Working with Biological Toxins and Laboratory Chemicals

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Outline

- Introduction to Toxins
- Botulinum Toxins
- Ricin Toxin
- SEB
- T2
- Chemical issues
- Reproductive concerns
Introduction to Toxins

• Any toxic substance of natural origin produced by an animal, plant, fungus, or bacteria.
  – Unlike a biological agent – toxins are NOT self-replicating.
  – Unlike a chemical agent – toxins are NOT “man-made”.

• Non-volatile – thus, not a persistent battlefield threat and no person-to-person transfer.

• NOT dermally active (mycotoxins are exception).

• More toxic per weight than chemical agents.

• Some show up on both chem agent and bio agent lists (ricin & saxitoxin).
Toxins on the CDC Select Agent List (partial list)

- Abrin
- Botulinum neurotoxins
- Conotoxins
- Ricin
- Saxitoxin
- Shigatoxin
- Staphylococcal Enterotoxins
- Tetrodotoxin
- T2 Toxin

Note – CDC Select Agent list also includes bacteria, viruses, fungi, and prions.

Complete list at:
http://www.cdc.gov/od/sap/docs/salist.pdf
The United States is one of 175 States Parties to the Chemical Weapons Convention (CWC), which prohibits the development, production, stockpiling, and use of chemical weapons (CW). The CWC does not prohibit production, processing, consumption, or trade of related chemicals for peaceful purposes, but it does establish a verification regime to ensure such activities are consistent with the object and purpose of the treaty.

April 25, 1997 = ratified by the United States

For more information see: http://www.cwc.gov/archives/regulations_cfr15_part-712-s1.html
## Comparison of Toxins and Chemical Agents (Table 30-1)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Toxins</th>
<th>Chemical Agents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Origin</td>
<td>Natural</td>
<td>Man-made</td>
</tr>
<tr>
<td>Production</td>
<td>Difficult, small-scale</td>
<td>Large-scale industrial</td>
</tr>
<tr>
<td>Volatility</td>
<td>None volatile</td>
<td>Many volatile</td>
</tr>
<tr>
<td>Relative Toxicity</td>
<td>Many are more toxic</td>
<td>Less toxic than many toxins</td>
</tr>
<tr>
<td>Dermal Toxicity</td>
<td>Not dermally active</td>
<td>Dermally active</td>
</tr>
<tr>
<td>Use</td>
<td>Legitimate medical use</td>
<td>No use other than as weapons</td>
</tr>
<tr>
<td>Odor and Taste</td>
<td>Odorless and tasteless</td>
<td>Noticeable odor or taste</td>
</tr>
<tr>
<td>Toxic Effects</td>
<td>Diverse toxic effects</td>
<td>Fewer types of effects</td>
</tr>
<tr>
<td>Immunogenicity</td>
<td>Many are effective immunogens</td>
<td>Poor immunogens</td>
</tr>
<tr>
<td>Delivery</td>
<td>Aerosol delivery</td>
<td>Mist/droplet/aerosol delivery</td>
</tr>
</tbody>
</table>
# Toxicity: Toxins vs. Chemical Agents

