I. Overview (30 m)
   a. Brief history, aerosol exposures
   b. Equipment/animals
   c. Class III cabinets
   d. Procedural video

II. Aerosol generation (15 m)
   a. Overview of generation technologies
   b. Collison nebulizer
   c. Viability

III. Sampling & characterization (15 m)
   a. Methods of sampling (impinger, filter, etc.)
   b. Particle sizing
   c. Deposition and retention

IV. Dose (15 m)
   a. Definition of dose
   b. Calculation
   c. Importance of the 'spray factor'

BREAK

V. Emerging Technology (30 m)
   a. Genesis of the automated technology
   b. Application

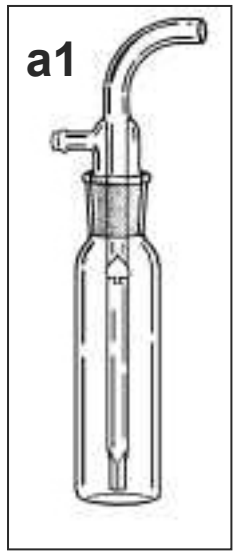
VI. Examples: aerosol exp. of animals (30 m)
   a. Yersinia pestis
   b. Bacillus anthracis
   c. Staphyloccocal enterotoxin B

Sampling

- why sample during exposures
  - characterize experimental atmosphere
    - physical parameters
      - particle size
      - number
    - concentration
      - » viability, total organisms
- considerations
  - type & time of sample
  - method of sample analysis
    - precision and accuracy of method
    - efficiency of sampling method
      - effect on viability of captured bioaerosol
    - sensitivity of assay
    - least detectable quantity

bioaerosol sampling

- Samplers
  - *Impingers*; AGI (a1); biosampler (a2)
  - *Slit-style impactors* (b)
  - *Various filters* (c2)
  - *Cascade impactors*; single-stage ‘N6’(d); Seven stage cascade impactor (d2)



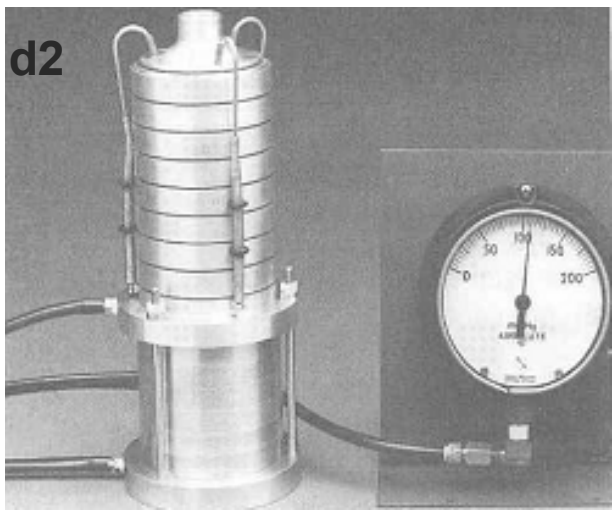


TABLE J-2. Outline for Selecting a Sampler

Sampling Location	Collection of Viable Particles or Cells	Separation of Particles by Size	Aerosol Concentration*	Slit-to-agar (1)‡	Sieve Impactors: 1-Stage Portable (2a)	Sieve Impactors: 1-Stage N-6 (2b)	Sieve Impactors: 2-Stage (2c)	Sieve Impactors: 4- or 6-Stage (2d)	Sieve Impactors: Personal 8-Stage (2e)	Centrifugal Impactor (3)	Impingers: AGI-30 (4a)	Impingers: AGI-4 (4b)	Impingers: Personal (4c)	Impingers: Multi-stage (4d)
Indoors	Particles	No size separation	Low	H/S	H/S	H/S	H/S	H/S	A/A′	H/S	—	—	—	—
			Interm.	H/S	H/S	H/S	H/S	H/S	A/A′	H/S	A′	A′	—	A′
			High	H/S	—	H/S	H/S	H/S	A/A′/H/S	—	A′	A′	A′	A′
		Size separation	Low	—	—	—	H/S	H/S	A/A′	—	—	—	—	—
			Interm.	—	—	—	H/S	H/S	A/A′	—	A′†	A′†	—	A′
			High	—	—	—	H/S	H/S	A/A′/H/S	—	A′†	A′†	—	A′
	Cells	No size separation	Low	H/S§	H/S§	H/S§	H/S§	H/S§	A/A′	—	—	—	—	A′/H/S
			Interm.	H/S§	H/S§	H/S§	H/S§	H/S§	A/A′	—	A′/H/S	A′/H	—	A′/H/S
			High	H/S§	—	H/S§	H/S§	H/S§	A/A′/H/S§	—	A′/H/S	A′/H	A′/H/S	A′/H/S
		Size separation	Low	—	—	—	H/S§	H/S§	A/A′	—	—	—	—	A′/H/S
			Interm.	—	—	—	H/S§	H/S§	A/A′	—	A′/H/S†	A′/H†	—	A′/H/S
			High	—	—	—	H/S§	H/S§	A/A′/H/S§	—	A′/H/S†	A′/H†	—	A′/H/S
Outdoors	Particles	No size separation	Low	H/S	H/S	H/S	H/S	H/S	A/A′	H/S	—	—	—	—
			Interm.	H/S	H/S	H/S	H/S	H/S	A/A′	H/S	A′	A′	—	A′
			High	H/S	—	H/S	H/S	H/S	A/A′/H/S	—	A′	A′	A	A′
		Size separation	Low	—	—	—	H/S	H/S	A/A′	—	—	—	—	—
			Interm.	—	—	—	H/S	H/S	A/A′	—	A′†	A′†	—	A′
			High	—	—	—	H/S	H/S	A/A′/H/S	—	A′†	A′†	—	A′
	Cells	No size separation	Low	H/S§	H/S§	H/S§	H/S§	H/S§	A/A′	—	—	—	—	A′/H/S
			Interm.	H/S§	H/S§	H/S§	H/S§	H/S§	A/A′	—	A′/H/S	A′/H	—	A′/H/S
			High	H/S§	—	H/S§	H/S§	H/S§	A/A′/H/S§	—	A′/H/S	A′/H	A′/H/S	A′/H/S
		Size separation	Low	—	—	—	H/S§	H/S§	A/A′	—	—	—	—	A′/H/S
			Interm.	—	—	—	H/S§	H/S§	A/A′	—	A′/H/S†	A′/H†	—	A′/H/S
			High	—	—	—	H/S§	H/S§	A/A′/H/S§	—	A′/H/S†	A′/H†	—	A′/H/S

A = aeroallergens (microscopic identification)
A′ = aeroallergens (immunoassay)
H = hardy microorganisms, e.g., spore-forming bacteria and fungi
S = sensitive microorganisms, e.g., vegetative cells

*Low concentration: < 100 CFU/m^3; e.g., clean rooms and operating rooms; collect > 0.5 m^3 (500 L) air.
Intermediate concentration: 100 to 1000 CFU/m^3; e.g., general indoor and outdoor concentrations; collect 0.25 to 1 m^3 (250–1000 L) air.
High concentration: > 1000 CFU/m^3; e.g., animal and plant handling areas, outdoor construction and excavation; collect < 0.25 m^3 (250 L) air.
‡Numbers refer to sampler listing in first column of Table J-1.
†Used with a pre-impinger or cyclone (as appropriate); see text.
§Particles washed from surface, or glycerol/gelatin or other soluble medium used; see text.

