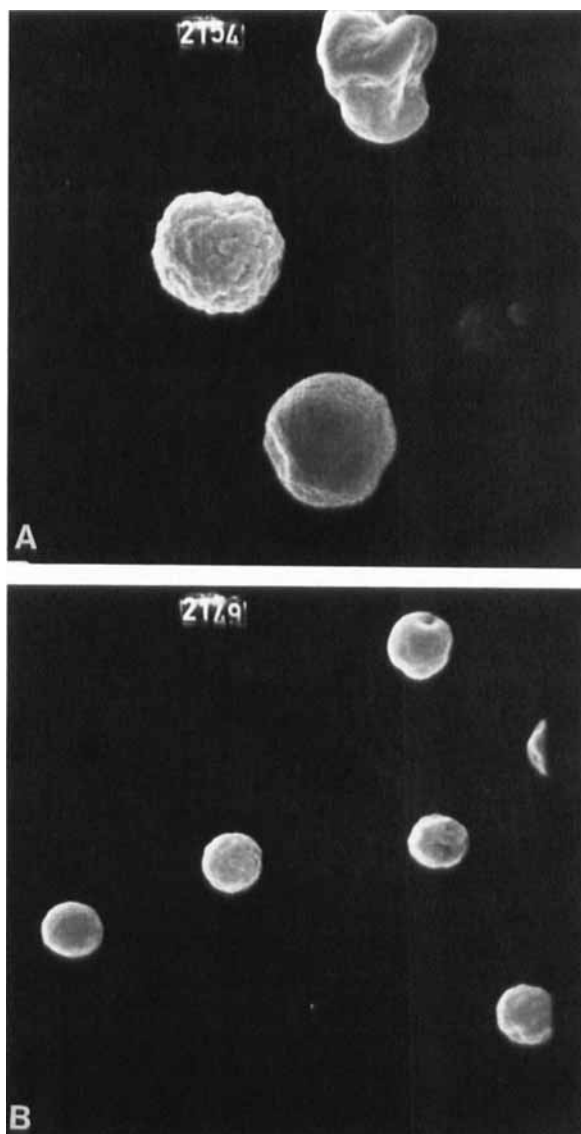


Figure 2 Scanning electron micrographs of (a) 1.7- μm and (b) 0.8- μm $^{57}\text{Co}_3\text{O}_4$ particles used for the inhalation study (magnification 16k).

Since the rats were intubated during inhalation, extrathoracic and pelt contamination was avoided, resulting in tracheobronchial and alveolar deposition only of the aerosol particles. The mean initial ^{57}Co activities (ILA) and particle masses M in the lungs determined from sacrifices immediately after inhalation are given in Table 2. These ^{57}Co activities were comparable to those in rats of the previous study. As discussed there [1], the masses and the activities of Co administered were well below the levels at which any chemical toxic or radiotoxic effect, would be expected.

Table 2 ^{57}Co Activity (ILA) and $^{57}\text{Co}_3\text{O}_4$ Particle Mass Initially Deposited in the Lungs of Those Rats That Were Kept for Long-term Clearance Studies

	Rat					
	1	2	3	4	5	6
1.7-μm $^{57}\text{Co}_3\text{O}_4$						
^{57}Co ILA (kBq)	7.4	9.3	11.9	13.1	6.4	4.9
$^{57}\text{Co}_3\text{O}_4$ mass (ng)	740	930	1190	1310	640	490
Fast cleared fraction	0.38	0.34	0.41	0.41	0.22	0.44
Biol. half-life (d)						
In thorax	59	34	29	49		
In lungs	61	33	29	49		
0.8-μm $^{57}\text{Co}_3\text{O}_4$						
^{57}Co ILA (kBq)	9.9	13.5	27.9	7.2	7.2	
$^{57}\text{Co}_3\text{O}_4$ mass (ng)	110	150	310	80	80	
Fast cleared fraction	0.37	0.25	0.52	0.21	0.54	
Biol. half-life (d)						
In thorax	24	15	15	22		
In lungs	22	16	15	21		

Note. Fast-cleared particle fraction estimated from lung retention measurements and excretion analysis which was cleared from the lungs to the GI tract during the first 4 days after inhalation. Biological half-life of long-term retention starting from day 3 after inhalation.

Short-term Phase of Particle Clearance

The short-term phase of particle clearance was determined from fecal excretion fractions during the first 3 days after inhalation. This clearance fraction averaged for all rats was 0.37 ± 0.07 and 0.38 ± 0.13 of ILA for 1.7- and 0.8- μm particles, respectively. Excretion fractions for each rat are given in Table 2. Although it was assumed that short-term clearance would be terminated after 3 days, daily fecal excretion fractions continued to decrease during the first week until they became constant in the second week.

Long-term Retention

Mean long-term thorax retention $\text{Th}(t)$ as a fraction of thorax content at day 3 and the calculated lung retention $L(t)$ are given in Table 3. In each rat long-term thorax retention was described by an exponential function whose biological half-life is given in Table 2. Mean exponential rate ($\pm$ standard error) and half-life derived from retention data of all rats were $0.028 \pm 0.0007 \text{ d}^{-1}$ and 25 d for the 0.8- μm particles and $0.013 \pm 0.0004 \text{ d}^{-1}$ and 53 d for the 1.7- μm particles.

