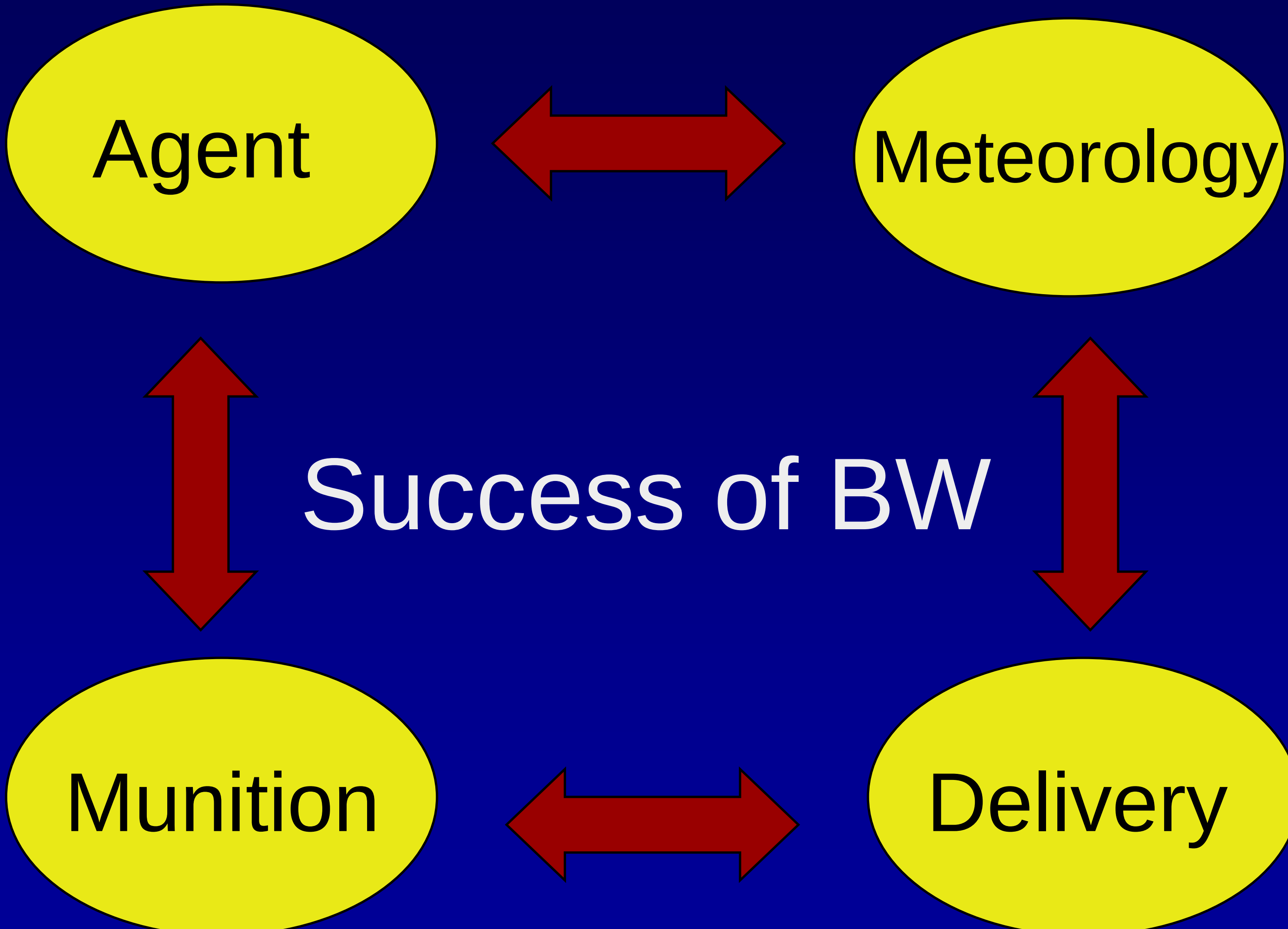
BioThreats Assessment

William C. Patrick III


That's odd. This bottle of Viagra was full two days ago.
Reynolds


Agent
Meteorology
Success of BW
Munition
Delivery

Atmospheric Conditions

Aerosol disseminated in bright sunlight will not remain at ground level

- Rises immediately into atmosphere

Atmospheric Conditions

(continued)

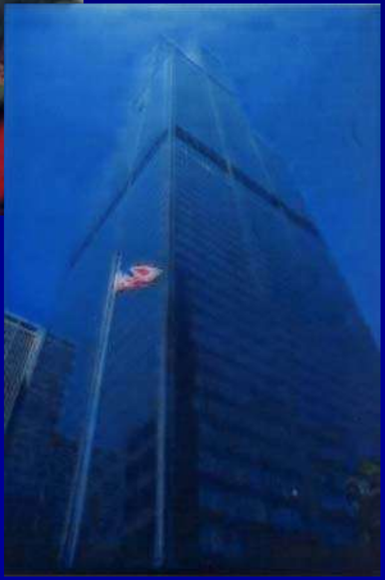
BW attacks usually pre-dawn, sunset, or night

- Temperature inversions more likely

Wind

Important factor in preplanning BW attack

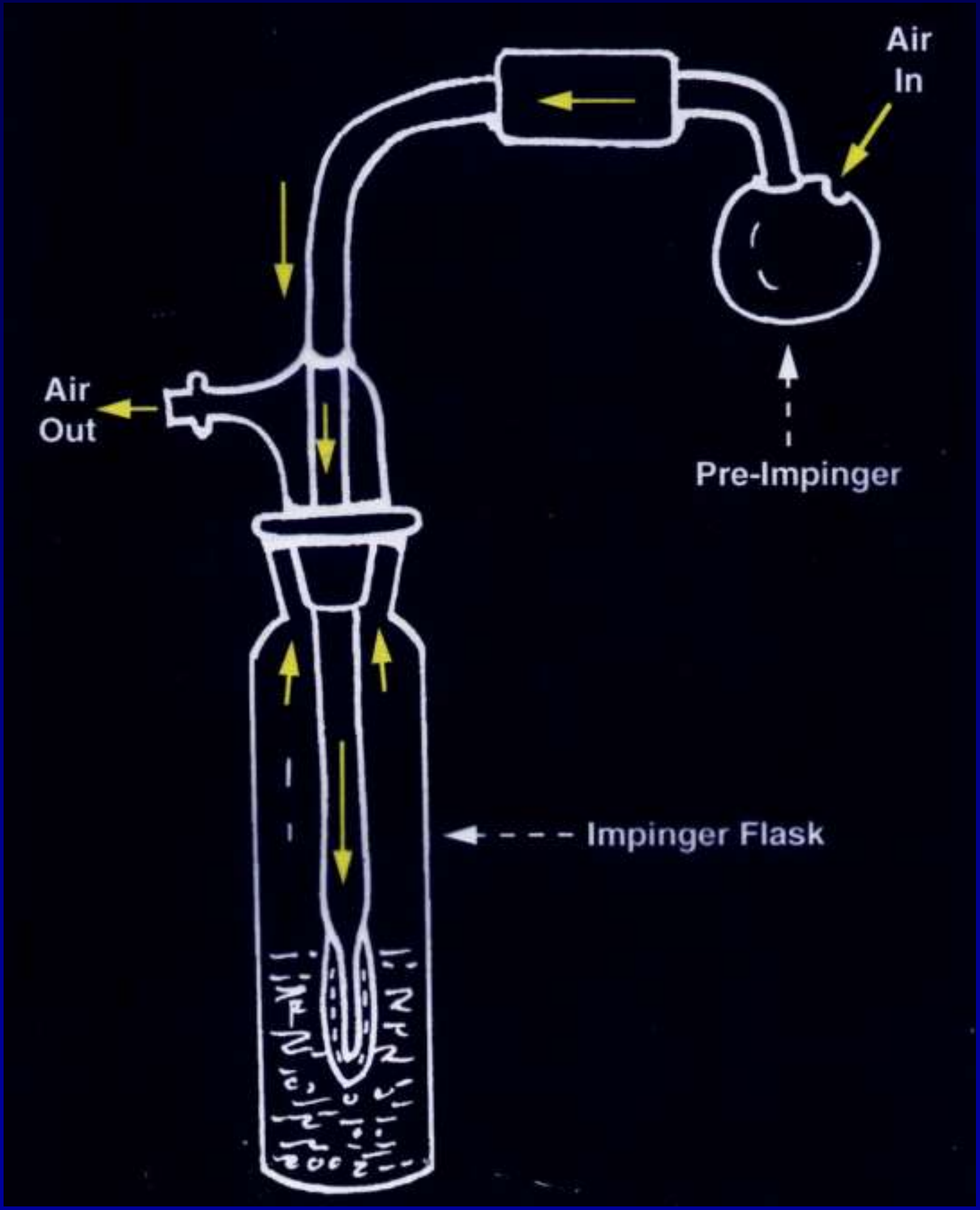
- If less than 5 mph, aerosol will be limited in coverage
- If more than 30 mph, aerosol disintegrates and loses integrity
  - Results on target unpredictable

