Aerosol Particle Size and Infectivity

- Today's presentation will describe two important variables and how they interact to cause infections in primary aerosols

- Particle Size and Agent Concentration

- Much of the data to be presented are derived from the extensive studies of Dr. William C. Day, Experimental Aerobiology Division, Former U.S. Offensive BW Program.

- I had the privilege of working with Bill Day in that he requested my division, Product Development, to supply him with unique liquid and dry agents.

- Bill Day made an extensive survey of particle size in the scientific literature while he was receiving his many immunizations around 1953.

- He found that lots of information was available on particle size in many different environments…office buildings, hospital wards, operating rooms, dental offices and even sewage disposal plants.

- These extensive studies indicated that in ambient air, the average particle size that contained viable organisms was <u>12 to 13 microns</u>, MMD.

- **Only a small fraction of small particles, less than 5 microns, was found in the ambient air.**

  - **and those particles less than 5 microns contained only a few viable organisms.**

- **From these studies it could be inferred that MOTHER NATURE does not usually create small particle highly infectious aerosols.**

- **If she did, perhaps we would not have survived as a species.**

- **It is the artificial manipulation of agents to create small particle infectious aerosols that should cause real concern.**

- **Mother Nature simply does not effectively address those laboratory procedures and protocols found in the laboratory ...**

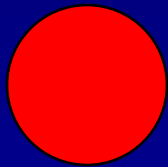**Blending   Centrifugation**

Manipulation of small particle dried agent powders

- **It is these types of laboratory operations that produce the majority of infections via the respiratory tract.**
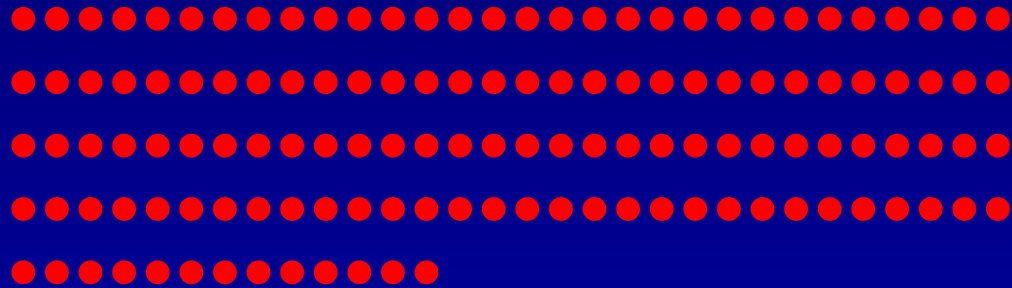
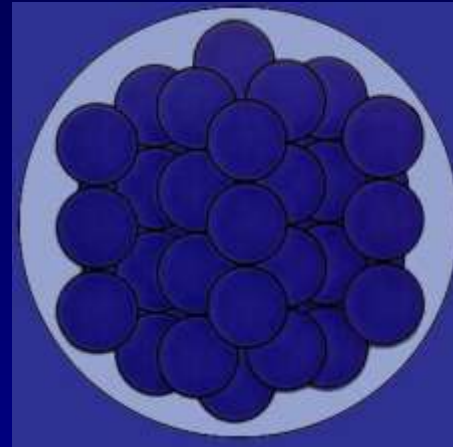# Particle Size: Microns, Mass Median Diameter

5μ   1μ
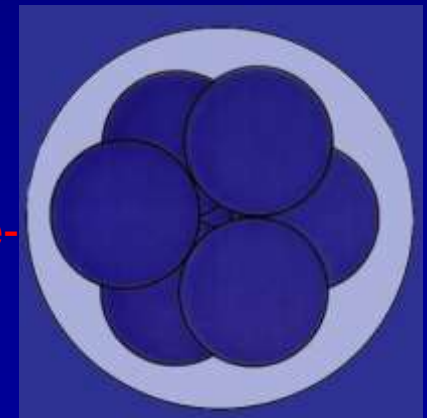
Size *DOES* Matter

- For successful weaponization, agent that can be disseminated into small particle aerosol must be developed

53 one-micron spheres in a five-micron sphere

- More efficient to place 53 one-micron particles in a 5 micron aerosol particulate than 15 two-micron particles in the five micron particulate

15 two-micron spheres in a five-micron sphere

Influence of Particle Size on Respiratory Virulence of 5 Agents to Guinea Pigs (LD$_{50}$)