Table 3 Mean Measured Thorax Retention Data $Th(t)$ ($\pm$ SD) and Lung Retention Data $L(t)$ Corrected for Physical Decay and Normalized to the Lung Retention at Day 3 for Both 1.7 and 0.8- μ m particles

Days	1.7 μ m		0.8 μ m	
	$Th(t) \pm$ SD	$L(t) \pm$ SD	$Th(t) \pm$ SD	$L(t) \pm$ SD
3	1.000	1.000	1.000	1.000
5	0.936 $\pm$ 0.019	0.945 $\pm$ 0.000	0.955 $\pm$ 0.000	0.965 $\pm$ 0.095
6	0.943 $\pm$ 0.038	0.963 $\pm$ 0.019	0.951 $\pm$ 0.094	0.971 $\pm$ 0.054
7	0.905 $\pm$ 0.050	0.924 $\pm$ 0.039	0.932 $\pm$ 0.053	0.951 $\pm$ 0.080
8	0.857 $\pm$ 0.035	0.874 $\pm$ 0.051	0.880 $\pm$ 0.078	0.897 $\pm$ 0.049
9	0.843 $\pm$ 0.024	0.860 $\pm$ 0.036	0.829 $\pm$ 0.048	0.845 $\pm$ 0.078
11	0.810 $\pm$ 0.029	0.827 $\pm$ 0.024	0.805 $\pm$ 0.076	0.820 $\pm$ 0.075
12	0.761 $\pm$ 0.022	0.776 $\pm$ 0.030	0.718 $\pm$ 0.073	0.731 $\pm$ 0.070
13	0.772 $\pm$ 0.036	0.788 $\pm$ 0.023	0.713 $\pm$ 0.069	0.725 $\pm$ 0.078
14	0.737 $\pm$ 0.034	0.751 $\pm$ 0.037	0.718 $\pm$ 0.077	0.730 $\pm$ 0.095
16	0.703 $\pm$ 0.031	0.717 $\pm$ 0.035	0.622 $\pm$ 0.093	0.632 $\pm$ 0.069
20	0.659 $\pm$ 0.040	0.671 $\pm$ 0.031	0.520 $\pm$ 0.068	0.527 $\pm$ 0.051
22	0.605 $\pm$ 0.042	0.616 $\pm$ 0.040	0.522 $\pm$ 0.051	0.528 $\pm$ 0.082
26	0.543 $\pm$ 0.047	0.553 $\pm$ 0.042	0.428 $\pm$ 0.081	0.432 $\pm$ 0.059
30	0.501 $\pm$ 0.057	0.509 $\pm$ 0.048	0.386 $\pm$ 0.059	0.389 $\pm$ 0.057
34	0.525 $\pm$ 0.058	0.533 $\pm$ 0.058	0.318 $\pm$ 0.056	0.320 $\pm$ 0.044
37	0.440 $\pm$ 0.077	0.447 $\pm$ 0.059	0.275 $\pm$ 0.043	0.276 $\pm$ 0.052
42	0.402 $\pm$ 0.062	0.408 $\pm$ 0.078	0.236 $\pm$ 0.052	0.236 $\pm$ 0.062
48	0.369 $\pm$ 0.068	0.375 $\pm$ 0.063	0.186 $\pm$ 0.061	0.185 $\pm$ 0.049
56	0.326 $\pm$ 0.071	0.330 $\pm$ 0.069	0.135 $\pm$ 0.049	0.134 $\pm$ 0.038
69	0.259 $\pm$ 0.069	0.262 $\pm$ 0.072	0.087 $\pm$ 0.038	0.085 $\pm$ 0.024
83	0.216 $\pm$ 0.065	0.218 $\pm$ 0.070	0.042 $\pm$ 0.025	0.042 $\pm$ 0.017
103	0.184 $\pm$ 0.043	0.185 $\pm$ 0.066	0.041 $\pm$ 0.018	0.039 $\pm$ 0.001
131	0.081 $\pm$ 0.086	0.162 $\pm$ 0.043	0.021 $\pm$ 0.021	0.038 $\pm$ 0.006
133	0.086 $\pm$ 0.092	0.171 $\pm$ 0.041		
135	0.078 $\pm$ 0.082	0.171 $\pm$ 0.047	0.010 $\pm$ 0.010	0.018 $\pm$ 0.004
141	0.071 $\pm$ 0.076	0.155 $\pm$ 0.047	0.005 $\pm$ 0.006	0.009 $\pm$ 0.003
156	0.059 $\pm$ 0.061	0.140 $\pm$ 0.035	0.005 $\pm$ 0.005	0.008 $\pm$ 0.001
161	0.119 $\pm$ 0.016	0.116 $\pm$ 0.038	0.008 $\pm$ 0.009	0.014 $\pm$ 0.002
167	0.121 $\pm$ 0.022	0.117 $\pm$ 0.025	0.014 $\pm$ 0.001	0.011 $\pm$ 0.001
177	0.105 $\pm$ 0.017	0.119 $\pm$ 0.016	0.012 $\pm$ 0.002	0.010 $\pm$ 0.002
180	0.095 $\pm$ 0.005	0.103 $\pm$ 0.021	0.010 $\pm$ 0.001	0.008 $\pm$ 0.001

Table 4 shows the percentages of total-body ^{57}Co found in the lungs and other organs and tissues of sacrificed animals at various times points. Percentages of skeleton and soft tissue were estimated from measurements of bone and muscle samples. Initially the activity found outside the lungs was mainly associated with the GI tract and resulted from short-term

Table 4 Organ Distribution of Co at Various Days After Inhalation for Both 1.7- and 0.8- μ m Particles