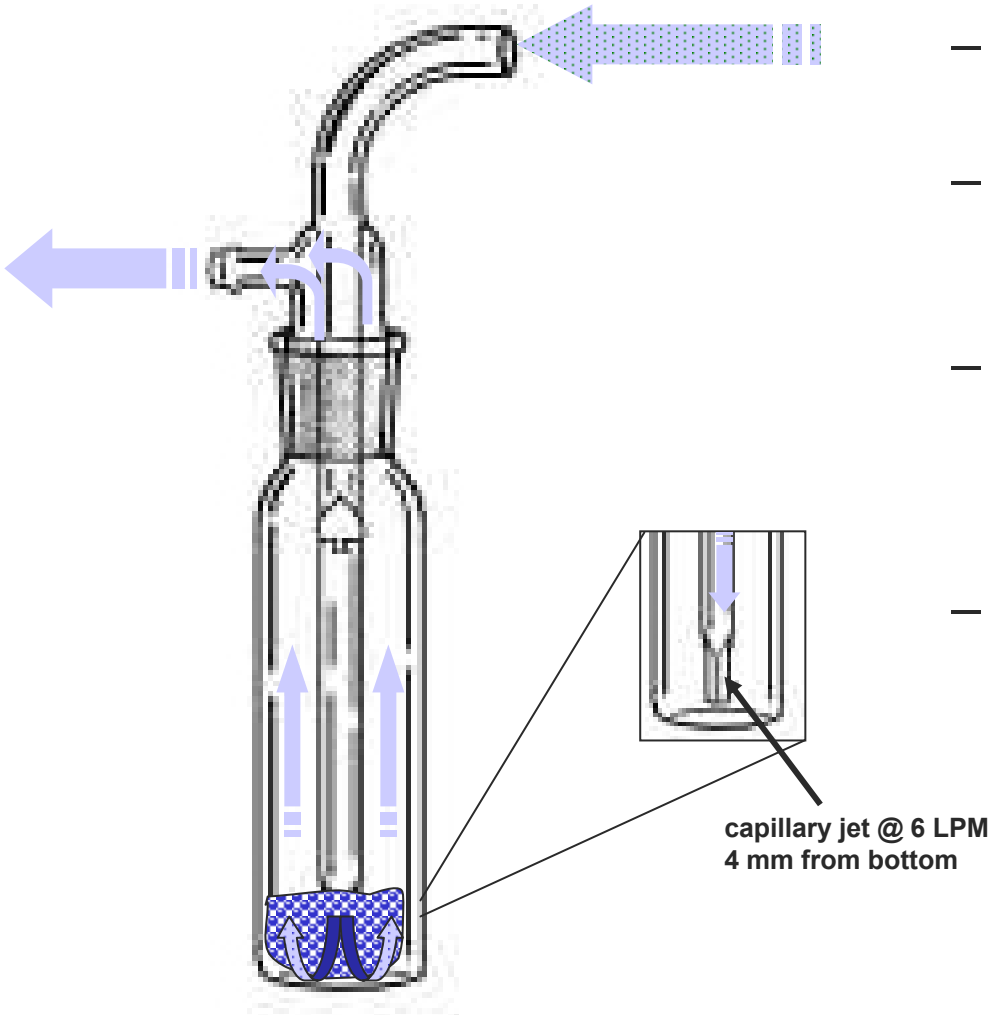
From: Chatigny, M.A. et al. Chapter J: Sampling Airborne Microorganisms and Aeroallergans. In: Air sampling Instruments, ACGIH, Cincinnati,OH.

TABLE J-1. Samplers Recommended for Collecting Viable Microbiological Aerosols and Aeroallergens (see Chapter P and Q for further details)

Sampler[C]	Operation	Sampling Rate (L/min)	Recommended Sampling Time for Viable Recovery (min)	Manufacturer/ Supplier[A] Descriptions[B] in Other Chapters	Applications and Remarks
1. Slit or slit-to agar impactor (a, b — some models)	Impaction onto agar in a 10-cm or a 15-cm plate on a rotating surface	30–700	1–60, depending on model and sampling situation	NBR[A] (P-50)[B] CAS (P-49)	Provides information on aerosol concentration over time. Available with a single or with multiple slits and variable rotation speeds. Bulky; AC operation.
2. Sieve impactors:					
a. single-stage, portable impactor (b)	Impaction onto agar in a "rodac" plate	90 or 180	0.5–5	SPR (P-53)	Portable, useful for making preliminary estimates of aerosol concentrations. Flow rate is not easily checked. Approximately 40% as efficient as the slit impactor.
b. single stage (N-6) impactor (a, c)	Impaction onto agar in a 10-m plate	28	1–30	AND (P-46)	Approximately as efficient as the slit impactor. Bulky; AC operation.
c. two-stage impactors (a, c)	See 2b above	28	1–30	AND (P-47)	See 2b above. Divides samples into respirable and nonrespirable fractions.
d. four-stage and six-stage impactors (a, c)	See 2b above	28	1–30	AND (P-48)	See 2b above. Provides information on particle size distribution.
e. personal cascade impactor (a)	Impaction onto filters or onto media in a special tray; see text	2	≤ 60 with filters, 5–30	AND (P-15)	Eight stages available. For viable recovery, sampler is useful only in highly contaminated environments.
3. Centrifugal sampler (b)	Impaction onto agar in plastic strips	40±	0.5	BDC (P-54)	Sampler is small, portable, and useful for making preliminary estimates of aerosol concentration. Flow rate is not easily checked. Does not collect particle below 3 μm efficiently.
4. Impingers:					
a. All-glass impinger/ AGI-30 (a, c)	Impingement into liquid, jet 30 mm above impaction surface	12.5	1–30	AGI (P-55)	Cells on or in larger particles are broken apart. Suitable for viral particle collection.
b. All-glass impinger/ AGI-4 (a, c)	See 4a above; jet 4 mm above impaction surface	12.5	1–30	AGI (P-55)	See 4a above. More vigorous impaction than 4a above.
c. Personal impinger (a)	See 4a above	1.5	5–15	DAC (P-43)	See 4a above. Provides information on personal exposures. Useful in highly contaminated areas.
d. Multistage impinger (a)	See 4a above	55	1–30	DIX	Provides information on particle size distribution. Three stages with cut points of ≥ 7, ≥ 3, and ≥ 1 μm. Limited availability.
5. Filters:					
a. Cassette filters (a)	Filtration	1–2	5–60	GEL, MFC, NUC; also see Table OI-1 and Chap. K and O	Some viable loss of microorganisms due to dessication. Samplers are easily portable, inexpensive, and can be used for personal monitoring. Useful for collecting large amounts of aeroallergens.
b. High-volume filters (a)	Filtration	140–1400	5–60		

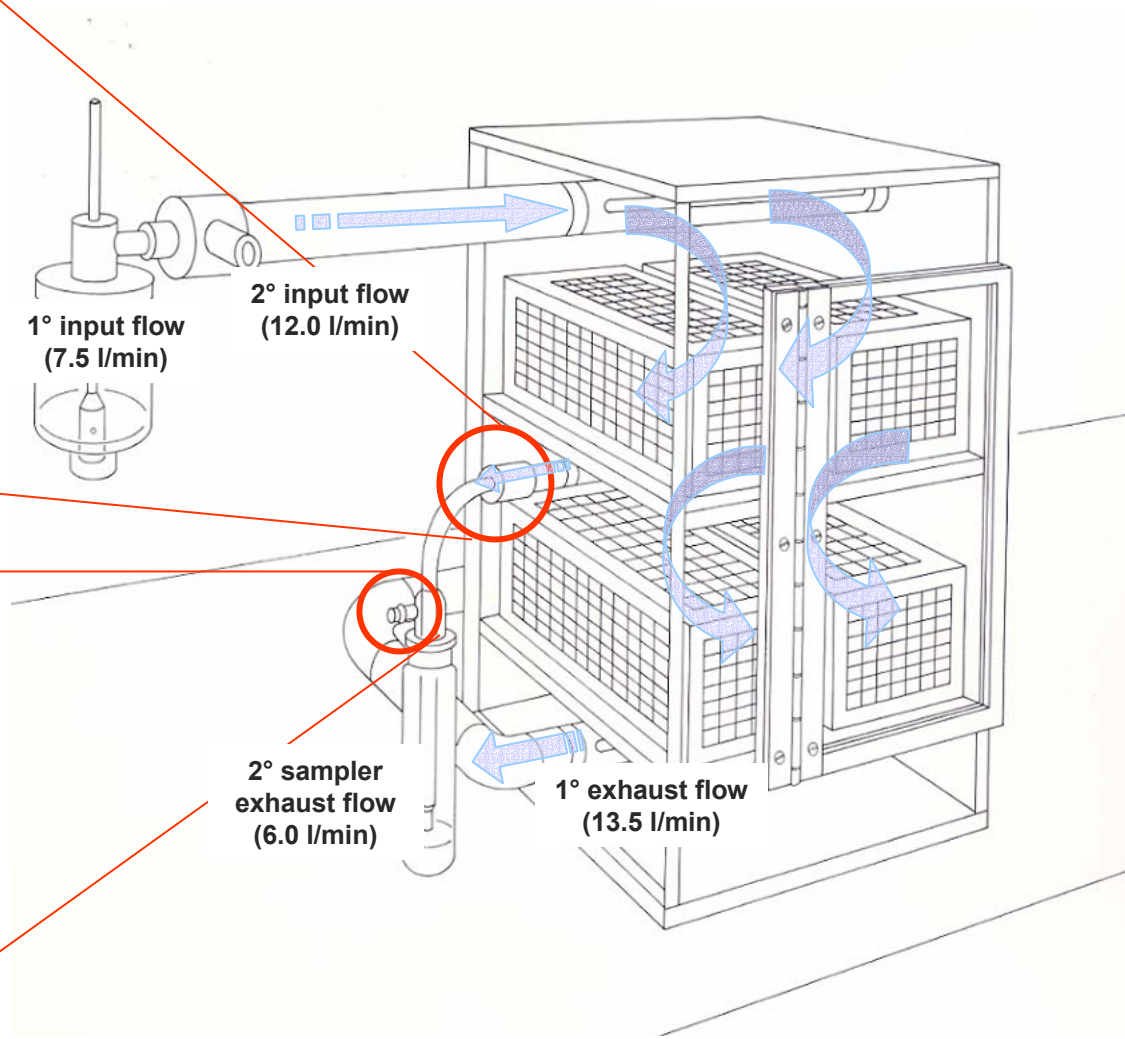
From: Chatigny, M.A. et al. Chapter J: Sampling Airborne Microorganisms and Aeroallergans. In: Air sampling Instruments, ACGIH, Cincinnati,OH.

