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)

Aerosol Parameters (Historical)

- Standardized Exposures - 10 min duration
- Nonhuman primates
  - anesthetized
  - Respiratory parameters (MV, RR) measured by whole-body plethysmograph
- Rodents
  - awake
  - Respiratory parameters calculated from published formulas
- No assumptions made about retention
- Doses delivered – “Presented dose”
  - Inhaled dose estimation : aerosol concentration . MV . Time

Example of Bacterial Infectivity Data

Animal Model	LD_{10} cfu	LD_{50} cfu	LD_{99}(# LD_{50}) cfu	LCt_{50} cfu.min.m^3
Guinea Pig (Hartley)	6.9×10^3	7.9×10^4	2.1×10^5 (2.7)	
Rabbit (NZW)	8.8×10^4	1.1×10^5	1.4×10^5 (1.3)	
Monkey (Rhesus)	3.4×10^4	5.5×10^4	1.3×10^5 (2.3)	5.2×10^7

cfu - colony forming units

Bacterial Infectivity Data

Animal Model	LD_{50} (cfu)	Probit Slope
Guinea Pig (Hartley)	7.9×10^4	2.4
Rabbit (NZW)	1.1×10^5	8.5
Monkey (Rhesus)	5.5×10^4	6.3

cfu - colony forming units

A comparative efficacy study using an aluminum based adjuvant

Animal Models:

- Hartley Guinea pig
- NZW Rabbit
- *Macaca mulatta* (Rhesus)

Study Design:

- Vaccination: 2 doses, IM, 0 and 4 weeks
- Aerosol Challenge: 16 weeks

Survival Results

	Guinea pig	Rabbit	Rhesus
Challenge Dose (LD_{50})	30	63	147
Vaccine X Control	5/20 (20%)	9/10 (90%)	5/5 (100%)
50 µg Vaccine Y	7/20 (35%)	10/10 (90%)	9/9 (100%)
5 µg Vaccine Y	6/20 (30%)	9/10 (90%)	9/10 (90%)
0.5 µg Vaccine Y	8/20 (40%)	6/10 (60%)	9/10 (90%)
Adjuvant Only	0/19 (0%)	0/10 (0%)	0/6 (0%)

Example of a Bacterial Natural History Study

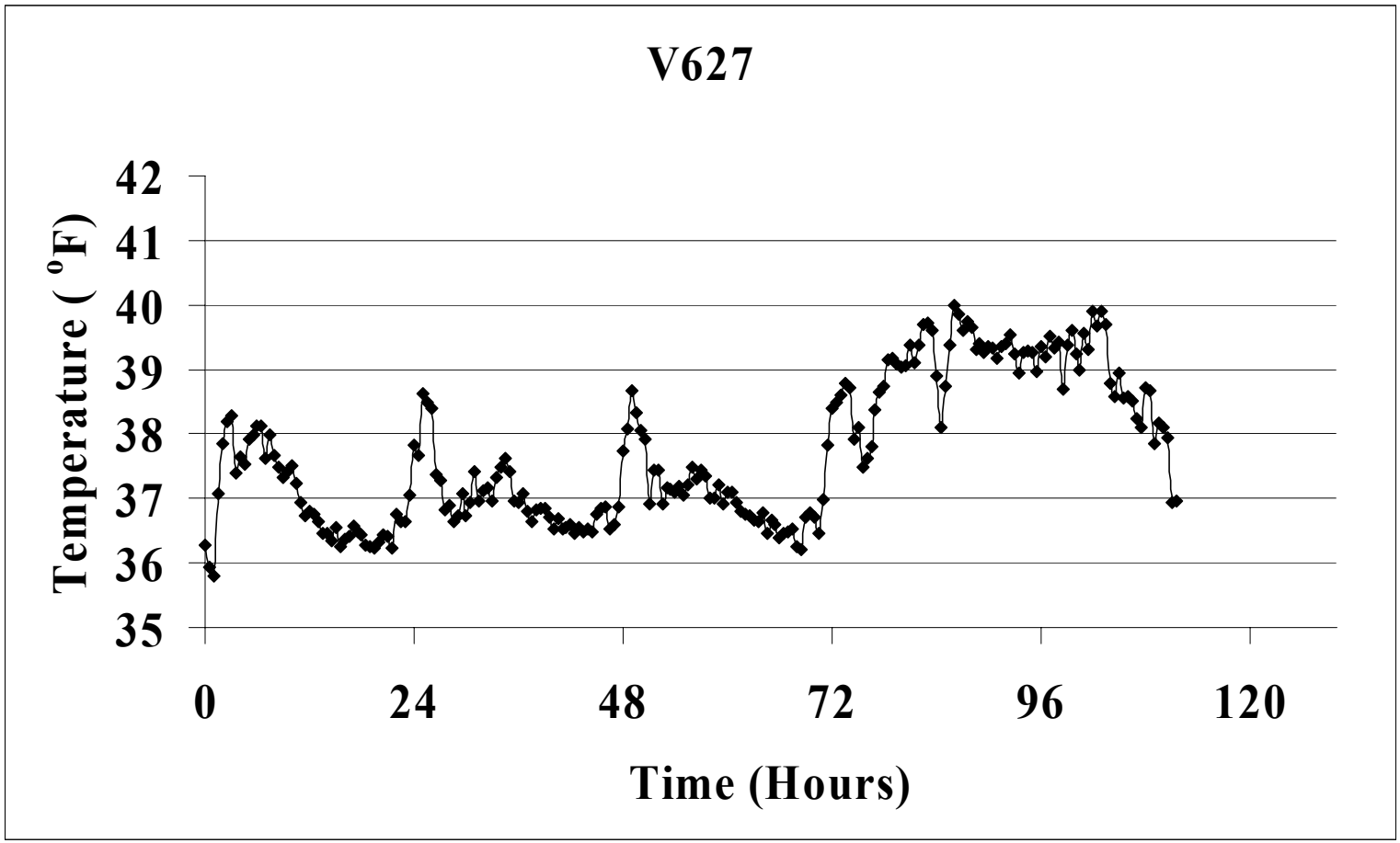
Animal Model: *Cercopithecus aethiops* (African green monkey)