Percentage of Co ($\pm$ SD) in organ after inhalation of 1.7 μ m particles			
Day of sacrifice:	3	100	180
Number of rats:	2	2	2
Lung	95.55 $\pm$ 0.02	96.94 $\pm$ 0.00	91.39 $\pm$ 0.02
Liver	0.13 $\pm$ 0.00	0.11 $\pm$ 0.00	0.17 $\pm$ 0.00
Kidney	0.04 $\pm$ 0.00	0.07 $\pm$ 0.00	0.13 $\pm$ 0.00
GI tract	2.47 $\pm$ 0.61	0.73 $\pm$ 0.28	0.74 $\pm$ 0.05
Skeleton	0.30 $\pm$ 0.30	0.00 $\pm$ 0.00	5.63 $\pm$ 3.67
Soft tissue	1.07 $\pm$ 0.58	1.53 $\pm$ 0.09	1.38 $\pm$ 1.38
Percentage of Co ($\pm$ SD) in organ after inhalation of 0.8- μ m particles			
Day of sacrifice:	3	100	180
Number of rats:	1	2	2
Lung	81.13	89.25 $\pm$ 0.06	51.79 $\pm$ 0.08
Liver	0.27	1.42 $\pm$ 0.01	0.20 $\pm$ 0.00
Kidney	0.07	0.85 $\pm$ 0.01	0.12 $\pm$ 0.00
GI tract	17.81	1.03 $\pm$ 0.34	2.33 $\pm$ 0.11
Skeleton	0.13	5.79 $\pm$ 5.79	21.50 $\pm$ 4.69
Soft tissue	0.43	1.19 $\pm$ 1.19	17.19 $\pm$ 2.09

Note. Values are mean percentages ($\pm$ SD) of two animals.

particle clearance out of the lungs. The fraction of the total-body ^{57}Co found in the lungs generally exceeded 80% between 3 days and 6 months after inhalation. Thus, for most of the period of thorax retention measurements only small corrections were needed to estimate the ^{57}Co lung content. However, substantial retention outside the lungs was found for the 0.8- μ m particles 180 days after inhalation. This was also indicated by long-term thorax retention measurements starting from 140 days after inhalation, which had changed into the next phase showing a flat pattern. During this period only 1% of the initial long-term retention $L(3)$ was retained in the lungs.

Long-term Lung Clearance Mechanisms

The results of the supplementary experiment to characterize retention and excretion of ^{57}Co entering the bloodstream are needed to calculate the kinetics of the clearance mechanisms according to equation 2 from the

measurements made in the clearance study. One day after $^{57}\text{Co}(\text{NO}_3)_2$ injection 70% of the injected ^{57}Co was found in urine and 10% was found in feces. Seven days after injection 79% was found in urine and 16% was found in feces. After 16 days only 1.9% of the initial dose was found to be retained in the body and the rest had been excreted. The retained fraction was similar to those found in the strains of rats used in the previous interspecies comparison after 3 weeks. The transfer factors are given in Table 5.

Urinary and fecal excretion rates $U(t)$ and $F(t)$ as a fraction of the contemporary lung content $L(t)$ are given in Table 6. From the excretion rates, the rates of translocation $S(t)$ and mechanical particle clearance $M(t)$ were calculated according to equation 2 and are also given in Table 6.

Translocation from the 1.7- μm particles to blood increased significantly from initially 0.005 d^{-1} to 0.008 d^{-1} ($p < .0001$, by linear regression analysis). Likewise, translocation from the 0.8- μm particles increased significantly from 0.012 (excluding the first 3 days) to a maximum rate of 0.05 d^{-1} at about 50 days after inhalation ($p < .0001$, by linear regression analysis) and declined thereafter to about 0.02 d^{-1} at 140 days after inhalation.

No significant difference between long-term particle transport of the two particle sizes was found. The mean rate $M(t)$ decreased from about 0.03 d^{-1} one week after inhalation to 0.004 d^{-1} at 140 days after inhalation.

DISCUSSION

The present study was carried out one year after the previous interspecies comparison and, thus, one year after particle production. At the same time this study was performed, Collier and coworkers [9] carried out a second lung clearance study on HMT rats using the same test particles in order to investigate the effect of aging of the particles. Lung retention, mechanical particle transport, and the characteristic pattern of translocation of dissolved ^{57}Co did not differ significantly from the first clearance study in HMT rats, showing that the particles had not changed properties affecting their clearance from the lungs [9].

Table 5 Transfer Coefficients from Blood to Urine b_u and Feces b_f and from the GI tract to Urine g_u and Feces g_f

Particle Size	b_u	b_f	g_u	g_f
0.8 mm	0.804	0.177	0.02	0.98
1.7 mm	0.804	0.177	0.004	0.996

Table 6a Mean Daily Excreted Co Percentage in Urine $U(t)$ Normalized to the Contemporary Lung Burden and Calculated Translocation Rates $S(t)$ for Both 1.7 and 0.8- μm Particles