Aerosol Particle Size (Microns)	*Bacillus anthracis*	*Francisella tularensis*	*Yersinia pestis*	Q Fever	VEE Virus
0.3 - 1.5	23,000	2.5	12,000	10^6	20
4.6 - 6.5	221,000	6,500	250,000	52×10^6	19,000
8.5 - 13	700,000	19,500	450,000	$>2 \times 10^6$	280,000

Particle Size and Infectivity

- **Information on how organisms behave during dissemination and as aerosol was sparse or fragmented in early years of U.S. Offensive Program**

  - **Scientists at then Camp Detrick invented science of "aerobiology"**

Particle Size and Infectivity
(continued)

- **Early aerosol studies frustrating**

  - **Exposure of animal models to infectious particles produced inconsistent results**

  - **Program did not advance until disseminators with sharp particle-size profiles selected**

Three Disseminators
Particle Size Distribution
### Particle Range (Microns)

Disseminator	1-1.9	2.0-5.3	5.4-10	10.5-15.0
Vaponefrin Nebulizer	5842	516	0	0
Collison Atomizer	4145	1266	0180	6
Spinning Disc	0	0	3432	180

Table 4: Relationship of Aerosol Particle Size Distribution to Respiratory LD$_{50}$ Values for Rhesus Monkeys Obtained with *P. tularensis*

Aerosol Particle Size (microns)	Aerosol Particle Diameters Defined in Microns											Monkey Respiratory LD$_{50}$ (cells)
	1.4	1.9	2.7	3.8	5.4	7.6	10.8	12.5	17.6	24.9	35.0	
1.0	52.2*	24.9	13.3	6.4	1.4	0.4	0.2	0.0	0.0	0.0	0.0	14
6.5	0.0	0.0	0.0	0.3	4.8	85.4	9.5	0.0	0.0	0.0	0.0	178
11.5	0.0	0.0	0.0	0.0	0.0	0.5	7.8	83.8	7.0	1.0	0.0	672
22.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	3.3	82.6	13.8	3447