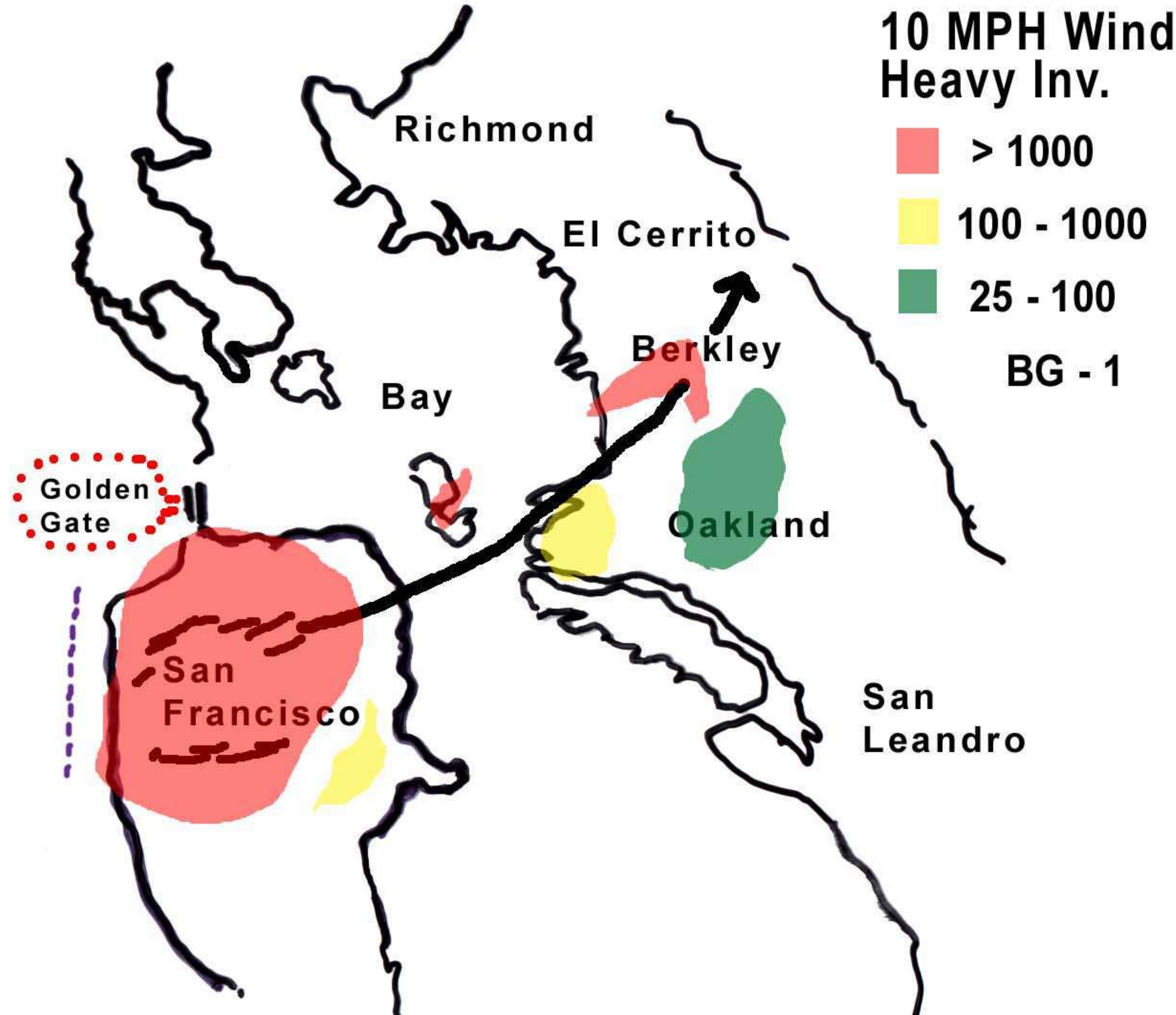



Richmond
El Cerrito
Berkley
Bay
Golden
Gate
Oakland
San
Francisco
San
Leandro

All Glass Impinger

With Pre-Impinger





10 MPH Wind
Heavy Inv.
> 1000
100 - 1000
25 - 100
BG - 1
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Bay
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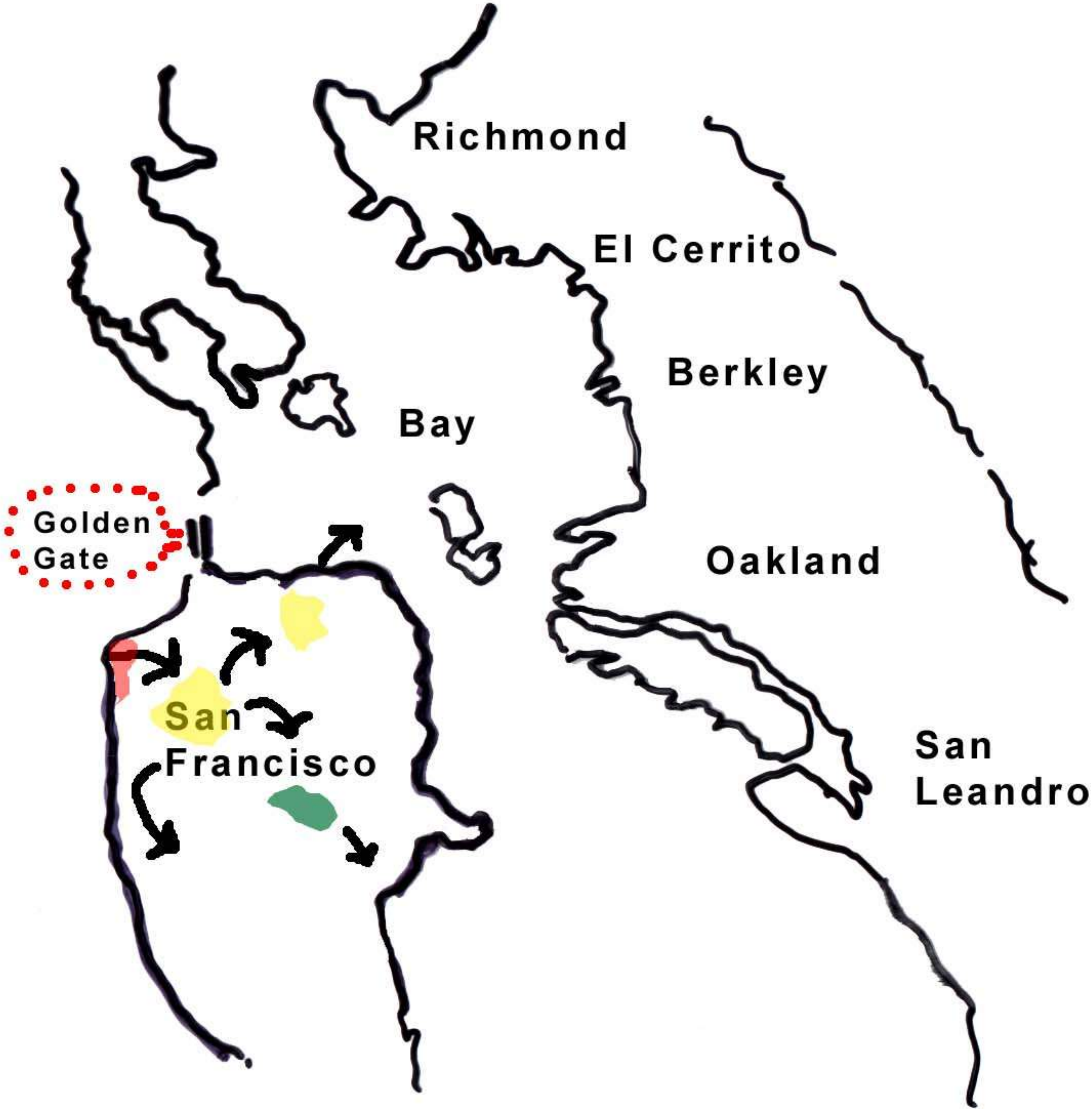
Gate
Oakland
San
Francisco
San
Leandro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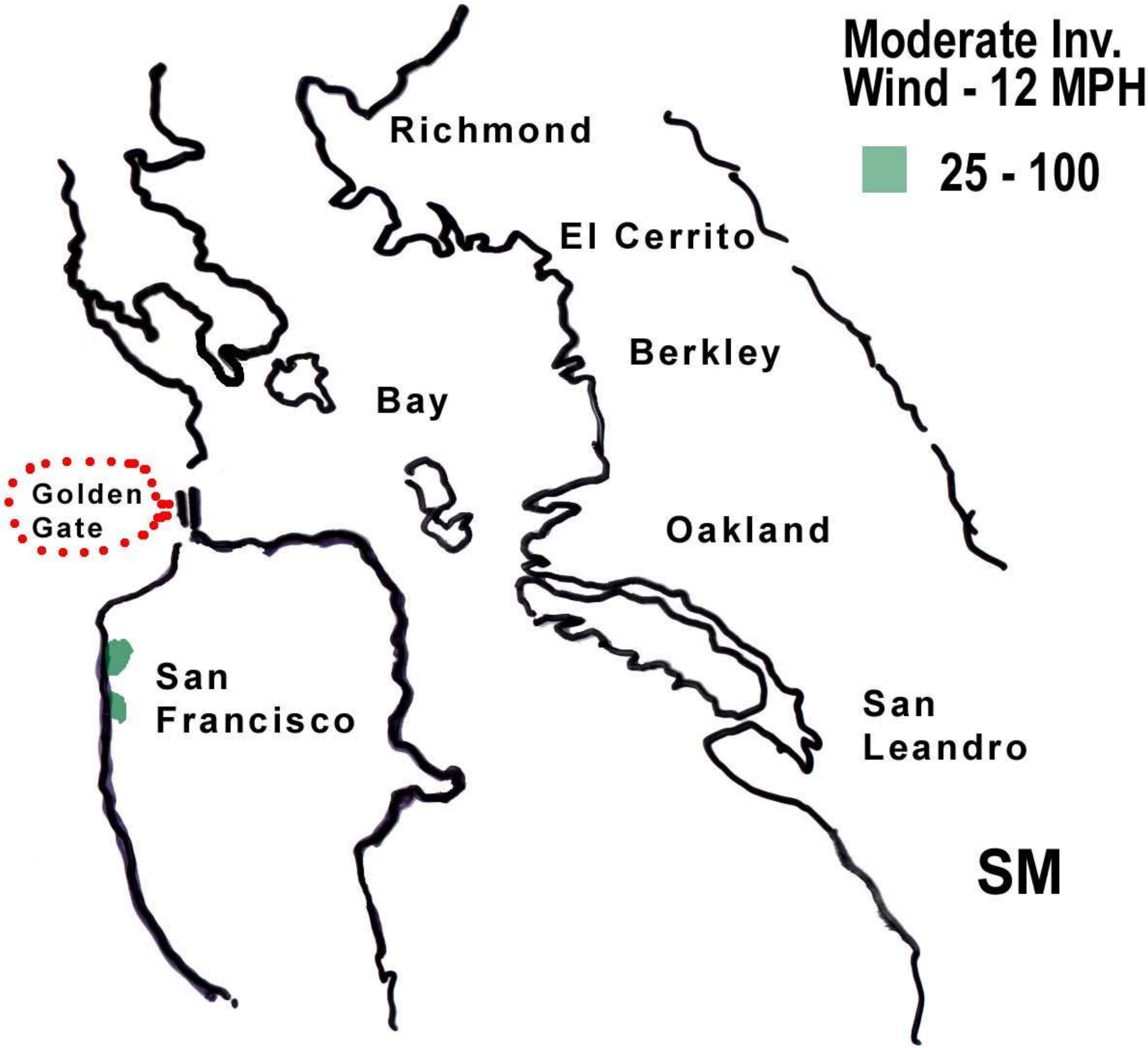

Moderate Inv.
Wind - 12 MPH
25 - 100
Richmond
El Cerrito
Berkley
Bay
Golden Gate
Oakland
San Francisco
San Leandro
SM

Scenario: Attack on New York Subway System

- One of the most important vulnerability studies conducted by the former U.S. Offensive Program concerned the N.Y. subway system.

- A unique simulant BG powder was prepared that had good *secondary aerosol properties*

- Light bulbs were filled with the special powder. Three light bulbs (filled with a small amount of powder) were thrown onto the tracks from the rear car during passage through each subway tunnel.

- A total of 3 North/South tunnels were attacked.
- The BG quickly spread through each tunnel by passage of the trains over the powder.
- BG penetrated all test trains and remained in high concentration for 1.5 hours. Thereafter, the spore concentration in the subway cars dropped markedly and was not a factor after 2 hours.
- Risk of infection and exposure levels were shown to have been highest for personnel using the subway near the site of the powder dissemination and within the first hour after dissemination.

- Studies showed that the average time on the train during rush hour in AM and PM was 8 minutes.
- Studies also showed that in 1965 approximately one million workers used the subways daily in the mid-Manhattan business district to reach their work during rush hours.
- Less than one kilogram of dried anthrax would produce 50% casualties throughout the entire NY subway system.
- If ridership today of 1,000,000 passengers per day during AM and PM rush hours, it seems logical to conclude that 500,000 infections would occur.

- Since the window for initiating treatment for pulmonary anthrax is quite short, perhaps as many as 90% of infected patients would die.

- This level of deaths simply cannot be conceived…all deaths occurring in 3 to 5 days post attack.