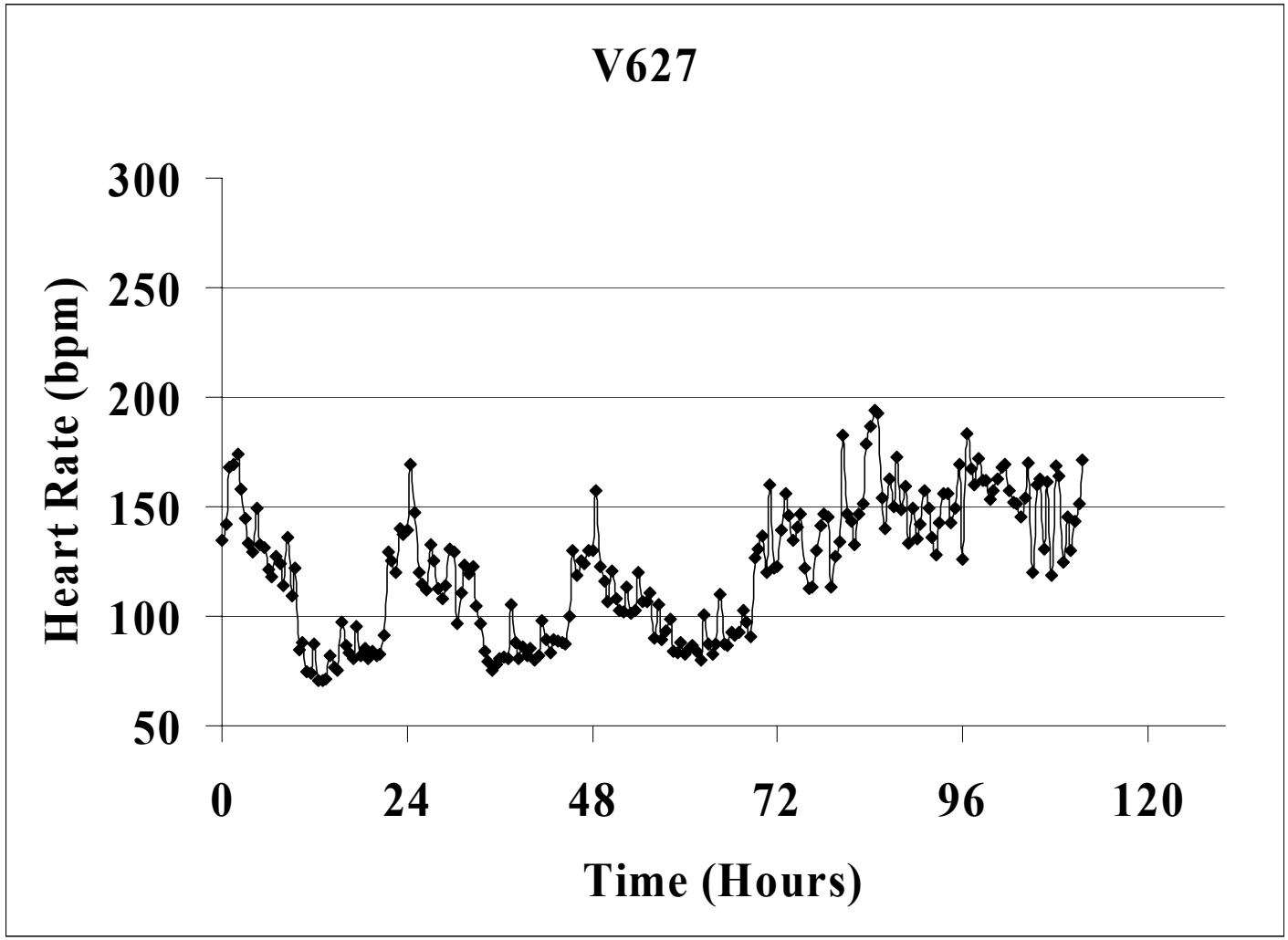
Exposure Data

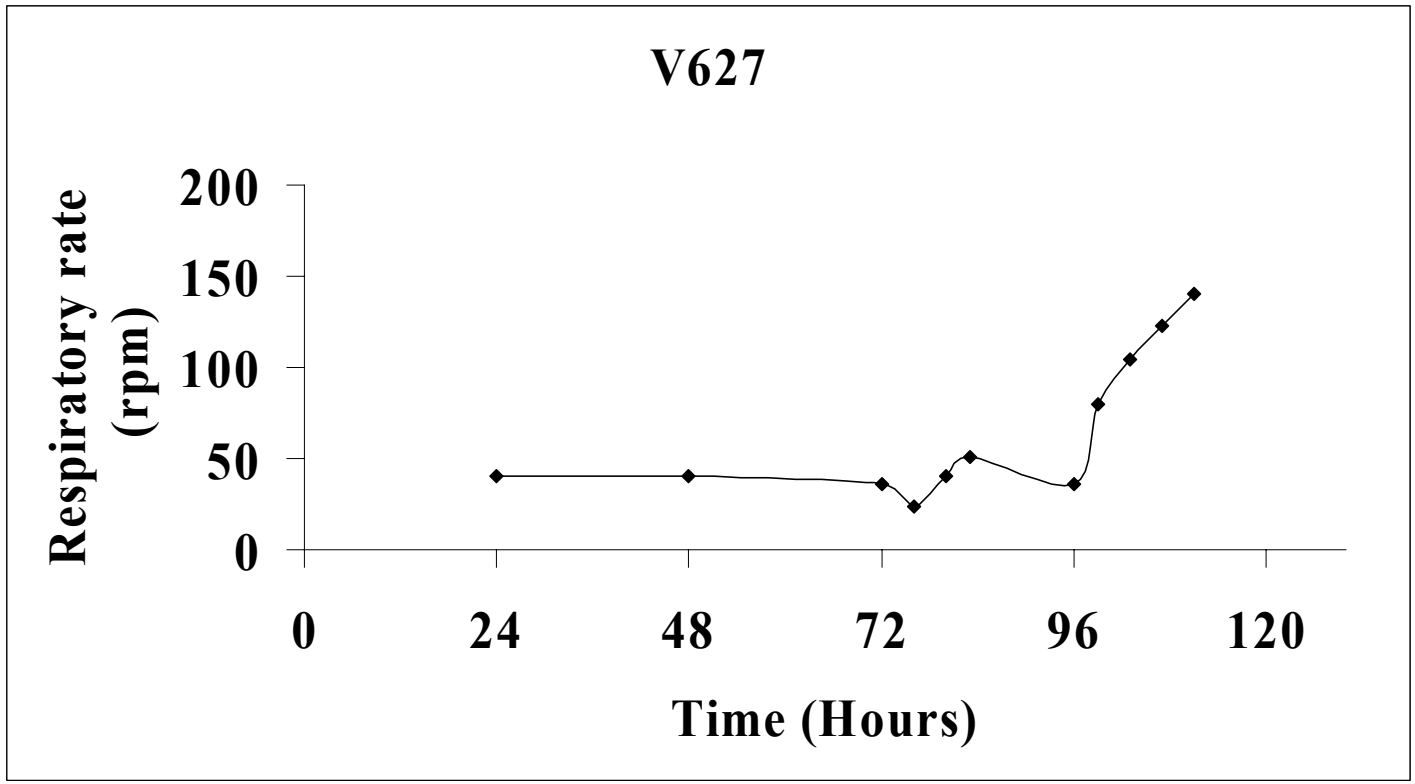
			Inhaled Dose		
Run #	Animal #	MV (l)	cfu	log	LD_{50}
1	V627	0.78	1.90E+04	4.3	57
2	V514	0.52	1.00E+04	4	30
3	V569	0.63	8.00E+03	3.9	23
4	V113	0.72	7.20E+03	3.9	21
5	V605	0.71	3.10E+03	3.5	9
6	V521	0.64	4.30E+03	3.6	12