Particle Stability
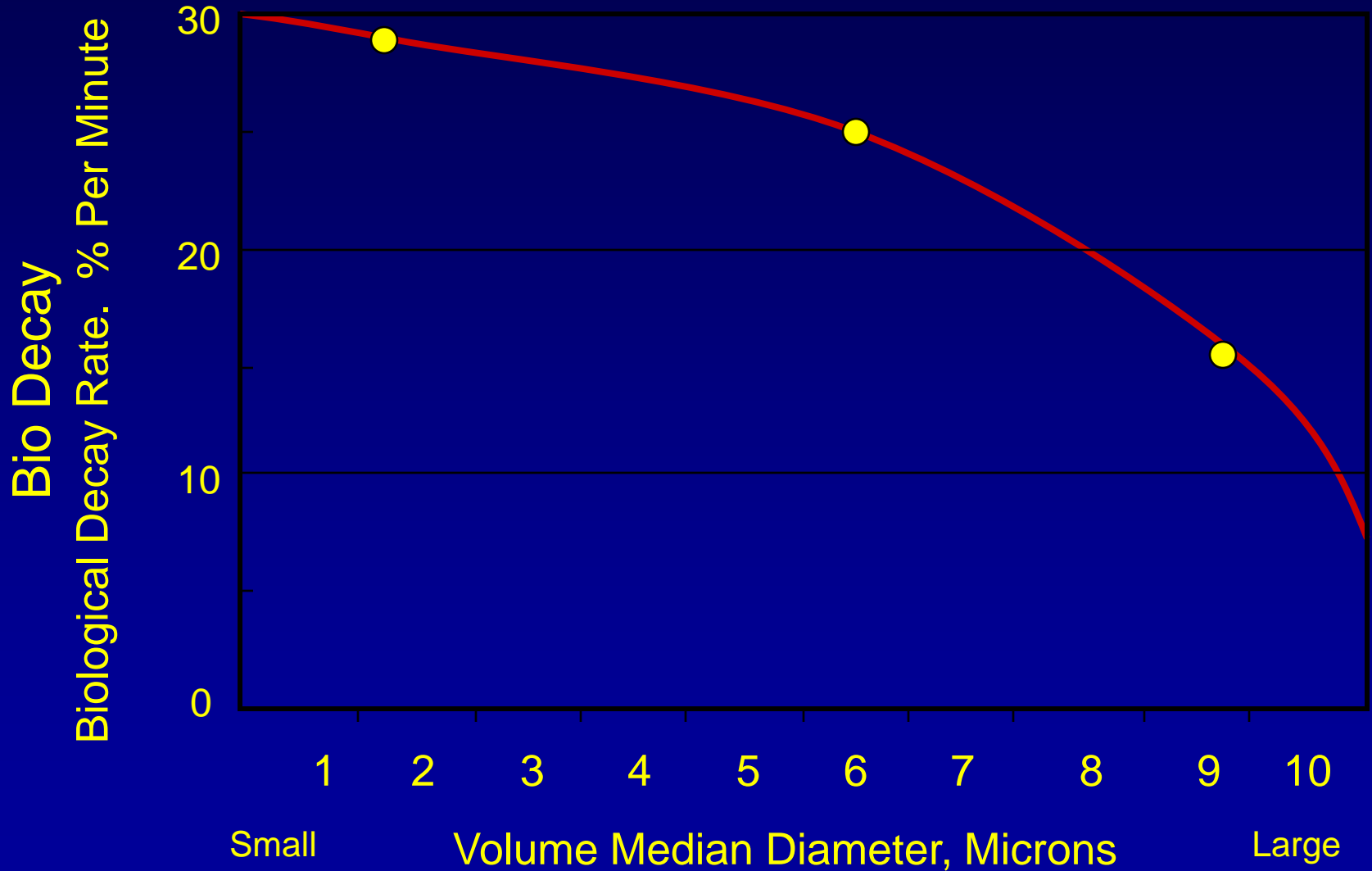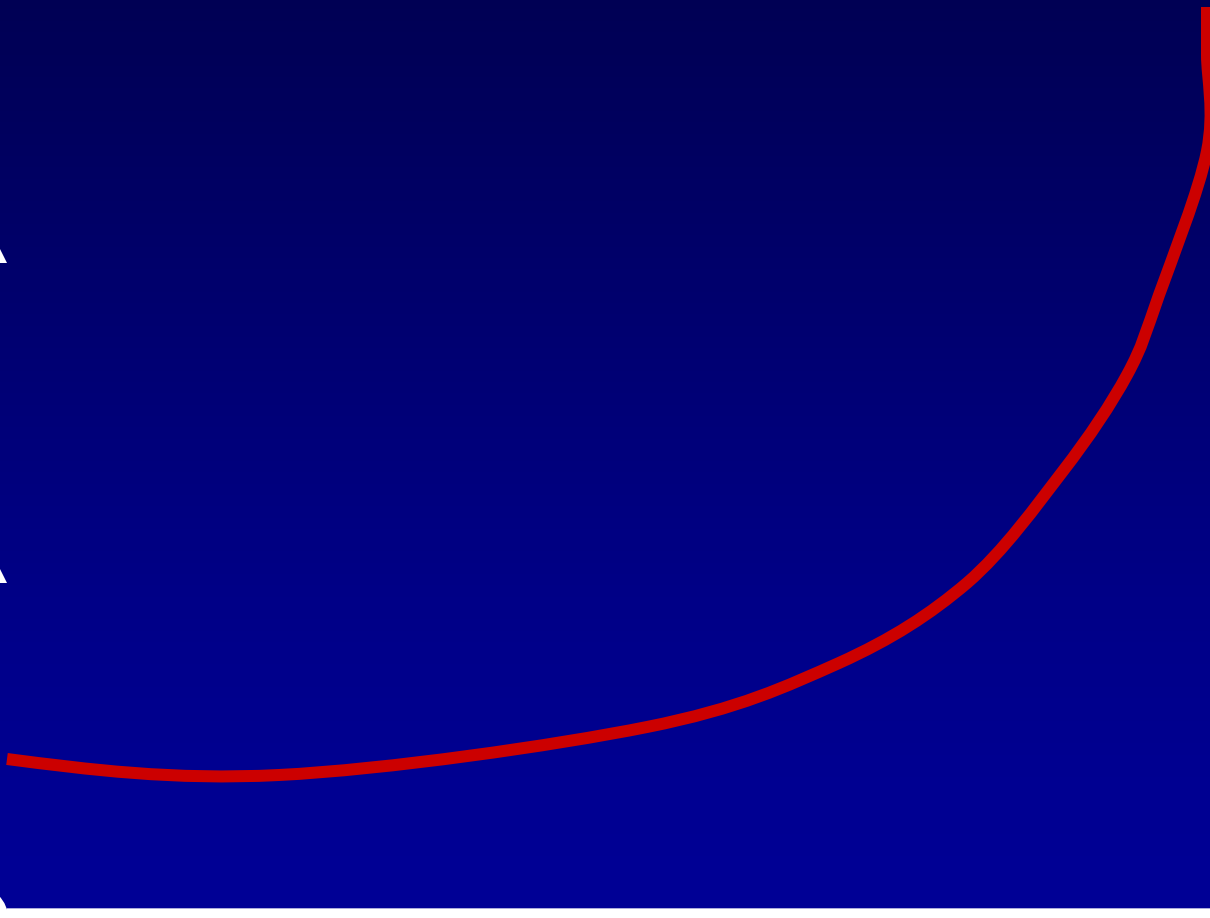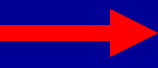
Dry SM: Particle Size, Viable Cells per Particle, Viable Cells per 1000 Particles