All glass impinger



- Impingers
  - Operate similar to impactors
    - V can reach 60 m/s
  - Originally designed for dust counting; now standard in collection of microbial aerosols
  - Collects by both wetted bottom surface in collection vessel and bubbling action caused by flow
  - Effective for particles between 1 and 20 μm
    - Lower size dependant on stokes number
    - Upper size (greater than 20 μm) cannot follow air stream into impinger

Experimental set-up – modified impinger with whole-body exposure chamber

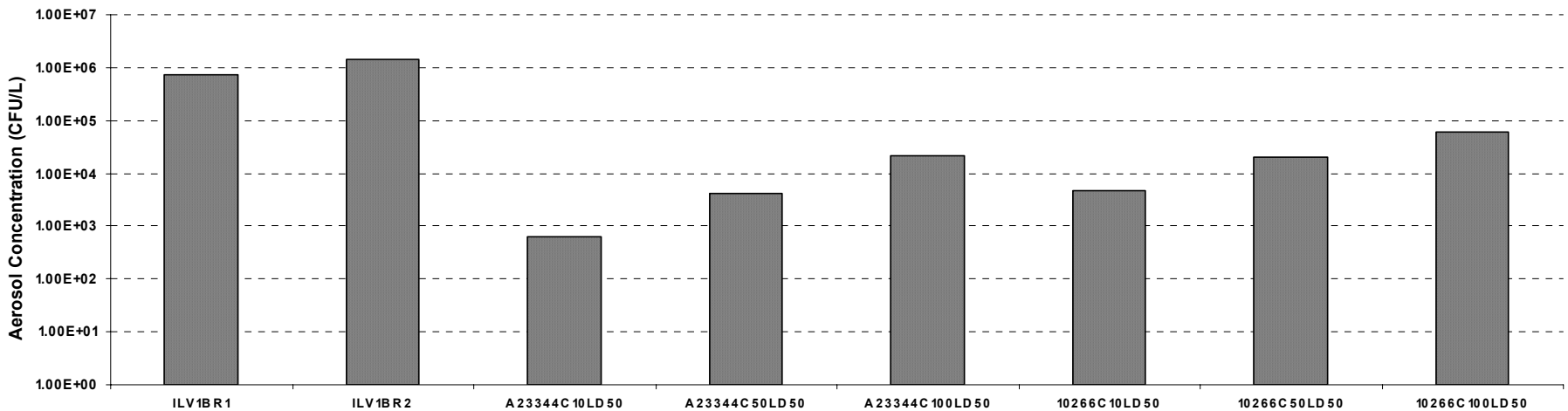


establishing aerosol concentration

Calculating aerosol concentration
From liquid impinger sample

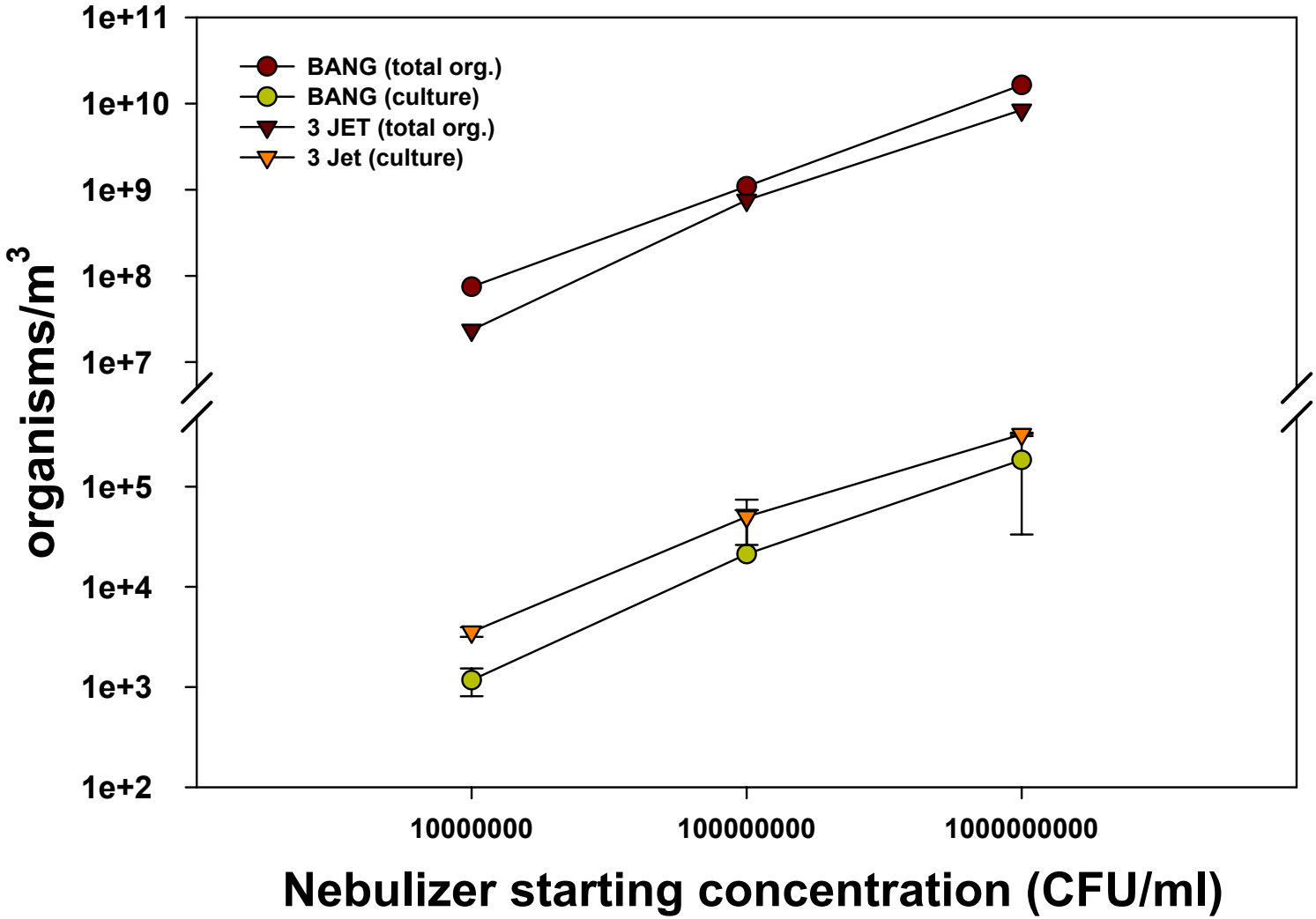
$$C = \frac{C_{agi} \times V_{agi}}{Q_{agi} \times t_{\exp}}$$

	nebulizer		AGI				
	C_A	SC	VS	C_S	T_S	F_S	AC
Group #	nebulizer conc	nebulizer conc	AGI volume	AGI conc.	sample time	AGI flow	aerosol conc
	(cfu/ml)	(cfu/l)	(ml)	(cfu/ml)	(min)	(l/min)	(cfu/l)
	D11*C11	E11*1000	VS=10[1]	G11*H11	= T_E= 10[1]	= 6[1]	(I11*J11)/ (K11*L11)
ILV1BR1	1.1E+10	1.10E+13	8.5	5.10E+06	10	6	7.23E+05
ILV1BR2	1.1E+10	1.10E+13	8.5	1.00E+07	10	6	1.42E+06
A23344C10LD50	2.0E+08	2.00E+11	8.5	4.50E+03	10	6	6.38E+02
A23344C50LD50	1.0E+09	1.00E+12	8.5	3.00E+04	10	6	4.25E+03
A23344C100LD50	2.0E+09	2.00E+12	8.5	1.50E+05	10	6	2.13E+04
10266C10LD50	3.5E+06	3.50E+09	8.5	3.30E+04	10	6	4.68E+03
10266C50LD50	6.9E+06	6.90E+09	8.5	1.40E+05	10	6	1.98E+04
10266C100LD50	3.5E+07	3.50E+10	8.5	4.30E+05	10	6	6.09E+04

