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Toxic Effects of a Whole-body Inhalation Sarin (GB) Vapor Exposure in the Gottingen Minipig

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U.S. Army RDECOM

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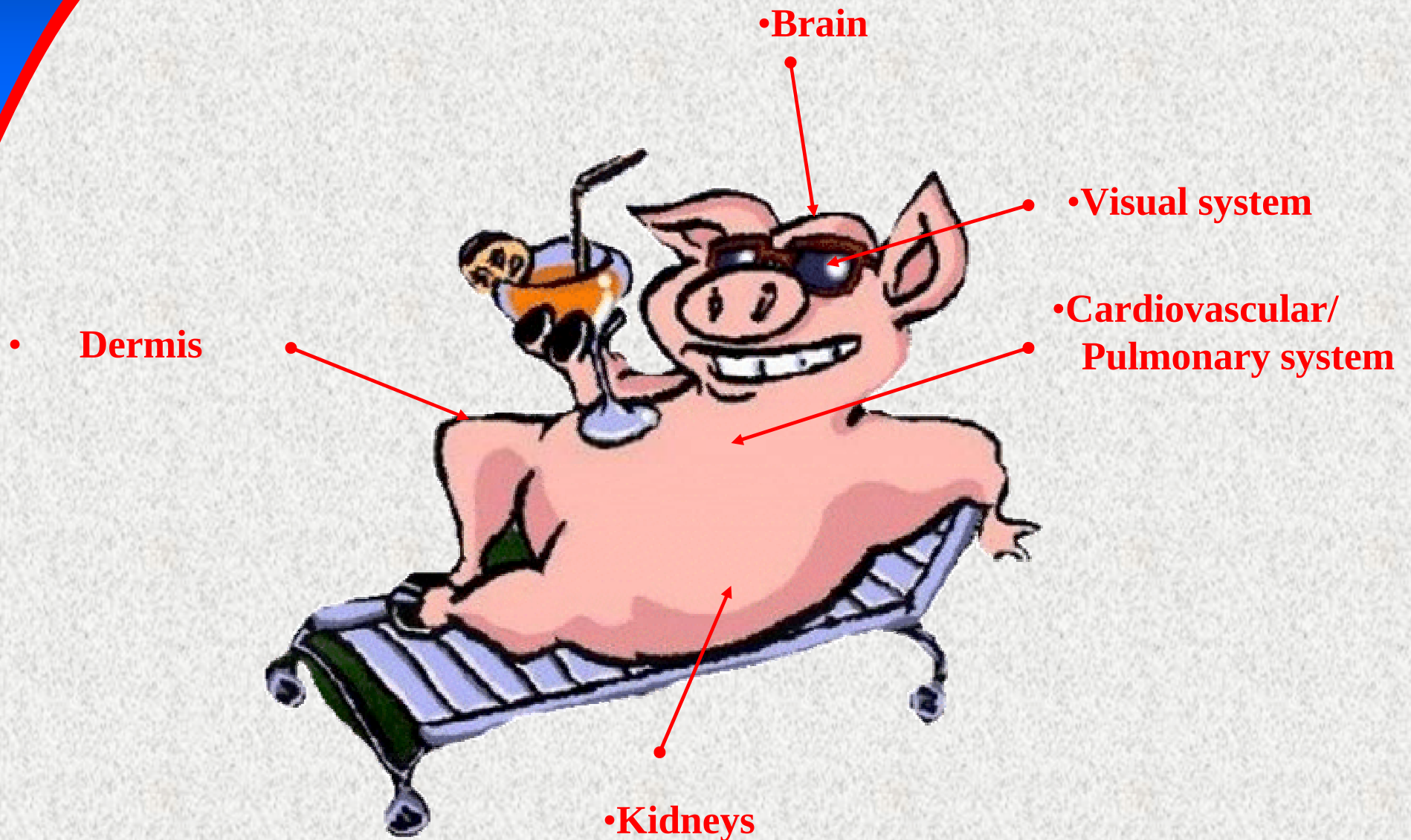
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Benefits of the minipig

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Gottingen Minipigs

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- Well defined genetic background and health status
- Small size at sexual maturity
 - Males 7-9 kg (3-4 months)
 - Females 9-11 kg (4-5 months)

Experimental preparations

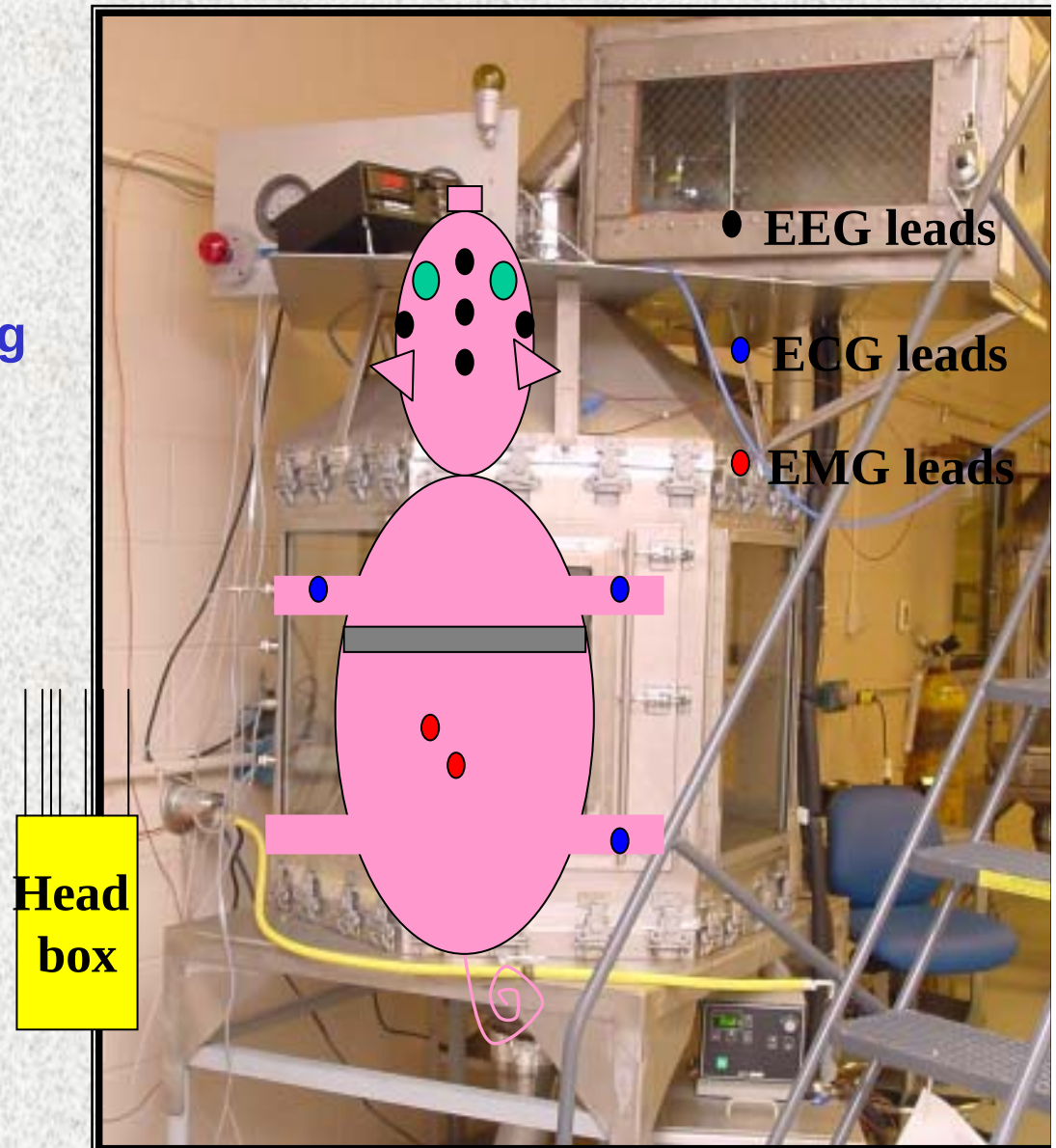
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- Exposure chambers modified/set-up for whole-body exposure of minipigs.
- Gottingen (Ellegaard) minipigs (10-15 Kg)
 - Pigs surgically prepared with jugular cannulas for serial blood sampling during exposure
 - IR/digital video camera and digital image capture system developed to monitor and calculate pupil size
 - Real-time monitoring of first noticeable effect (FNE) during exposure

Vapor GB generation chamber

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- Pig placed in sling
- Respiratory belt, ECG leads and EEG leads attached to pig and leading to Bio-logic headbox.
- 1000 L dynamic airflow chamber
- GB generation system contained in glove box
 - Vapor Sampling / Analysis
- Jugular catheter passed through ports
- IR images of pupil taken through plexiglass



Exposure

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- 5.35 mg/m³ vapor GB for 10 minutes (Ct=53.5 mg.min/m³)
- Infrared images captures continuously
- Blood samples collected every 2 minutes during exposure
- Electrophysiology system collects signals throughout exposure
- A score sheet is used to rate pigs on activity and onset/progression of signs

Data collected

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- Miosis onset, Time-to-maximum miosis
- RBC and plasma cholinesterase activity
- Internal GB (GB regeneration assay)
- Blood Chemistry
 - Lactate, glucose, creatinine, hematocrit, hemoglobin, Sodium, Potassium, pH, HCO₃, SaO₂, PO₂, PCO₂, TCO₂, base excess
- Electrocardiogram
 - Heart rate (R-R interval), PR interval, QT interval, QTc interval, ST interval, QRS interval
- EEG, EMG, airflow, eye movements, respiratory rate,
- Distribution of GB in tissue

- First and only studies looking at the global toxicological affects of a whole-body nerve agent vapor exposure in real-time
- Large scale operation that requires the help and expertise of many people

Data collection methods / Biological endpoints

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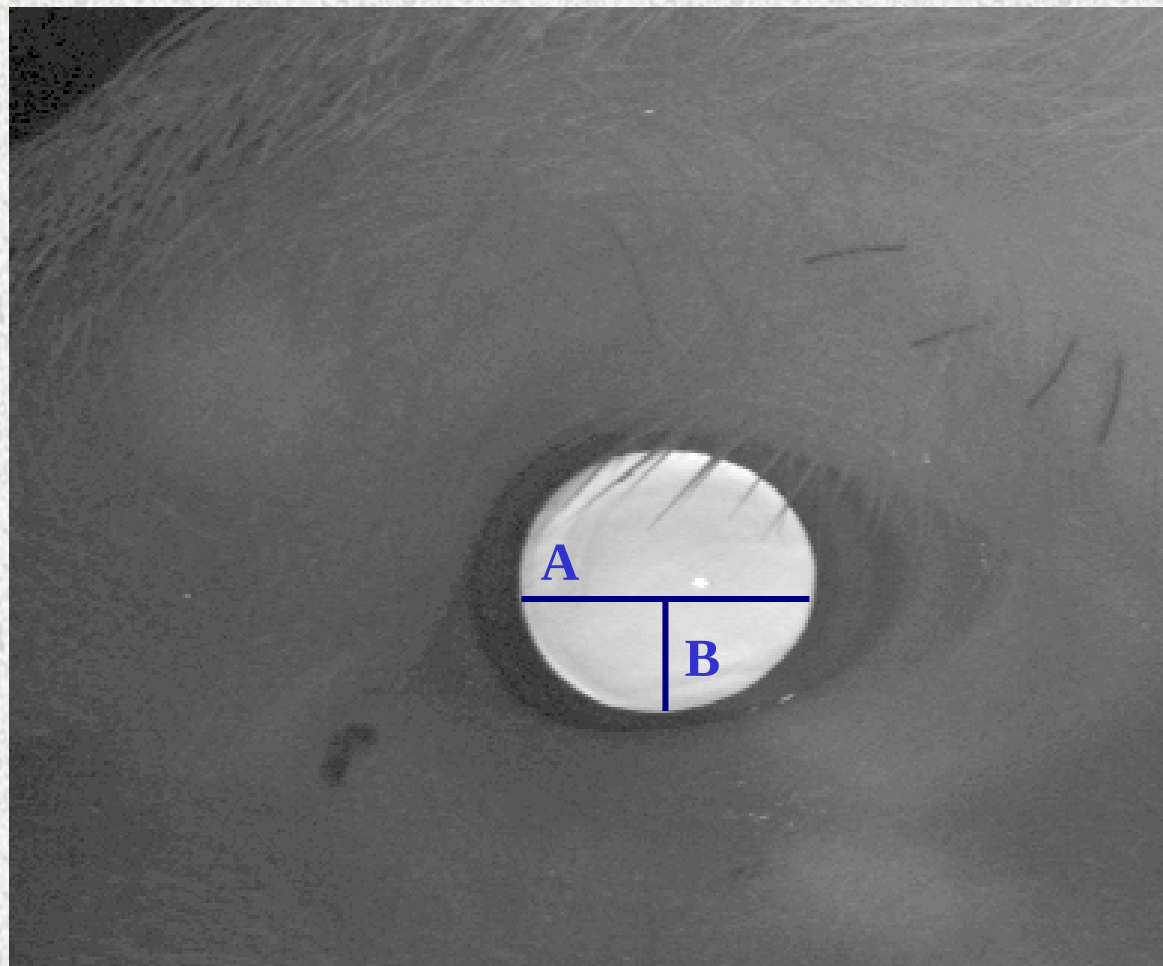
- Infrared camera
 - Allows images under dim light conditions
 - Uses pupil areas to plot time-to-miosis
- Insertion of external jugular catheter
 - Serial blood samples during “real-time” exposure
 - RBC and plasma cholinesterase
 - GB regeneration assay
 - Blood Chemistry (iStat)
- Physiological monitoring (Bio-logic, Inc.)
 - EEG, EKG, EMG, airflow, eye movements, respiratory rate, heart rate, SaO₂
- Necropsy
 - Tissue GB levels