Day	1.7 μm		0.8 μm	
	$U(t) \pm \text{SD}$ (% day ⁻¹)	$S(t) \pm \text{SD}$ (% day ⁻¹)	$U(t) \pm \text{SD}$ (% day ⁻¹)	$S(t) \pm \text{SD}$ (% day ⁻¹)
1	0.47 $\pm$ 0.10	0.48 $\pm$ 0.20	0.66 $\pm$ 0.24	0.75 $\pm$ 0.28
2	0.43 $\pm$ 0.11	0.38 $\pm$ 0.10	0.90 $\pm$ 0.25	0.26 $\pm$ 0.75
3	0.35 $\pm$ 0.11	0.39 $\pm$ 0.13	0.75 $\pm$ 0.16	0.58 $\pm$ 0.29
4	0.54 $\pm$ 0.23	0.64 $\pm$ 0.29	1.58 $\pm$ 0.27	1.52 $\pm$ 0.34
5	0.47 $\pm$ 0.20	0.56 $\pm$ 0.25	1.19 $\pm$ 0.25	1.31 $\pm$ 0.31
6	0.42 $\pm$ 0.08	0.50 $\pm$ 0.10	1.07 $\pm$ 0.11	1.18 $\pm$ 0.17
7	0.42 $\pm$ 0.09	0.50 $\pm$ 0.11	1.59 $\pm$ 0.33	1.86 $\pm$ 0.47
8	0.38 $\pm$ 0.05	0.46 $\pm$ 0.06	1.43 $\pm$ 0.10	1.75 $\pm$ 0.13
9	0.37 $\pm$ 0.03	0.45 $\pm$ 0.04	1.80 $\pm$ 0.16	2.16 $\pm$ 0.18
10	0.44 $\pm$ 0.06	0.54 $\pm$ 0.08	1.79 $\pm$ 0.38	2.17 $\pm$ 0.47
11	0.45 $\pm$ 0.06	0.55 $\pm$ 0.08	1.86 $\pm$ 0.40	2.25 $\pm$ 0.49
12	0.45 $\pm$ 0.12	0.56 $\pm$ 0.15	1.82 $\pm$ 0.44	2.20 $\pm$ 0.54
13	0.46 $\pm$ 0.05	0.56 $\pm$ 0.07	1.89 $\pm$ 0.05	2.28 $\pm$ 0.07
14	0.41 $\pm$ 0.06	0.50 $\pm$ 0.07	2.16 $\pm$ 0.27	2.64 $\pm$ 0.35
15	0.52 $\pm$ 0.08	0.63 $\pm$ 0.11	2.45 $\pm$ 0.37	3.00 $\pm$ 0.45
16	0.47 $\pm$ 0.07	0.57 $\pm$ 0.09	2.75 $\pm$ 0.51	3.37 $\pm$ 0.63
27	0.52 $\pm$ 0.07	0.63 $\pm$ 0.09	3.17 $\pm$ 0.49	3.90 $\pm$ 0.59
28	0.45 $\pm$ 0.10	0.54 $\pm$ 0.13	3.03 $\pm$ 0.62	3.71 $\pm$ 0.75
29	0.52 $\pm$ 0.06	0.64 $\pm$ 0.08	3.48 $\pm$ 0.40	4.28 $\pm$ 0.48
30	0.45 $\pm$ 0.09	0.56 $\pm$ 0.12	3.20 $\pm$ 0.68	3.93 $\pm$ 0.85
32	0.48 $\pm$ 0.10	0.58 $\pm$ 0.12	3.83 $\pm$ 0.65	4.72 $\pm$ 0.81
33	0.49 $\pm$ 0.10	0.59 $\pm$ 0.12	3.97 $\pm$ 0.71	4.90 $\pm$ 0.87
48	0.48 $\pm$ 0.22	0.58 $\pm$ 0.27	3.79 $\pm$ 1.03	4.69 $\pm$ 1.28
49	0.51 $\pm$ 0.14	0.62 $\pm$ 0.17	3.92 $\pm$ 0.66	4.85 $\pm$ 0.81
50	0.60 $\pm$ 0.13	0.73 $\pm$ 0.16	4.70 $\pm$ 1.79	5.82 $\pm$ 2.22
51	0.54 $\pm$ 0.17	0.67 $\pm$ 0.22	3.73 $\pm$ 0.88	4.61 $\pm$ 1.09
52	0.67 $\pm$ 0.22	0.83 $\pm$ 0.27	4.95 $\pm$ 1.14	6.13 $\pm$ 1.41
69	0.63 $\pm$ 0.26	0.77 $\pm$ 0.32	2.94 $\pm$ 1.18	3.64 $\pm$ 1.49
70	0.60 $\pm$ 0.15	0.75 $\pm$ 0.18	3.00 $\pm$ 0.79	3.71 $\pm$ 0.98
71	0.58 $\pm$ 0.12	0.71 $\pm$ 0.15	2.79 $\pm$ 1.26	3.43 $\pm$ 1.56
75	0.58 $\pm$ 0.18	0.72 $\pm$ 0.22	3.03 $\pm$ 0.96	3.72 $\pm$ 1.18
76	0.59 $\pm$ 0.18	0.73 $\pm$ 0.22	3.15 $\pm$ 1.02	3.87 $\pm$ 1.25
98	0.54 $\pm$ 0.30	0.67 $\pm$ 0.37	2.28 $\pm$ 1.96	3.76 $\pm$ 2.06
99	0.74 $\pm$ 0.33	0.91 $\pm$ 0.41	2.64 $\pm$ 0.75	3.23 $\pm$ 0.90
100	0.60 $\pm$ 0.15	0.74 $\pm$ 0.18	2.97 $\pm$ 1.59	3.67 $\pm$ 1.96
101	0.42 $\pm$ 0.30	0.52 $\pm$ 0.37	1.59 $\pm$ 0.25	1.97 $\pm$ 0.30
138	0.75 $\pm$ 0.36	0.93 $\pm$ 0.44	1.32 $\pm$ 0.02	1.39 $\pm$ 0.02

(table continued on next page)

Table 6a. (Continued)

Day	1.7 μm		0.8 μm	
	$U(t) \pm \text{SD}$ (% day ⁻¹)	$S(t) \pm \text{SD}$ (% day ⁻¹)	$U(t) \pm \text{SD}$ (% day ⁻¹)	$S(t) \pm \text{SD}$ (% day ⁻¹)
139	0.69 $\pm$ 0.31	0.85 $\pm$ 0.39	2.62 $\pm$ 0.91	3.23 $\pm$ 1.14
140	0.69 $\pm$ 0.31	0.86 $\pm$ 0.39	2.70 $\pm$ 0.94	3.33 $\pm$ 1.18
141	0.76 $\pm$ 0.33	0.95 $\pm$ 0.41	3.28 $\pm$ 0.12	3.98 $\pm$ 0.16
142	0.62 $\pm$ 0.38	0.77 $\pm$ 0.47	1.24 $\pm$ 1.00	1.54 $\pm$ 1.24
143	0.63 $\pm$ 0.38	0.78 $\pm$ 0.48	1.28 $\pm$ 1.03	1.59 $\pm$ 1.28

Inhalation Using Endotracheal Intubation

Since rats are obligatory nose breathers, rather high fractions of aerosol particles above 2 μm aerodynamic diameter will be deposited in the extrathoracic airways due to impaction. Virtually no particles larger than 3 μm aerodynamic diameter will be deposited in the pulmonary region [13]. However, inhalation via an endotracheal tube allows the inhalation of large particles and their deposition in the pulmonary region of rats since extrathoracic airways are bypassed. Hence, this inhalation technique allows rat studies on hazardous airborne materials beyond 3 μm aerodynamic diameter, which can be deposited in the human lungs. Forced ventilation of the animals and a breathholding pause were additionally included in this study to increase deep lung deposition.