<table>
<thead>
<tr>
<th>AGENT</th>
<th>LD50 (g/kg)</th>
<th>MOLECULAR WEIGHT</th>
<th>SOURCE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Botulinum toxin</strong></td>
<td>0.001</td>
<td>150,000</td>
<td>Bacterium</td>
</tr>
<tr>
<td><strong>Shiga toxin</strong></td>
<td>0.002</td>
<td>55,000</td>
<td>Bacterium</td>
</tr>
<tr>
<td><strong>Tetanus toxin</strong></td>
<td>0.002</td>
<td>150,000</td>
<td>Bacterium</td>
</tr>
<tr>
<td><strong>Abrin</strong></td>
<td>0.04</td>
<td>65,000</td>
<td>Plant (Rosary Pea)</td>
</tr>
<tr>
<td><strong>Diphtheria toxin</strong></td>
<td>0.10</td>
<td>62,000</td>
<td>Bacterium</td>
</tr>
<tr>
<td><strong>Ciguatoxin</strong></td>
<td>0.40</td>
<td>1,000</td>
<td>Marine Dinoflagellate</td>
</tr>
<tr>
<td><strong>C. perfringens toxins</strong></td>
<td>0.1 - 5.0</td>
<td>35,000-40,000</td>
<td>Bacterium</td>
</tr>
<tr>
<td><strong>Rcin</strong></td>
<td>3.0</td>
<td>64,000</td>
<td>Plant (Castor Bean)</td>
</tr>
<tr>
<td>alpha-Conotoxin</td>
<td>5.0</td>
<td>1,500</td>
<td>Cone Snail</td>
</tr>
<tr>
<td>Tetrodotoxin</td>
<td>8.0</td>
<td>319</td>
<td>Puffer Fish</td>
</tr>
<tr>
<td>alpha-Tityustoxin</td>
<td>9.0</td>
<td>8,000</td>
<td>Scorpion</td>
</tr>
<tr>
<td>Saxitoxin</td>
<td>10.0 (Inh 2.0)</td>
<td>299</td>
<td>Marine Dinoflagellate</td>
</tr>
<tr>
<td>VX</td>
<td>15.0</td>
<td>267</td>
<td>Chemical Agent</td>
</tr>
<tr>
<td><strong>SEB (Rhesus/Aerosol)</strong></td>
<td>27.0 (ED50~pg)</td>
<td>28,494</td>
<td>Bacterium</td>
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<tr>
<td>Anatoxin-A(s)</td>
<td>50.0</td>
<td>500</td>
<td>Blue-Green Algae</td>
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<tr>
<td>Microcystin</td>
<td>50.0</td>
<td>994</td>
<td>Blue-Green Algae</td>
</tr>
<tr>
<td>Soman (GD)</td>
<td>64.0</td>
<td>182</td>
<td>Chemical Agent</td>
</tr>
<tr>
<td>Sarin (GB)</td>
<td>100.0</td>
<td>140</td>
<td>Chemical Agent</td>
</tr>
<tr>
<td>Aconitine</td>
<td>100.0</td>
<td>647</td>
<td>Plant (Monkshood)</td>
</tr>
<tr>
<td><strong>T-2 Toxin</strong></td>
<td>1,210.0</td>
<td>466</td>
<td>Fungal Mycotoxin</td>
</tr>
</tbody>
</table>
Safety in Chem Agent Labs

- Annual medical examinations
- Biological monitoring (cholinesterase)
- Butyl gloves and aprons
- Respirators available
- All work in Chemical fume hood
- Antidotes for self-aid and buddy aid
- Regular emergency drills with decon and hospital transport
Key Concepts

• Biological toxins are hazardous chemical agents
• Biological laboratories do not approach toxins in the same manner as chemical laboratories approach hazardous chemicals
• Fortunately, toxins do not pose a vapor or dermal hazard under most circumstances
• Toxins present a percutaneous exposure hazard from sharps/animal handling
• Aerosol generation is extremely hazardous
Routes of Exposure

- Intravenous (IV)
- Intramuscular (IM)
- Intraperitoneal (IP)
- Intradermal (ID)
- Inhale – Aerosol, breath it in
- Inject – Puncture through skin
- Ingestion – Eat it
- Dermal – Get it on your skin
  - Only important for mycotoxins

Used in the lab to study the toxin.

Potential lab exposure

Which of these routes is most severe, in general?
Aerosol Particle Size is Important

This particle is too small:
• **<0.5 µm** – Are inhaled, but most are exhaled.

This particle is too big:
• **5-15 µm** – Lodge in the nasal passages or trachea and do not reach the lung.
• **15-20 µm** – Drop harmlessly to the ground.

This particle is just right:
• **0.5-5 µm** – Retained in the lung. This is the size needed for an effective attack.

Size = Diameter
Aerosol Toxicity vs. Toxin Required for an Effective Open-Air Exposure

Threat = toxicity + ease of production + stability

Similar to Figure 30-1.
How to Classify Toxins?