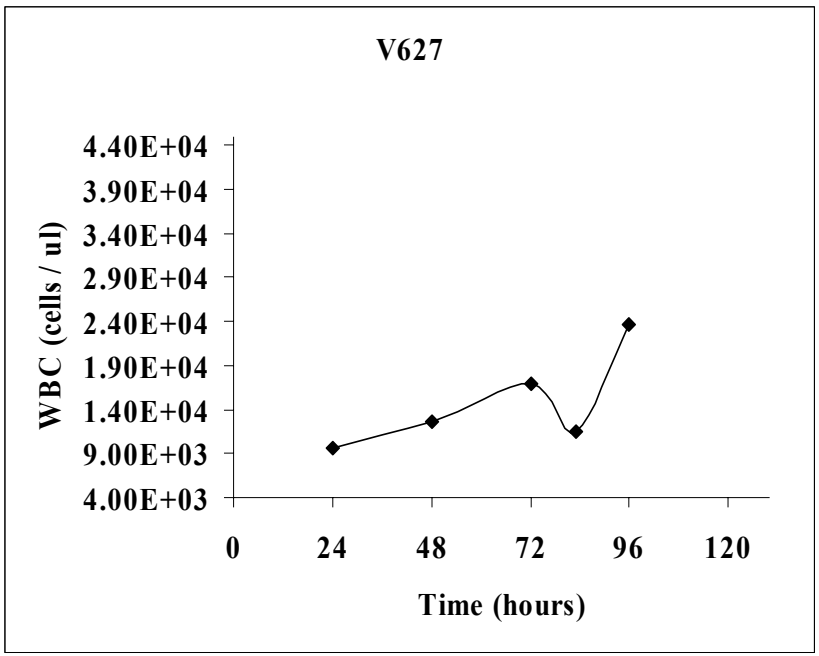
Bacteremia Summary

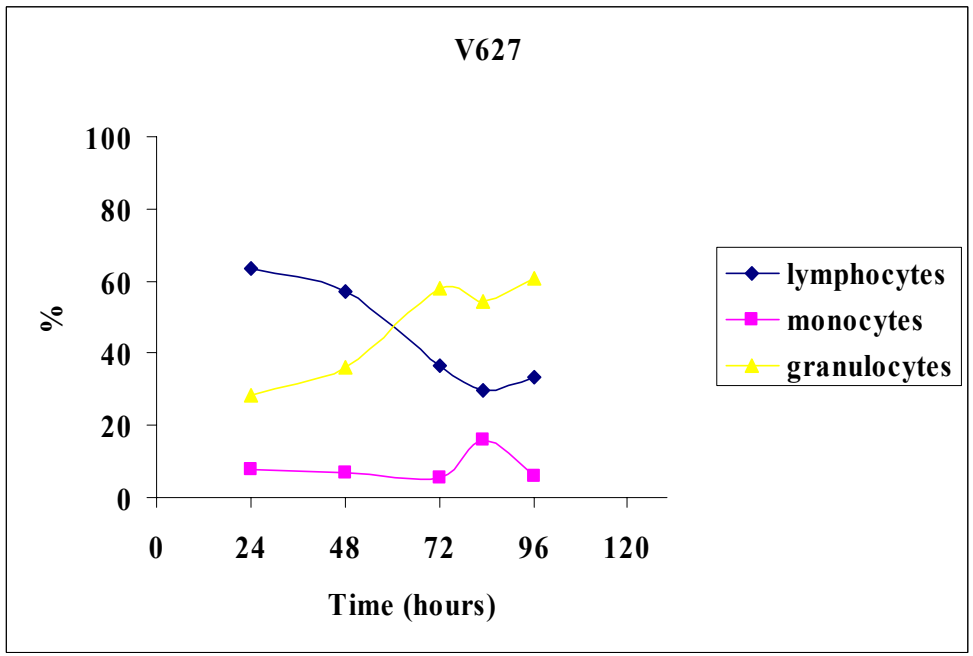
		Time (hours)						
Animal No.	Inhaled Dose (LD_{50})	24	48	72	80	83	96	At euthanasia (cfu / ml)
V627	57	-	-	+	nd	+	+	8E+07 (111.5 hr)
V514	30	-	-	+	+	nd	+	3E+06 (111 hr)
V569	23	-	-	+	nd	+	+	9E+08 (99.5 hr)
V113	21	-	+	+	nd	+	+	1E+08 (125 hr)
V605	9	-	-	-	nd	nd	-	
V521	12	-	-	-	nd	nd	-	

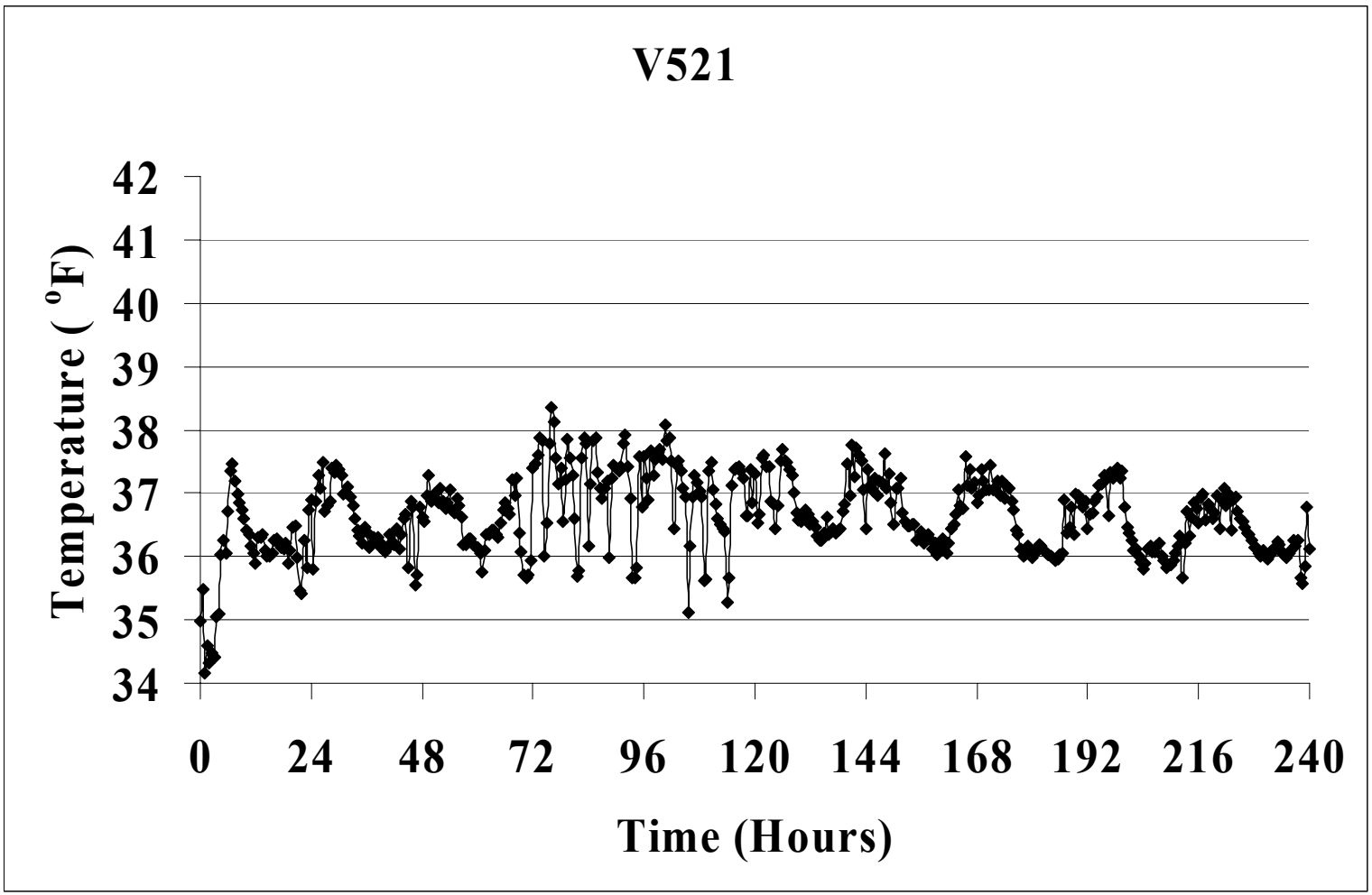

V627
Temperature (°F)
42
41
40
39
38
37
36
35
0
24
48
72
96
120
Time (Hours)