Short-term Particle Clearance

Short-term clearance from the pulmonary region of individual rats was investigated, since there was no contamination of the pelt and the extrathoracic airways. In an earlier study [13] short-term particle clearance was determined in Long-Evans rats after nose-only exposure to monodisperse particles of 1, 2, and 3 μm aerodynamic diameter. The short-term cleared fractions were calculated from lung measurements of rats sacrificed immediately and 20 h after inhalation, excluding extrathoracic deposition. The fractions of 0.24 ± 0.13 , 0.18 ± 0.31 , and 0.51 ± 0.23 , respectively, showed large intersubject variability and did not clearly increase with particle size, as would be expected from the predominant deposition mechanisms of sedimentation and impaction for these particles. While in the former study short-term clearance was evaluated and averaged from measurements on different animals, we determined the short-term cleared fraction of each individual animal as a result of the advanced inhalation technique using an endotracheal tube. The fractions of short-term particle clearance of 0.37 ± 0.07 and 0.38 ± 0.13 for the

Co_3O_4 particles with an aerodynamic diameter of 1.4 and 2.7 μm , respectively, showed again no clear increase with particle size. We found less intersubject variability but the mean fractions were in reasonable agreement with those reported in the former study [13]. It should be noted, however, that these fractions are considerably higher than those of about 5 and 10%, respectively, reported for humans [19].

The large fractions resulted most likely from a high deposition probability in the airways as a result of the externally enforced ventilation of the animals, including rapid inhalation and a subsequent 1-s breathhold. The absence of any abrupt difference in the fecal excretion, the smooth transition from the high rates at day 1 through 3 to those a few days later, and rather constant rates during the second week after inhalation (Table 6b) indicated both fast particle clearance and a slower phase from the airways, as suggested by other studies as well [20, 21]. A smooth transition to an even more slowly clearing alveolar compartment would then not be surprising.

Long-term Translocation of Dissolved Co from Lungs to Blood

Since deposited particles are phagocytized within less than a day [22], long-term particle clearance is macrophage mediated. Conceivably, the translocation of dissolved Co consists of several steps, i.e., dissolution inside phagolysosomes of alveolar macrophages followed by elimination of ionic or protein bound Co. As discussed earlier [23, 24], the rate-determining process in the translocation of dissolved ^{57}Co from the dogs' lungs was the intracellular particle dissolution in alveolar macrophages. In previous lung clearance studies on the translocation of dissolved Co in dogs using monodisperse Co_3O_4 particles of various sizes and porosities [25–27], the translocation rate was proportional to the specific surface area, i.e., the total surface area including the inner surface area of porous particles per particle mass [28].

Hence, the clearance process of intracellular particle dissolution can be deduced from the kinetics of the translocation rate of dissolved $^{57}\text{Co}_3\text{O}_4$ particles (Fig. 4). The initial translocation rate of dissolved ^{57}Co from 0.8- μm particles was twice that for 1.7- μm particles according to the twofold specific surface area of the former. It should be noted that the initial translocation rates were 1–2 orders of magnitude larger than the dissolution rates in saline, which was determined as a substitute for the epithelial lining fluid or blood [8]. As discussed earlier [1, 29] the increase was expected to be due to the increasing specific surface area of the dissolving particles. A maximum was reached when the fraction of particles available for this clearance mechanism approached zero, while other long-term clearance mechanisms such as long-term retention of nonpar-

Table 6b Mean Daily Excreted Co Percentage in Feces $F(t)$ Normalized to the Contemporary Lung Burden and Calculated Mechanical Particle Transport Rates $S(t)$ for Both 1.7- and 0.8- μm Particles