NMD μ	Cells per Particle	Viable Cells per Particle	Viable Cell Frequency/1000 Particles
0.8	1.8	0.001	0.5
1.3	4.2	0.01	2.6
3.0	18.0	0.2	15.6
6.5	73	2.5	38
11.5	195	7.7	14
16.0	350	11	6
23.0	670	16	3

Classical Experiment:
Man – Monkey – Guinea Pig:

Influence of Particle Size on Tularemia Infectivity

Aerosol Particle Diameter (microns)	Number of Tularemia Cells for:			
	Guinea Pig RLD_{50}	Monkey RLD_{50}	Man RID_{50} Mean	Range
1	2.5	14	15	10 – 52
6.5	4,700	178	88	14 – 162
11.5	23,000	672	130*	—
18	125,000	3447	10,000*	—
22	230,000	>8500	No Data	

* Data from Dr. Bill Sawyer

Influence of Aerosol Particle Size on Severity of Illness in Monkeys

Aerosol Particle Size (microns)	Number of Cells	Mean Day of Illness (Post Exposure)	Severity of Illness	Fever (°F)	Death
1	14	4	5+	105+	Yes
6.5	178	6	5+	104-105	Yes
11.5	672	9	3+	103-105	Yes/No
18	3447	15	2+	102-103	Maybe
22	>8500	22	1+	101-102	No

Volunteer Study with Tularemia: Severity of Infection

Number of Cells	Days Incubation (Post Exposure)	Fever (°F)	Percent Infected	Numerical Rating
26	4-5	103	86	4+
30	4-5	103	85	4+
38,000	3	105	100	5+
52,000	2	105	100	5+

Influence of Aerosol Particle Size on Development of Lung Lesions in Monkeys
(Time Following Exposure)

| Particle Size (microns)	Appearance of Lesions on Lungs (hours following exposure)
1	24 hours
8	48 hours
11.5	96 hours

Particle Size, Spore Concentration, Lung Retention: Anthrax / Guinea Pig

Aerosol Size (m)	Conc./ml $x10^8$	Calculated Inhaled Dose	Viable Spores Retained	Percent Retained
1	5	1×10^4	4×10^2	2.5
1	50	20×10^4	4×10^4	21
1	100	40×10^4	17×10^4	43
5	5	8×10^4	3×10^2	0.4
5	50	91×10^4	5×10^4	6
11	50	89×10^4	5×10^2	0.06
11	500	720×10^4	4×10^4	0.54