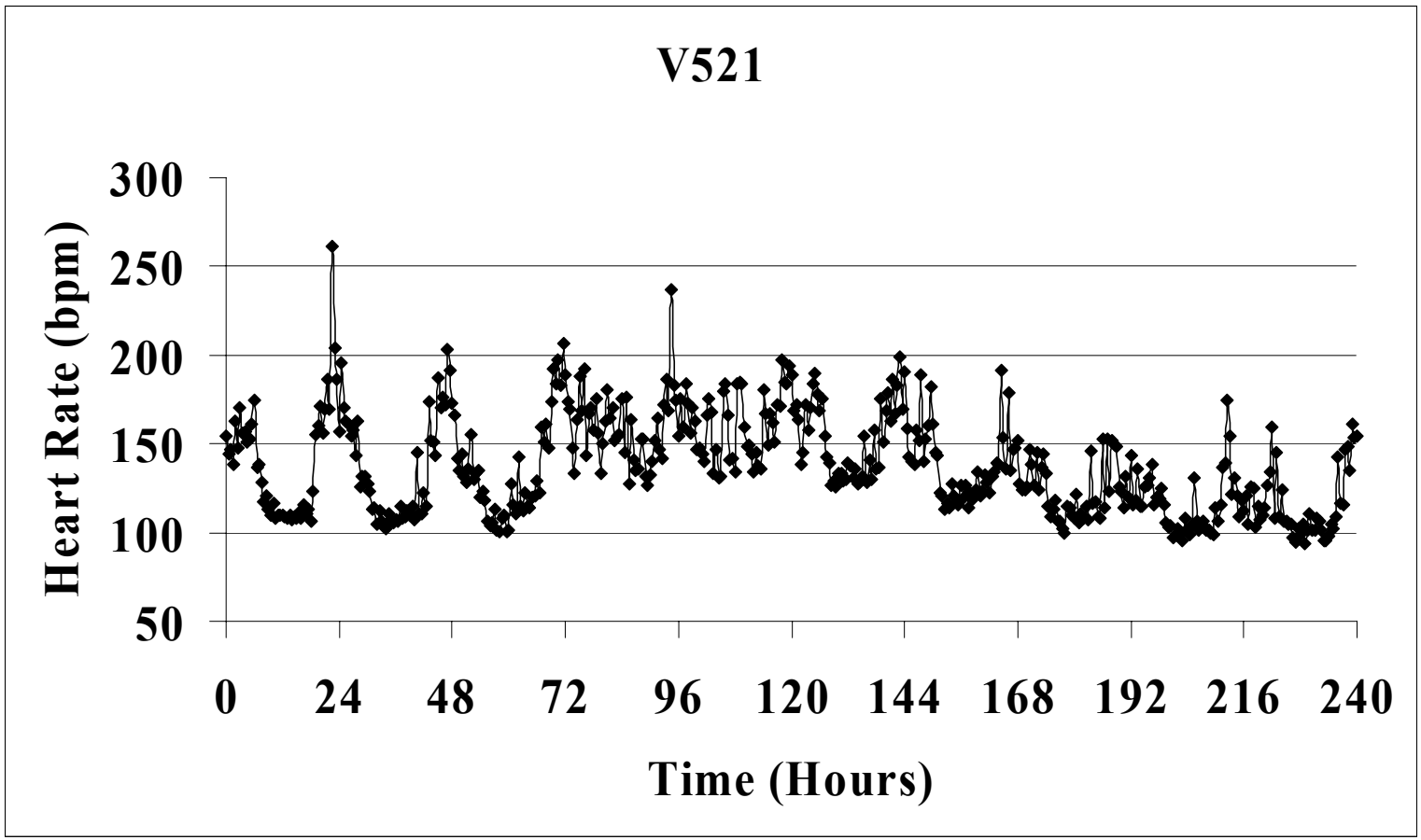

V627
300
250
200
150
100
50
Heart Rate (bpm)
0
24
48
72
96
120
Time (Hours)

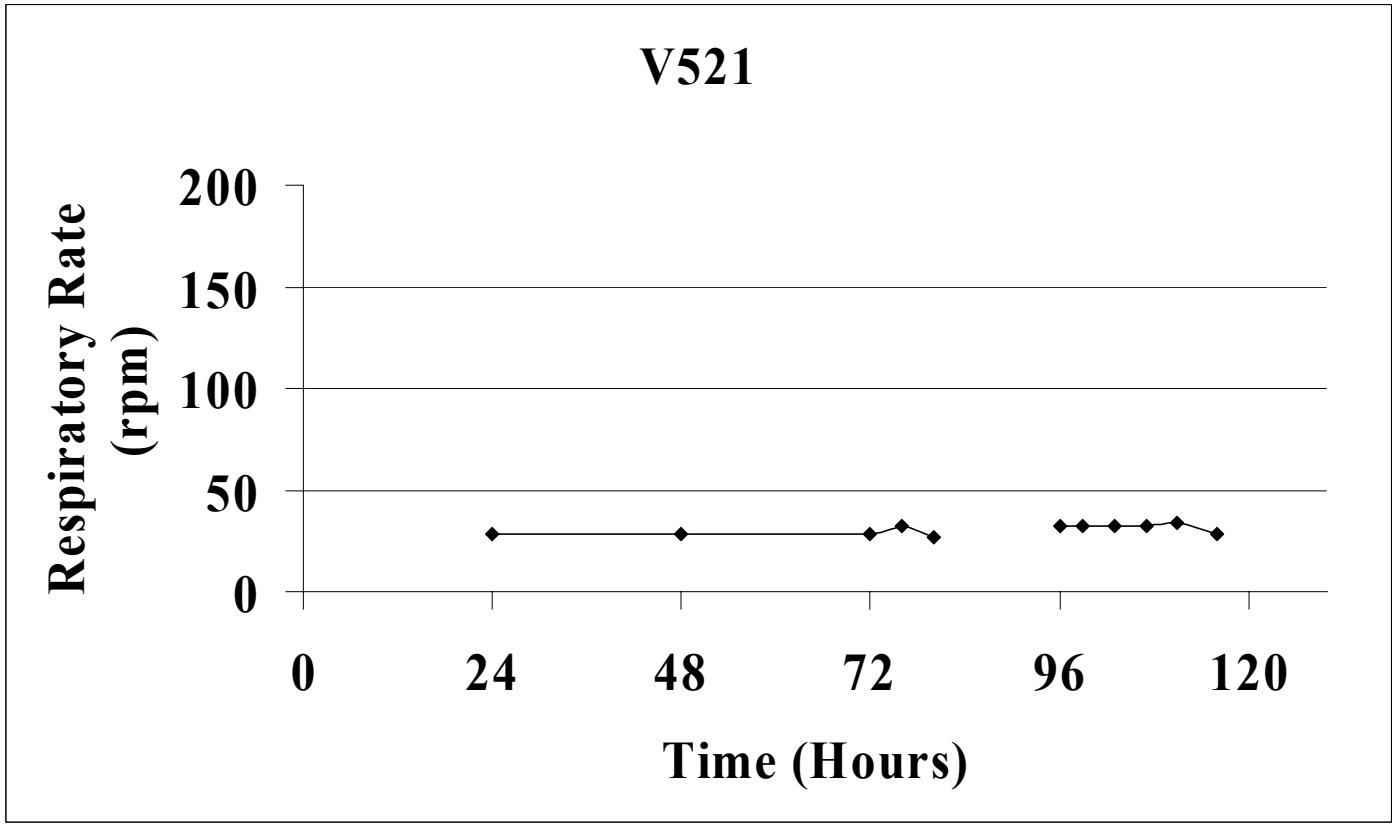

V627
Respiratory rate (rpm)
200
150
100
50
0
0
24
48
72
96
120
Time (Hours)