Infrared pupillometry

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Baseline

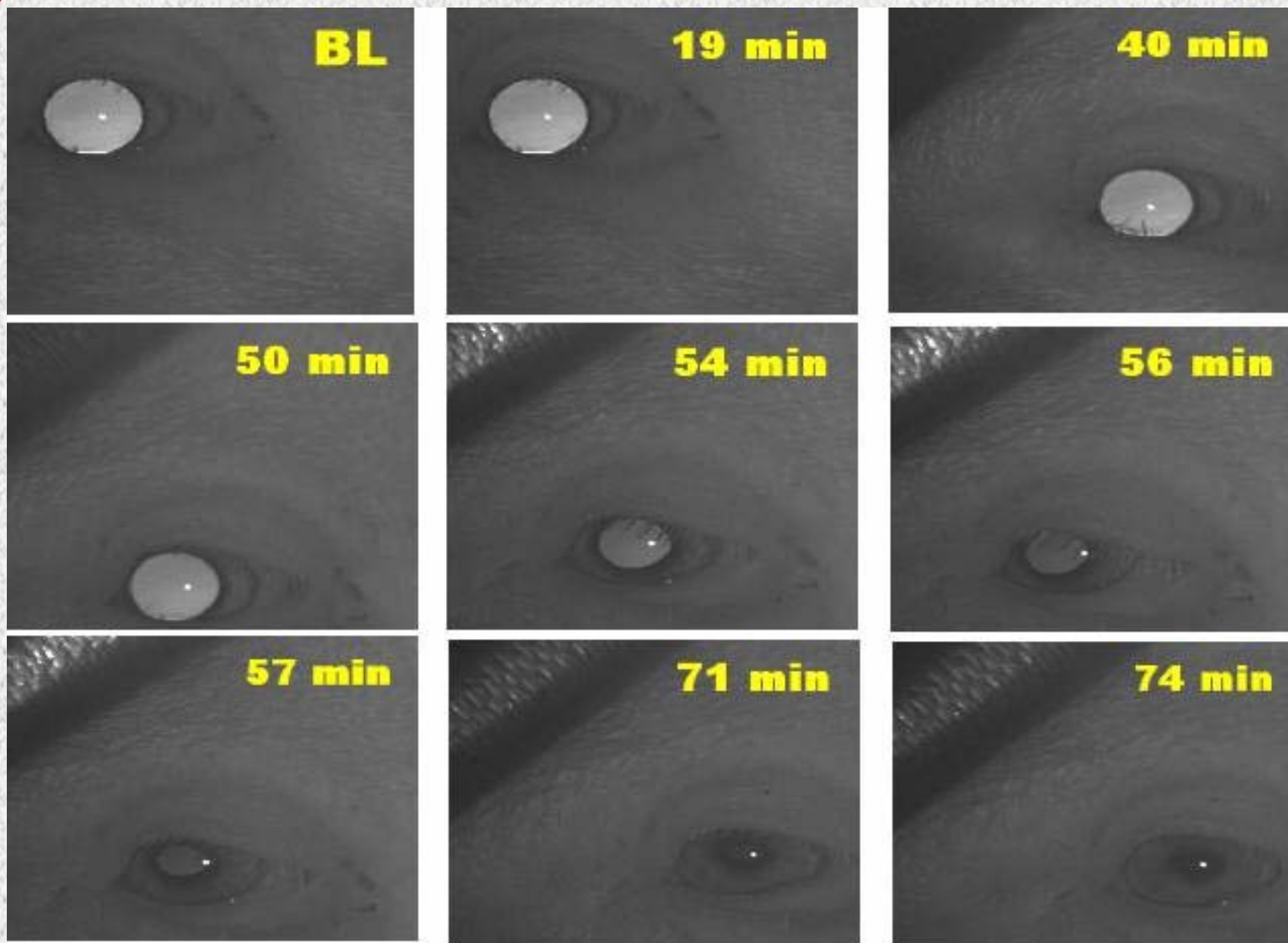


- Infrared light reflects off of the retina
- Pupil area = $A*B*\pi$

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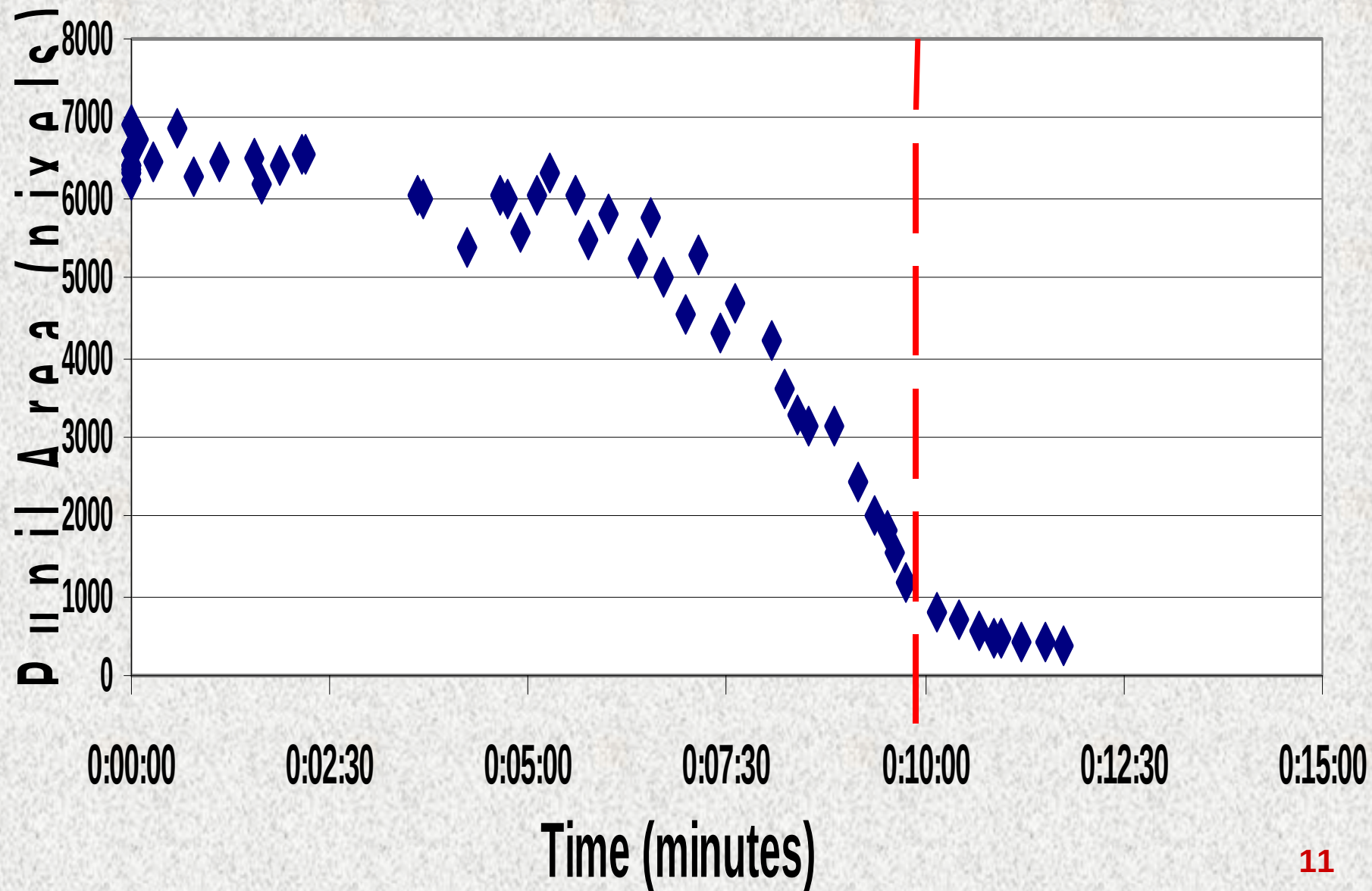
60 minute GB exposure- 0.047 mg/m³

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Pupil Constriction

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Data collection methods / Biological endpoints

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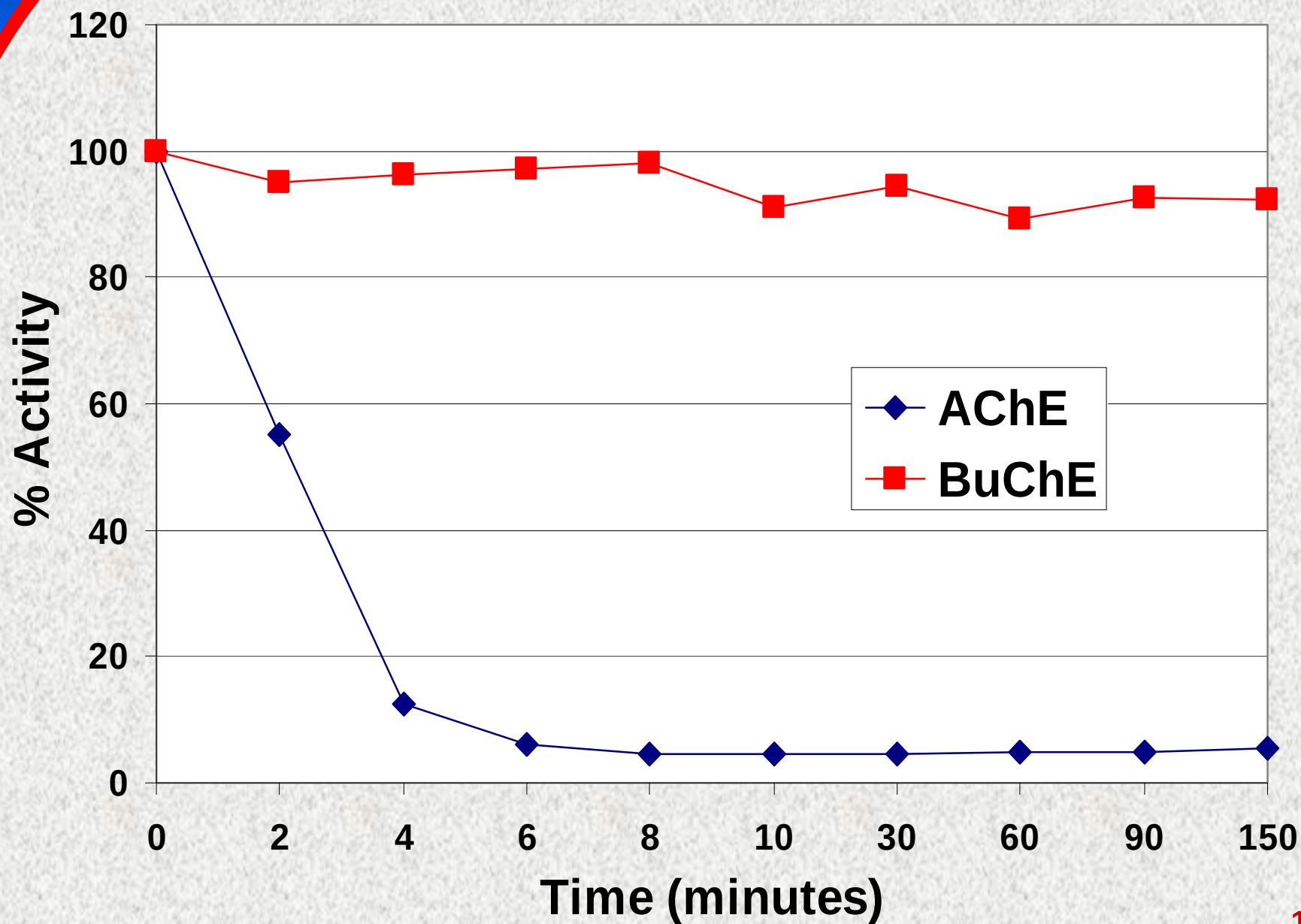
Data collection methods / Biological endpoints