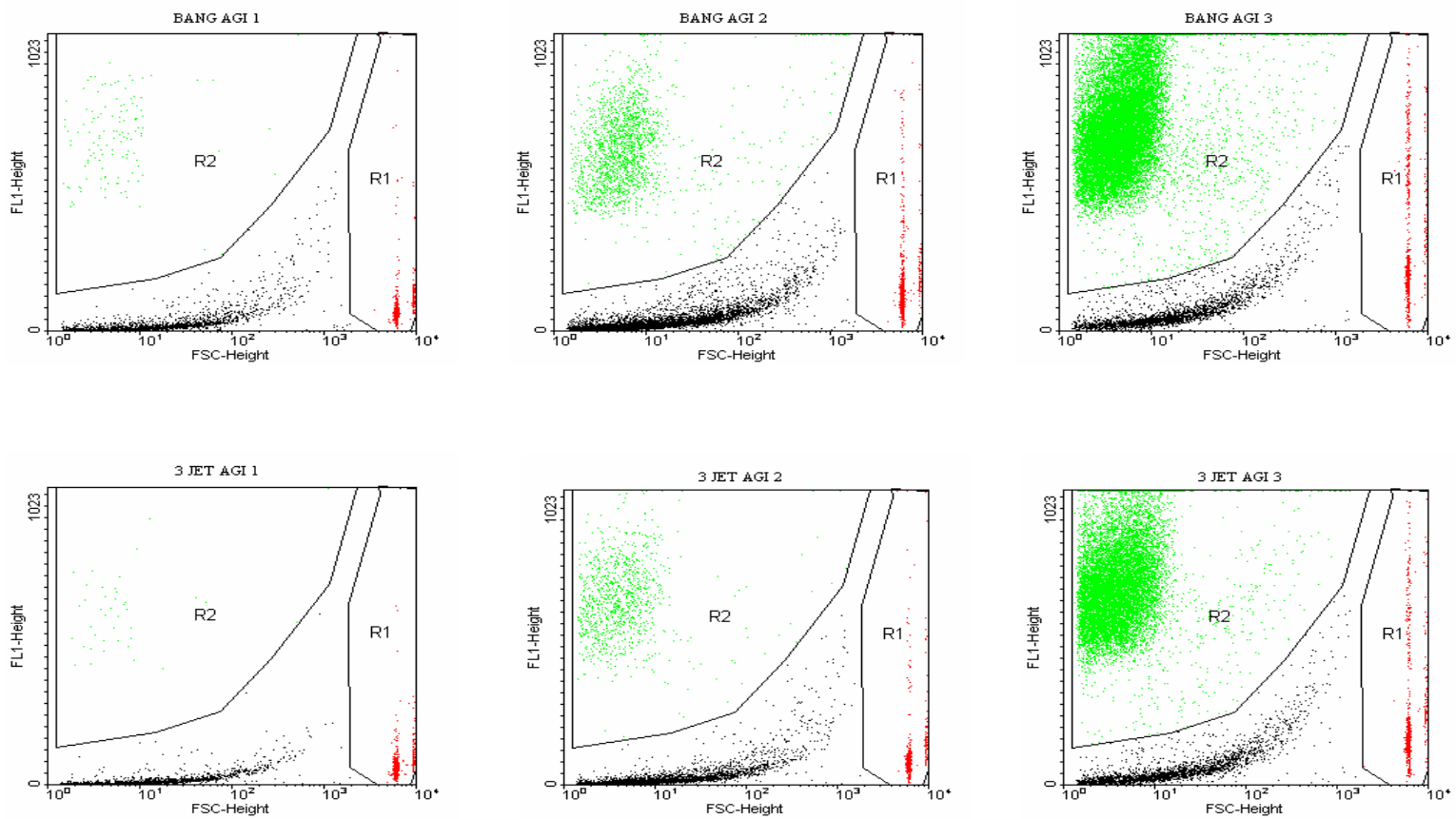


Differential Bioaerosol Viability

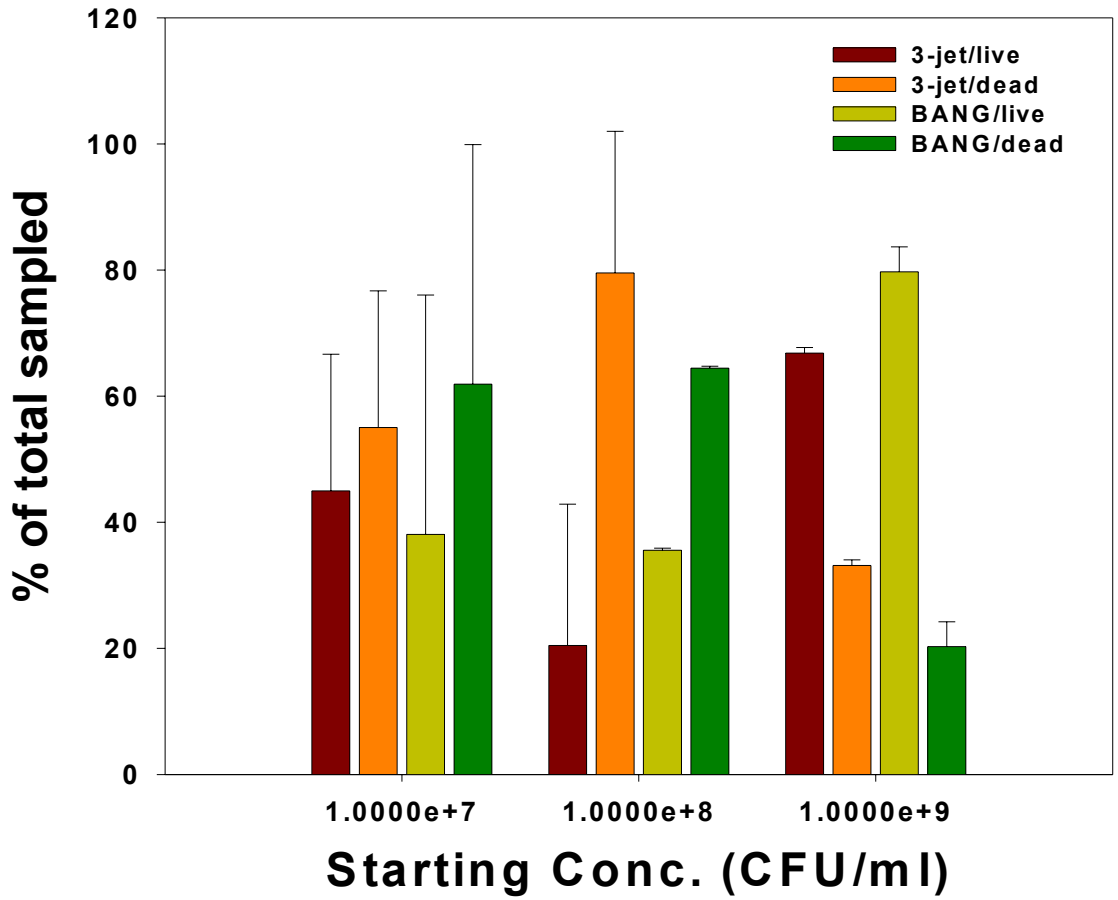
- *Rationale*
  - In animal aerosol challenge, is an “inhaled dose” the true dose?
    - How does aerosolization effect
      - viability
      - infectivity
- *Objective*
    - assess the impact of nebulization comparing using two different nebulizers
- *Aerosols*
  - *Pseudomonas aeruginosa* (ATCC, Rockville, MD)
- *Nebulizers: Collison v. BANG*
- *Characterization & sampling*
  - particle sizing (APS Model 3320; APS 3375 (UV/APS))
  - Continuous sampling of chamber by AGI
  - Culture/TSA
    - analysis by flow cytometry
      - bacterial counting kit (Molecular Probes)
      - Live/dead viability kit (MP)



Counts of total and culturable bacteria. Data collected by flow cytometry and cultured bacteria from the AGI samples collected during the spray show that there is no significant difference between the BANG and 3 jet collison.



Dot plot graphs showing the changes in bacteria total counts in the 3 jet collison and BANG. The AGI samples were analyzed by the bacteria counting kit. The six dot plots above show the increase in total bacteria counted as the starting concentration increases. The 3 jet collison and BANG show similar counts.



Percentage of bacteria sampled shows the live/dead of the BANG and 3 Jet collison. Comparison of the live/dead to the concentration of starting solution show that there is a slight increase in live bacteria when a more concentrated spray agent is used.