Physics of Aerosol

Lou Dixon

And

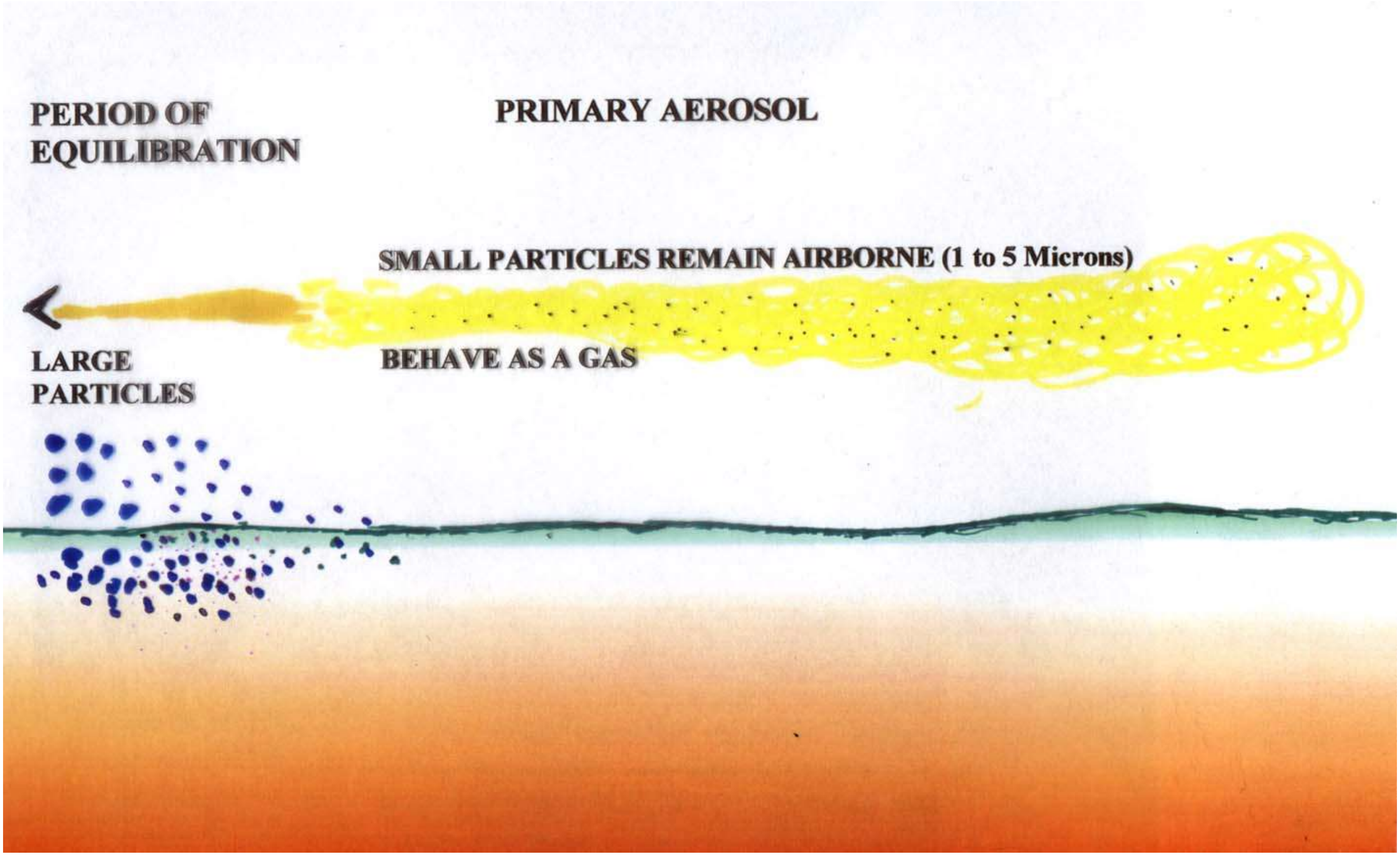
The Gas Mask

Mask Protection for Individuals

Type of Protection	Filter Efficiency (%)**
HEPA	**99.99**
Dust/Mist	**99.7**
Sub-Micron Surgical Mask	**96**
Handkerchief - 5 folds	**94**
Toilet Paper - 3 layers	**91**
Bath Towel - 2 layers	**85**
Cotton Shirt - 2 layers	**65**

NOISH estimates that leakage around the seals is the dominant factor. **0.3 micron particle

Physics Of Primary Aerosol



Man - Monkey - Guinea Pig: Influence of Particle Size on Tularemia Infectivity

Number of Tularemia Cells

Aerosol Particle Diameter (microns)	Guinea Pig RLD_{50}	Monkey RLD_{50}	Man RID_{50}
1	2.5	14	10-52
6.5	4700	178	14-162

BG Simulant Tests: Interim Report 113*

When HRS-2 helicopters land in area previously contaminated by BG fallout from primary aerosol, there will be little or no contamination and personnel receive little or no respiratory exposure while moving through dust created by rotor movement.

* DTIC Recovery Number AD222-773

Physics of Aerosols

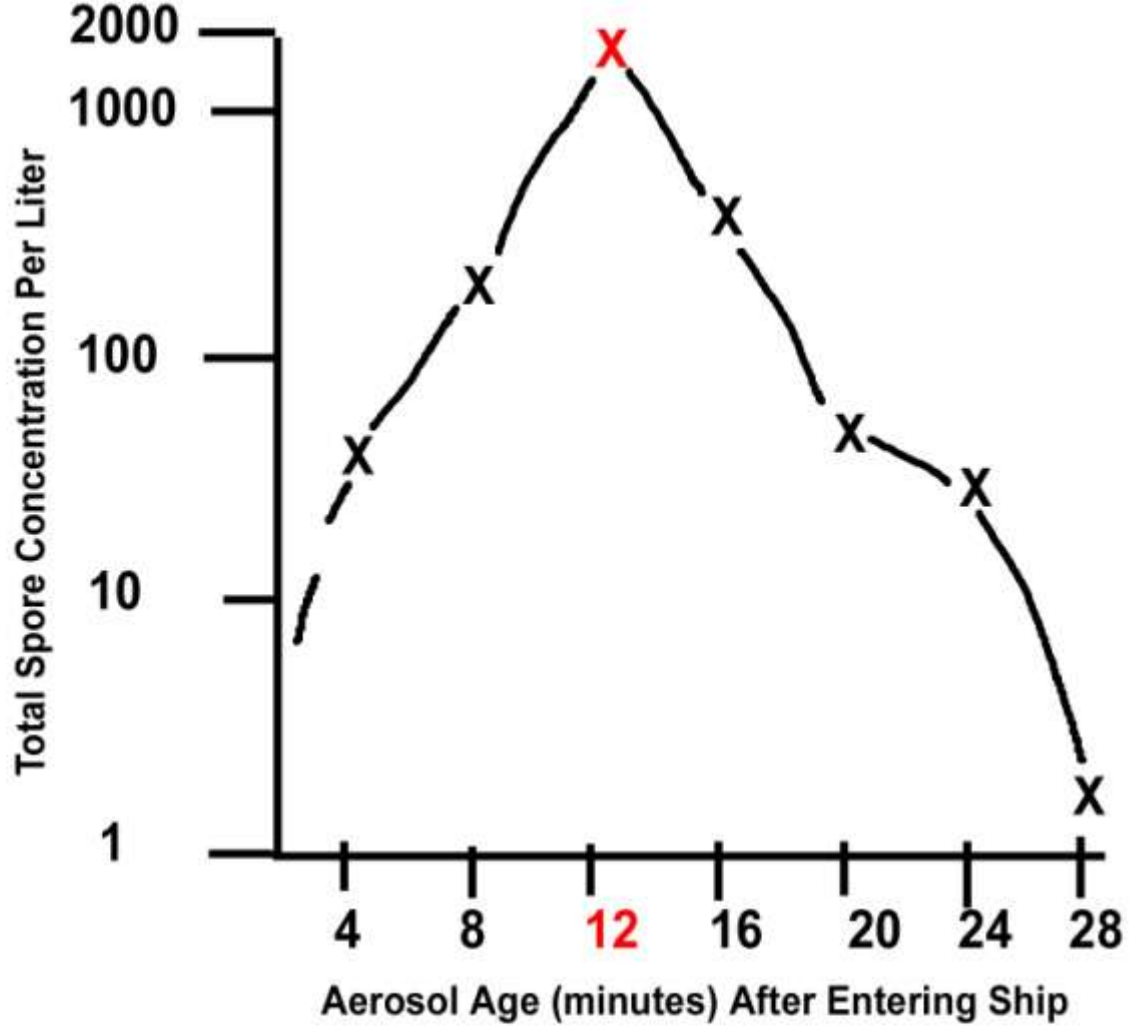
Residual Hazards Ref Primary Aerosols

- Copper Head Test in Arctic: Aero 14 sprayed simulate BG 20 miles upwind of Naval test ships
- Impinger samplers indicated large number of spores per liter of air in interior of ships
- Particularly heavy concentrations present in air circulating in engine rooms where air sucked in to dissipate heat

- Exterior and interior of ship surfaces showed marginal contamination

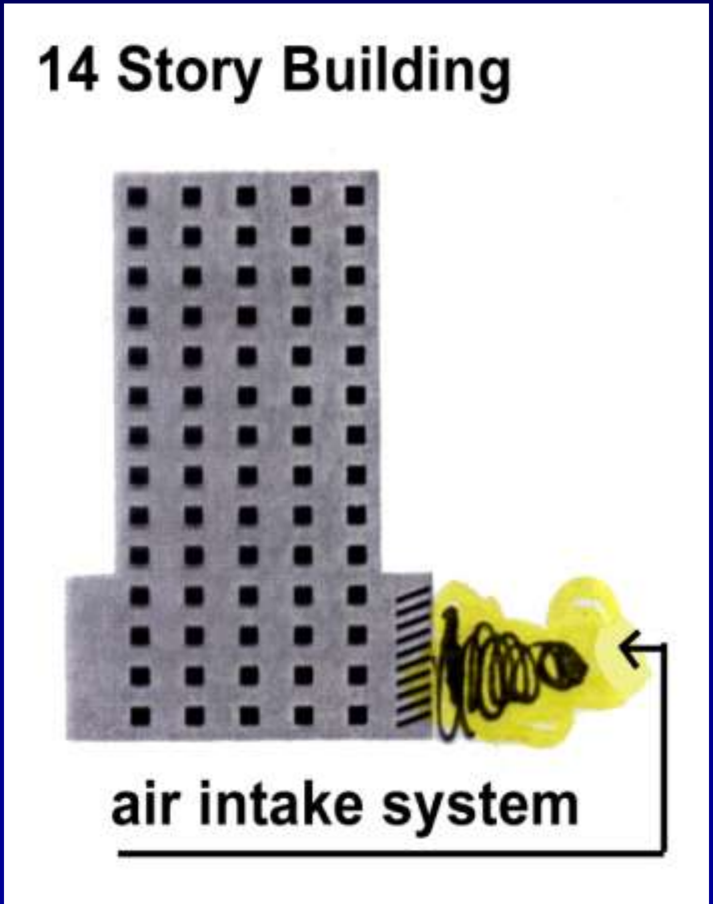
- Sea water wash was effective in removing the light concentration

Penetration of Destroyer by Primary Aerosol of BG Spores Released Up-Wind



Primary Aerosol Behaves As A Gas

- In 1960s the Federal Civil Defense Administration requested Ft. Detrick to assess the vulnerability of buildings to biological attacks
- Impinger samplers distributed throughout all floors
- Building contained 3 million cubic feet of air (84 million liters)