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Cholinesterase Activity

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Data collection methods / Biological endpoints

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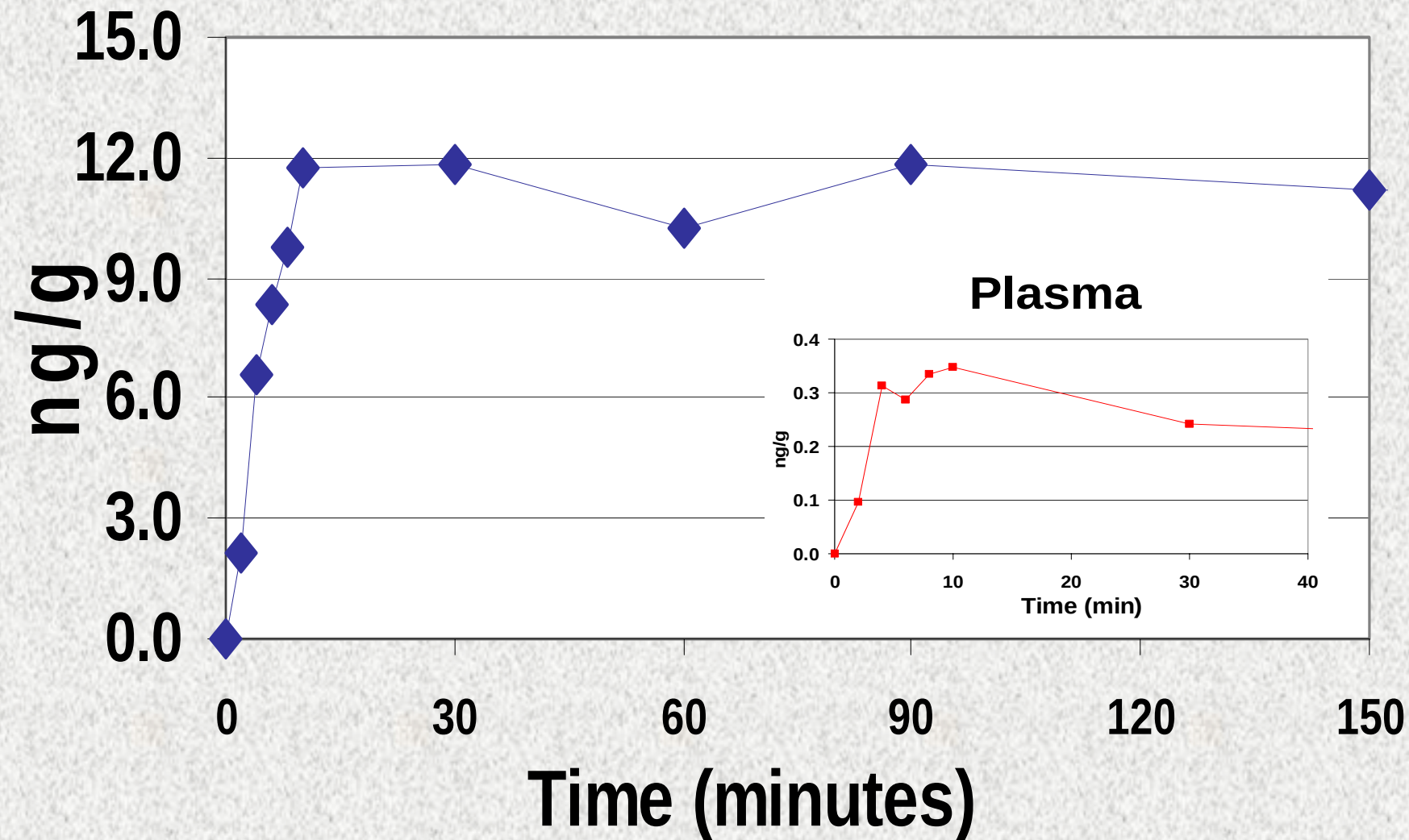
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- Necropsy
 - Tissue GB levels
 - Changes in gene transcription



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RBC REGENERATED GB



Data collection methods / Biological endpoints

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- Necropsy
 - Tissue GB levels
 - Changes in gene transcription

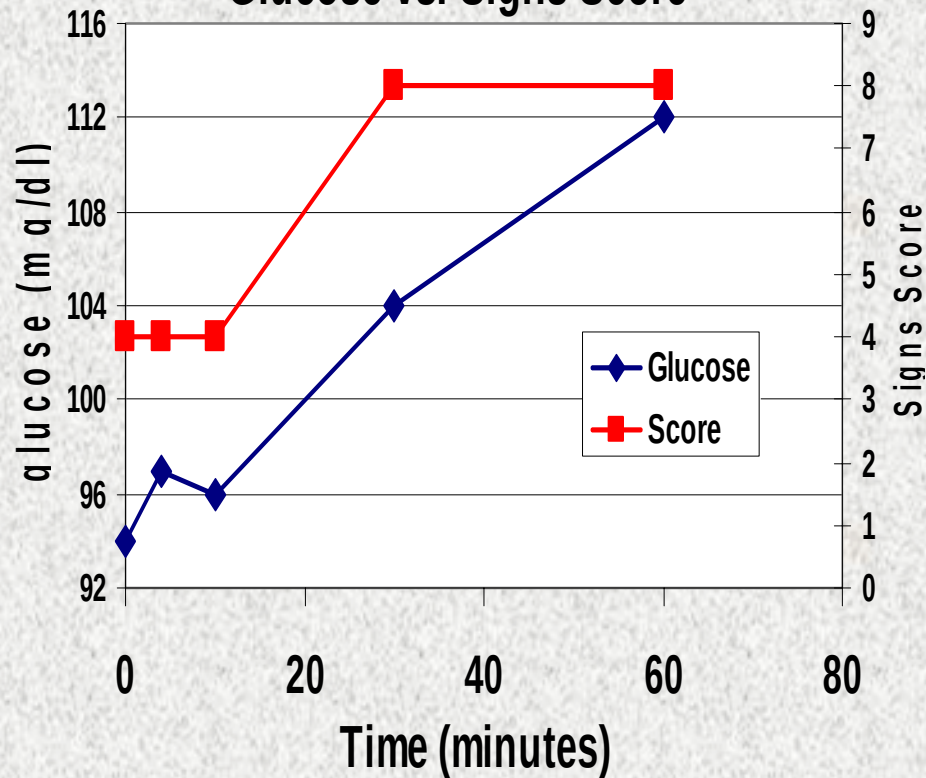


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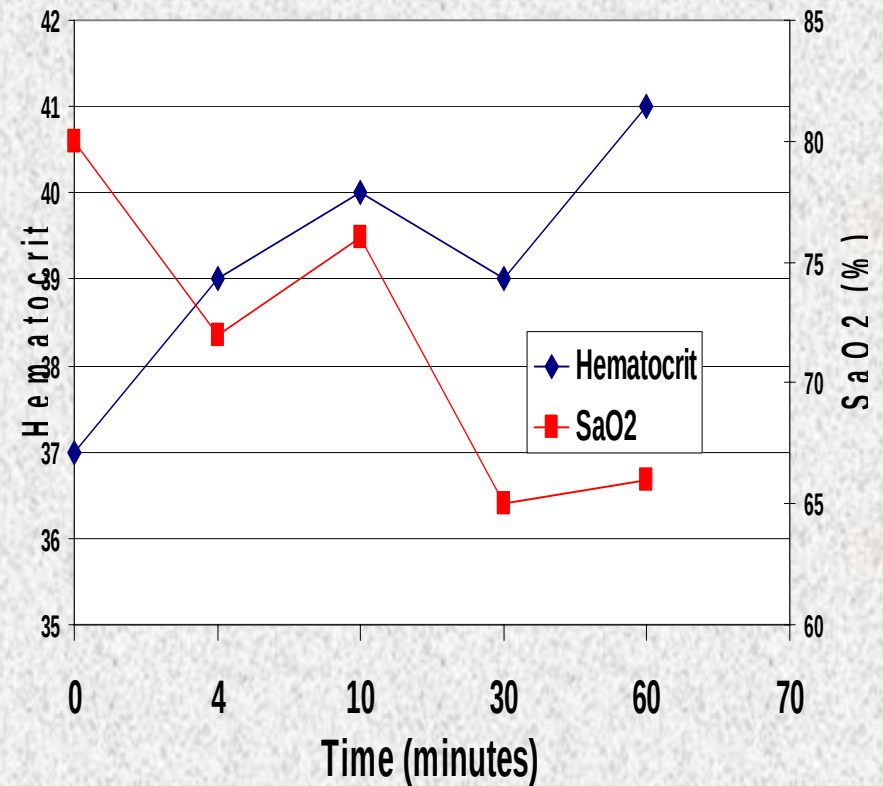
iStat blood analyzer