Particle Size

- In most cases size cannot be directly measured
- Particle size must be determined from measurement of a behavior or property that is a function of size
- Equivalent diameter: diameter of a sphere having the same value of a physical property as the particle being measured

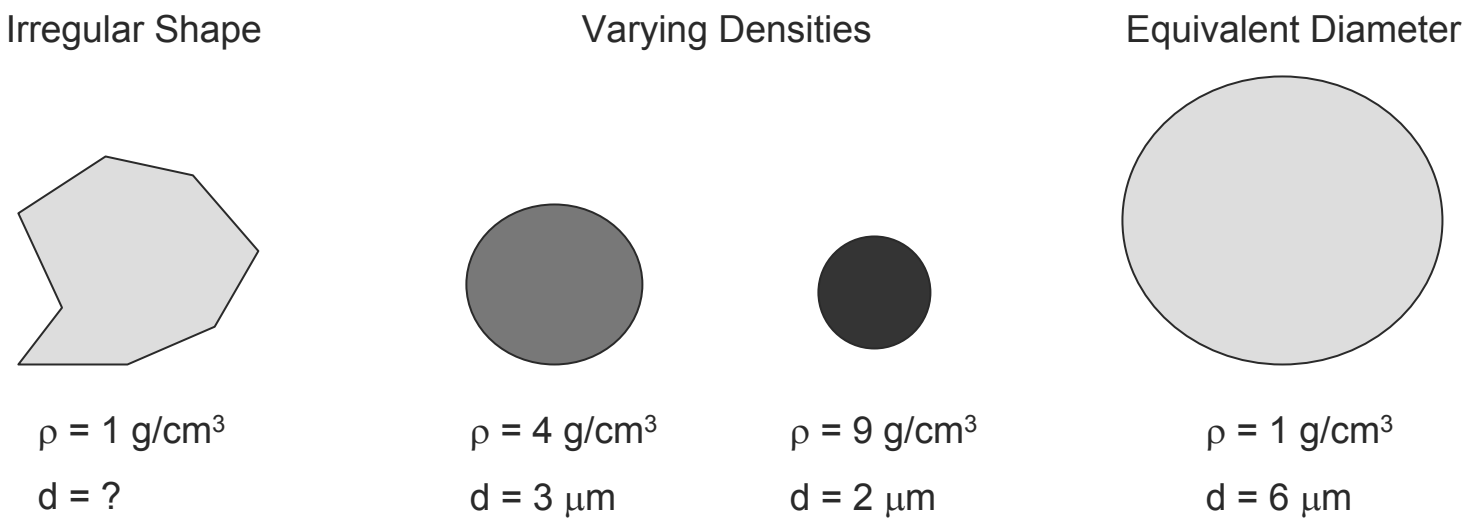
Particle Size

- In most cases size cannot be directly measured
- Particle size must be determined from measurement of a behavior or property that is a function of size
- Equivalent diameter: diameter of a sphere having the same value of a physical property as the particle being measured

Property or Behavior	Equivalent Diameter
Brownian Motion	Diffusion
Gravity	Aerodynamic
Inertia	Aerodynamic
Light Scattering	Optical

Biological Aerosol Size

- Use equivalent diameter that derives from particle property relevant to bioaerosol exposures
  - Mechanism of deposition
  - Particle size
- Aerodynamic diameter: diameter of a unit-density sphere having the same gravitational settling velocity as the particle being measured



Size Distribution

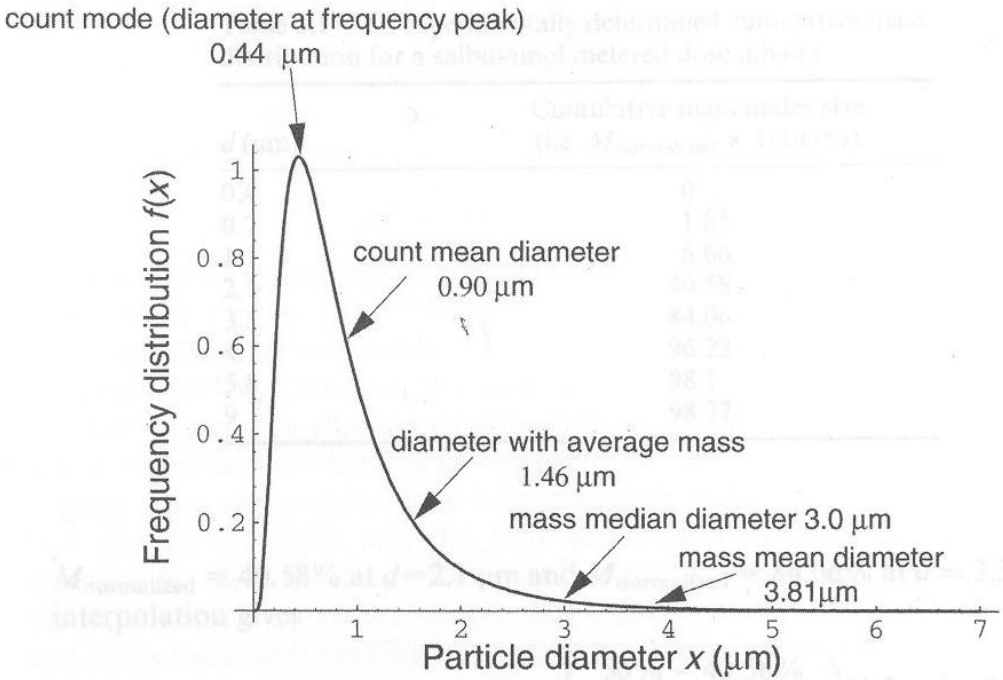


Fig. 2.2 The frequency distribution $f(x)$ is shown for a log-normal distribution with mass median diameter of 3.0 μm and geometric standard deviation $\sigma_g = 2.0$, along with the values of various other diameter definitions.

Figure from Warren H. Finlay, *The Mechanics of Inhaled Pharmaceutical Aerosols*, Academic Press, 2001

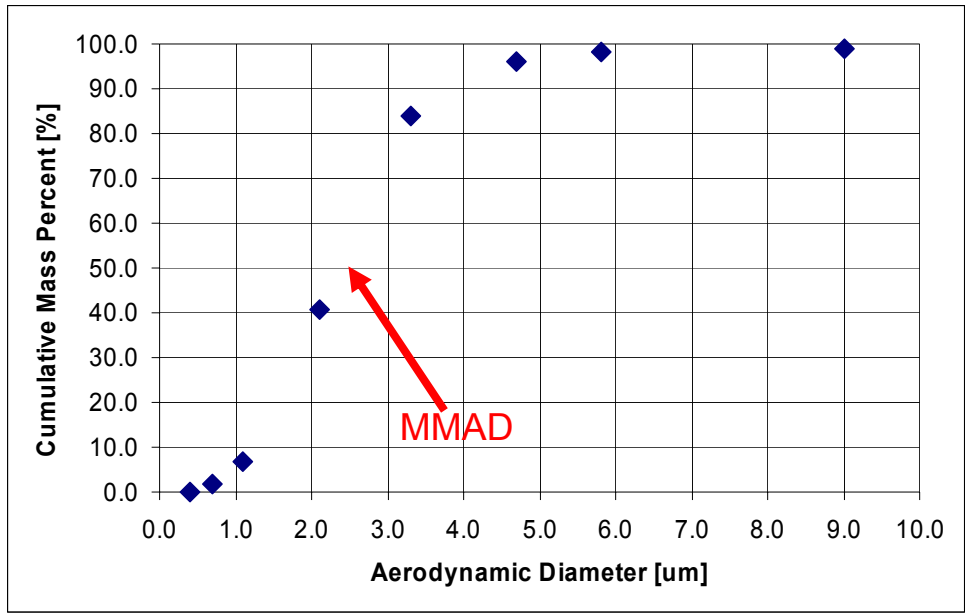
MMAD

- Mass median aerodynamic diameter: aerodynamic diameter such that half the cumulative mass of all particles is contained in particles with smaller (or larger) diameters
- $M_{Normalized}(MMAD)=1/2$
- Most directly measured with cascade impactor
- Geometric standard deviation for log-normal distributions:
  - $\sigma_g = MMAD/d_{16} = d_{84}/MMAD$
- MMAD and σ_g describe aerosol distribution for bioaerosol studies

Example

d [um]	Cum %
0.4	0.0
0.7	1.9
1.1	6.7
2.1	40.6
3.3	84.1
4.7	96.2
5.8	98.1
9.0	98.8

Experimentally determined cumulative mass distribution for a salbutamol metered dose inhaler.