Day	1.7 μm		0.8 μm	
	$F(t) \pm \text{SD}$ (% day ⁻¹)	$M(t) \pm \text{SD}$ (% day ⁻¹)	$F(t) \pm \text{SD}$ (% day ⁻¹)	$M(t) \pm \text{SD}$ (% day ⁻¹)
1	22.79 $\pm$ 14.36	22.83 $\pm$ 14.39	6.22 $\pm$ 4.69	6.35 $\pm$ 5.03
2	29.25 $\pm$ 8.59	29.30 $\pm$ 8.61	34.19 $\pm$ 22.07	34.84 $\pm$ 22.64
3	8.68 $\pm$ 2.58	8.64 $\pm$ 2.57	13.69 $\pm$ 6.08	13.87 $\pm$ 6.24
4	5.63 $\pm$ 0.77	5.54 $\pm$ 0.76	17.80 $\pm$ 14.09	17.89 $\pm$ 14.41
5	4.88 $\pm$ 1.11	4.80 $\pm$ 1.10	6.99 $\pm$ 7.13	6.89 $\pm$ 7.30
6	3.66 $\pm$ 0.65	3.59 $\pm$ 0.65	6.08 $\pm$ 3.59	5.99 $\pm$ 3.68
7	2.60 $\pm$ 0.82	2.52 $\pm$ 0.82	4.72 $\pm$ 2.75	4.48 $\pm$ 2.87
8	2.42 $\pm$ 0.66	2.35 $\pm$ 0.67	1.62 $\pm$ 0.84	1.33 $\pm$ 0.86
9	2.50 $\pm$ 0.61	2.43 $\pm$ 0.62	3.39 $\pm$ 1.71	3.06 $\pm$ 1.73
10	2.38 $\pm$ 0.41	2.29 $\pm$ 0.41	2.64 $\pm$ 0.90	2.30 $\pm$ 0.90
11	2.42 $\pm$ 0.42	2.33 $\pm$ 0.42	2.75 $\pm$ 0.96	2.40 $\pm$ 0.95
12	2.24 $\pm$ 0.66	2.15 $\pm$ 0.68	2.80 $\pm$ 0.78	2.46 $\pm$ 0.77
13	1.91 $\pm$ 0.62	1.82 $\pm$ 0.63	2.83 $\pm$ 0.60	2.47 $\pm$ 0.62
14	2.16 $\pm$ 0.52	2.08 $\pm$ 0.52	2.35 $\pm$ 0.83	1.92 $\pm$ 0.87
15	1.73 $\pm$ 0.95	1.63 $\pm$ 0.96	2.61 $\pm$ 0.98	2.12 $\pm$ 0.98
16	1.89 $\pm$ 0.37	1.80 $\pm$ 0.36	2.41 $\pm$ 0.70	1.85 $\pm$ 0.63
27	1.80 $\pm$ 0.52	1.70 $\pm$ 0.51	2.32 $\pm$ 0.62	1.67 $\pm$ 0.53
28	2.07 $\pm$ 0.82	1.98 $\pm$ 0.82	3.06 $\pm$ 1.16	2.46 $\pm$ 1.09
29	1.93 $\pm$ 0.54	1.83 $\pm$ 0.54	2.62 $\pm$ 0.95	1.91 $\pm$ 0.88
30	1.73 $\pm$ 0.51	1.64 $\pm$ 0.51	2.52 $\pm$ 0.75	1.86 $\pm$ 0.77
32	1.80 $\pm$ 0.81	1.70 $\pm$ 0.80	2.37 $\pm$ 0.51	1.57 $\pm$ 0.44
33	1.83 $\pm$ 0.83	1.74 $\pm$ 0.82	2.47 $\pm$ 0.55	1.63 $\pm$ 0.46
48	1.82 $\pm$ 0.58	1.72 $\pm$ 0.55	1.92 $\pm$ 0.48	1.12 $\pm$ 0.42
49	1.37 $\pm$ 0.66	1.27 $\pm$ 0.65	1.99 $\pm$ 0.63	1.15 $\pm$ 0.54
50	1.56 $\pm$ 0.61	1.44 $\pm$ 0.61	1.71 $\pm$ 0.88	0.70 $\pm$ 0.72
51	1.37 $\pm$ 0.28	1.25 $\pm$ 0.28	1.88 $\pm$ 0.70	1.08 $\pm$ 0.54
52	1.67 $\pm$ 0.56	1.53 $\pm$ 0.52	1.93 $\pm$ 0.54	0.86 $\pm$ 0.35
69	1.55 $\pm$ 0.64	1.42 $\pm$ 0.60	1.22 $\pm$ 0.57	0.58 $\pm$ 0.74
70	1.20 $\pm$ 0.52	1.07 $\pm$ 0.50	1.63 $\pm$ 0.37	0.99 $\pm$ 0.35
71	1.40 $\pm$ 0.48	1.28 $\pm$ 0.46	1.99 $\pm$ 0.50	1.41 $\pm$ 0.32
75	1.32 $\pm$ 0.41	1.19 $\pm$ 0.38	2.20 $\pm$ 1.28	1.57 $\pm$ 1.18
76	1.34 $\pm$ 0.42	1.22 $\pm$ 0.39	2.29 $\pm$ 1.35	1.64 $\pm$ 1.24
98	0.70 $\pm$ 0.24	0.59 $\pm$ 0.23	1.94 $\pm$ 1.15	1.47 $\pm$ 1.15
99	1.17 $\pm$ 0.62	1.01 $\pm$ 0.61	2.57 $\pm$ 1.89	2.04 $\pm$ 1.78
100	1.01 $\pm$ 0.54	0.89 $\pm$ 0.52	1.61 $\pm$ 1.16	0.98 $\pm$ 0.91
101	0.42 $\pm$ 0.05	0.33 $\pm$ 0.11	0.89 $\pm$ 0.49	0.55 $\pm$ 0.44
138	0.44 $\pm$ 0.10	0.27 $\pm$ 0.02	0.95	0.89

Table 6b. (Continued)

Day	1.7 μm		0.8 μm	
	$F(t) \pm \text{SD}$ (% day ⁻¹)	$M(t) \pm \text{SD}$ (% day ⁻¹)	$F(t) \pm \text{SD}$ (% day ⁻¹)	$M(t) \pm \text{SD}$ (% day ⁻¹)
139	0.55 $\pm$ 0.12	0.40 $\pm$ 0.05	1.58 $\pm$ 0.22	1.03 $\pm$ 0.43
140	0.55 $\pm$ 0.12	0.40 $\pm$ 0.05	1.63 $\pm$ 0.22	1.06 $\pm$ 0.44
141	0.61 $\pm$ 0.10	0.44 $\pm$ 0.02	4.87 $\pm$ 0.61	4.25 $\pm$ 0.65
142	0.45 $\pm$ 0.14	0.32 $\pm$ 0.05	1.05	0.57
143	0.46 $\pm$ 0.14	0.32 $\pm$ 0.05	1.080	0.585

ticulate Co in cartilageneous structures [25, 30, 31] continued. Hence, a maximum translocation rate of dissolved ⁵⁷Co from 1.7- μm particles would be expected at later times, as seen previously in dogs [1, 8].