Influence of Aerosol Particle Size on: % Retention in Lower and Upper Respiratory Tracts; % Viability of SM; SM Population Density
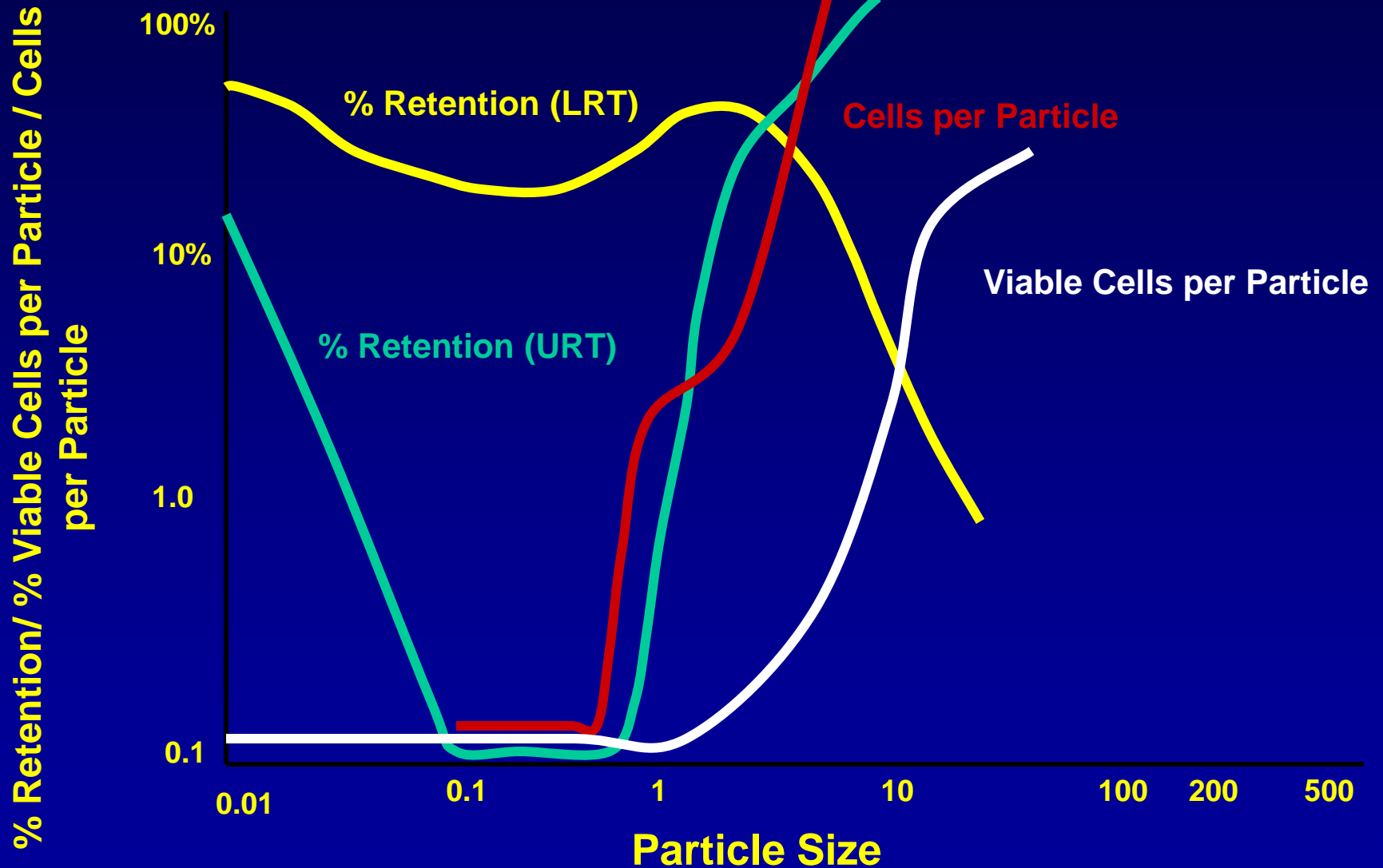
Anthrax Spores vs. Tularemia Cells in Aerosol

SPORES
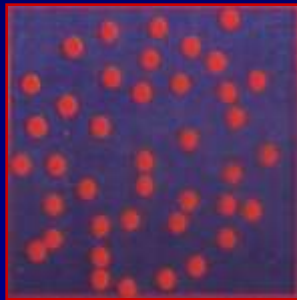
CELLS

Mean Respiratory Dose for Volunteers as a Function of Aerosol Age
(Liquid Tularemia Not Stabilized)

Post Dissemination

4 Min.	120 Min.	180 Min.
15	250	3,000

Tularemia Aerosol, Particle Size and Type of Infection

18 - 20 micron particles fall out of aerosol, lodge in eye

15 - 18 micron particles lodge in pharynx

7 - 12 micron particles lodge in trachea

4 - 6 micron particles lodge in bronchiole

1 - 3 micron particles lodge in alveolus

Particle Size (Micron, Mass Median Diameter)