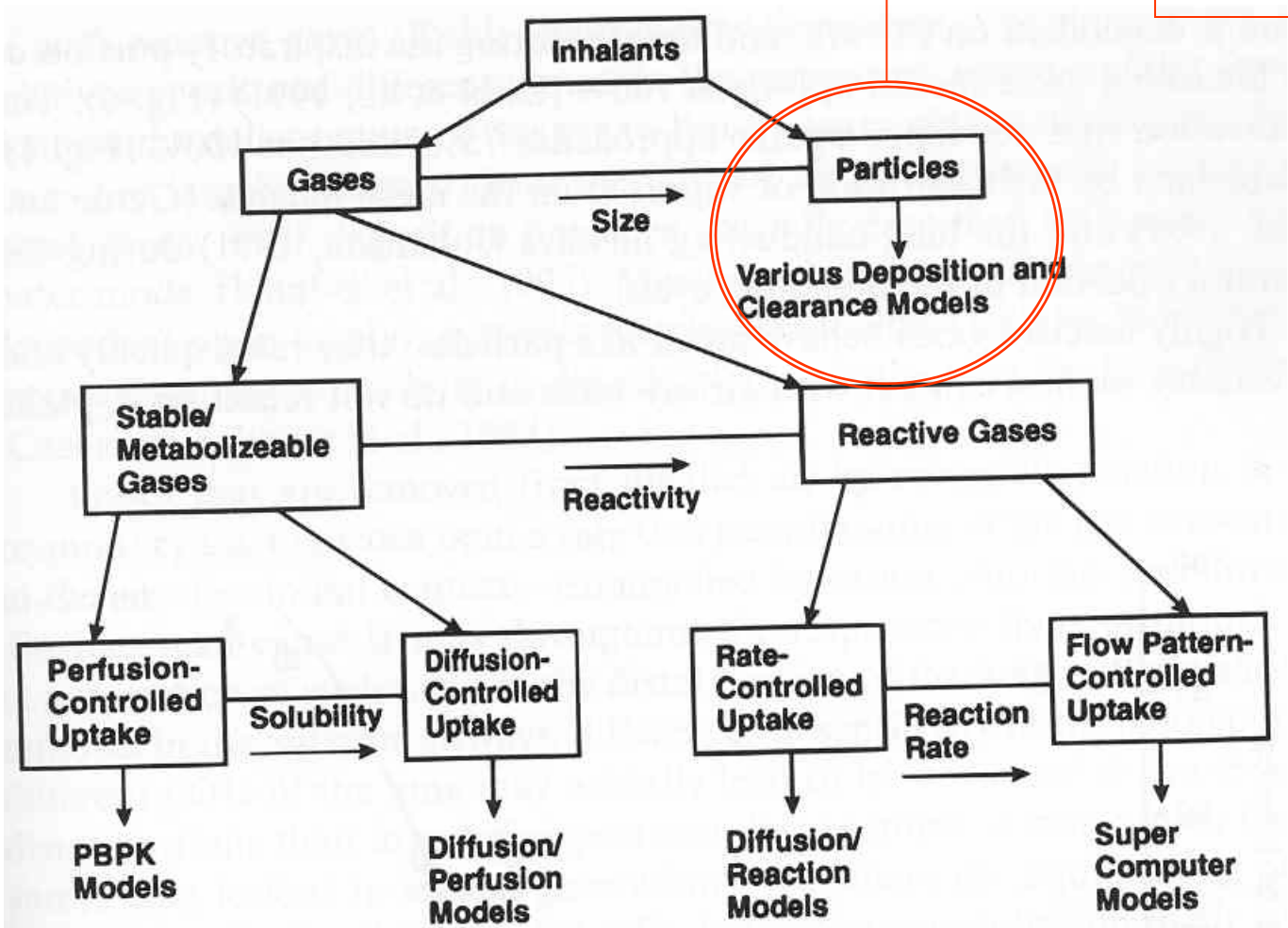


MMAD = 2.4 μm

$\sigma_g = d_{84}/MMAD = 3.3\mu m/2.4\mu m = 1.4$

Data from Warren H. Finlay, *The Mechanics of Inhaled Pharmaceutical Aerosols*, Academic Press, 2001

bioaerosols



From Scheslinger, R. In: Inhalation Toxicology

Factors Affecting Particle Deposition

- Five important mechanisms
  1) Inertial impaction
  2) Sedimentation
  3) Diffusion
  4) electrical charge
  5) Interception

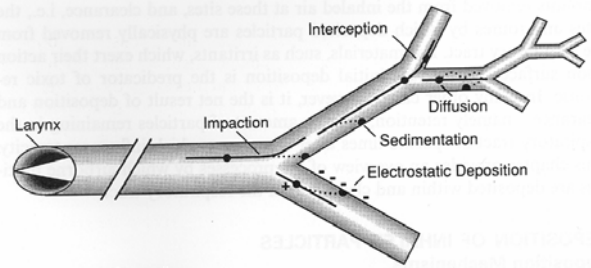


Figure 1 Schematic diagram of particle deposition mechanisms.

- Particle characteristics
  - Aerodynamics (size, shape, distribution, hydroscopicity, charge)
  - Respiratory anatomy
    - Ventilation
    - Breathing pattern (modality,flow rate/velocities)
  - Other
    - Airway reactivity, preexisting disease, age, gender

What do we really know about D&R of threat agents?

Respiratory deposition is well-defined for particles; but not for infectious agents.

Available comparative path. data with respect to aerosol size?