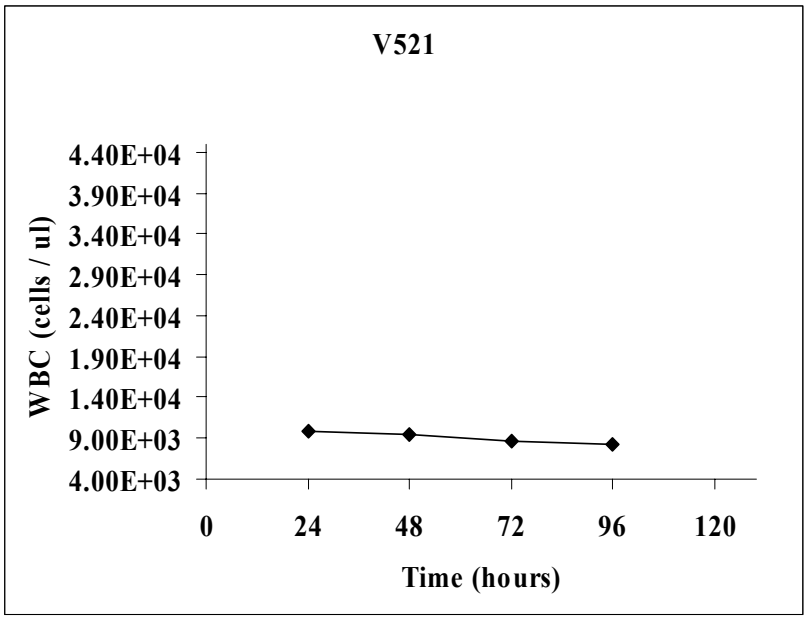

V627
WBC (cells / ul)
4.40E+04
3.90E+04
3.40E+04
2.90E+04
2.40E+04
1.90E+04
1.40E+04
9.00E+03
4.00E+03
0
24
48
72
96
120
Time (hours)

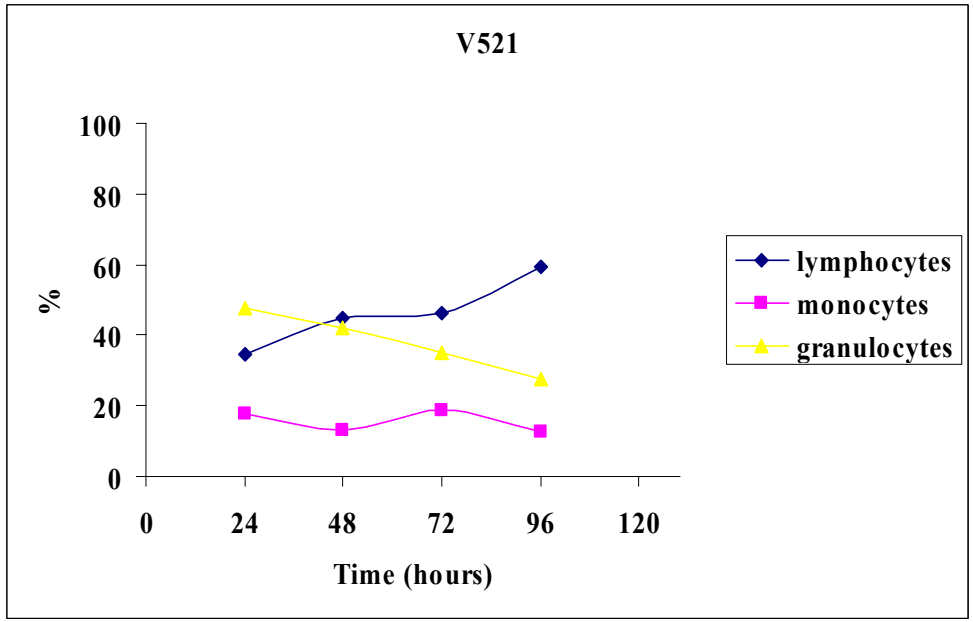

V627
%
100
80
60
40
20
0
0
24
48
72
96
120
Time (hours)
lymphocytes
monocytes
granulocytes


V521
Temperature (°F)
42
41
40
39
38
37
36
35
34
0
24
48
72
96
120
144
168
192
216
240
Time (Hours)


V521
Heart Rate (bpm)
300
250
200
150
100
50
0
24
48
72
96
120
144
168
192
216
240
Time (Hours)


V521
Respiratory Rate (rpm)
200
150
100
50
0
0
24
48
72
96
120
Time (Hours)