Interstrain Comparison of Long-term Retention

Figure 3 shows the lung retention found in the three strains of rats (Long-Evans, Fischer-344, HMT) for both particle sizes as a result of the effective overall clearance. Long-Evans rats and HMT rats showed an initially faster decrease of lung retention than Fischer-344 rats for both particle sizes ($p < .001$, approximate F -test from nonlinear regression). While this persisted for the 0.8- μm particles for 6 months after inhalation, in agreement to the study of Ferin and Morehouse [14], lung retention of 1.7- μm particles decreased slowest in Long-Evans rats 2 months after inhalation. As a result, the retained fraction of 1.7- μm particles was similar in Long-Evans rats and Fischer-344 rats 6 months after inhalation, and these fractions were larger than that observed in HMT rats ($p < .01$). One interpretation is that mechanical particle clearance is similar between Long-Evans rats and Fischer-344 rats, whereas translocation of the dissolved material is similar between Long-Evans rats and HMT rats.

Interstrain Comparison of Translocation of Dissolved Co from the Lungs to Blood

Figure 4 shows the translocation rates found in three strains of rats (Long-Evans, Fischer-344, HMT) for both particle sizes. The initial translocation rates (as measured in Long-Evans rats and extrapolated for Fischer-344 and HMT rats) were not significantly different between the three strains for a given particle ($p = .69$ for 0.8 μm ; $p = .09$ for 1.7 μm), but differed by a factor of about 2 between the two particle sizes in all three strains, the 1.7- μm particles having the lower initial rates (Long-Evans rats $p <$

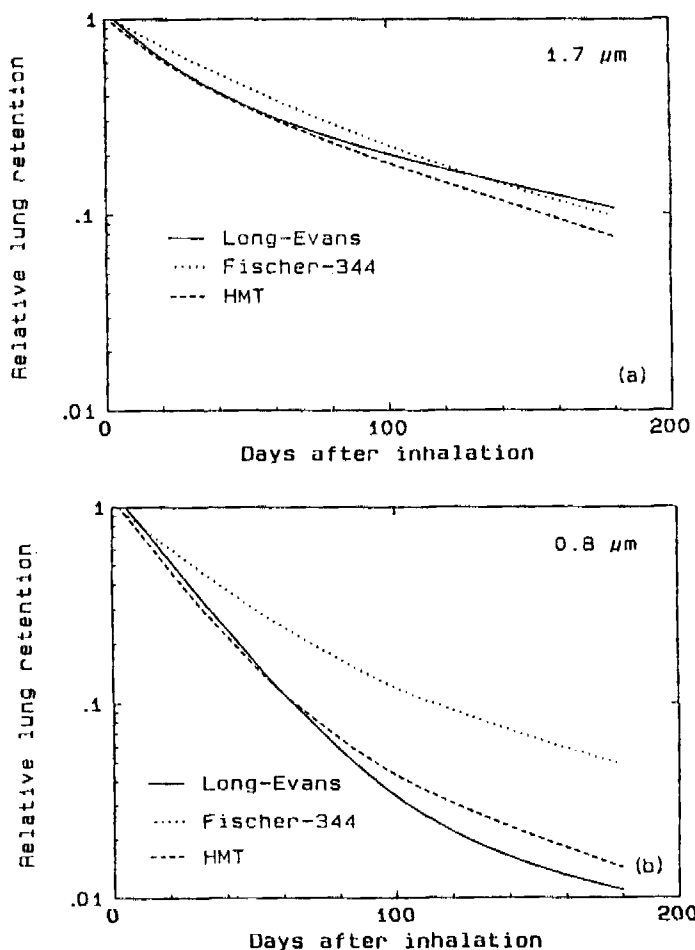


Figure 3 Long-term lung retention of (a) 1.7- μm and (b) 0.8- μm $^{57}\text{Co}_3\text{O}_4$ particles as a fraction of retention $L(3)$ at day 3 found in three strains of rats: Long-Evans, Fischer-344, HMT.

.0015; Fischer-344 rats $p < .0001$; HMT rats $p < .008$). Similarly, this ratio was observed in all species studied previously [1, 6–12], although the initial translocation rates differed significantly between the species studied. Translocation was fastest in Long-Evans rats for both particles compared to those of the other strains. The pattern observed in HMT rats for 0.8 μm was even more pronounced in Long-Evans rats, showing a steeper initial increase and a higher maximum at a similar time of about 50 days after inhalation and a subsequent decrease. Since the translocation of dissolved Co from lungs to blood is alveolar macrophage mediated, as discussed above, the differences found between the three strains suggest functional differences of the mechanism of intracellular particle dissolution between the alveolar macrophages of the three strains.