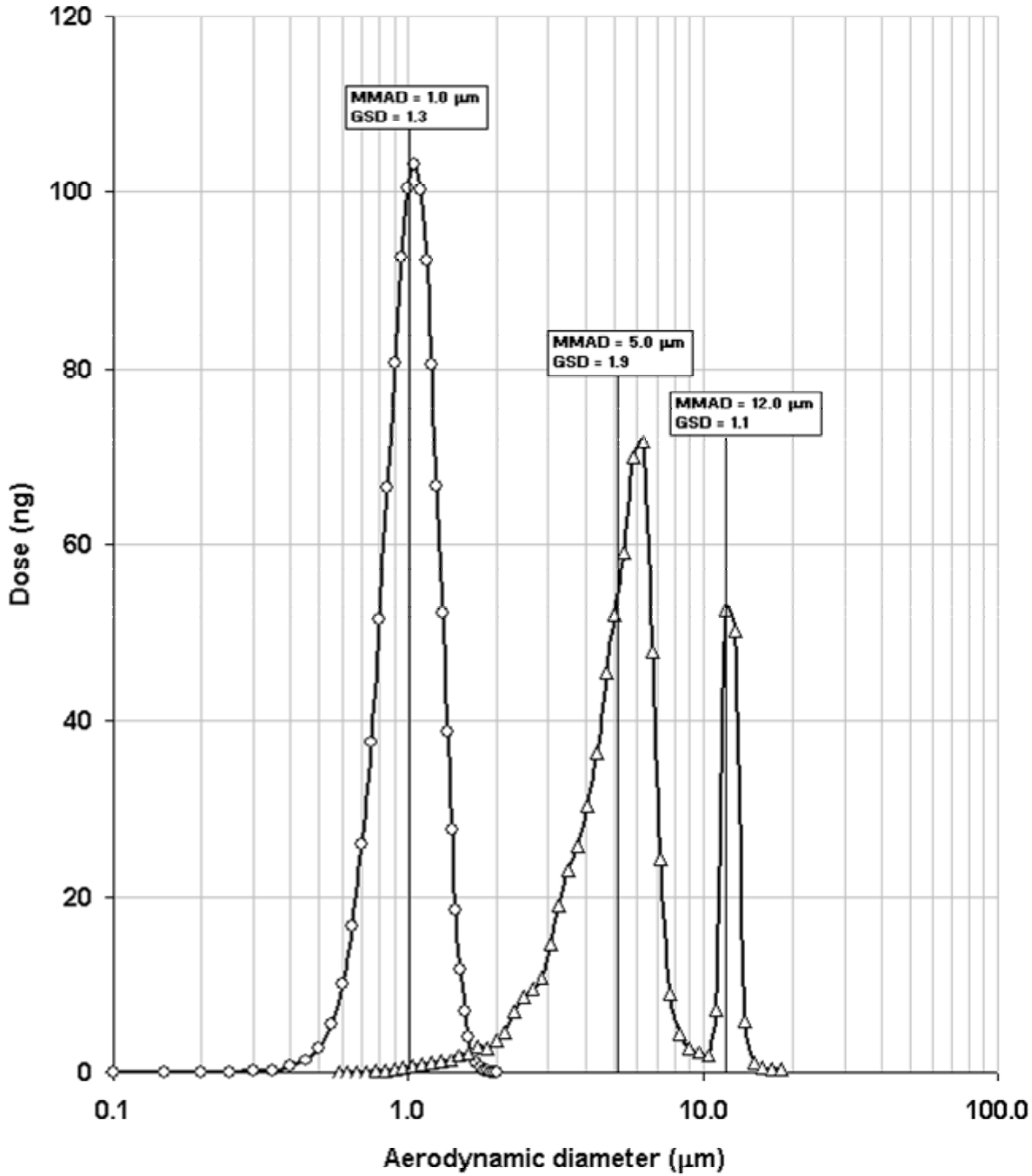
Comparative dose/infectivity

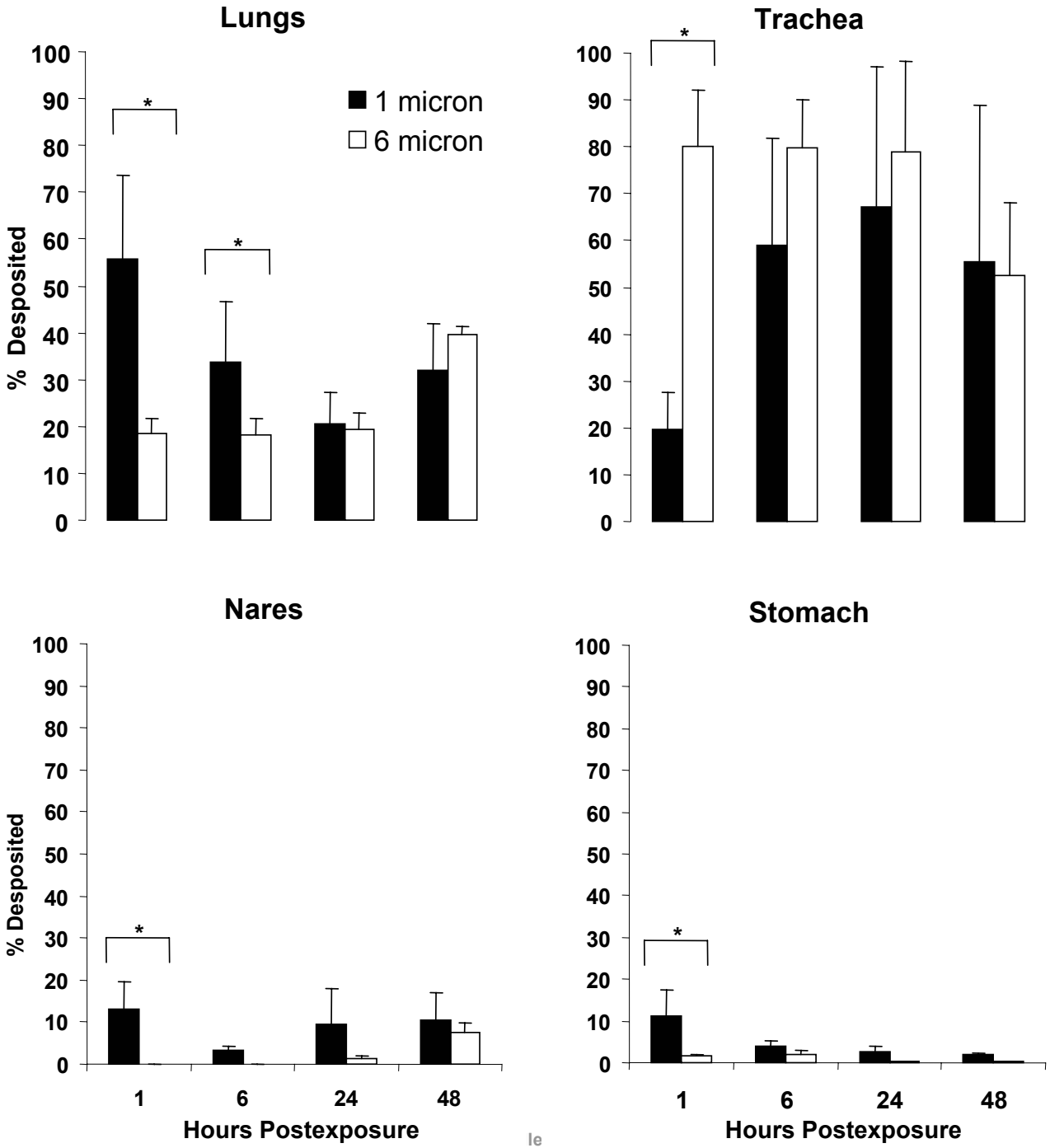
- Comparison of ± 1 μm v. 12 μm aerosols in guinea pigs (Druett et al., 1954)
  - *Yersinia pestis*
    - LCt_{50} - 2.5 X less infectious
  - *Bacillus anthracis*
    - LCt_{50} - 17X less infectious
- Ongoing (comparison of 1μm v. >3 μm) (Roy et al., 2003)
  - Toxin
    - Diff. LD_{50} at diff. aerosol sizes
    - Differences in deposition in respiratory tract
    - impact on pathogenesis
  - Virus
    - No major differences in LD_{50} and MTD regardless of aerosol size
    - Across two species

Aerodynamic size characterization of ricin aerosols generated by the collison nebulizer (circles) and the STAG (triangles) at a corresponding estimated inhaled dose of 55 and 36 μg/kg, respectively.

The bimodal distribution of the STAG is attributed to satellite aerosol formation (first peak of the STAG) in the primary generation process, which composes 85% of the cumulative mass of the output from this device.

(Roy et al., 2003)





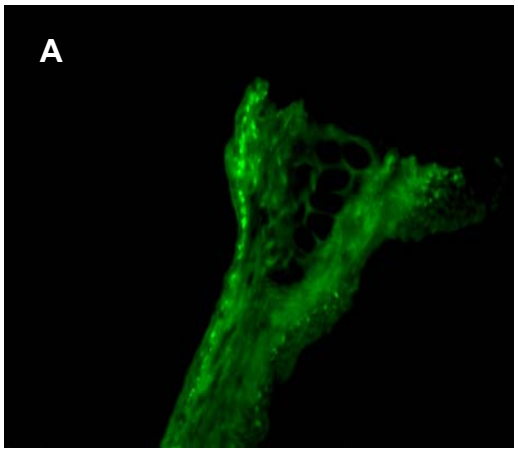
Agent in selected tissues of mice as a percentage of total body dose.

Values are group means; error bars represent standard error

Significantly different values at $p<0.01$ are denoted by an asterisk

horizontal brackets indicate the comparative groups

Roy *et al.*, 2003



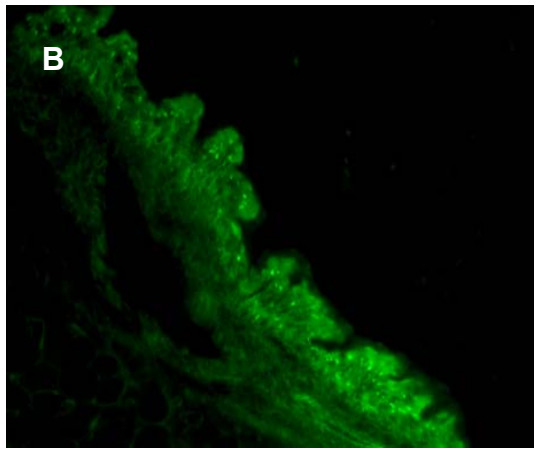
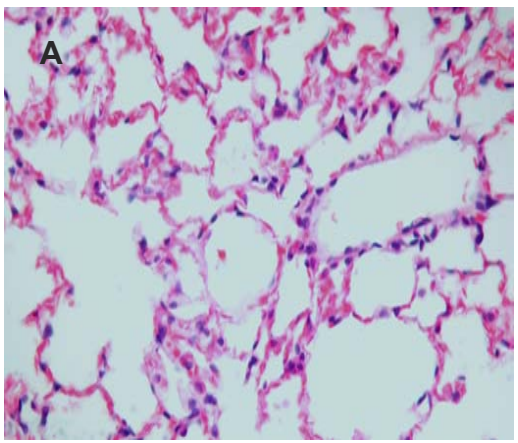


Figure 5 (A and B). Nasal turbinates (A) and olfactory epithelium (B) of a mouse exposed to 5 μm aerosols by whole-body chamber configuration. Epifluorescent particles localized to the olfactory epithelium in the turbinates (A; 40X) whereas particles are localized to all levels of the olfactory epithelium (B; 100X).