• Source (Natural vs. Synthetic)

• Molecular Structure and Size
  – Low Molecular Weight Toxins – <1 kDa or ~ 10 amino acids.
  – Protein Toxins – >10 amino acids
    - Share characteristics of enzymes
      - Denatured by heat, acid, and proteolytic enzymes
      - Act catalytically
      - Specificity of action – attacks a specific substrate

• Mechanism of Action
  – Neurotoxins
  – Membrane-damaging Toxins
Medical approach to toxins

• Prophylaxis
  – PPE
  – Active Immunization

• Treatment
  – Diagnostics
  – Passive immunotherapy
  – Chemotherapy
  – Supportive

• Decontamination
  – Not a primary problem
  – Non-volatile aerosols
  – Risk to Health Care providers is low

• Not all casualties are mortalities

• Symptomatic care may be sufficient
Sources of Toxins

- Bacteria
- Marine
- Fungal
- Plant
- Animal (venom)
Bacterial Toxins

• Most toxic of biological materials.

• Neurotoxins
  – Botulinum Toxins (7 types)
    - Most toxic substances known to man.
  – Tetanus Toxin (30-40% homologous to botulinum toxins)

• Membrane-Damaging
  – Diphtheria Toxin
  – Staphylococcal Enterotoxins (7 types) (e.g. SEB)
  – Hemolysins (E. coli)
  – Cytolysins and Phospholipases (Aeromonas, Pseudomonas, and Staphylococcus)
  – Lower toxicity than neurotoxins.
Marine Toxins

- Difficult to produce and/or low toxicity
  - Not a major battlefield threat

- Most are low molecular weight toxins

- **Saxitoxin** – Shellfish (mussels, clams, scallops) infected with dinoflagellates (paralytic shellfish poisoning). Neurotoxin – Na channel blocker

- **Tetrodotoxin** – Puffer fish, Neurotoxin

- **Palytoxin** – *Palythoa tuberculosa* (soft coral). Extremely toxic and stable, but hard to produce in large quantities. Thus, not a major terror threat.

- **Brevetoxins** – “Red tide” dinoflagellate blooms. Limited toxicity.
  - Neurotoxic shellfish poisoning – mild compared to paralytic shellfish poisoning
Mycotoxins (Fungal Toxins)

- Produced by fungi – mostly molds.
- Aflatoxins
- Rubratoxins
- Ochratoxins
- Fumonisins
- Trichothecenes
Trichothecene Mycotoxins

- Low molecular weight toxins (< 500 Da)
- Produced by
  - *Fusarium*
  - *Mycotecium*
  - *Trichoderma*
  - *Cephalosporium*
  - *Verticimonosporium*
  - *Stachybotrys*
- **T2 Toxin** – Believed to be the cause of “yellow rain” in Southeast Asia in late 1970’s/early 1980’s (~10,000 deaths).
  - Laos (6310), Kampuchea (981), Afghanistan (3042)
  - Very stable
  - Dermally active (unusual for toxins)
Plant Toxins

• Easy to produce in large quantities at minimal costs. Thus, a potential threat.

• **Ricin** – From the bean of castor plants (*Ricinus comminus*).

• **Abrin** – From *Abras precatorius* (rosary pea). Similar to ricin.
Animal (Venom) Toxins

- **Ion Channel Toxins** – rattlesnake, scorpion, and cone snail
- **Presynaptic phospholipase A$_2$ Neurotoxin** – banded krait, Mojave rattlesnake, Australian taipan snake
- **Postsynaptic Neurotoxin** – coral, mamba, cobra, sea snake, and cone snail
- **Membrane-damaging Toxins** – Formosan cobra and rattlesnake
- **Coagulation/Anticoagulation Toxins** – Malayan pit viper and carpet viper
Mechanisms of Action: Neurotoxins

- Effects nerve and muscle function without damaging tissues.
- Effects are reversible (if you do not die!!).
- **Saxitoxin/Tetrodotoxin**
  - Block nerve conduction directly and cause death by paralyzing muscles of respiration. (Na channel blocker.)
  - Death within minutes if inhaled.
  - Thus, only prophylaxis (immunization or pretreatment) is effective at protection.
  - More toxic if inhaled than if given IV or oral (death in minutes vs. hours for saxitoxin).
- **Botulinum Toxin**
  - Must enter nerve terminals before they can block the release of neurotransmitters.
  - Inhibit muscle contractions.
  - Kill by slow onset respiratory failure (hours to days).
  - Possible to treat with antitoxin if injected within hours of the original exposure.
  - Less toxic if inhaled than if given IV.
Mechanisms of Action: Membrane-Damaging Toxins

- Cause microscopic damage to tissues and organs.
- Damage is NOT reversible.
- Damage happens within minutes to a few hours. Thus, early prophylaxis is critical.