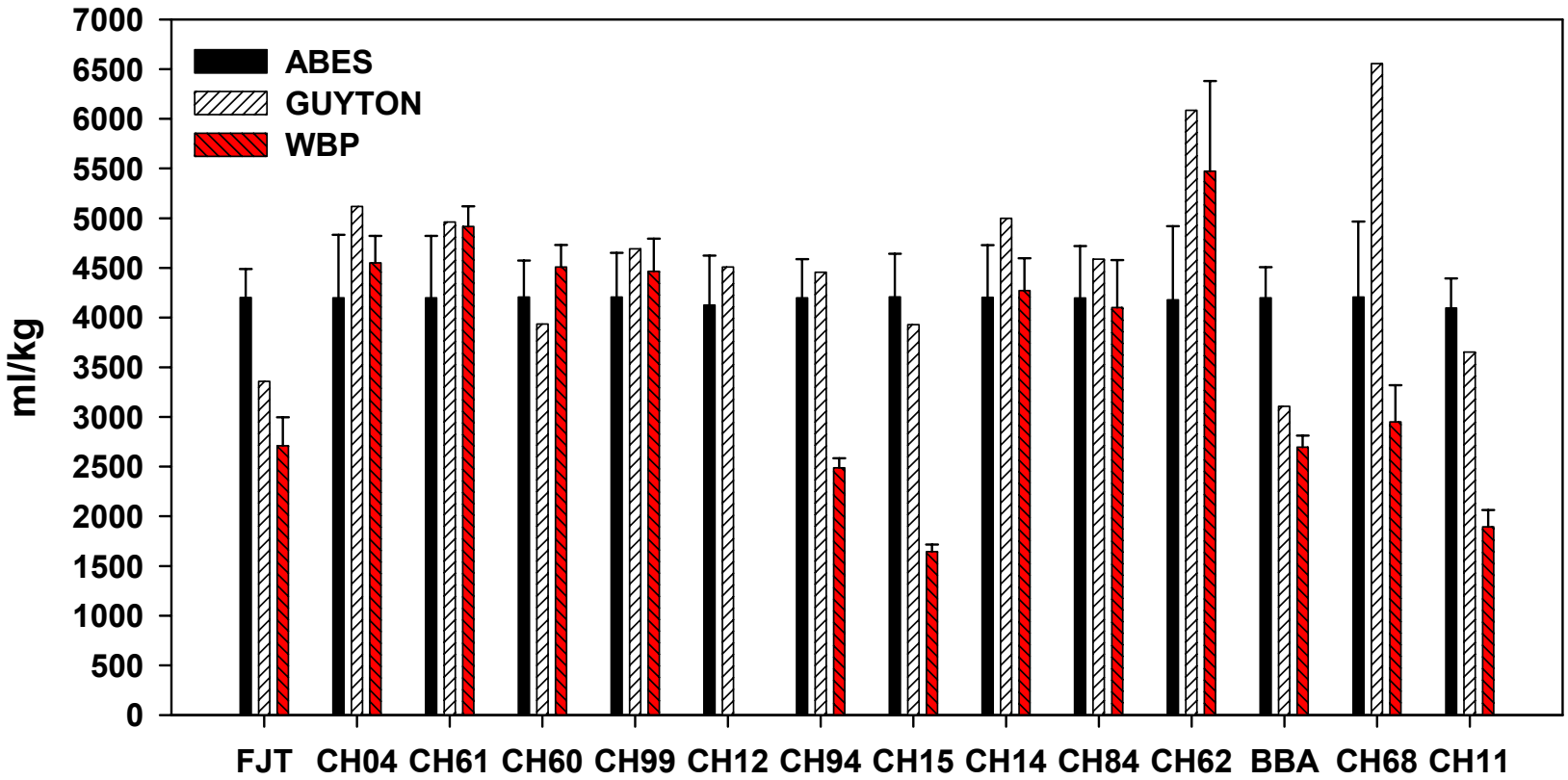

V521
WBC (cells / ul)
4.40E+04
3.90E+04
3.40E+04
2.90E+04
2.40E+04
1.90E+04
1.40E+04
9.00E+03
4.00E+03
0
24
48
72
96
120
Time (hours)


V521
%
100
80
60
40
20
0
0
24
48
72
96
120
Time (hours)
lymphocytes
monocytes
granulocytes

Vaccine Efficacy Study

- Comparative efficacy study to determine if alternative delivery of vaccine would protect against lethal aerosol challenge
  - Both precision and accuracy of challenge dose especially important because of the comparative nature of the study
- Selected challenge dose: 20 LD_{50}s (550 μg/kg)
- Primates (n=16) were anesthetized (5-6 mg/kg Telazol)
  - Whole body plethysmography performed prior to challenge
  - The automated system w/ real-time dosimetry function used for challenge
  - Guyton's formula used to calculate dose based on exposure time (for comparison purposes)
  - Primates weights (6.4 ± 0.8 kg)

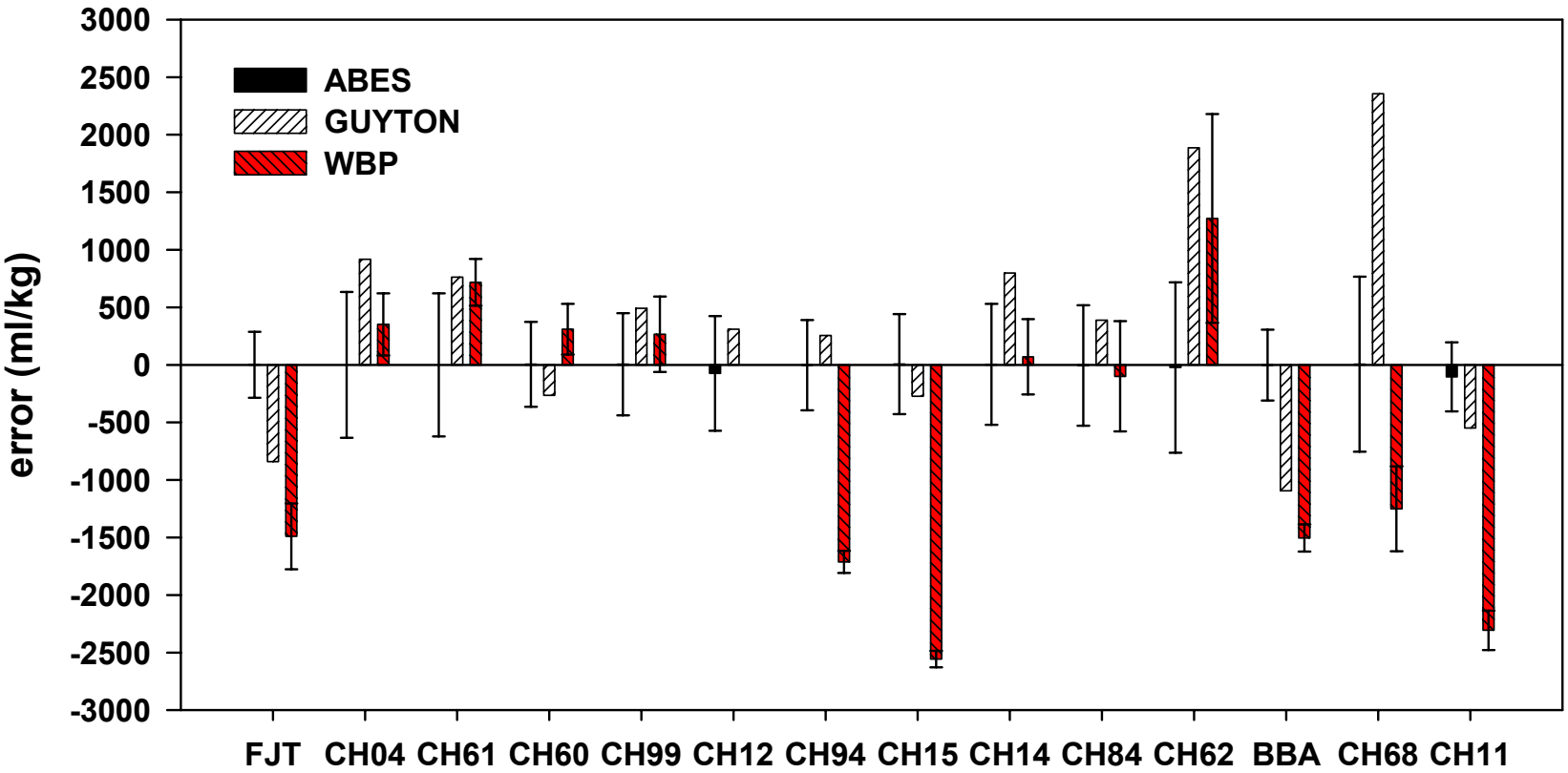
Respiratory parameters and dose calculation for exposures using ABES respiratory monitoring, Guyton's formula, and WBP