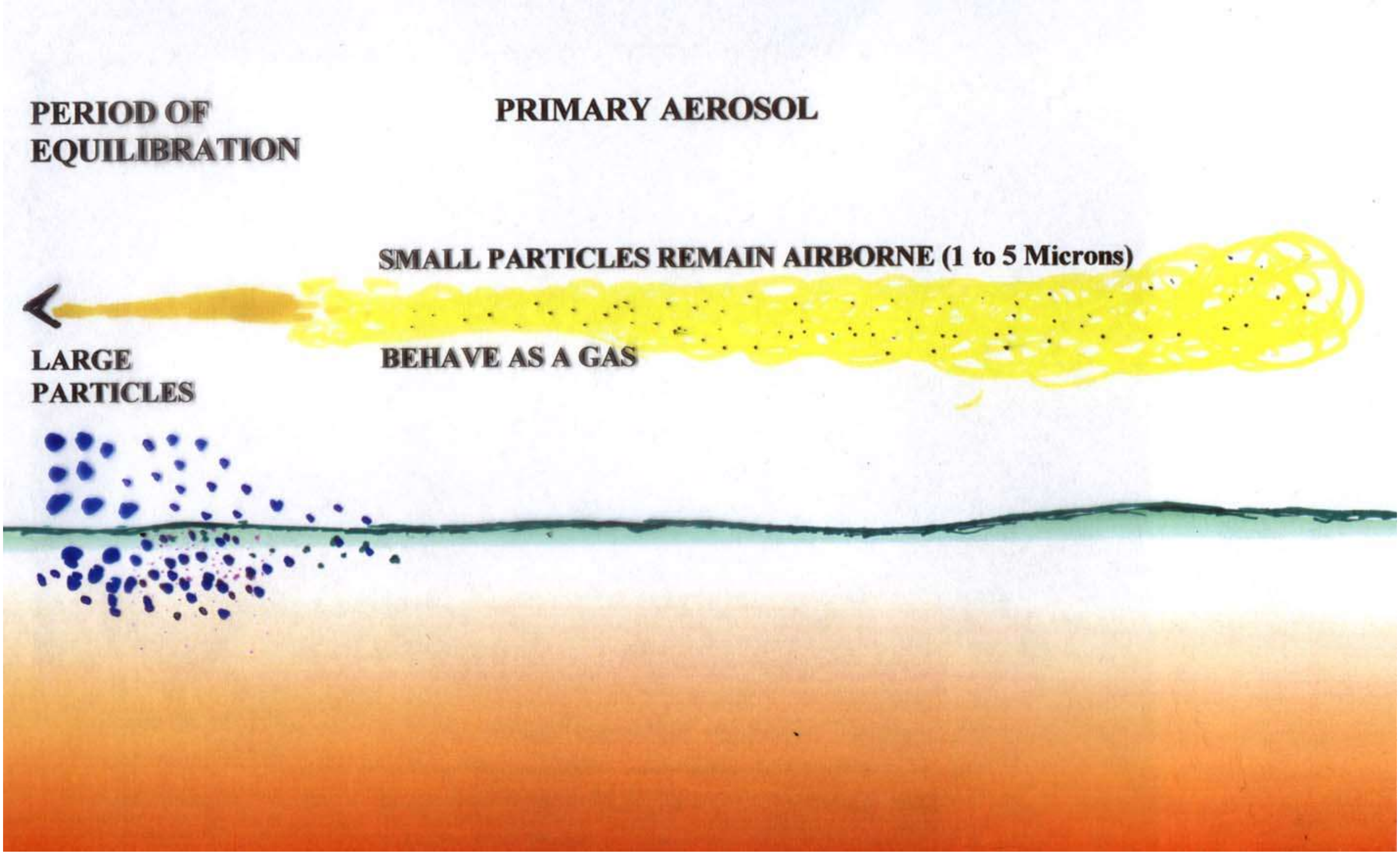


- After 2 hours, spores not detected in building air
- Spore concentration was extremely light on floors, walls and ceilings

Conclusion

Building air system brings primary aerosol into building and then removes it, leaving little or no evidence of its passage.

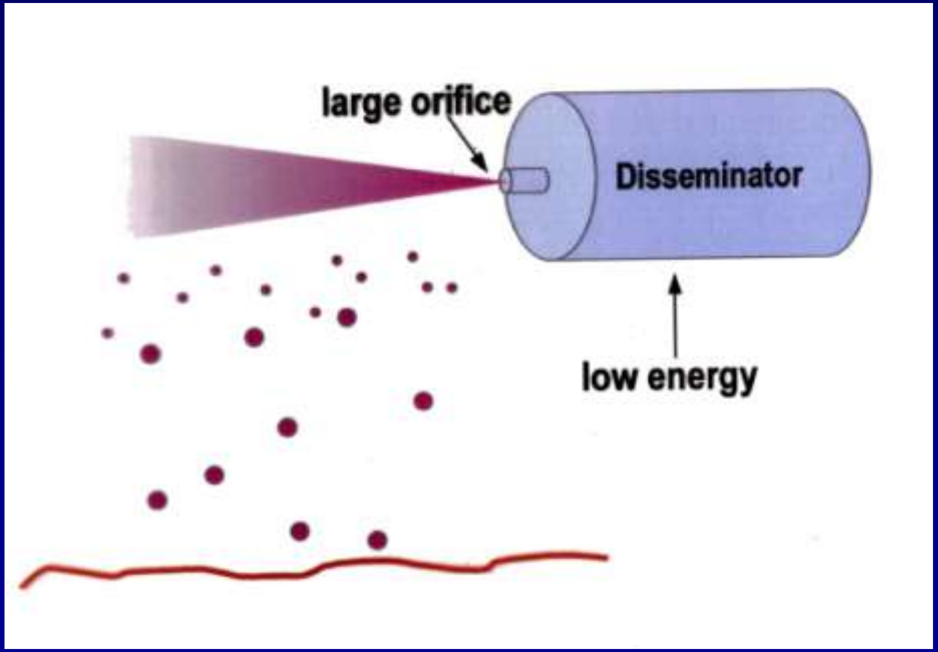
Physics of Primary Aerosol



Fate of Large Particles That Fall Out Of Aerosol During Equilibration

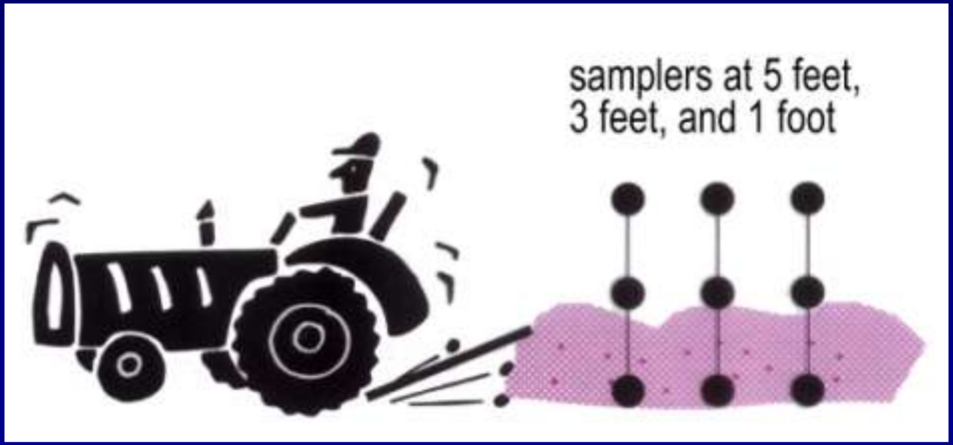
- In the 1950s, Wagner deliberately generated large particle aerosols to study terrain contamination and secondary aerosol relationships

- Disseminated 60 liters of BG slurry over a small grid



Efforts to Create Secondary Aerosol

- Wagner drove a tractor with large sheet of rubber beating the ground over BG-contaminated terrain creating lots of dust



- Sampled dust with impinger samplers located throughout the grid at 1, 3 and 5 feet above the ground, one hour and five hours after BG dissemination

Fallout of BG Spores During Aerosol Equilibrium: Sand

Concentration of Spores on Sand

Spores per Meter^2	Post Hours	1 Foot	3 Feet	5 Feet
$1x10^4$	1	67	2	1
	5	0	0	0
$6x10^7$	1	2150	62	22
	5	58	3	1

Efforts to Create Secondary Aerosol *(continued)*

- Wagner also sprayed BG slurry directly onto the terrain in order to achieve very high levels of contamination
- This method produced concentrations as high as 15 billion spores per square meter

Direct Spray of BG Spores Onto Sand

Concentration of Spores on Sand

Spores per Meter^2	Post Hours	1 Foot	3 Feet	5 Feet
$6x10^9$	1	158,000	3,250	3,180
	5	61,200	2,300	1,610
	12	34,000	3,100	286

Particle Size - Aerosol - Tularemia

Particle Diameter Microns	Number of Cells For Guinea Pig RLD_{50}	Monkey RLD_{50}	Man RID_{50}
1	2.5	14	10 - 52
6.5	4700	178	14 - 162
11.5	23,000	672	No data
18	125,000	3447	No data
22	230,000	>8500	No data

Agent Particle Size

Therefore, agents that fall out during aerosol equilibrium are not of primary concern because:

- Many large particles are required to cause respiratory infection
- Strong adhesive forces between agent and terrain

Secondary Aerosols *(continued)*

- **Special BW agent powders can be prepared**
  - **Overcome adhesive forces and form good secondary aerosols**
    - Require special processing
    - Much more difficult to handle safely than ordinary dry agent

Show film that demonstrates

Primary and Secondary Aerosols

from Dry Powders and Liquid Agents

Estimate Human Anthrax Doses Airborne

Based on 20 grams *Bacillus Globigii* powder: Disseminated from smashed Christmas tree ball

Conc. Of Simulant (per gram)		Volume Airborne (grams)		% Volume in 1 to 5 (microns)		Human LD_{50} Dose (spores)		% Lung Retention of Particles		Total Doses Available (time 0)
800×10^9	x	**13.8**	x	**50**	÷	**8000**	x	**40**	=	**2.76×10^8**

- 276,000,000 infectious doses airborne represent a catastrophic level of contamination
- Based on this concept, 2.76×10^8 doses would infect a building roughly the size of the former World Trade Center

Estimate Human Anthrax Doses Airborne

By Fanning Pool of Powder Following Smash of Christmas Tree Ball

Conc. Of Simulant (per gram)		Volume Airborne (grams)		% Volume in 1 to 5 (microns)		Human LD_{50} Dose (spores)		% Lung Retention of Particles		Total Doses Available (time 0)
800×10^9	x	**5.1**	x	**35**	÷	**8000**	x	**40**	=	**14×10^6**

- 14,000,000 infectious doses rendered airborne as a secondary aerosol; very serious level of contamination
- 33 HVAC systems would require closure to seal and isolate contamination, based on one HVAC per 150,000 cubic feet

The two previous experiments just shown were the types of studies we performed in 1965 that provided the basis of the New York Subway Trials in 1966.

AGENT SELECTION

Criteria for Potential BW Agents

- Pathogenic for humans (animals or plants)
- Cause a severe disability or lethality
- Highly infectious but generally not contagious
- Prophylactic and/or treatment measures generally available
- Infectious by the aerosol route
- Stable as a small particle aerosol
- Stable during logistical operations
- Readily and rapidly produced
- Weaponized in munitions and delivery systems
- Produce desired effects on the target

What Constitutes An Effective BW Agent?

- **Many organisms that appear on "BW Lists" would be very difficult to weaponize.**
- **The properties of "The Disease" desired on the target do not necessarily reflect the inherent problems of weaponizing the agent.**
- **Two diseases can be illustrated:**
  - **Influenza virus, until recently (?) could not be stabilized with respect to virulence**
  - ***Yersina pestis*, frequently used in today's scenarios, is an extremely difficult organism to grow. It is difficult to stabilize virulence and decays rapidly in both logistics storage and as an aerosol.**
- **Both of these organisms require sophisticated programs and money to meet target requirements**

What Constitutes An Effective BW Agent?

- In modern times (2004), a panel of BW experts was convened to discuss new potential BW agents.
- Hanta virus was one agent under consideration.
- The problem of growing this virus was discussed.
- Some of these experts concluded that growing this virus was not a problem.
  - The virus could be consistently grown to titers of $1x10^7$ infectious units per ml.
- This level of growth places a tremendous burden on the purification - concentration aspects of the process:
  - The process should increase concentration from 100 to 500 times over growth.
  - If agent stability is a factor, this increase in concentration becomes a significant problem.