- **Microcystin**
  - Produced by blue-green algae.
  - Covalently binds to phosphatase in liver cells. No other cells are affected.
  - Irreversible damage within 15-60 minutes.
  - Toxicity the same by all routes of exposure.

- **Ricin**
  - Protein synthesis inhibitor.
  - Toxicity depends on the route of exposure.

- **Trichothecene Mycotoxins**
  - The only membrane-damaging toxin that is dermally active.
  - T2
  - Cause skin lesions and systemic illness without being inhaled. Route of exposure = skin and ingestion.
Botulinum Toxins

Overview

- Description of the Agent
- Epidemiology
- History
- The Toxin
- Pathogenesis
- Clinical Disease
Description of the Agent

- **Clostridium botulinum**
  - Gram positive
  - Spore forming
  - Obligate anaerobe
  - Soil bacteria (ubiquitous)
  - Resistant to heat, light, drying, & radiation

- Botulinum toxins = botulism (food poisoning)
- Canning and food preservation problems (esp. high pH foods)
- Modern commercial procedures have greatly improved the situation.
- Easier to kill the toxin than the spores.
Botulinum Toxins

- Produced by *C. botulinum*
  - 30-40% homologous to tetanus toxin (*C. tetani*)
- Seven toxins (A-G)
- Highly toxic
  - More toxic when ingested compared to inhaled
  - Most lethal substance known to man (LD50 = 1 ng/kg, IV)
- Cause the same disease after inhalation, oral ingestion, or injection. This is unlike other threat agents.
- Causes food poisoning
- Likely biological warfare agent.
  - Very toxic
  - Easy to produce
  - Public not mass vaccinated
Types of Botulism (Epidemiology)

**Foodborne** (food poisoning)
- Incubation 12-36 hours
- Types A, B, E
- **Infant**
  - Nearly all type A
  - 3 weeks to 8 months of age

**Wound** (Types A & B)
- Incubation 4-18 days

**Inhalation** (Man-made)
- Incubation 24-36 hours

**Note** - No person-to-person transmission!
No dermal threat!
Human Cases in US

• In U.S., average <200 cases each year
  — ~70% infant form
  — ~15% foodborne form
  — ~15% wound form

• Case-fatality rate (1995)
  — 5-10%

• Infective dose - few nanograms
Laboratory-Confirmed Botulism 1995-2001

Foodborne
Infant
Wound
History

- 1793, Justinius Kerner
  - “Wurstgift” = sausage poison
- “Botulus” = Latin for sausage
- 1895, Emile von Ermengem
  - Isolated *C. botulinum* during Belgium outbreak
- U.S. outbreaks led to improved food industry processing
History – Bioagent Related

- CDC Video
  [http://emergency.cdc.gov/training/historyofbt/05botulism.asp](http://emergency.cdc.gov/training/historyofbt/05botulism.asp)
- First agent to be considered as a biological agent.
- Japan – Unit 731 (1930’s)
- Agent X (WWII)
  - US, Germans, and Japanese
- Early work focused on isolation, purification, and mechanism of action. Also worked on a vaccine.
- Former US and USSR programs
- Iraq, Iran, Syria, North Korea
Structure of Botulinum Toxins

- 7 Serotypes (A-G)
- Similar pathological effects
- Immunologically different (no cross-neutralization of antibodies)
- Humans susceptible to A, B, E, and F (rare).
- Wildlife and domestic animals = Types C and D.
- Each have been cloned and sequenced.
- Natural function of the neurotoxins is unknown.