18 - 20

15 - 18

7 - 12

4 - 6
Bronchioles

1 - 3
alveoli
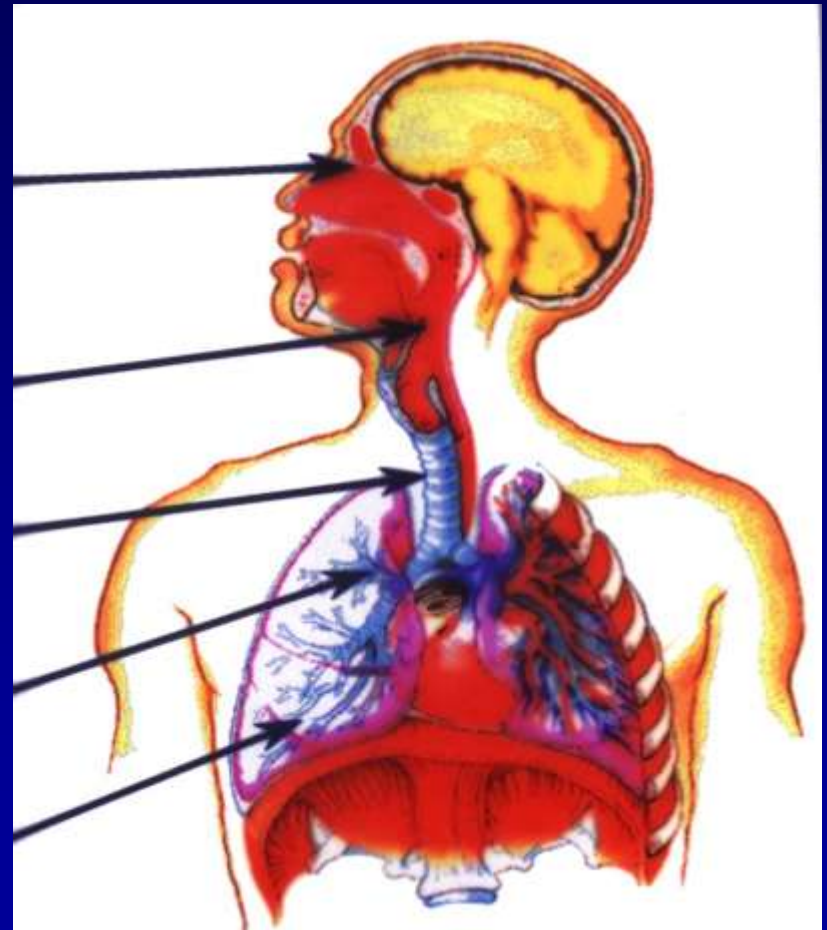
Vaccine Protection to Aerosol Challenge

- **Killed vaccines do not protect animals or people to virulent aerosol challenge**

- **This is demonstrated by volunteers from the Seventh Day Adventist Church challenged with killed Tularemia vaccine (Forshay killed)**

- **Forshay killed vaccine provided volunteers some protection to intracutaneous challenge***

- **Forshay killed vaccine did <u>NOT</u> protect volunteers from aerosol challenge**

Test	Respiratory dose (cells)	Vaccinated Ill/Challenged	Non-Vaccinated Ill/Challenged
1	15	-	2/2
2	17	1/2	2/2
3	22	1/4	1/2
4	27	3/4	2/2
5	48	3/4	6/8
Means	26	8/14	13/16

*AD285-542: Eigelsback, et al.

- **The live attenuated Tularemia Vaccine (LVS) did protect volunteers to virulent aerosol challenge**

Respiratory Challenge of Volunteers Given Live Attenuated Vaccine (LVS)*

Test	Reparatory dose (cells)	Vaccinated Ill/Challenged	Non-Vaccinated Ill/Challenged
1	12	0/2	1/2
2	48	1/4	2/2
3	25	1/4	2/2
4	11	0/4	1/2
5	47	1/4	2/2
Means	29	3/18	8/10

*AD285-542: Eigelsback, et al.

- **There was a significant drop in the infection rate among "at risk" workers when the old killed vaccine were replaced with live attenuated vaccine.**