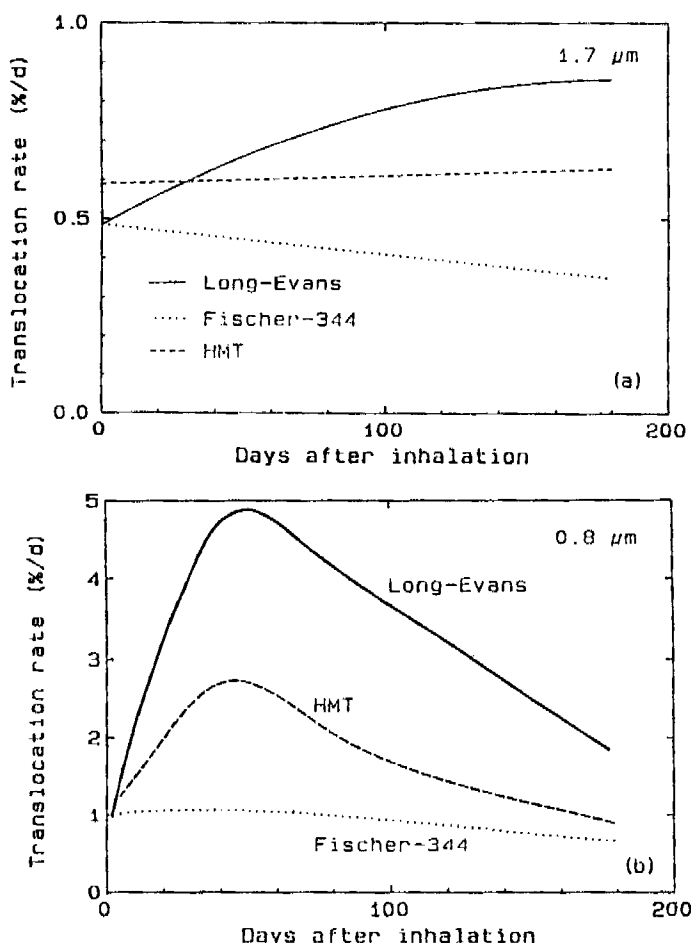


Figure 4 Translocation from (a) 1.7- μm and (b) 0.8- μm $^{57}\text{Co}_3\text{O}_4$ particles found in three strains of rats: Long-Evans, Fischer-344, HMT.

Interstrain Comparison of Particle Transport from Lungs to Larynx

No significant difference of the kinetics of the mechanical transport rate was found between 0.8- and 1.7- μm $^{57}\text{Co}_3\text{O}_4$ particles in Long-Evans rats (see Table 6b). This is in good agreement with the invariance of long-term particle transport to particle parameters, as has been discussed not only for various strains of rats but also for other species, including humans [32].

Figure 5 shows the long-term mechanical particle transport in three strains of rats (Long-Evans, Fischer-344, HMT) averaged for both particle sizes. There was not much of a difference of the particle transport pattern between the three strains, considering that the initial data points were not collected in HMT rats and Fischer-344 rats. Starting one month after

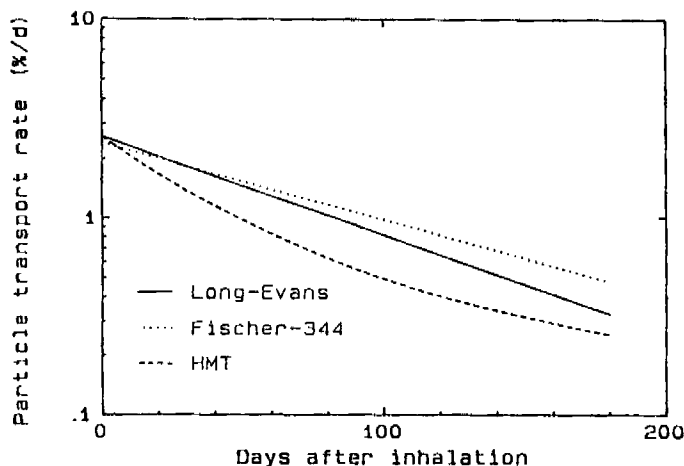


Figure 5 Long-term mechanical particle transport of $^{57}\text{Co}_3\text{O}_4$ particles from the lungs to the larynx found in three strains of rats: Long-Evans, Fischer-344, HMT. Data for both particle sizes were pooled.

inhalation, however, transport rates obtained from HMT rats were consistently lower than those of Fischer-344 rats ($p < .01$), with the transport rates obtained from Long-Evans rats in between.

The exponentially declining transport rate found in rats was also observed in other species as a general pattern [32]. The decreasing rate might be due to sequestration of particles in alveolar macrophages, epithelial and/or interstitial sites. It should be noted, however, that in all three strains of rats mechanical particle transport rates were about one order of magnitude higher than those in humans and large animal species; i.e., mechanical particle transport is an effective and important long-term clearance mechanism in rats, but not in humans and large animal species [32].

Comparing our results to those of the previous study on Long-Evans rats [14], we found similarly a faster overall clearance of particles in Long-Evans rats as opposed to Fischer-344 rats. Since highly insoluble TiO_2 particles were used in the previous study [14], we can assume the differences found earlier were due to differences in mechanical particle clearance rates between the two rat strains. However, Co_3O_4 particle clearance by mechanical transport was not different between Fischer-344 and Long-Evans rats, whereas clearance by Co_3O_4 particle dissolution was faster in Long-Evans rats, which explains the difference in the overall clearance between the two rat strains. Mechanical particle clearance was shown to be principally independent of particle material [1, 32]. The discrepancy in mechanical particle clearance (faster rates for TiO_2 particles in Long-Evans rats and no difference for Co_3O_4 particles in the two strains) might have been due either to the different particle size (polydis-

perse submicrometer TiO_2 particles versus monodisperse 0.8- and 1.7- μm Co_3O_4 particles) or to a higher lung burden in case of the TiO_2 particles, since the deposited lung burden was 155 μg per rat lung. This may have affected Fischer-344 rats more than Long-Evans rats. In contrast the Co_3O_4 particle study was not affected by lung burden, since these were on average only 0.88 and 0.15 μg for the 1.7- and 0.8- μm Co_3O_4 particles, respectively (see Table 2).

CONCLUSION

Aerosol inhalation via an endotracheal tube by forced ventilation proved to be a suitable method in rats and allowed retention and clearance measurements starting directly after inhalation, including both fast and slow phase. While mechanical particle transport is similar in the three rat strains studied, there are marked differences in the translocation of dissolved particle material to blood between the three strains. These data suggest that intracellular particle dissolution in alveolar macrophages is significantly different between the three rat strains and that alveolar macrophage-mediated particle transport toward the mucociliary escalator is similar in the three rat strains.

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