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

Stanley W. Hulet, Ph.D

prepared for
Joint Services Conference
20 Nov 2003
### Toxic Effects of a Whole-body Inhalation Sarin (GB) Vapor Exposure in the Gottingen Minipig

**Title and Subtitle:**
Toxic Effects of a Whole-body Inhalation Sarin (GB) Vapor Exposure in the Gottingen Minipig

**Authors:**
U.S. Army RDECOM Edgewood Chemical Biological Center
AMSSB-RRT-TT, Bldg. E3150 Aberdeen Proving Ground, Maryland, USA 21010-5424

**Abstract:**

**DISTRIBUTION/AVAILABILITY STATEMENT:**
Approved for public release, distribution unlimited.

**SUPPLEMENTARY NOTES:**

**ABSTRACT:**

**SUBJECT TERMS:**

**SECURITY CLASSIFICATION OF:**
- a. REPORT: unclassified
- b. ABSTRACT: unclassified
- c. THIS PAGE: unclassified

**LIMITATION OF ABSTRACT:**
UU

**NUMBER OF PAGES:**
39
Benefits of the minipig

- Brain
- Cardiovascular/Pulmonary system
- Visual system
- Dermis
- Kidneys
Gottingen Minipigs

Edgewood Chemical Biological Center

- 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

Edgewood Chemical Biological Center

- 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
Edgewood Chemical Biological Center

- 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

Edgewood Chemical Biological Center

- 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

Edgewood Chemical Biological Center

- 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**

Edgewood Chemical Biological Center

- **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

Baseline

- Infrared light reflects off of the retina
- Pupil area = $A \times B \times \pi$
60 minute GB exposure- 0.047 mg/m³

Edgewood Chemical Biological Center
Pupil Constriction

Edgewood Chemical Biological Center

Pupil Area (pixels)

Time (minutes)

0:00:00 0:02:30 0:05:00 0:07:30 0:10:00 0:12:30 0:15:00
Data collection methods / Biological endpoints

- **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, respiratory rate, heart rate, SaO₂

- **Necropsy**
  - Tissue GB levels
Data collection methods / Biological endpoints

- 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, respiratory rate, heart rate, SaO₂
- Necropsy
  - Tissue GB levels
Cholinesterase Activity
Edgewood Chemical Biological Center

% Activity

AChE
BuChE

Time (minutes)

0 2 4 6 8 10 30 60 90 150
Data collection methods / Biological endpoints

Edgewood Chemical Biological Center

- 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, respiratory rate, heart rate, SaO₂

- Necropsy
  - Tissue GB levels
  - Changes in gene transcription

M. Jakubowski
Data collection methods / Biological endpoints

Edgewood Chemical Biological Center

- 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, respiratory rate, heart rate, SaO₂
- Necropsy
  - Tissue GB levels
  - Changes in gene transcription

J. Forster
**Glucose vs. Signs Score**

- Glucose (mg/dl): 92, 96, 100, 104, 108, 112, 116
- Signs Score: 0, 2, 4, 6, 8, 10, 12

**Hematocrit vs SaO2**

- Hematocrit (%): 35, 36, 37, 38, 39, 40, 41
- SaO2 (%): 42, 41, 40, 39, 38, 37, 36

- Time (minutes): 0, 4, 10, 30, 60

Also can assess creatinine, lactate, pH, hemoglobin, Na, K, TCO₂, PCO₂, PO₂, HCO₃ and Base excess
Data collection methods / Biological endpoints

- 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, ECG, EMG, airflow, respiratory rate, heart rate, SaO₂
- Necropsy
  - Tissue GB levels
  - Changes in gene transcription
- Respiratory rate is depressed
- Breaths become deep, long and irregular
ECG analysis

Edgewood Chemical Biological Center

- 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
Heart Rate (bpm)

Time (minutes)

Sarin exposure

<table>
<thead>
<tr>
<th>0</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>150</td>
<td>200</td>
<td>250</td>
<td>300</td>
</tr>
</tbody>
</table>

* Suggests increased conduction through atria
* Suggests delayed ventricular repolarization

QTc = QT / (R-R_{1/2})

P-R interval (msec)

Time (minutes)

Sarin exposure

<table>
<thead>
<tr>
<th>0</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>60</td>
<td>80</td>
<td>100</td>
<td>120</td>
</tr>
</tbody>
</table>

Normal ECG

ST segment depression

Suggests delayed ventricular repolarization
EEG analysis

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

- 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 power spectral analysis

Baseline

6 minutes

20 minutes

35 minutes
Data collection methods / Biological endpoints

Edgewood Chemical Biological Center

- **Infrared camera**
  - Allows images under dim light conditions
  - Uses pupil areas to plot time-to-miosis
  - Calculation of EC₅₀ (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, respiratory rate, heart rate, SaO₂

- **Necropsy**
  - Tissue GB levels

---

M. Jakubowski
GB in blood plateau
Continued heart rate ↑
respirations regular
respiratory rate normalized
AUC normalized
30 minutes

GB in blood
2 minutes

EEG changes
6 minutes

QTc increase
10 min

Irregular resps
15 minutes

GB on

8 minutes
RBC ChE cellar
↑ in heart rate
↑ in resp. rate
↓ P-R interval
miosis

20 minutes
Muscle tremors
Salivation
↓ respiratory rate
↑ respiratory AUC

12 minutes
Pinpoint pupils

35 minutes
EEG changes dissipating

4 minutes
↑ in heart rate
↑ in QTc
↓ P-R interval
Onset pupil constriction

0 min
Thank You
Edgewood Chemical Biological Center

- Jacqueline Scotto
- Paul Dabisch
- Bernard Benton
- Ruth Way
- James Manthei
- Jeffry Forster
- Dennis Miller
- William Muse Jr.
- Charles Crouse
- Kathy Matson
- Bob Mioduszewski
- Sandra Thomson

- Jill Jarvis
- Dean Bona
- Carl Kurnas
- Melvin Ware
- E. Mike Jakubowski
- Jennifer Edwards
- Jeff McGuire
- Ron Evans
- David Burnett
- Bernardita Gaviola
- David McCaskey
- Jennifer Sekowski
- Dennis Johnson
Low Level Operational Toxicology
Edgewood Chemical Biological Center

Dr. Stanley Hulet
US Army ECBC

(410) 436-2685
Stanley.hulet@sbccom.apgea.army.mil
Effect of Perfusion on Tissue Levels

Edgewood Chemical Biological Center

Tissue Results at Lethal Levels:
Pig#41, Ct=89mg*min/m³ (perfused) vs Pig 44 Ct=95mg*min/m³

- Kidney
- Lung
- Liver
- Caudate
- Gyrus Rectus
- Brain
- Optic Nerve
- Frontal Cortex
- Parietal Cortex
- Temporal Cortex
- Eye
- Diaphragm
- Olfactory Bulb
- Dermis
- Muscle
- Adipose
- Heart
- Occipital Cortex

R-GB (ng/g)

pig 41(Perfused) pig 44