- **The next slide shows the infection rate for Tularemia and VEE infection before and after live vaccines replaced killed vaccines.**
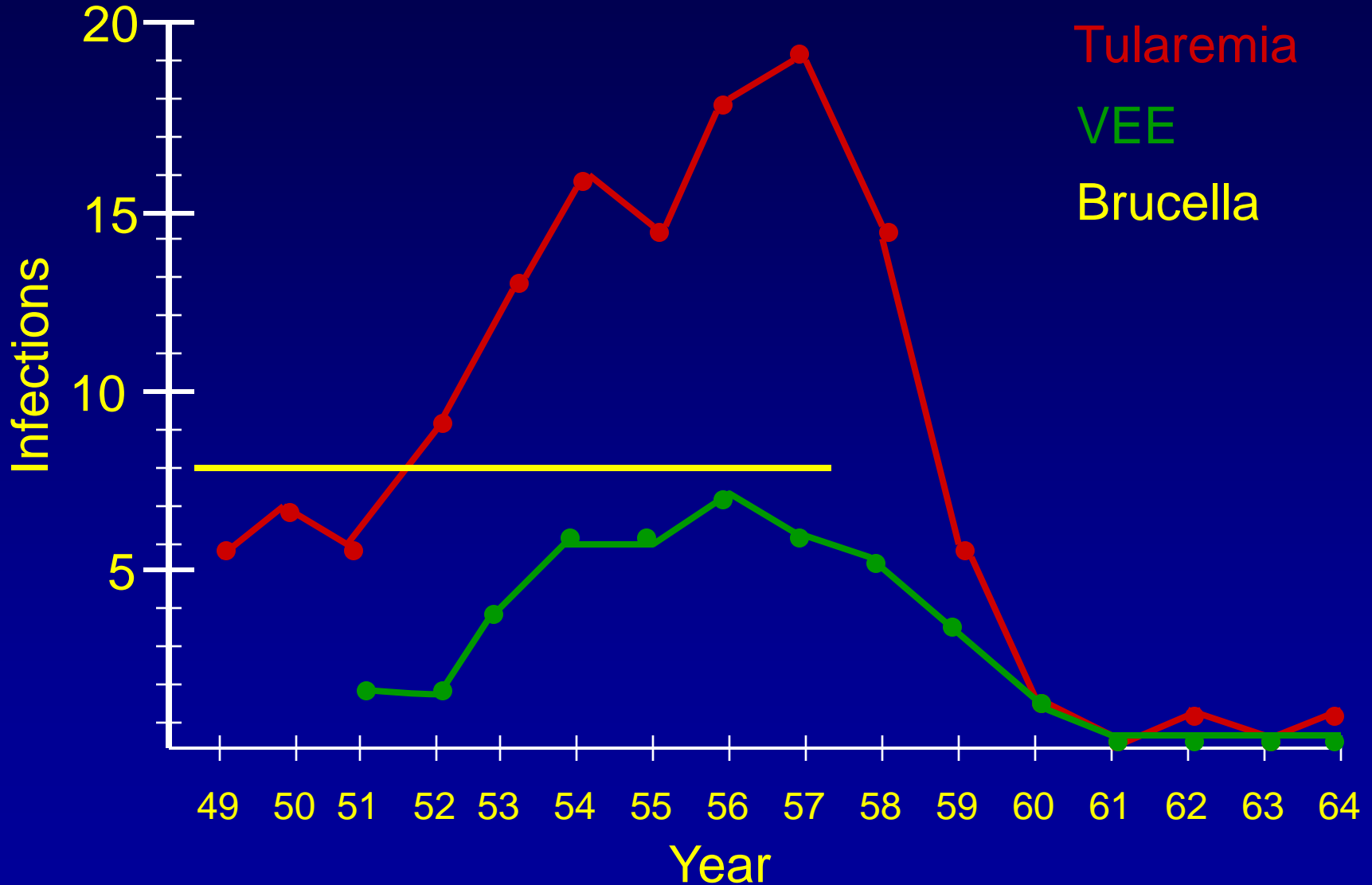
- **An effective vaccine was never developed for *Brucella suis***

- **The infection rate remained constant as long as this organism underwent R&D**

- **The data comparing infection rates for Tularemia and VEE, while dramatic, are not entirely clean**

- **The number of man hours devoted to the agent, safety protocols and the number of effective safety hood systems are a part of the information presented**

- **The anthrax skin infections follow the same pattern observed for the aerosol challenge of Tularemia and VEE**

- **Note, however, there are two respiratory anthrax infections that led to death**