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Glucose vs. Signs Score



Hematocrit vs SaO2

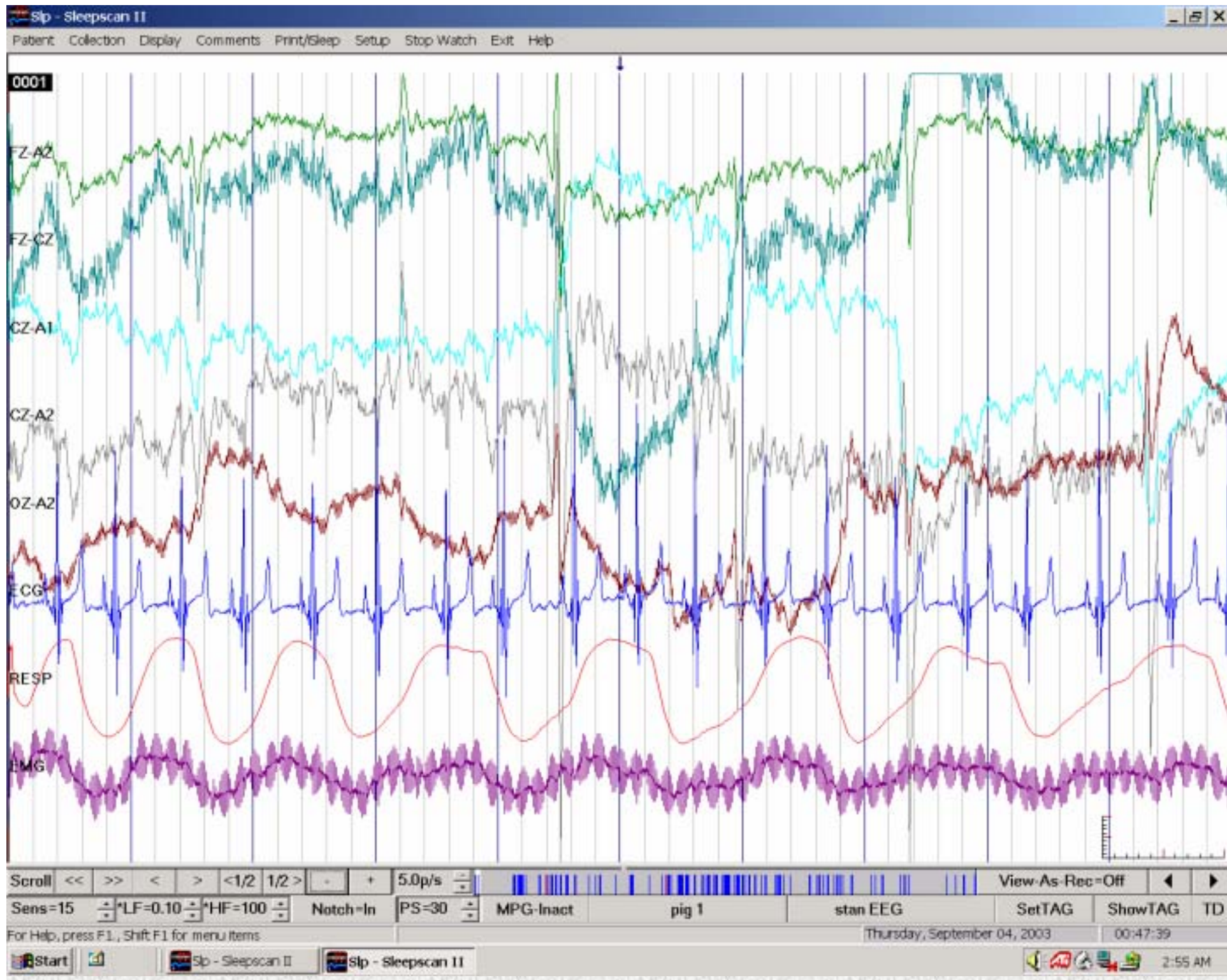


Also can assess creatinine, lactate, pH, hemoglobin, Na, K, TCO₂, PCO₂, PO₂, HCO₃ and Base excess

Data collection methods / Biological endpoints

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Time (min)

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Sarin Exposure

0

2

4

6

8

10

15

20

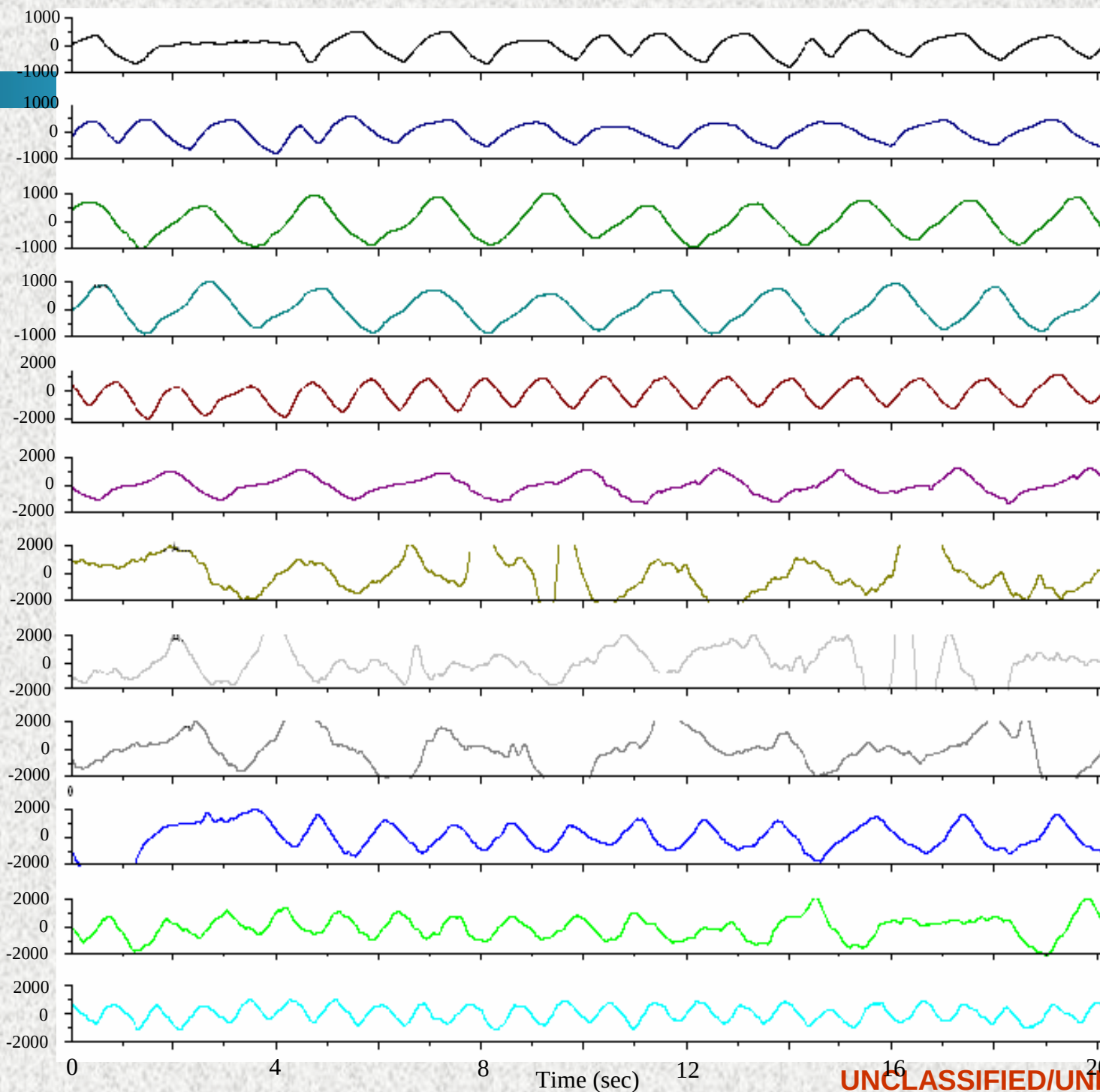
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30

35

40

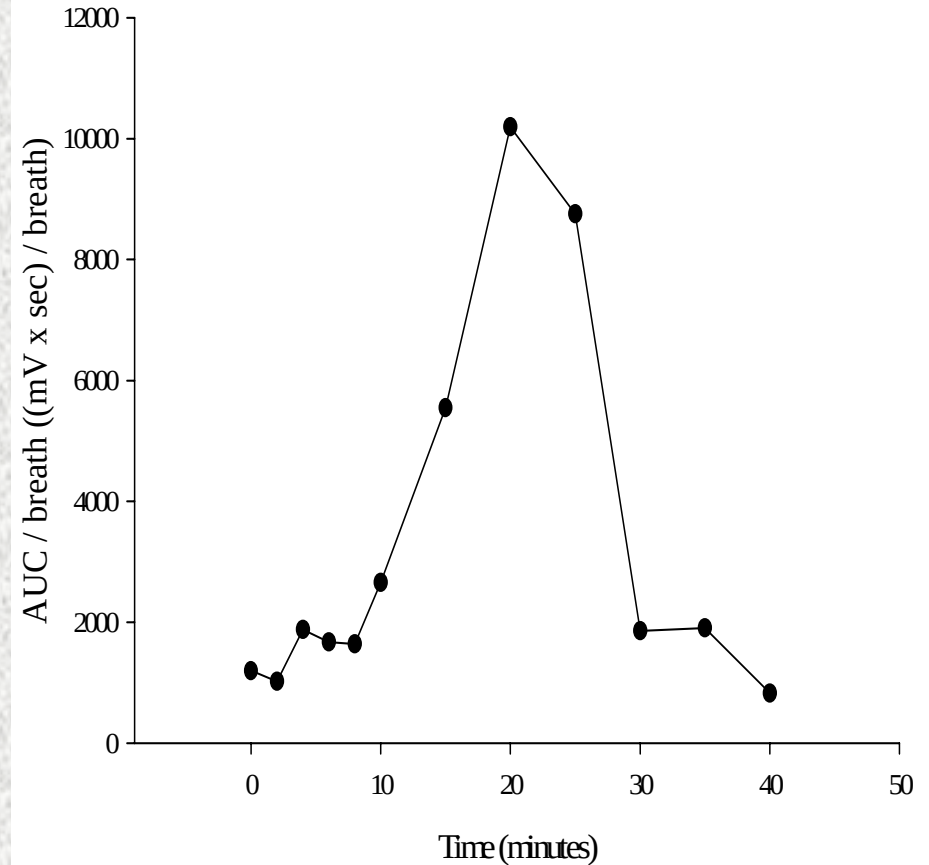
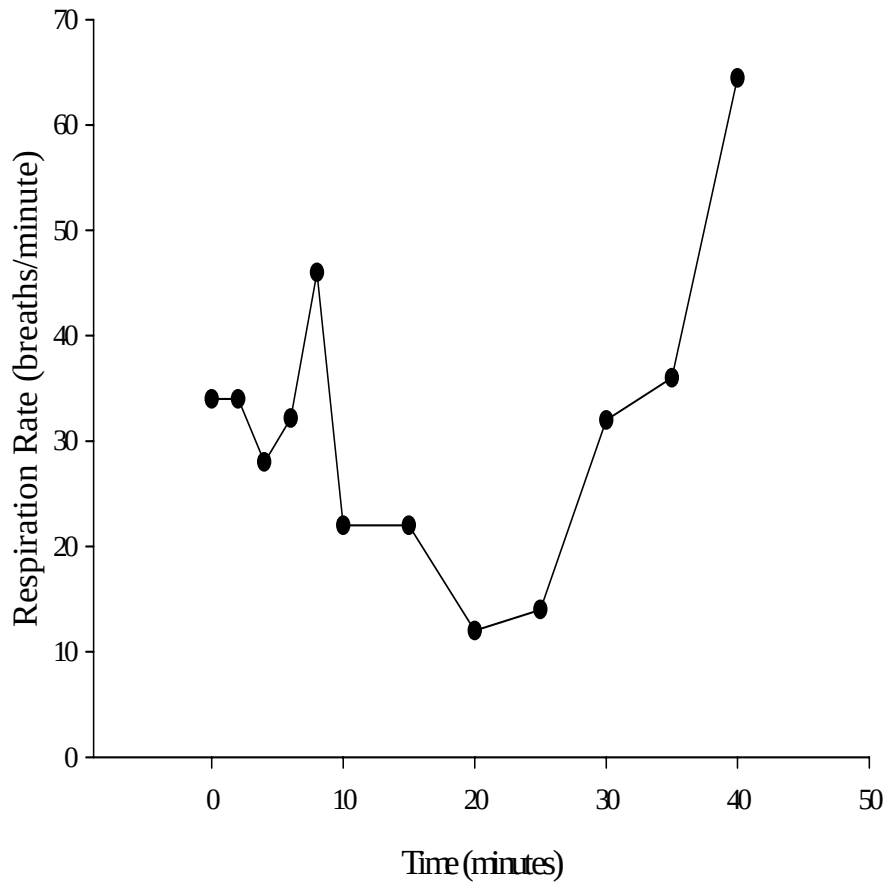
Amplitude (mV)



21

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- Respiratory rate is depressed
- Breaths become deep, long and irregular