What Constitutes An Effective BW Agent?

$$\left(\begin{matrix}\text{Product conc.}\\\text{Per ml/gm}\end{matrix}\right)\left(\begin{matrix}\text{Vol. Of}\\\text{1 ml/gm}\end{matrix}\right)\left(\begin{matrix}\text{\% Dissemination}\\\text{efficiency}\end{matrix}\right)\div\left(\begin{matrix}\text{Human}\\RLD_{50}\end{matrix}\right)$$ To achieve $1x10^7$ doses/meter

- Agent disseminated under unfavorable conditions: URBAN TARGET, poor meteorological conditions, average decay rate (2.5% per minute)

Downwind Distance (km)	Line Source Strength: LD_{50} doses per meter 10^5	10^6	10^7	10^8	10^9
0.5	1.6*	15.2*	80.7*	100*	100*
1.0	0.5	5.2	41.2	99.5	100
2.0	0.1	1.4	13.1	75.5	100
4.0	0.0	0.3	1.2	11.7	71.3
8.0	0.0	0.0	0.2	2.0	18.3
16.0	0.0	0.0	0.0	0.0	0.3

*** % Infections at points downwind**

Botulinum Toxin: A Potential BW Agent Via Aerosol?

- Grows to concentration of ± $1x10^6$ $MIPLD_{50}$ per ml
- Purify and concentrate: alternate precipitation-reconstitution to yield 50% purity
- Spray Dry: Powder contains on average $4x10^9$ $MIPLD_{50}$/gm
- Disseminate one kilo over one kilometer as line source, good met conditions; no biodecay, Urban target
- Total Doses = $(5x10^9)$ (1000) (25) ÷ 14,000 = $9x10^7$
- Doses per meter = $9x10^7$ ÷ 1000 = $\underline{9x10}^4$

Distance Downwind	% Infections
500 meters	0.33
1000 meters	0.15
1500 meters	0.1

WCP6

Why Did Bot Toxin Fail?

- Toxin is highly effective when injected into the gut or by the oral route

- Significantly less effective by the aerosol route

    - i.e. 1500 Mouse Gut Doses required for <u>1</u> (one) Mouse Aerosol Dose

U.S. vs. USSR: Dry Agent Production

(metric tons per year)

Agent	U.S.	USSR
SEB	1.9	0
Tularemia	1.6	1500
Q Fever	1.1	-
Anthrax	0.9	4500
VEE	0.8	150
Botulinum	0.2	0
Plague	0	1500
Smallpox	0	100
Glanders	0	2000
Marburg	0	250

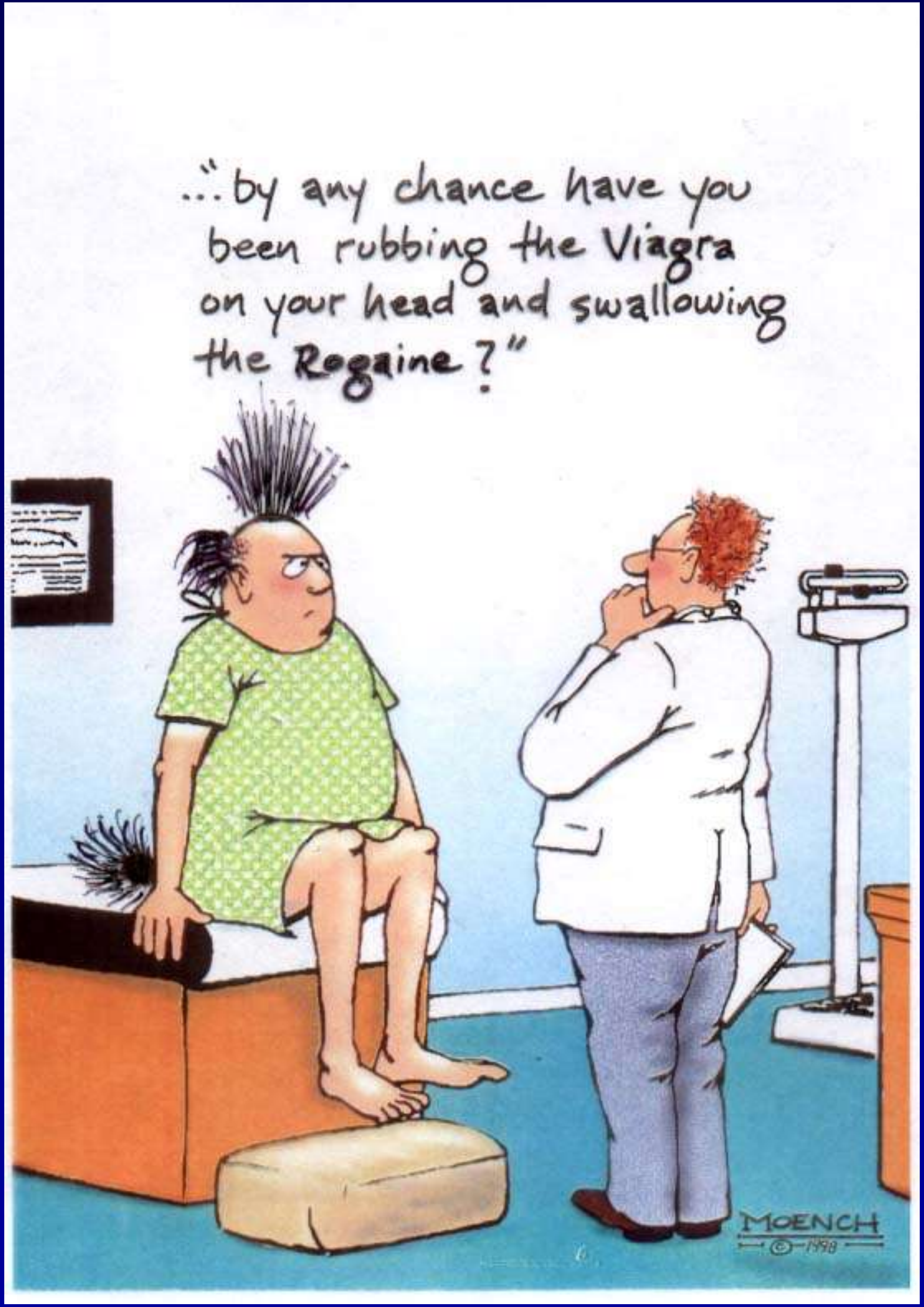
A Final Word About Agents:

U.S. vs. USSR Agent Production Capabilities

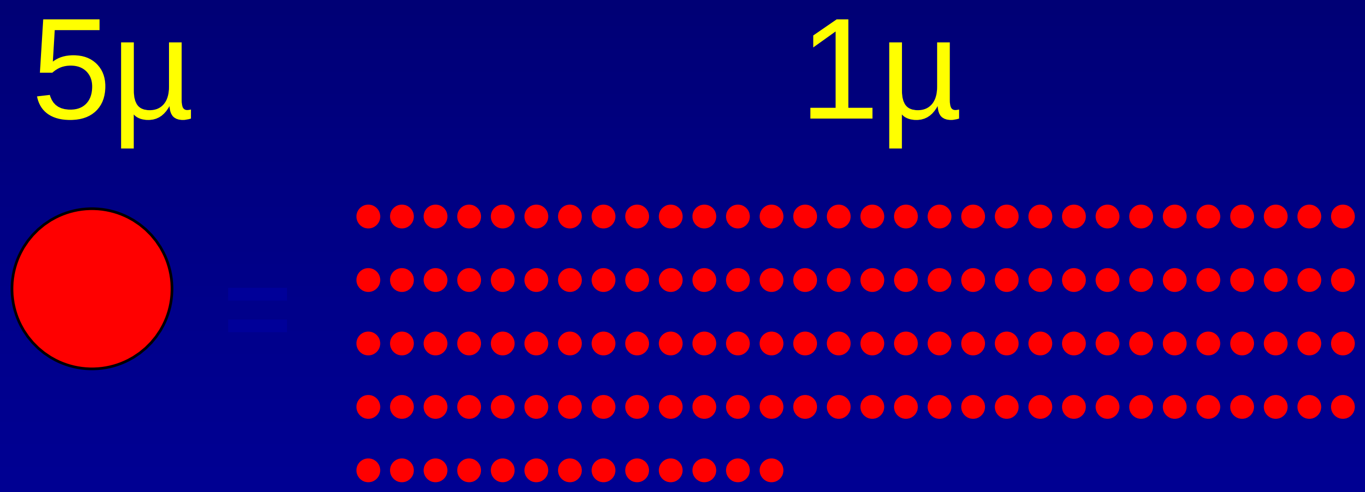
U.S. vs. USSR
Dry Agent Production

Agents

William C. Patrick III

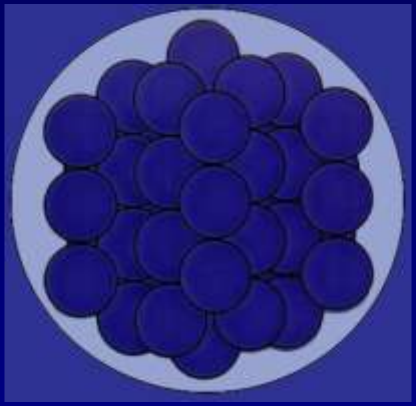

..."by any chance have you been rubbing the Viagra on your head and swallowing the Rogaine?"
MOENCH
© 1998

Particle Size: Microns, Mass Median Diameter



Size *DOES* Matter

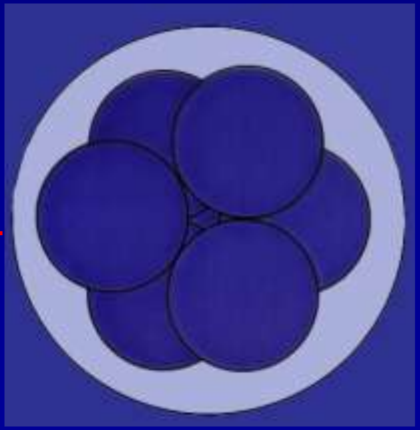
- For successful weaponization, agent that can be disseminated into small particle aerosol must be developed