inhaled volume per unit body weight (ml/kg) during exposure

From Hartings and Roy, *In Press*

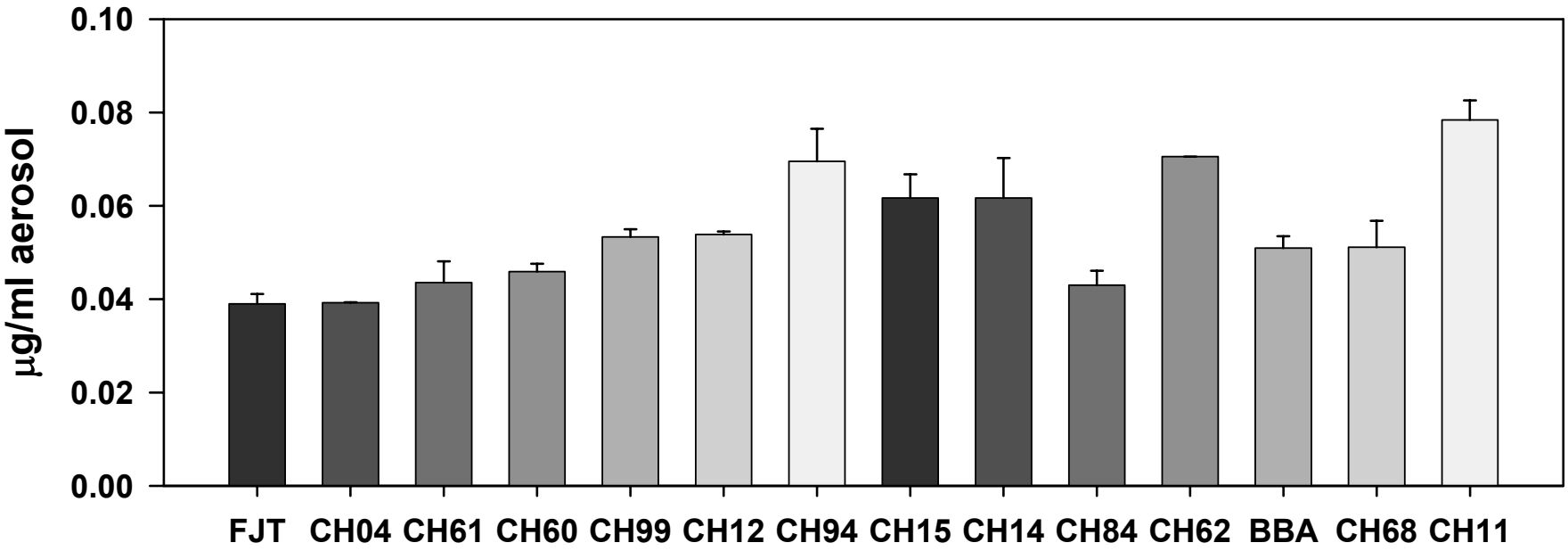
Respiratory parameters and dose calculation for exposures using ABES respiratory monitoring, Guyton's formula, and WBP



deviation of the inhaled volume per unit body weight (ml/kg) from the 4200 ml/kg required, based on estimated aerosol concentration, to achieve the desired dose of 550 μg/kg

From Hartings and Roy, *In Press*

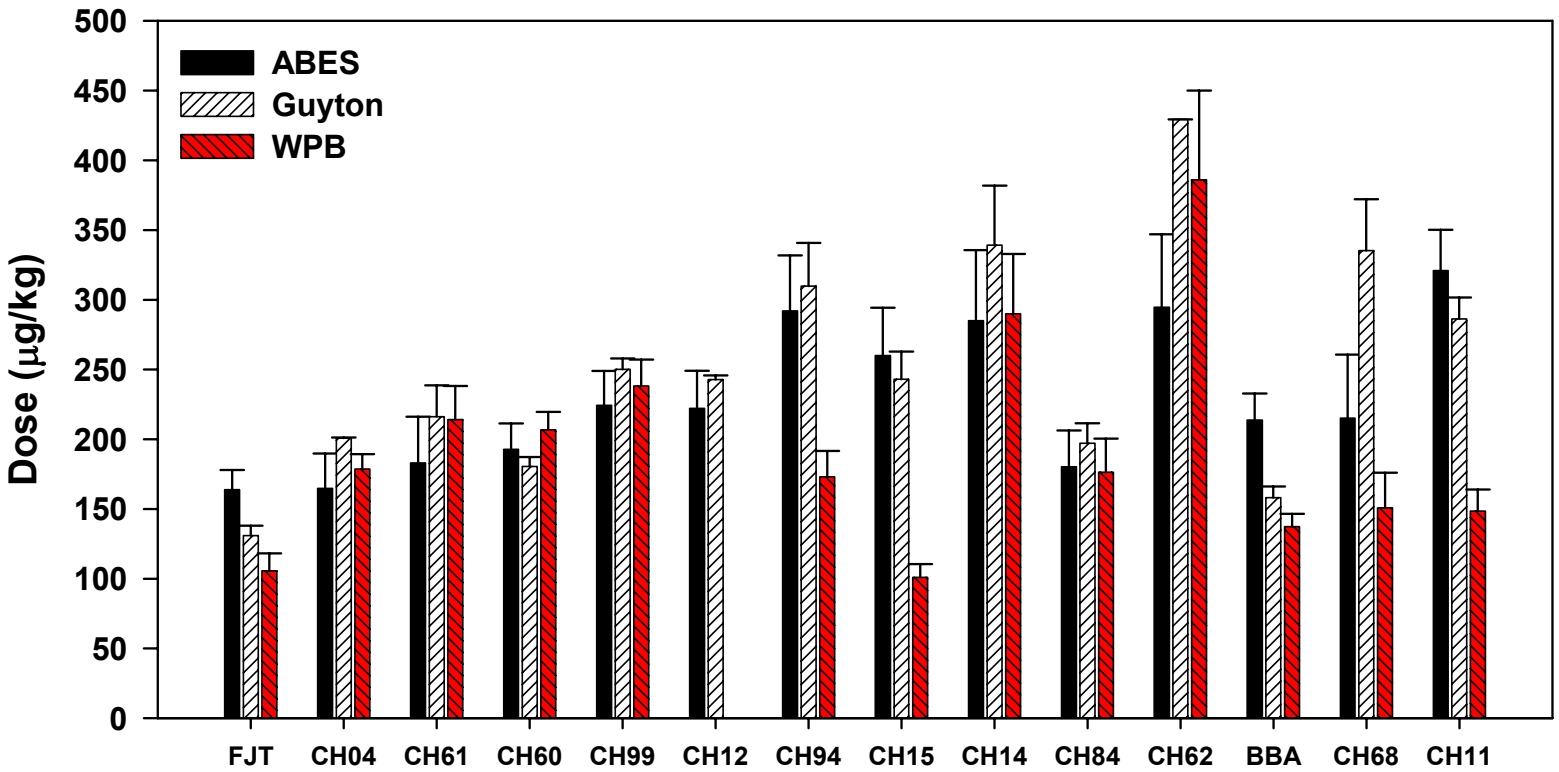
Respiratory parameters and dose calculation for exposures using ABES respiratory monitoring, Guyton's formula, and WBP