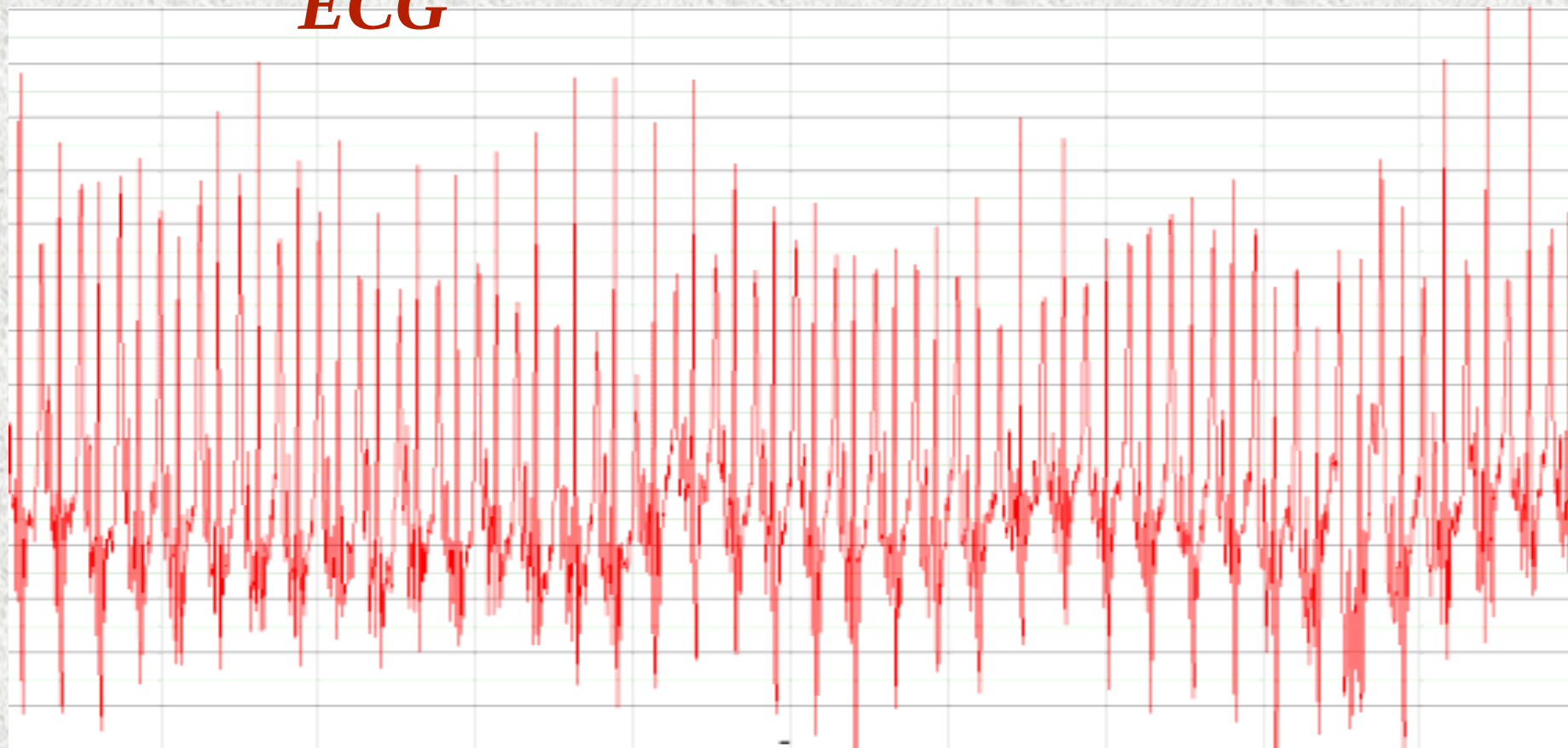
ECG analysis

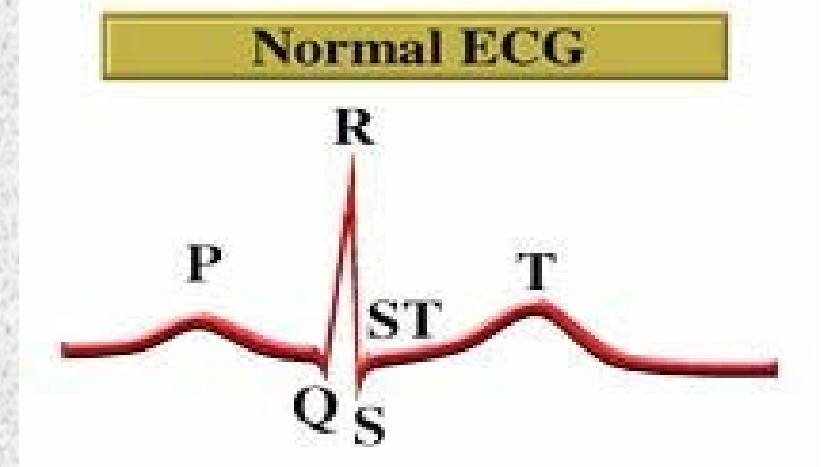
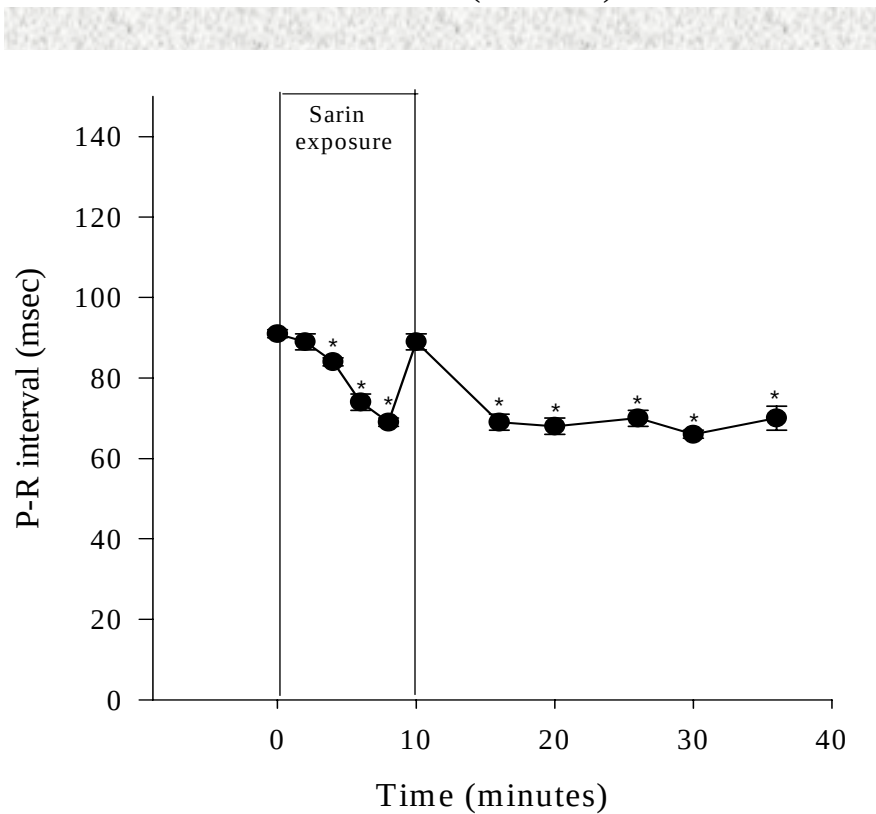
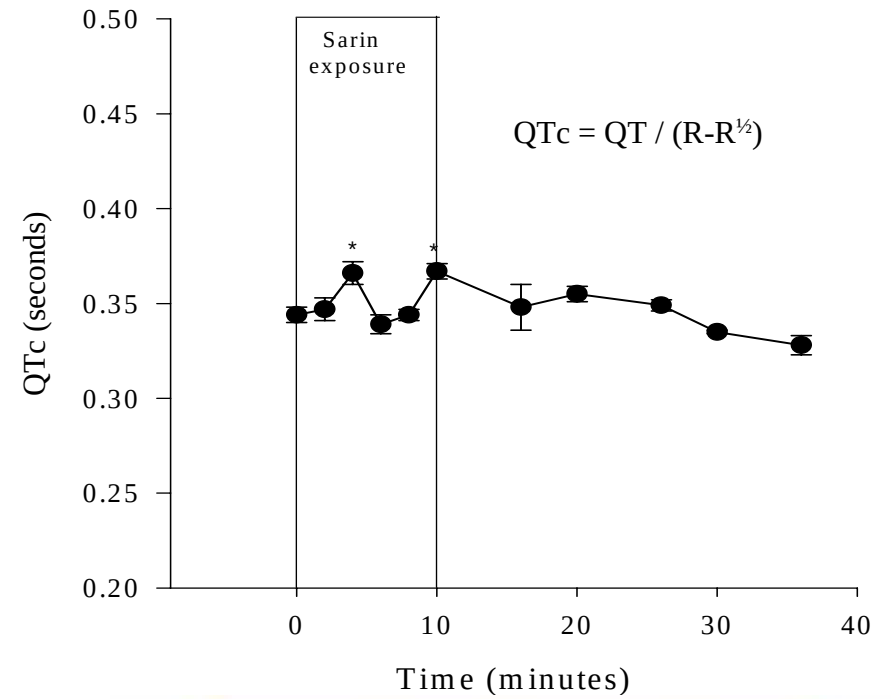
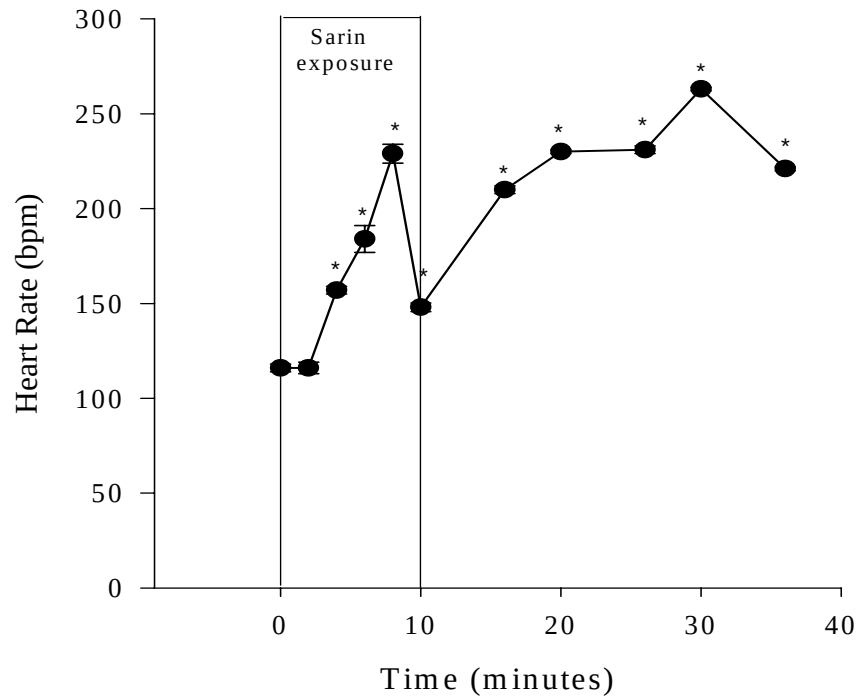
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- ECG signals recorded on Biologic Inc. system
- Standard Lead II configuration
 - Historical data available
- Exposure broken down into 2 minute segments
 - Approximately 30 second epochs during each segment analyzed
- Data analyzed using Dataquest ART and Statview
 - Heart rate (R-R interval)
 - PR interval
 - QT interval
 - QTc interval
 - ST interval
 - QRS interval