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

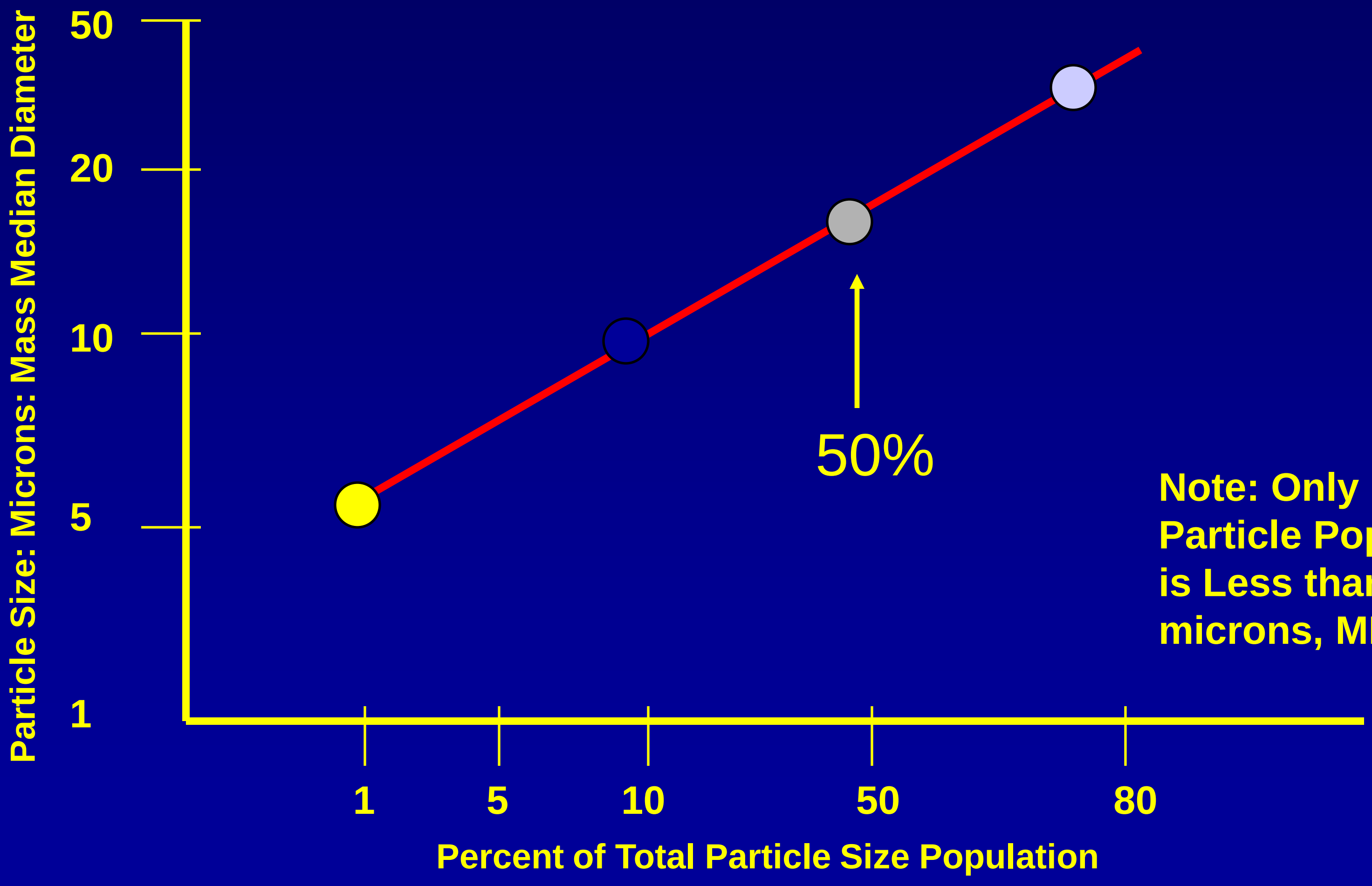
15 two-micron spheres in a five-micron sphere



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

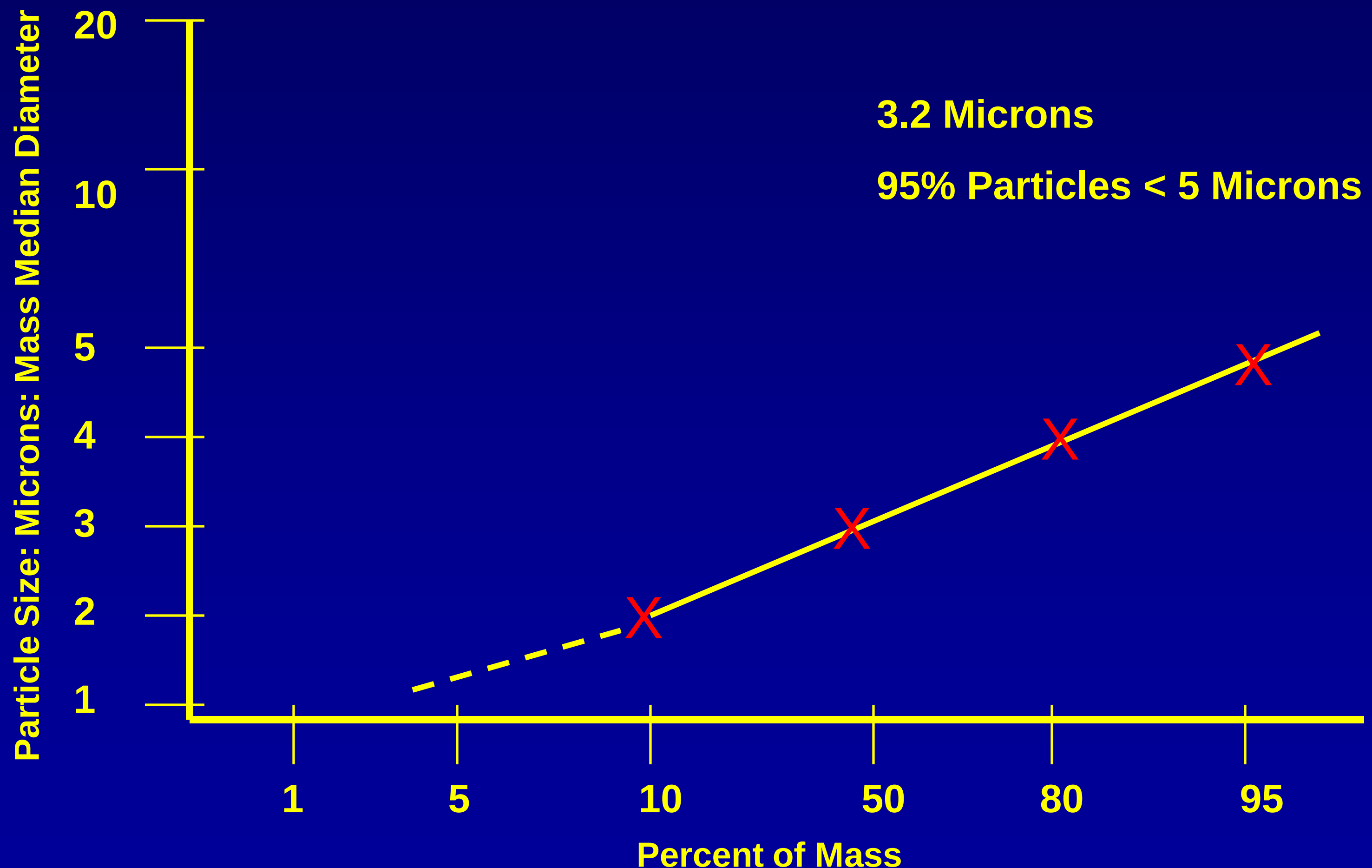
Aerosol Particle Size (Microns)	*Bacillus anthracis*	*Francisella tularensis*	*Yersinia pestis*	Q Fever	VEE Virus
0.3 - 1.5	23,000	2.5	12,000	10^6	20
4.6 - 6.5	221,000	6,500	250,000	$52x10^6$	19,000
8.5 - 13	700,000	19,500	450,000	$>2x10^6$	280,000

Particle Size Distribution of 18 Micron (MMD) BG Powder Using Whitby Centrifuge Technique