chamber aerosol concentrations for exposures as determined based on AGI samples

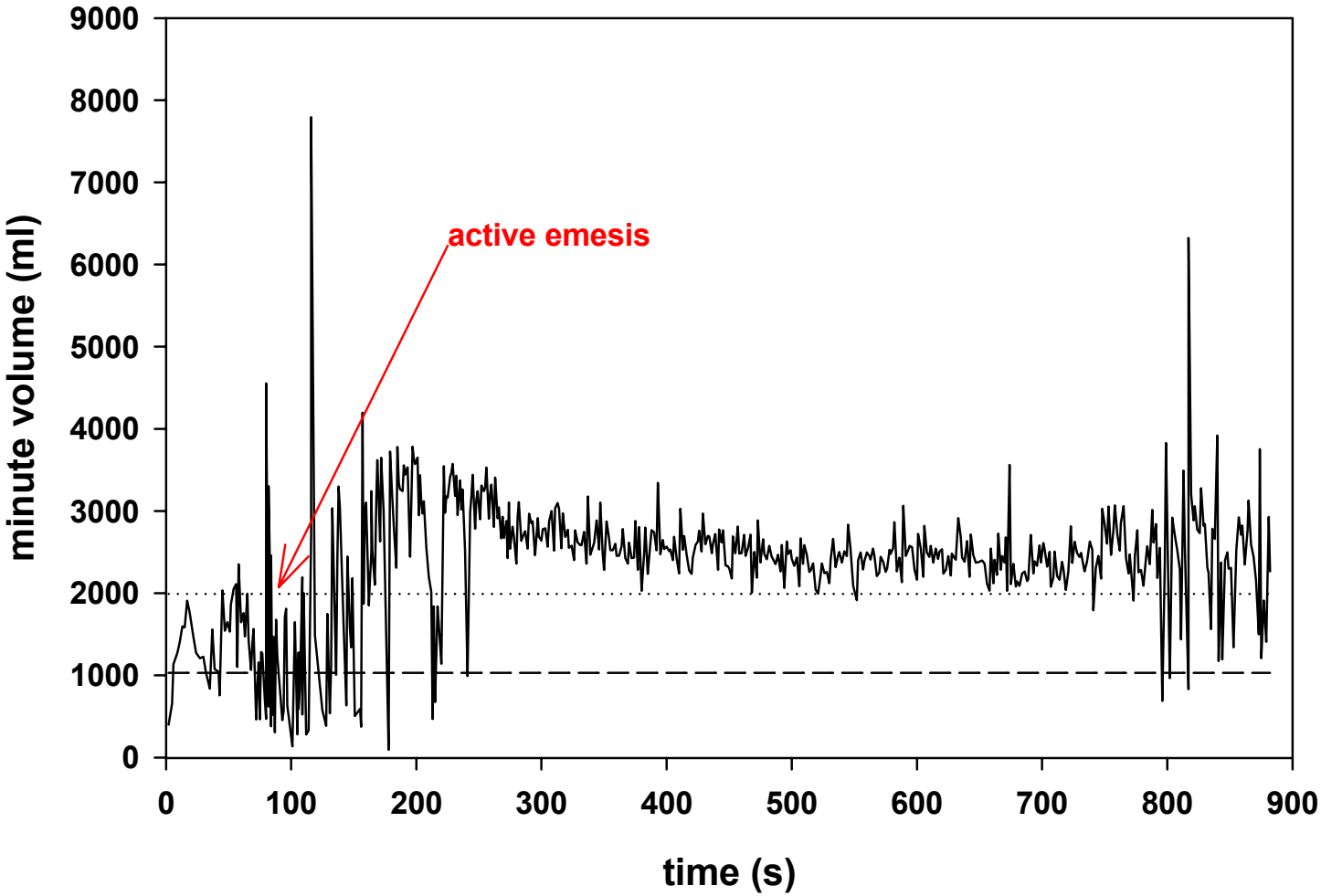
From Hartings and Roy, *In Press*

Respiratory parameters and dose calculation for exposures using ABES respiratory monitoring, Guyton's formula, and WBP



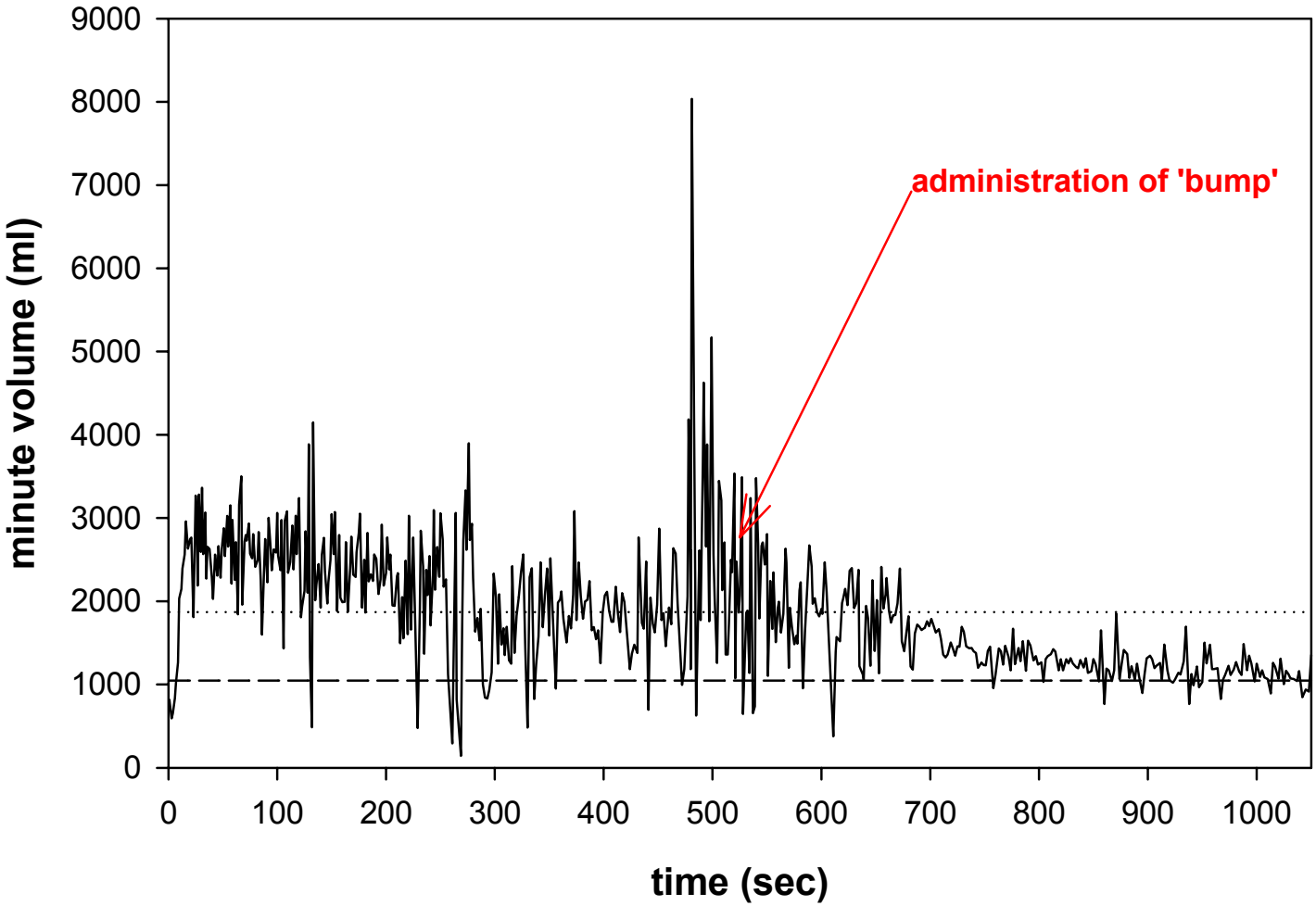
Inhaled doses for exposures as calculated based on the results shown from inhaled volume per body weight and determined aerosol concentration

From Hartings and Roy, *In Press*



Effect of emesis on minute volume during inhalation exposure of one rhesus monkey precipitated by acute enteric effects of challenge. The dashed (--) and dotted lines (···) represent minute volume estimates derived from predictive whole-body plethysmography or Guyton's formula, respectively.

From Hartings and Roy, *In Press*



Minute volume measurements for a NHP requiring administration of anesthetic during exposure. The dashed (--) and dotted lines (···) represent minute volume estimates derived from predictive whole-body plethysmography or Guyton's formula, respectively. A dose of 5-6 mg/kg of Telazol was administered at the point indicated by the arrow.

From Hartings and Roy, *In Press*