ECG



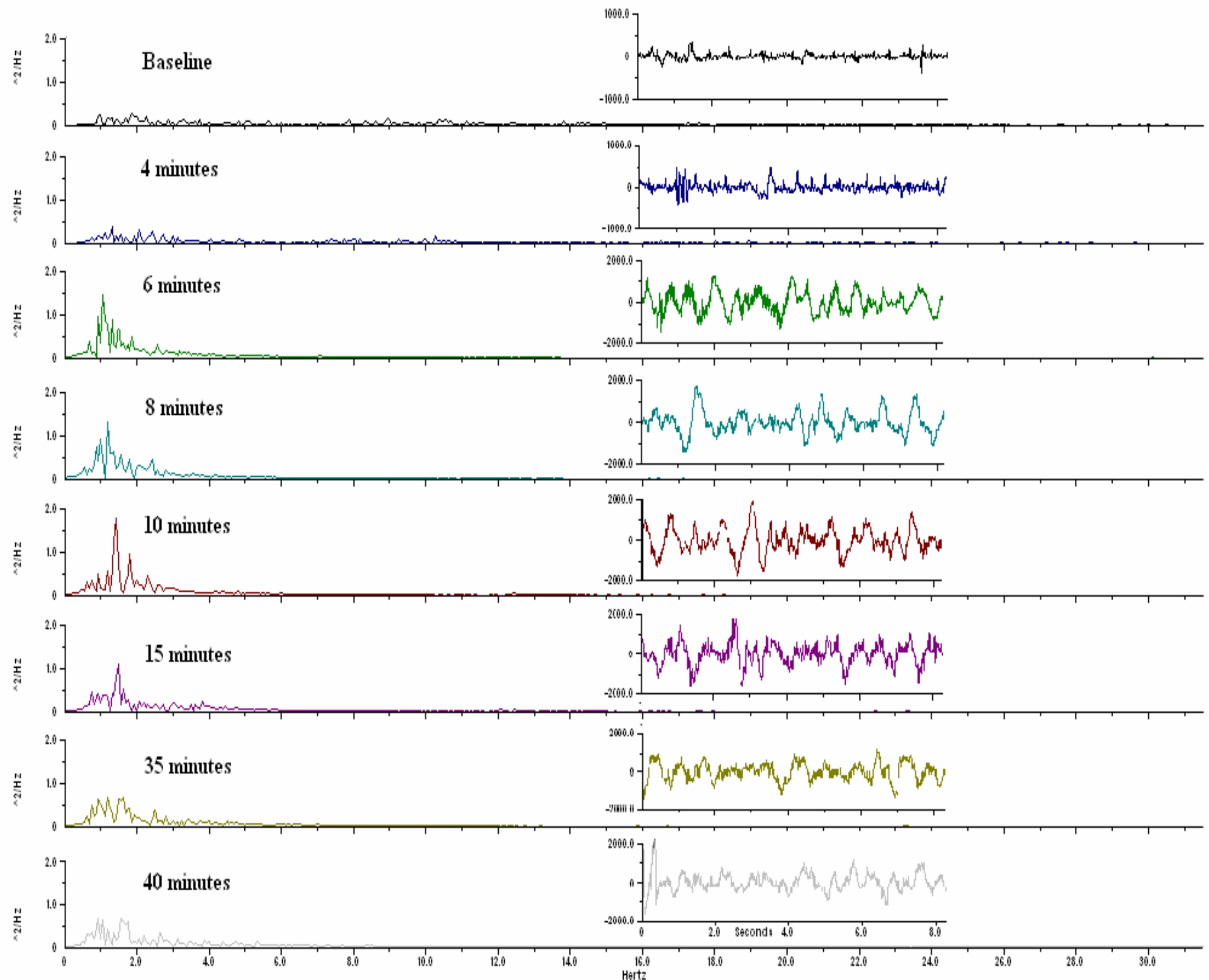


Suggests delayed ventricular repolarization

EEG analysis

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- EEG signals recorded on Biologic Inc. system
- Exposure broken down into 2 minute segments
 - Approximately 30 second epochs during each segment analyzed
- Power spectral density analysis using Dataquest ART
 - Bandpass filters: 1-35 Hz



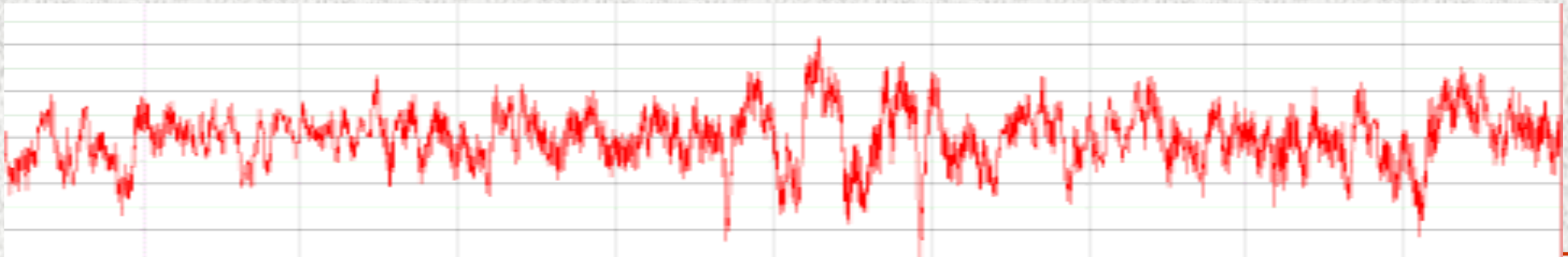
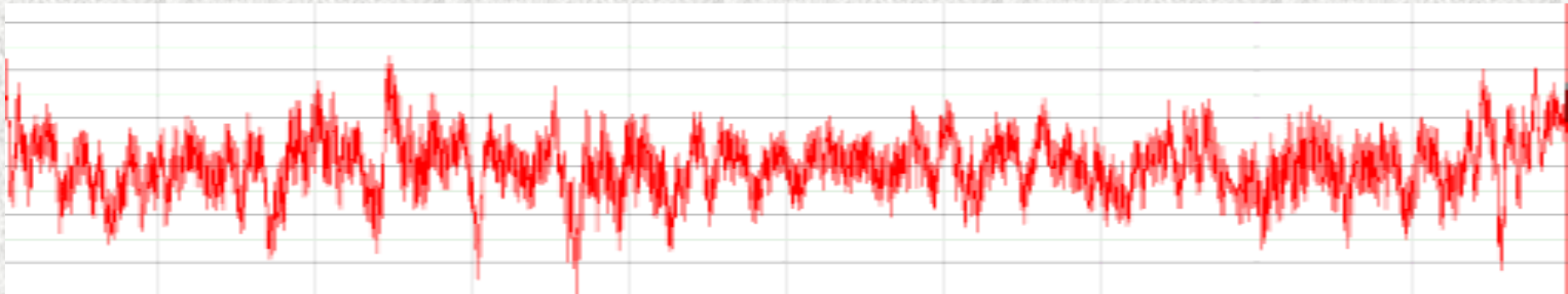
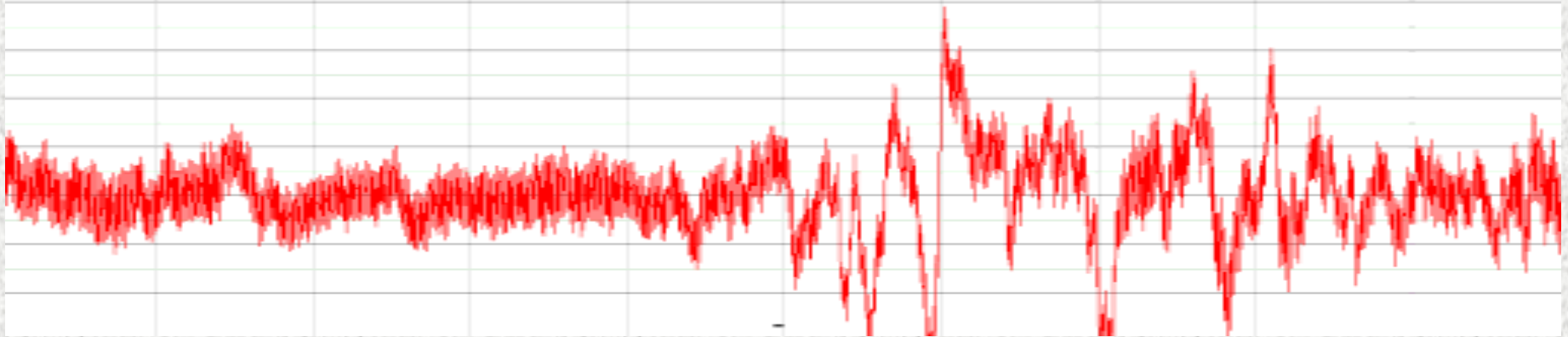
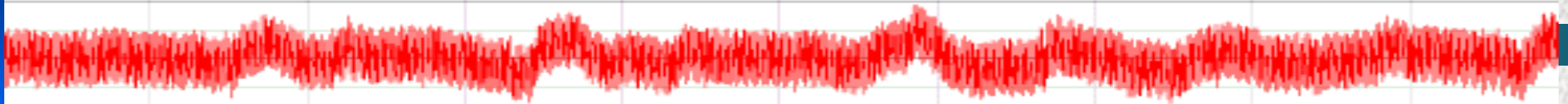
EMG analysis

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- EMG signals recorded on Biologic Inc. system
- Exposure broken down into 2 minute segments
 - Approximately 30 second epochs during each segment analyzed
- Power spectral density analysis using Dataquest ART

EMG

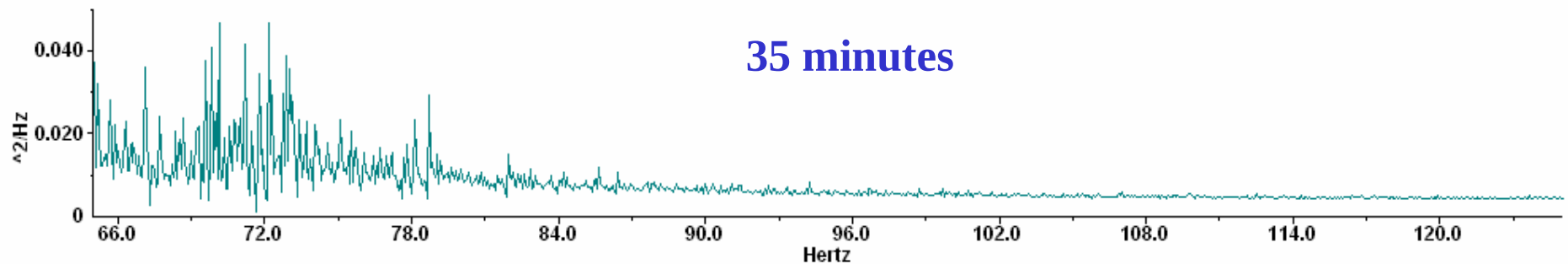
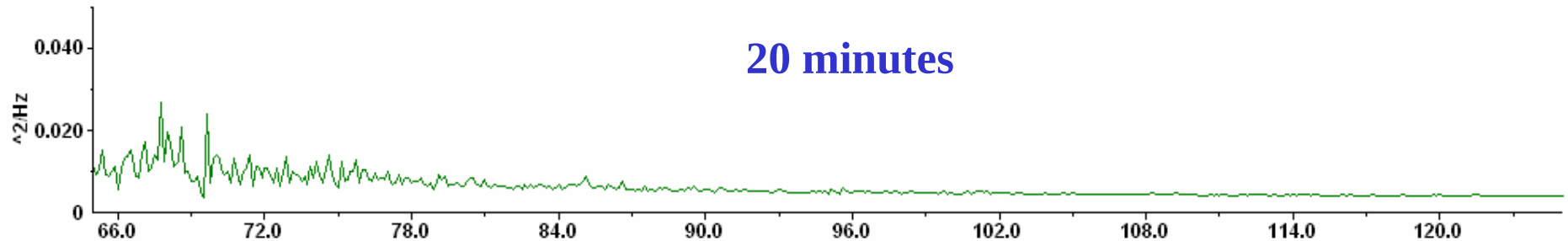
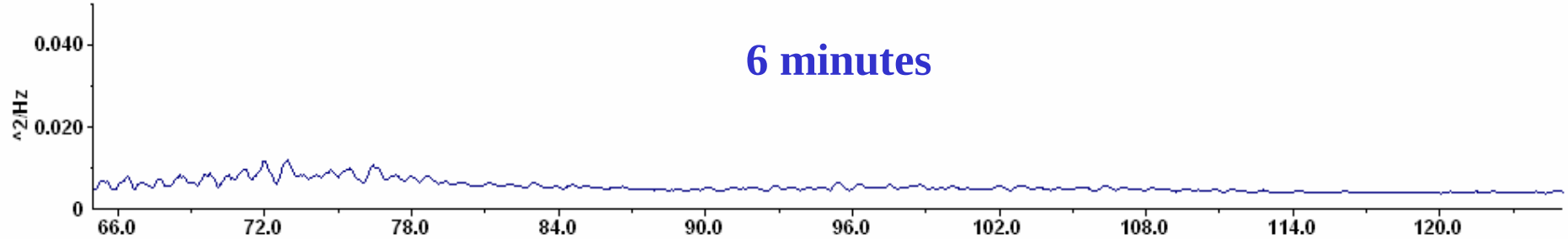
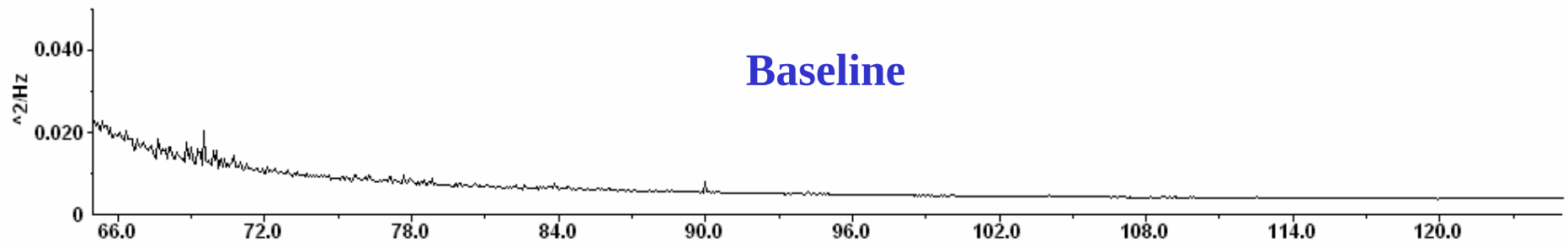
ED