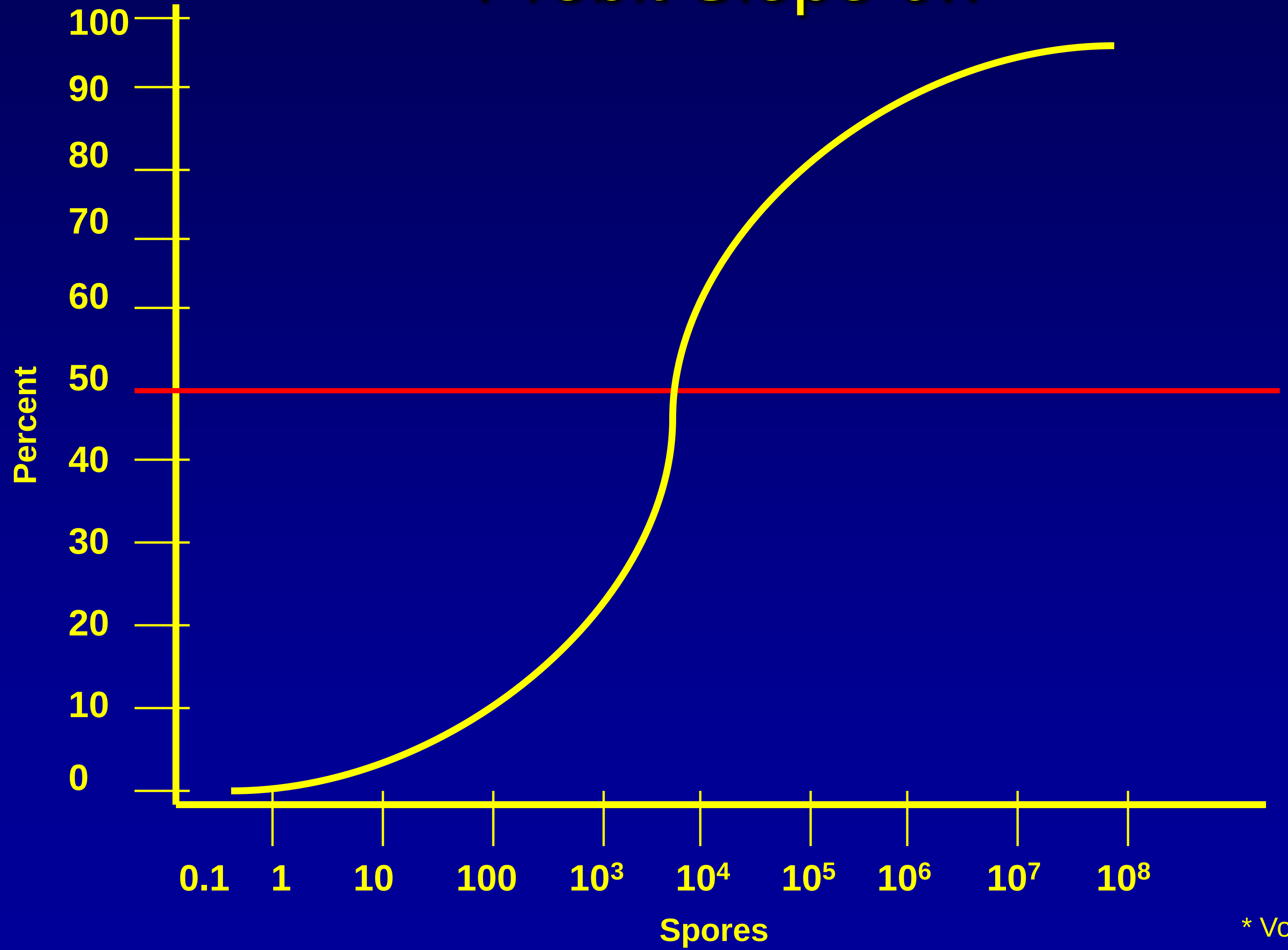


Note: Only 1% of Particle Population is Less than 5 microns, MMD

High Grade B.G. Powder



Anthrax*/Lethal Dose/Cyno Monkey
Probit Slope 0.7



* Vollum Strain

Human Dose: Vollum Strain: Cyno Monkey: Probit Slope 0.7

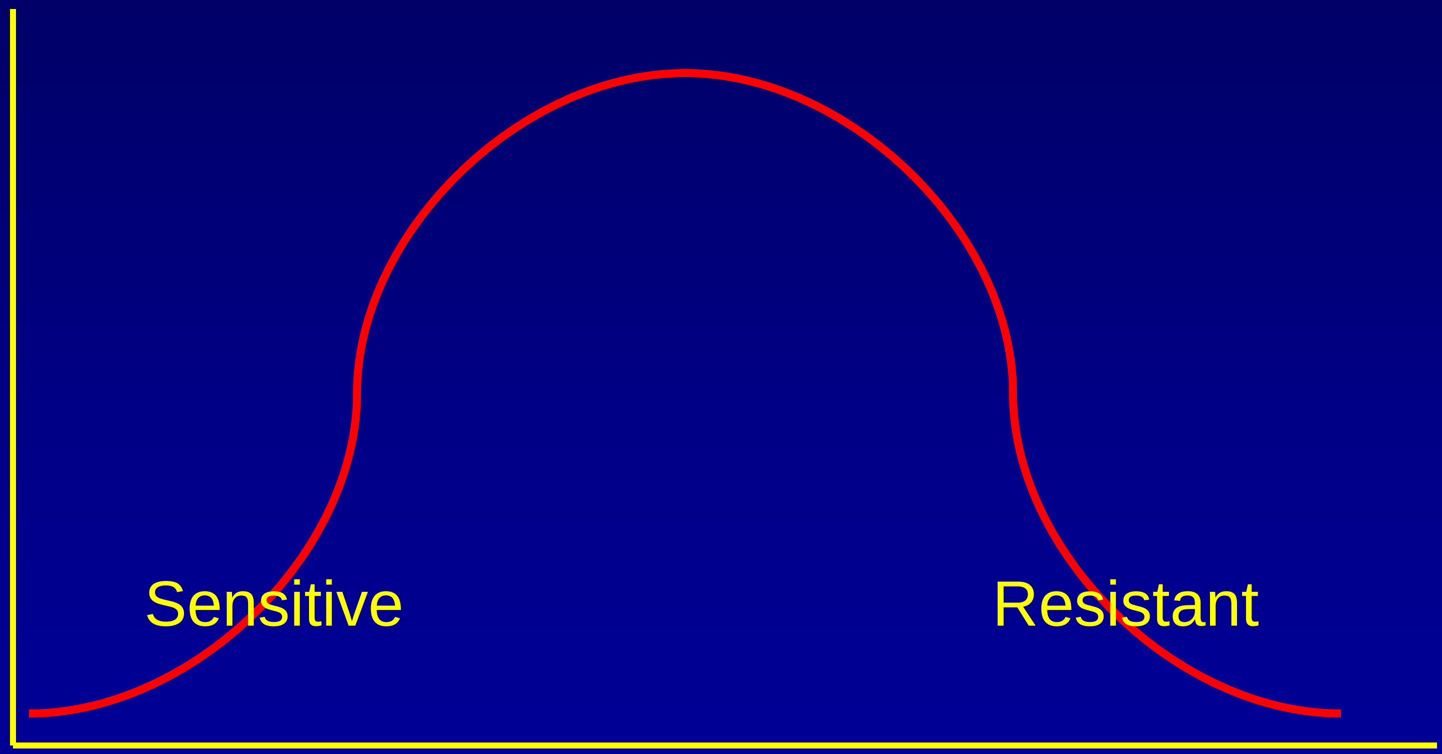
Lethal Dose	Number of Spores (Microns*)
10	120
20	500
30	1,400
40	3,500
50	8,000
60	18,000
70	45,000
80	130,000
90	540,000

*1 to 5 microns

RLD_{50} Anthrax Spores and Particle Size (Microns) For Man

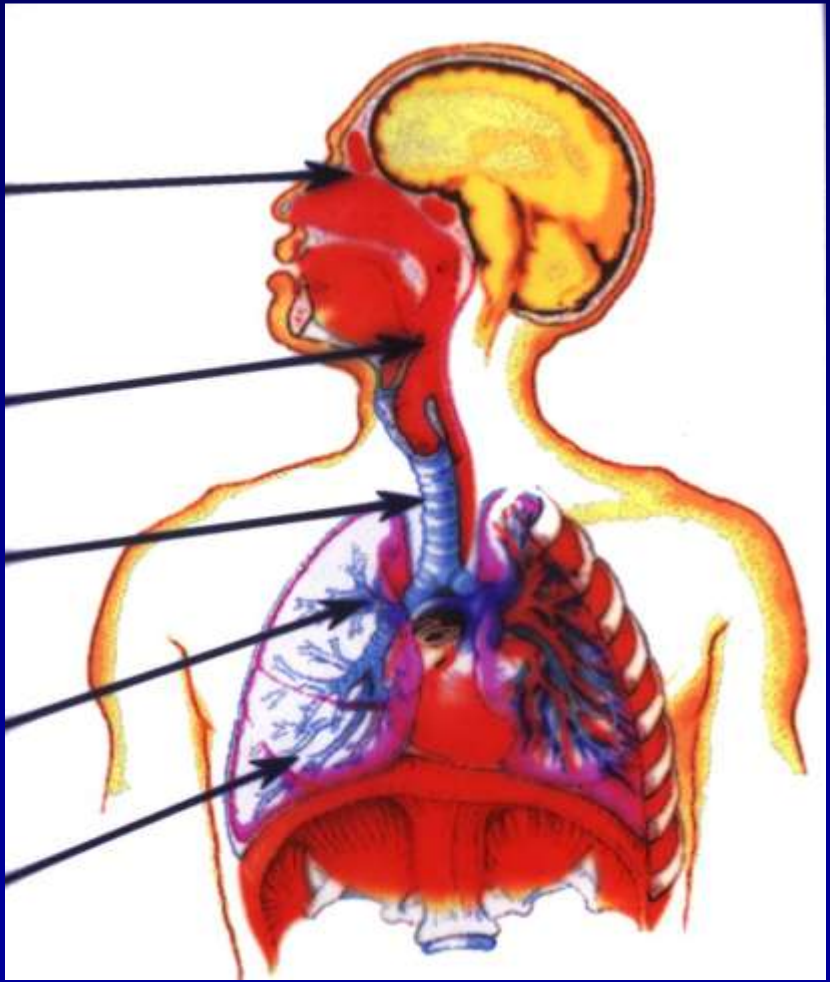
1 - 5	6.5 - 8.0	10 - 13
8,000	24,000	104,000

Bell Curve



Tularemia Aerosol, Particle Size and Type of Infection

	Particle Size (Micron, Mass Median Diameter)
18 - 20 micron particles fall out of aerosol, lodge in eye	**18 - 20**
15 - 18 micron particles lodge in pharynx	**15 - 18**
7 - 12 micron particles lodge in trachea	**7 - 12**
4 - 6 micron particles lodge in bronchiole	**4 - 6** Bronchioles
1 - 3 micron particles lodge in alveolus	**1 - 3** alveoli



Detrick Infections: 1943 - 1969 -- 456

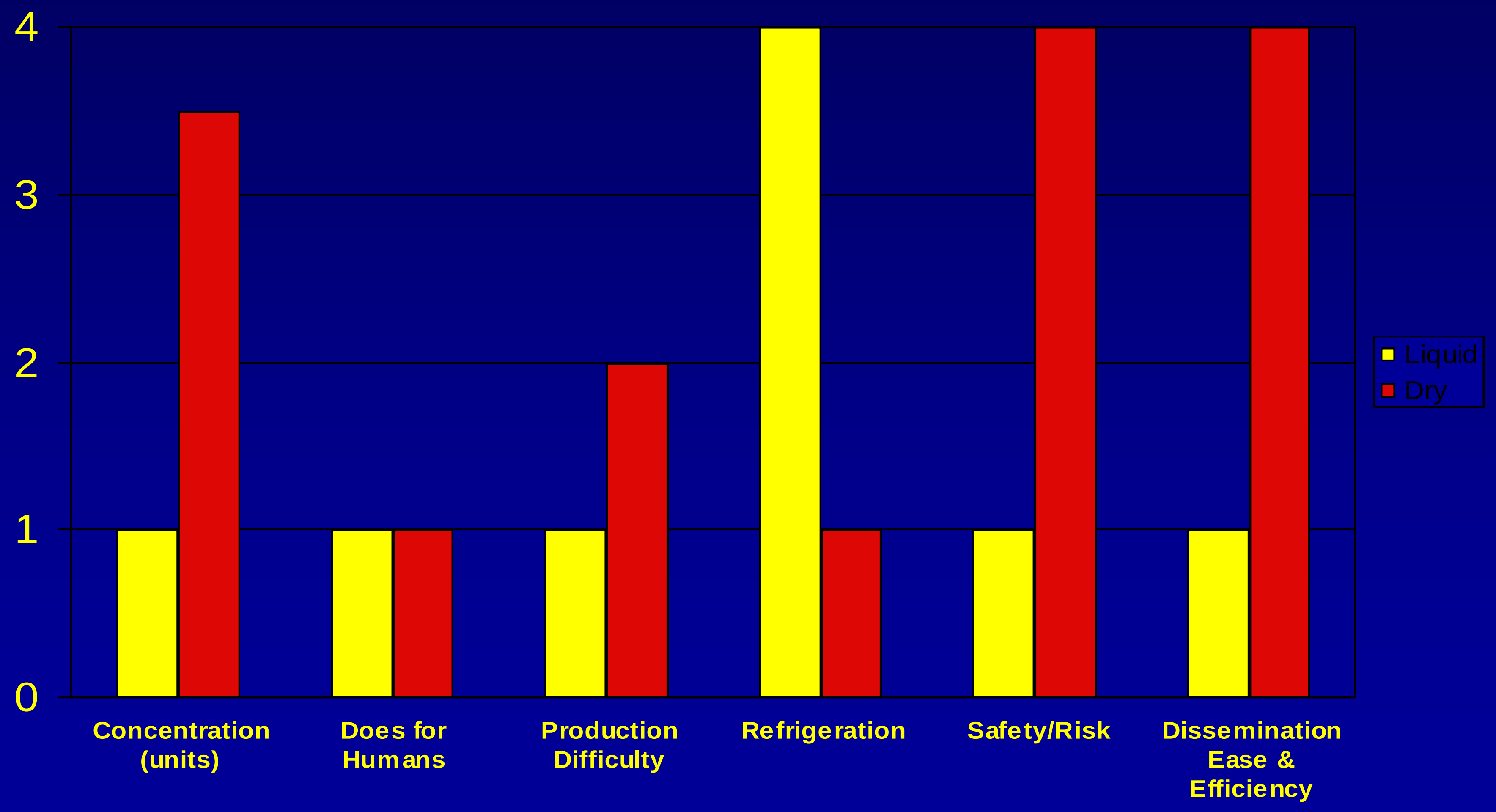
Tularemia*	153	Shigellosis	6
Brucellosis*	94	RMSF	5
Q Fever*	55	Newcastle	3
VEE*	43	BHF	1 **
Psittacosis*	32	Chikunguna	1
Anthrax*	31 **	Plague	1
SEB*	12	Salmonella	1
Coccidioidomycosis	9	Tuberculosis	1
Glanders	7	Blastomycosis	1

Bot Toxin* - 0

* Major Effort

**Lethal

Liquid/Dry Agent Formulation Comparisons and Characteristics



Relative Aerosol Potency for Agents with BW Potential

Agent	Respiratory Dose For Man (micrograms)
Q Fever	0.000002
Tularemia	0.0001
VEE	0.0004
Anthrax	0.008
SEB	0.025
Botulinum A	4.8
Nerve Agent VX	8,000.00

Less weight = better infectivity

More weight = worse infectivity

U.S. vs USSR: Comparison of Agent Products (kilo per one km^2)

Dry Agent	U.S.	USSR
Anthrax	4	5
Tularemia	3	4.5
Q Fever	2	-
Brucellosis	6	8 - 10
VEE	4	6
Botulinum Toxin	85	>100
Plague	-	3
Smallpox	-	3
Glanders	-	5
Marburg	-	0.2 to 0.8

U.S. vs USSR: Dry Agent Production
(metric tons per year)

Agent	U.S.	USSR
SEB	1.9	0
Tularemia	1.6	1500
Q Fever	1.1	-
Anthrax	0.9	4500
VEE	0.8	150
Botulinum	0.2	0
Plague	0	1500
Smallpox	0	100
Glanders	0	2000
Marburg	0	250

Crude Liquid Slurry/Not Stabilized

5 ml Disseminated from Single Fluid Nozzle at 75°F, 50% RH: In Darkness*

	Conc. Per mil	Organisms Per Liter of Aerosol			
	($x\ 10^9$)	4 Min	60 Min	120 Min	180 Min
Fresh Slurry at 0 Day	10	40,000**	2,000**	100**	6**
Monkey RLD_{50} (cells)		3	55	264	1370
Monkey doses per Liter		3333	127	-	-
Stored Slurry 4°C at 30 days	1	40	-	-	-
Monkey RLD_{50} (cells)		45	-	-	-
Monkey doses per Liter		0.88	-	-	-

* On overcast day - not bright sunshine, biological decay of tularemia is 20 to 30 percent per minute

**Biological decay for non-stabilized liquid tularemia in darkness is ±5% per minute

Tularemia Field Test in Marine Environment:
Line Source Dissemination of Stabilized Liquid*

Aerosol Age	Aerosol Traveled	Virulence for Monkey	Biodecay Over 144 Min.
30 min	14.5 kilometers	11 cells	1.7%/min
144 min	67 kilometers	57 cells	

*Sampling station not available beyond ±67 kilometers

- Liquid Tularemia, when properly cultivated, processed and stabilized was shown to be an *<u>outstanding</u>* agent in Field Tests in Pacific (1964).