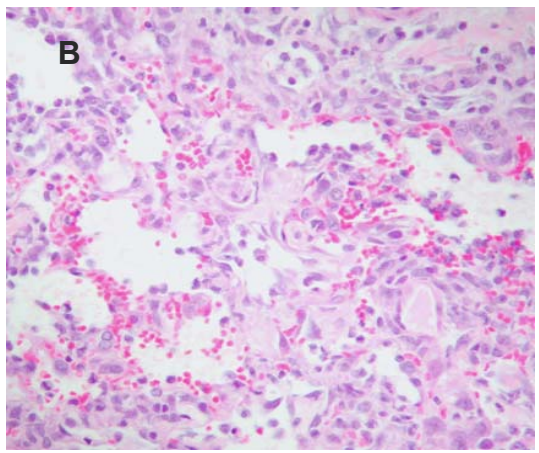


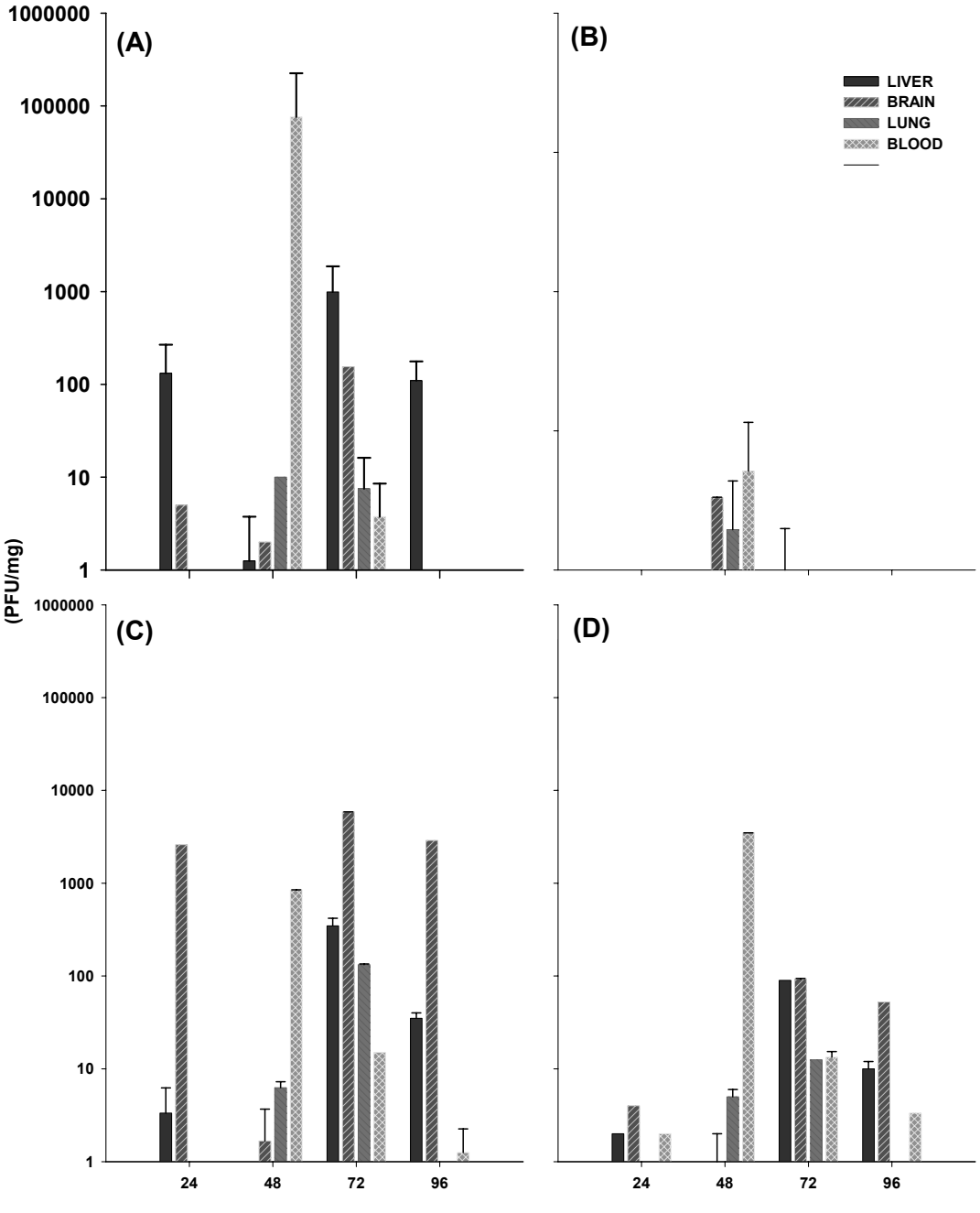
Figure 6 (A and B). Lung section of mouse exposed to 5 μm aerosols (A; 200X) or 1 μm particles (B; 400X). The lungs of the mouse exposed to the nonrespirable aerosol (A) shows no significant lesions. The lung of the mouse exposed to a respirable aerosol (B) indicates marked interstitial pneumonia with alveolar edema, fibrin and hemorrhage.

Roy *et al*., 2003

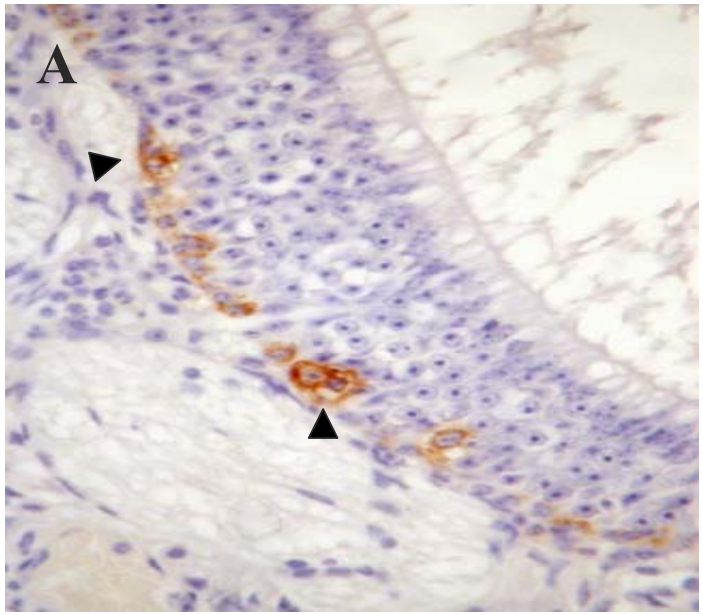
Table 1. Particle size and viral strain specific 50% lethal dose determination, by species

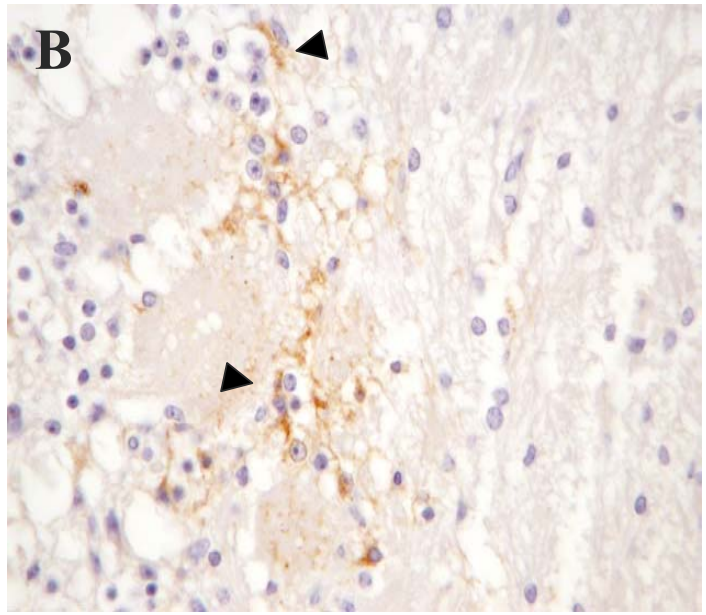
Species	Strain	Particle (μm)	LD_{50} (PFU)	95% fiducial limits		MTD[c] (days)
				lower	upper	
guinea pig *Hartley*	X	1	5.21E+03	2.93E+03	2.35E+05	5.0
		>3[b]	5.90E+04	8.70E+03	4.65E+11	5.0
	Y	1	1.08E+04	3.5E+03	8.93E+05	5.0
		>3	9.57E+03	-[a]	-	5.0
mouse *BALB/c*	X	1	4.61E+02	1.86E+02	1.66E+03	4.8
		>3	1.60E+04	-	-	4.6
	Y	1	6.16E+02	-	-	4.7
		>3	2.85E+03	1.07E+03	1.11E+04	5.0

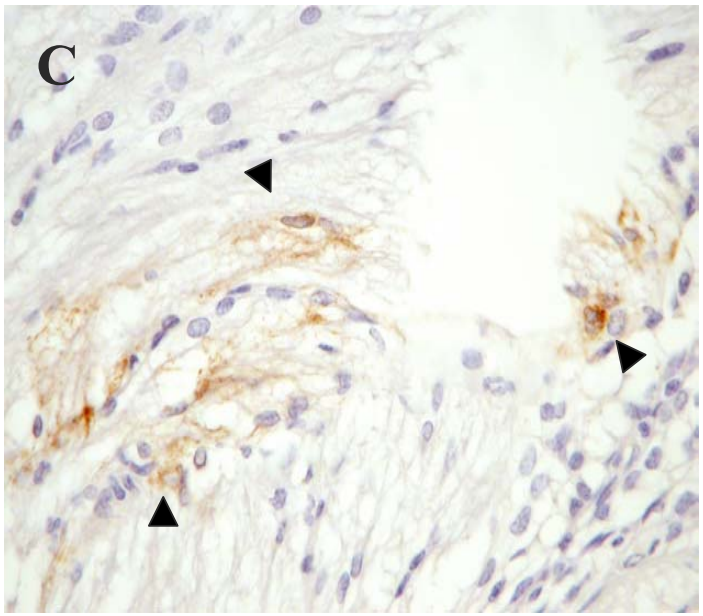
[a] the confidence limits were not determined due to lack of mortality in selected groups
[b] considered 'larger' particle distribution due to bimodal size distribution
[c] mean time to death



Viral loading in selected tissues when exposed to representative strains aerosolized at different particle size distributions. (A) and (B) graphs represent results from larger particle (<3 μm) exposures with Strain X and Y, respectively; (C) and (D) graphs represent results from respirable particles (≈1 μm) exposures with Strain X and Y, respectively. Bars represent group means; error bars represent standard deviation.







Guinea pig olfactory neuroepithelium (a), bulb (b) and nerve (c) 24 hours post exposure to aerosolized virus. Arrowheads indicate antigen reactivity.