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EMG power spectral analysis

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Data collection methods / Biological endpoints

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- **Infrared camera**
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 - Calculation of EC₅₀ (miosis)
- **Insertion of external jugular catheter**
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 - RBC and plasma cholinesterase
 - GB regeneration assay
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- **Necropsy**
 - Tissue GB levels

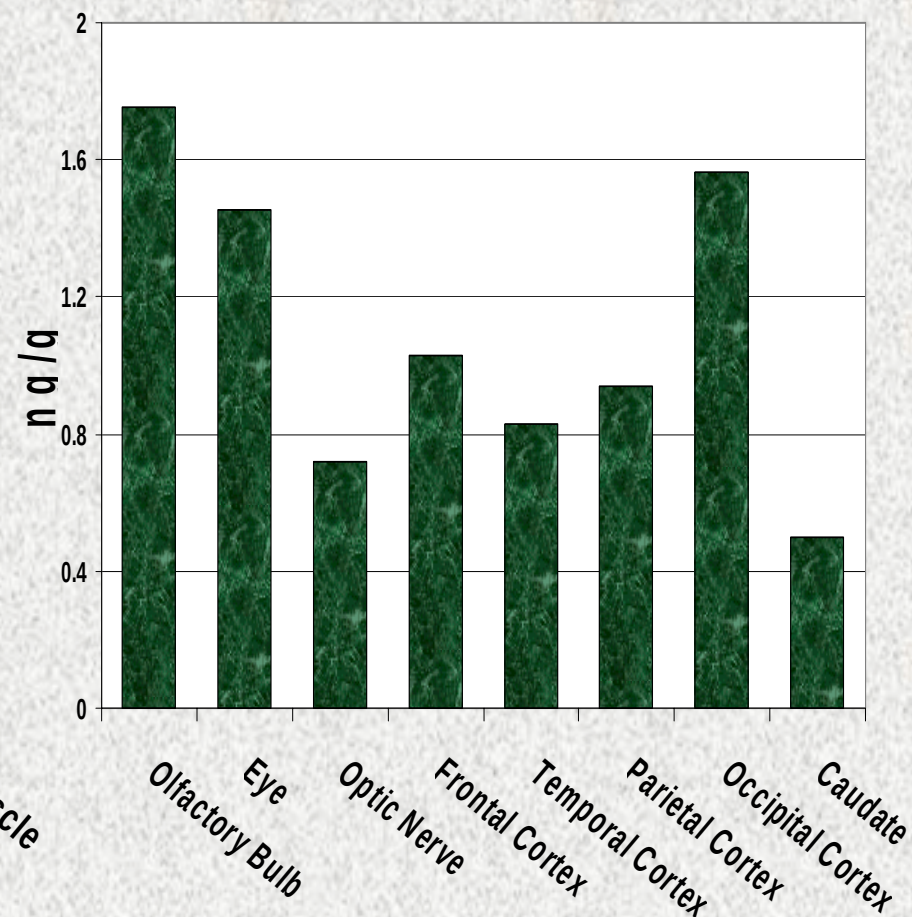
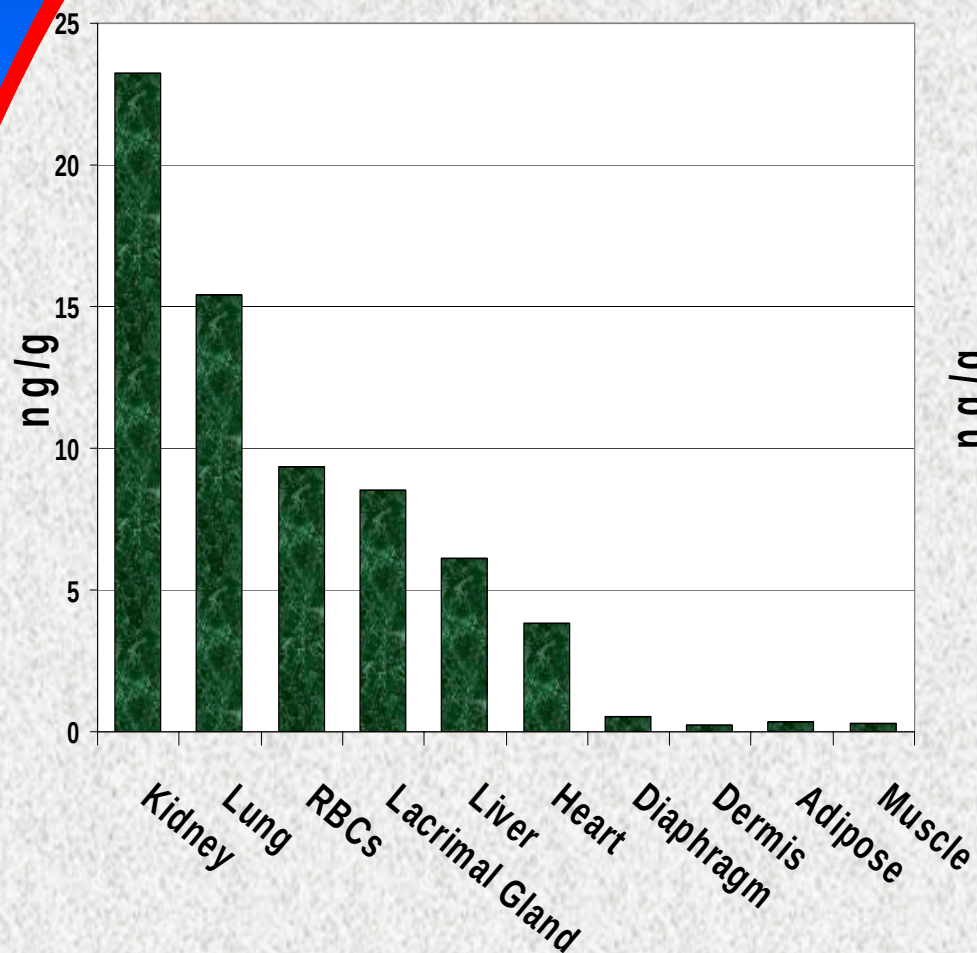


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GB Tissue Distribution

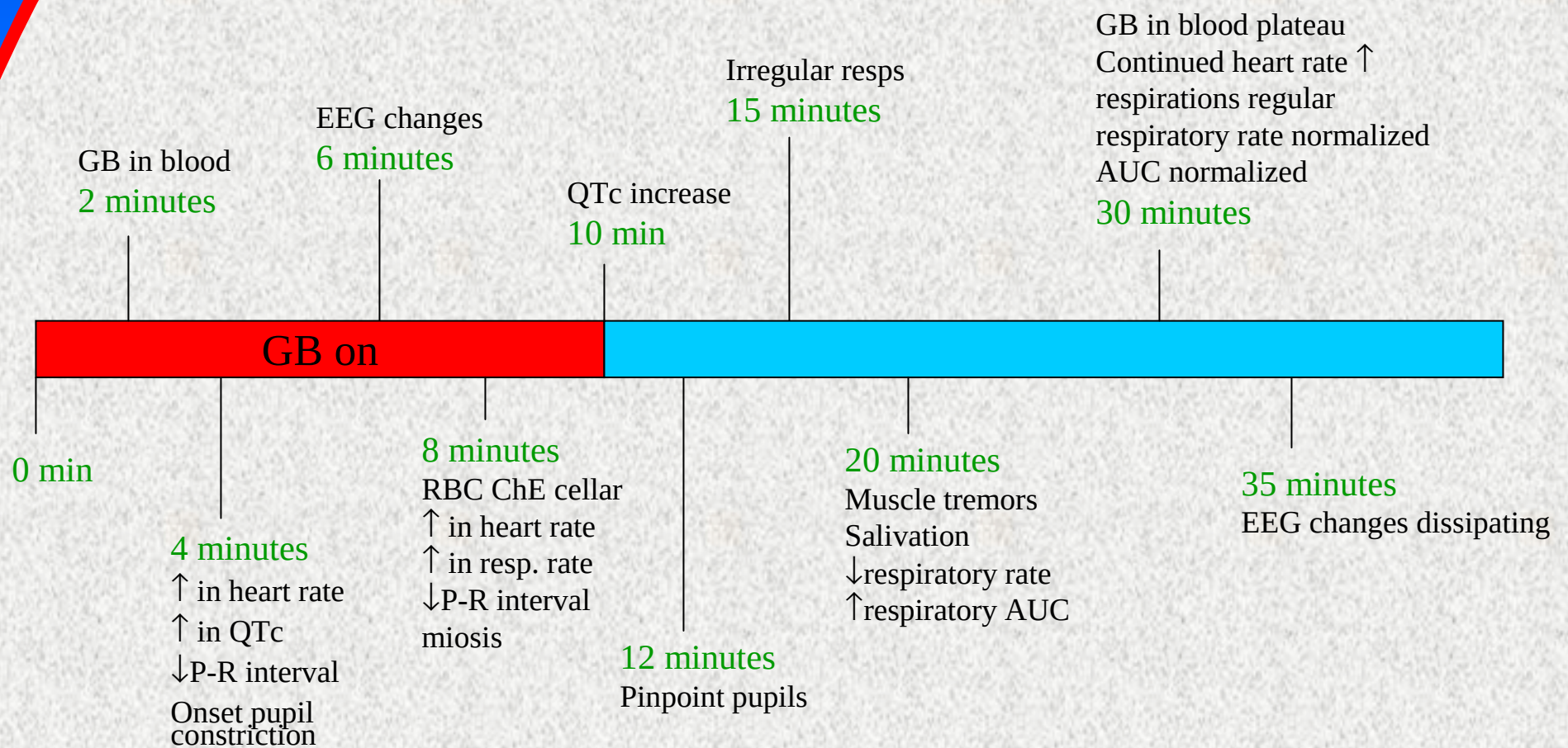
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Thank You

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- David McCaskey
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- Dennis Johnson