- Line Source dissemination, from high performance aircraft indicated 180 gallons could produce 50% infections over 9,000 $miles^2$

Realistic BW Agents & Common Misconceptions

	Bot A	**Plague**	**Anthrax**	**Tularemia**
Growth Conc. ($x10^9$)	0.001	35	1	35
Purification of Conc. ($x10^9$)	0.02	350	50	350
Dose for Human	14,000	3,000	8,000	50
Respiratory Dose (per ml)	143	1.2B	6.2M	7B
Logistical Stability	Fair	Poor	Outstanding	Good
Aerosol Stability	Fair	Poor	Outstanding	Good
Target (kilometers)	1	5	100,000	100,000

Botulinum Field Test: Horn Island; 10/28/1943

- 54 MK1 four-pound bombs filled with slurry
- Test grid contained stands of boxed Guinea Pigs
- Bombs were fired singly, then in combinations of 2, 3 and 4 bombs simultaneously
- None of the bombs, even in combination, killed a single Guinea Pig by inhalation

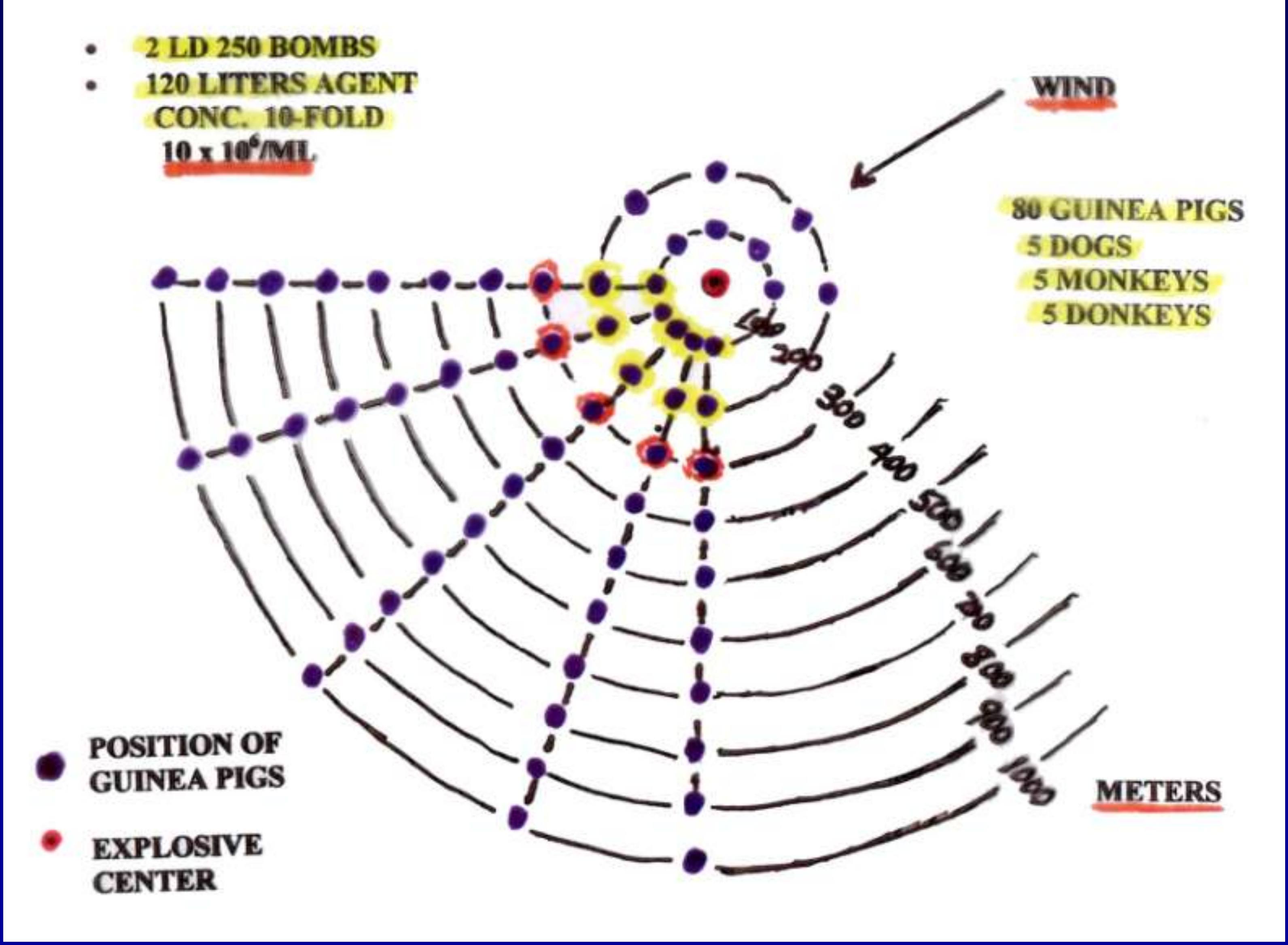
Botulinum Field Test: Horn Island; 10/28/1943 (continued)

- No trace of the toxin could be detected in the lungs during postmortem
- Only Guinea Pigs that died of Botulinum were those that licked the toxin off their own fur

CONCLUSION...

NOT AN EFFECTIVE LETHAL WEAPON

Iraqi Aerosol Test: Liquid Botulinum



Results of Iraqi Botulinum Aerosol Test

- **Guinea Pigs stationed at 100 and 200 meters downwind died TWO days post exposure**

- **Guinea Pigs stationed at 300 meters downwind became SICK but DID NOT DIE**

- **Guinea Pigs upwind of dissemination DID NOT BECOME SICK**

Results of Iraqi Botulinum Aerosol Test
(continued)

- **Monkeys, Donkeys and Dogs were not infected**

CONCLUSION...

The failure of 120 Liters to produce casualties only 200 - 300 meters downwind indicates that Botulinum Toxin is not an outdoor agent.

Pestis: LAB vs. PILOT PLANT

	Number of Cells for RLD_{50}	
	LAB	PILOT
Frozen Seed	3000 ±	3000 ±
25 ml Volume	3000 ±	3000 ±
200 ml Volume	3000 ±	3000 ±
12000 ml Volume	-	20,000
Small Seed Tank (15 gal)	-	800,000
Large Seed Tank		Not Done

Partial List of Organisms That Could Be Used in Oral Contamination

Organism	Growth Conc. ($x10^9$)	Effective Oral Dose (ED_{50})	Human Dose per mil
E. Coli -157.1 + >	40	2×10^1	2×10^9
Salmonella Quailes	30	1×10^7	6000
TY2-W	30	1×10^9	30
Meleagridis	50	4×10^7	400
Anatum	40	8×10^6	5000
Pullorum	20	$>1 \times 10^9$	±1
Shigella p.	50	50×10^9	±1
Brucella s.	40	1×10^6	40,000

Oral Dose (ED_{50}) In Volunteers*

Organism	Number of Organisms
Salmonella Anatum	6.5×10^7
Salmonella Newport	1.4×10^6
Salmonella Pullorum	1×10^9
Salmonella Typhosa	1×10^7
SEB	± 2.5 MCG
Shigella	1×10^8
Franciscella Tularensis	1×10^8

On average, these organisms grow to 35×10^9 cells per ml

* DTIC Recovery No. AD723-054

Contamination of Water Supply*

1. Salmonella Pullorum grows to conc. of $35x10^9$
2. Requires $1x10^9$ organisms to produce one ED_{50} (dose)
3. Therefore, 1 ml contains 35 doses or 0.028 ml per dose
4. Target: Reservoir contains $4.78x10^{10}$ gallons

Contamination of Water Supply* (continued)

5. Reservoir requires the addition of following GALLONS

   Salmonella Pullorum   148,444 gallons

*Based on Ft. Collins, CO. City of ±100,000 people. H_2O reservoir contains 150,000 acre foot of raw water x 328,000 gallons per acre foot.

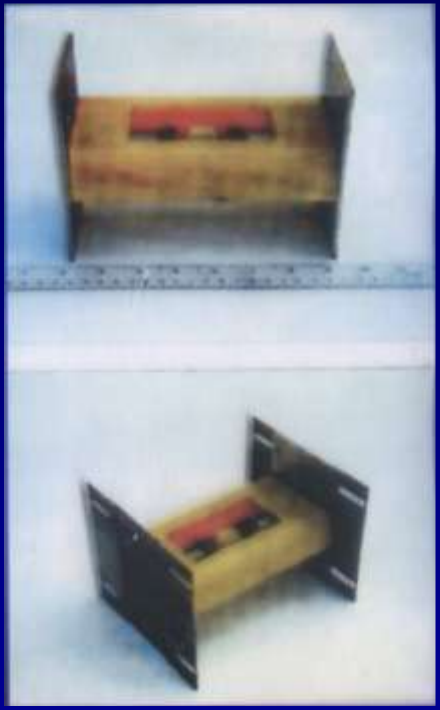
Dissemination

- Several means possible:
  - Aerosols most efficient
    - Droplets from liquid suspensions
    - Small particles from dry powders
  - Insect vectors
  - Contamination of food and water supplies

Examples of Point Source Bomblets

M114 Pipe Bomb

M143 Spherical Bomblet



Flettner Rotor Bomblet

Munitions (Terrorist)

Paint Sprayer

Trailed Sprayer

DRY

Leaf Blower

2-gallon garden sprayer

Munition Efficiency
(Aerosol Recovery)

- Defined as the number of organisms delivered airborne in the right particle size to cause respiratory infection
- Right particle size is 1 to 5µ, MMD

Munition Efficiency
(Aerosol Recovery) (*continued*)

- Example: 1,000 organisms available
  - Munition efficiency is <u>one</u> percent
  - Only 10 organisms in aerosol available to cause infection
  - Other 990 organisms killed or in large particles that quickly drop out of aerosol

Liquid Dissemination

- The generation of a small particle, infectious biological aerosol is a complex relationship between the device and the liquid
- This relationship is more complex for liquids than for powders
- For example, what is the disseminating efficiency of liquid tularemia using the line source tank the Aero 14 B tank?

Liquid Dissemination
(continued)

- **My response before providing an answer:**
  - What is the speed of the delivery vehicle?
  - What are the physical properties of the liquid; ie., viscosity, solids content, surface tension, etc.?
  - Is the agent stabilized?
- **These points will be described with experimental data**

Dissemination Efficiency of Dry Agent Powders

- Particle size and the absence of electrostatic charge are the important parameters that determine disseminating efficiency of the device (munitions)

- Quote from Don Falconer, Director of Munitions Development, former U.S. Offensive Program: "Dry agent (and suffering no loss of viability as a result of aerosolization) can be disseminated with efficiencies limited only by the proportion of the fill in the required particle size range."