- **The next slide shows the anthrax infections**
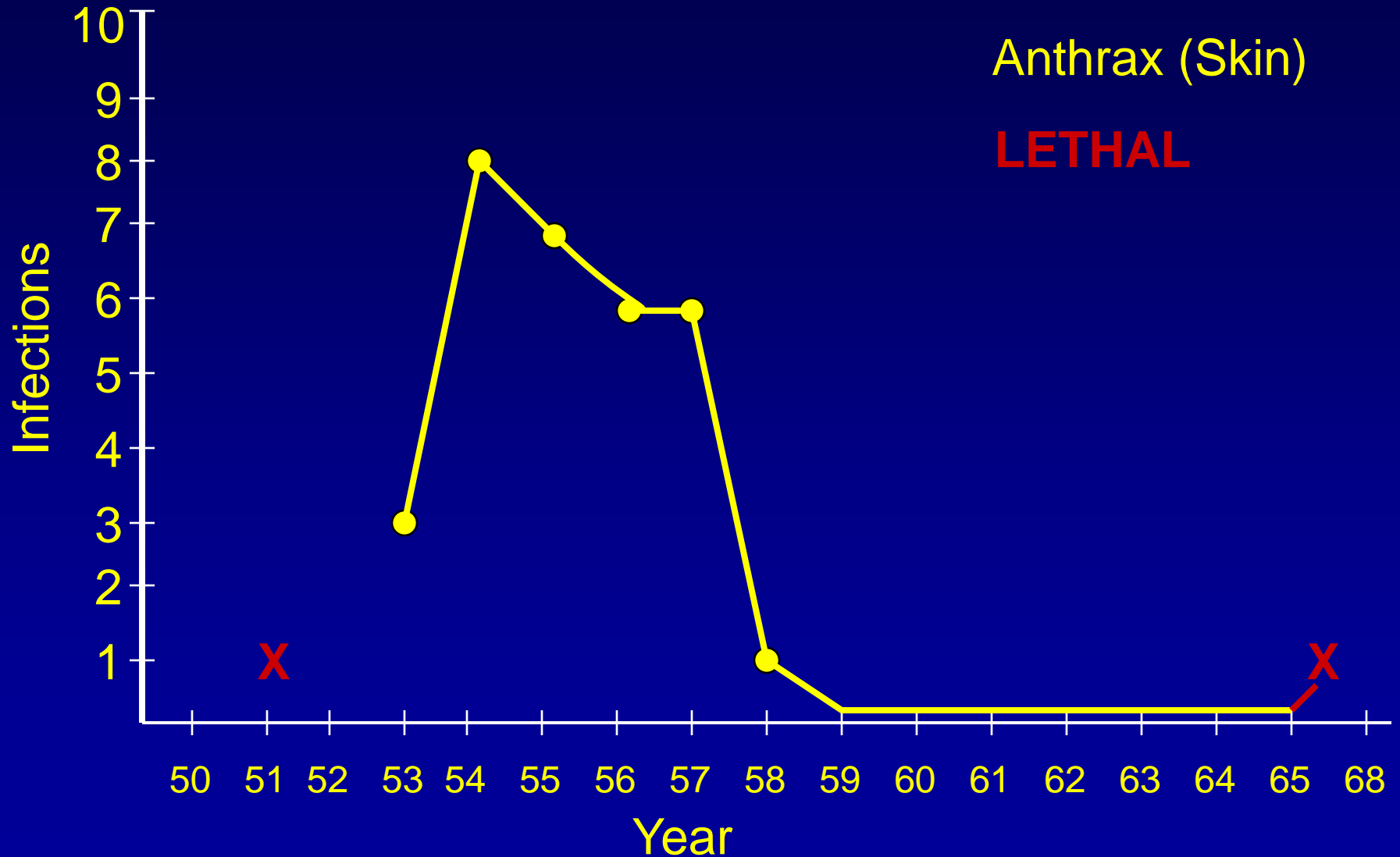
- **The significant impact of a good vaccine on aerosol protection is demonstrated in a large-scale field test of Tularemia.**

- **Non-immunized and LVS immunized Rhesus monkeys were stationed 5 kilometers downwind from the line of dissemination**

- **The Respiratory LD$_{50}$ was:**

Non Immunized	LVS Immunized
34 Cells	**14,600 Cells**

± 429 fold difference

Conclusions:

- **The appropriate vaccine significantly alters the impact of a biological warfare or bioterrorist attack**

- **Live vaccines, while providing good immunity, have serious limitations, particularly in females of child-bearing age**

- **The current anthrax vaccine, not a killed or attenuated agent, provides good protection because it is a chemical vaccine…neither live nor killed**

Three equations can be used to calculate the success of an enclosed operation, i.e. building

- **Equation 1: Calculate the total number of infectious units available.**

- **Equation 2: Calculate the number of liters of air available in the building.**

- **Equation 3: Divide total number of infectious doses by liters of building air. This provides the number of infectious doses per liter of building air.**

Equation 1: Total Infectious Doses Available (TIDA)

$$TIDA = \frac{\left(\begin{array}{c}\text{Product Conc.}\\ \text{per ml or gm}\end{array}\right)\left(\begin{array}{c}\text{Total Amount}\\ \text{of Agent ml/gm}\end{array}\right)\left(\begin{array}{c}\text{\% Dissemination}\\ \text{Efficiency of Device}\end{array}\right)}{\text{Human Infectious Dose}} \times 40\%$$

Example

A. Product Conc. = 1 x 10^9 C. 5% Dissemination Efficiency

B. 2000 ml of Agent D. Human Dose is 8,000 Cells

$$TIDA = \left[(1 \times 10^9/ml)\,(2000\ ml)\,(5\%) \div 8000\ cells\right] \times 40\%$$

$$TIDA - 1 \times 10^8$$

The information contained in this presentation
is the property of William C. Patrick III