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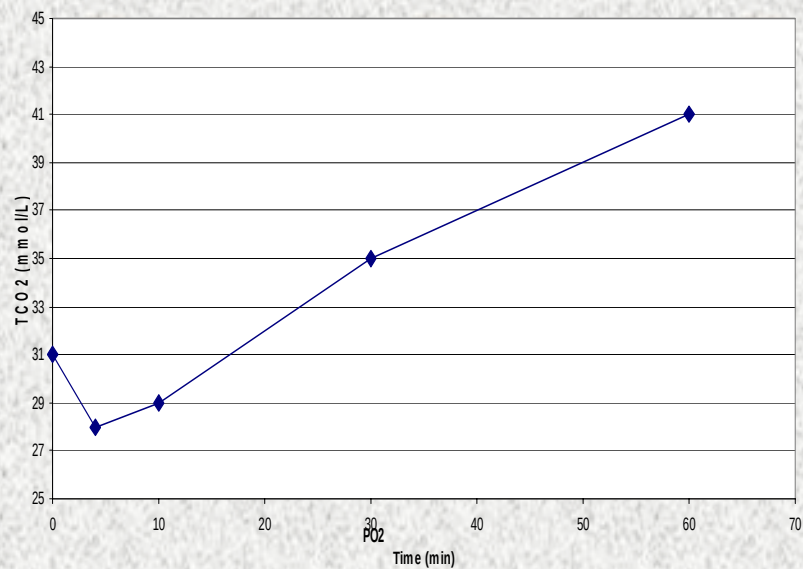
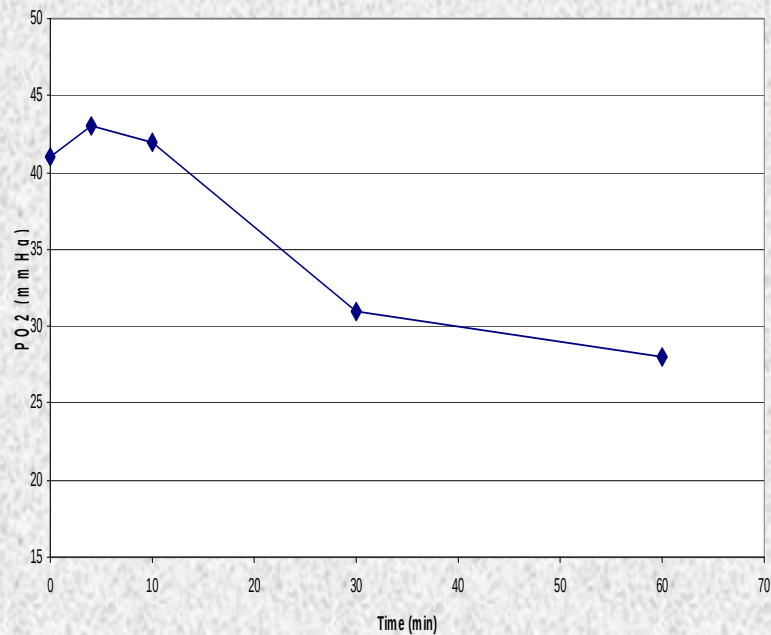
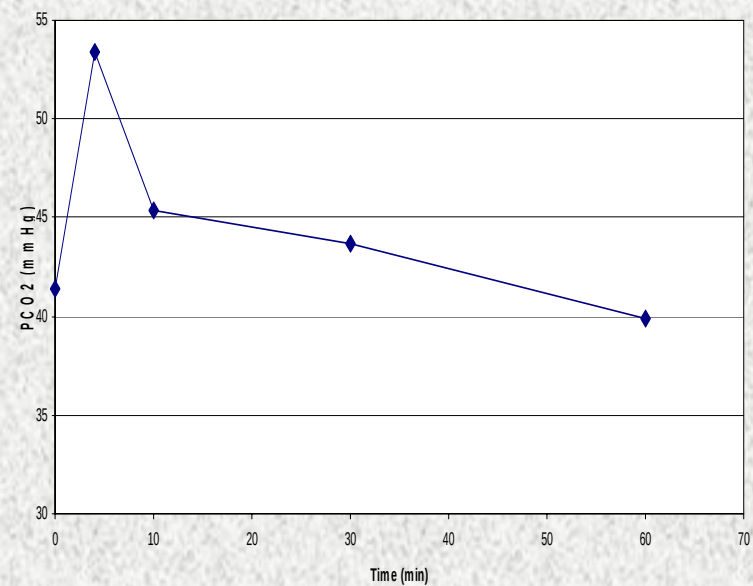
Low Level Operational Toxicology

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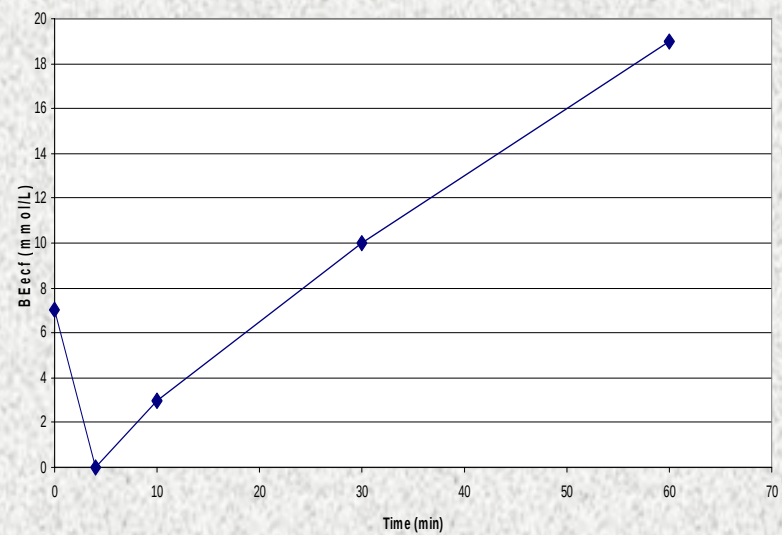
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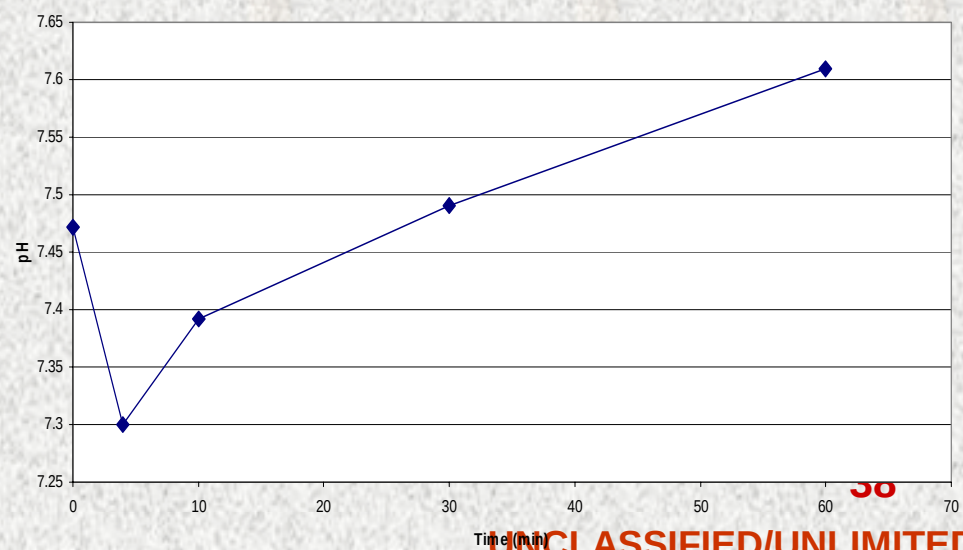
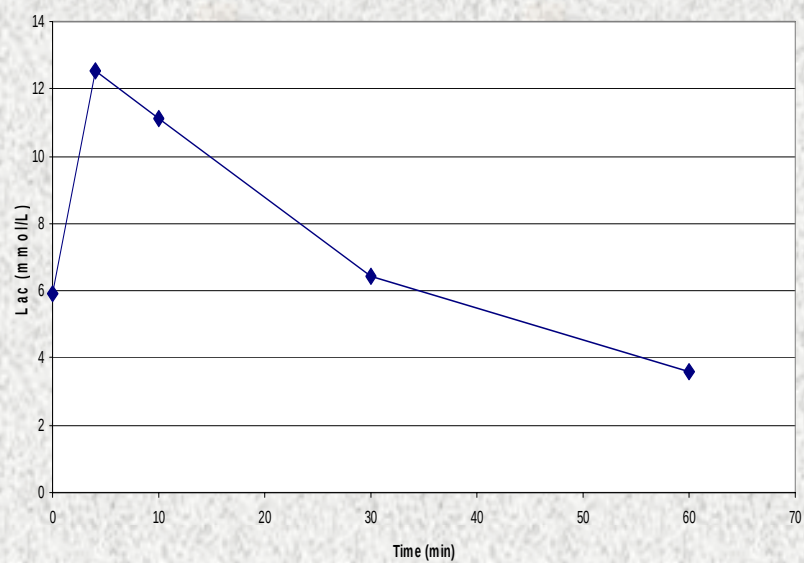
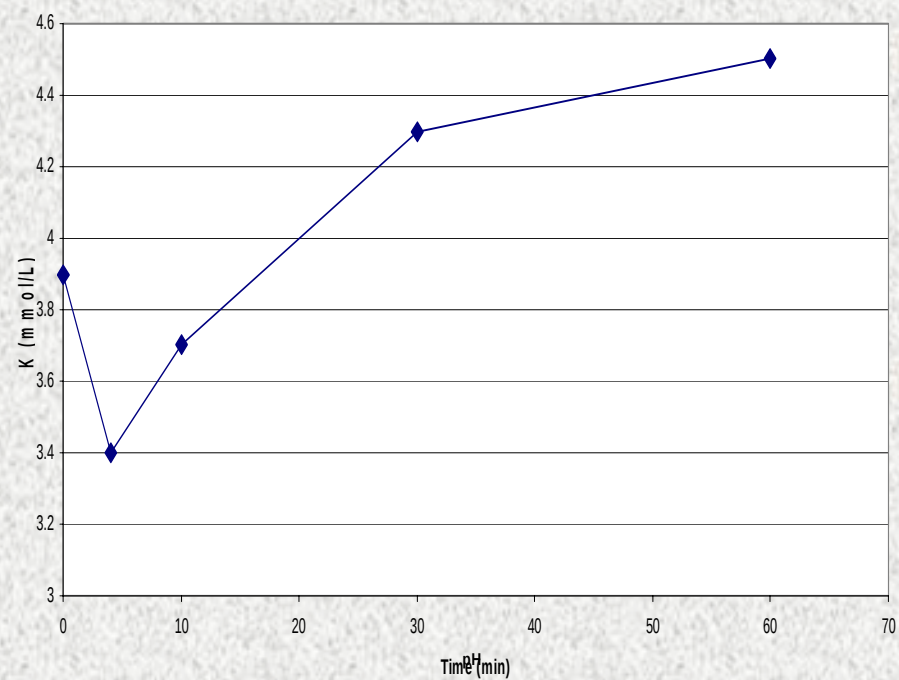
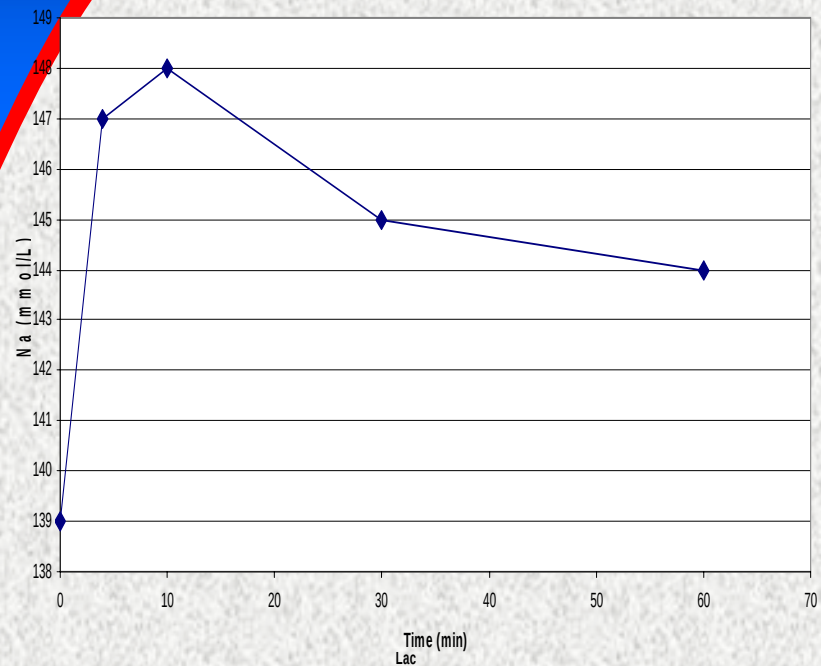
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TCO₂PCO₂

BEect



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Effect of Perfusion on Tissue Levels

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Tissue Results at Lethal Levels:

Pig#41, Ct=89mg*min/m3 (perfused) vs Pig 44 Ct=95mg*min/m3

■ pig 41(Perfused) ■ pig 44