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>G</td>
<td>Plasmid</td>
</tr>
<tr>
<td>C &amp; D</td>
<td>Bacteriophage</td>
</tr>
<tr>
<td>A, B, E, &amp; F</td>
<td>Bacteria</td>
</tr>
<tr>
<td></td>
<td>Chromosome</td>
</tr>
</tbody>
</table>
Pathogenesis (Mechanism of Action)

• Most poisonous substances known.
  – 1 microgram = kill 10 people
  – 250 grams = kill all the people on Earth

• LD$_{50}$ =
  – 1 ng/kg (IV, subcutaneous, IP)
  – 3 ng/kg (inhalation)

• Blood carries the toxin to peripheral cholinergic synapses.
  – Acts presynaptically to prevent the release of acetylcholine from neuromuscular junctions.
  – Muscles cannot receive signals to contract (flaccid paralysis)

• 3 stage mechanism of action
  – Binding
  – Internalization/Translocation
  – Enzymatic Activity
Normal Neurotransmitter Release

SNARE Proteins Form Complex

Synaptic Vesicle

SNAP-25

Syntaxin

(NERV TERMINUS)

Acetylcholine

Acetylcholine Receptor

Muscle Fiber Contracts
Botulinum Toxin Mechanism of Action
Clinical Disease

• Symptoms begin 12-36 hours after exposure.
• Classic symptoms
  – Acute symmetric cranial nerve paralysis
    - Blurred vision, droopy eyelids, double vision
  – Descending flaccid paralysis
    - Complete skeletal muscle paralysis
    - Respiratory failure
  – Nausea, vomiting, abdominal cramps, diarrhea (food borne)
  – Autonomic – Urinary retention, dry mouth
  – Difficulty swallowing
  – Difficulty speaking, sore throat
  – Difficulty breathing – leads to respiratory failure
  – Muscle weakness
  – General weakness
  – Constipation
  – Dizziness
• As the symptoms progress, respiratory distress begins.
  – Death caused by respiratory failure
• No fever
• No loss of mental abilities (toxin does not cross the blood/brain barrier.)
Diagnosis

• Laboratory Confirmation
  – Culture of stool or gastric aspirate – 5-7 days
  – Culture food if food borne exposure is suspected
  – Reference Labs (CDC + 20 nation wide)
  – Mouse Bioassay (detect 0.03 ng) – 48 hours
  – ELISA and PCR being developed

• On battlefield – multiple soldiers with symptoms (inhalation botulism)
Images courtesy of the CDC
Treatment

• Supportive Care
  – Mechanical ventilation, nutritional support (tube feed)
  – Prevention of secondary infections

• Passive Immunization (Antitoxin)
  – **Halts additional paralysis, doesn’t reverse existing paralysis**
  – Must be given ASAP (before toxin is internalized)
  – Equine antitoxin (Trivalent = A, B, and E)
    - Hypersensitivity may be a concern
    - Limited supplies (maintained by CDC)
  – Botulism immune globulin – infant cases of types A and B
    - Cangene
    - BabyBIG
    - 10/23/03
  – Heptavalent (A-G) Antitoxin (only one currently available)
    - Cangene - 2006
    - 200,000 doses in SNS ($362 million)
Vaccine – No longer available

• PBT (Pentavalent Botulinum Toxoid) (formalin-fixed)
  – No role for post-exposure prophylaxis
    - Immunity develops over months
  – Developed in the 1950’s (Fort Detrick)
  – Pentavalent (type A-E)
  – 0, 2, 12, 24 weeks + annual boosters
  – Controlled by CDC (IND)
  – Laboratory workers and military
  – Excellent efficacy
  – Few adverse effects
  – Not a candidate for mass vaccination of the general public
    - Need multiple doses
    - IND status
    - Limited Supply

• Recombinant vaccine (A & B) in development
  – Dynport Vaccine Company, only for the troops
Castor Bean Plant

- Native to tropical Africa, but grown worldwide.
  - Oil production (castor oil)
  - Ornamental
- Ricin + RCA
- The seeds are poisonous to people, animals, and insects because of ricin.
Ricin Toxin

- Isolated from the seeds.
- Part of the waste “meal” produced when castor oil is made (~5% = ricin).
- Oil is used as a lubricant and as medicine.
- Boiling destroys the toxin in the “meal”.
- “Meal” can then be used as animal feed.
Potential Terrorist Threat

• Available worldwide.
  – >1 million tons of beans processed each year.
• Easy to isolate.
• Relatively stable.
• Highly toxic.
  – 500 micrograms = lethal dose (head of a pin).
  – But still 1,000X less toxic than botulinum toxin.

Threat = toxicity + ease of production + stability
History and Military Significance

- Ricin is a choice toxin of terrorists/assassins, because without motive or witnesses, death appears to be from pneumonia. Death can be caused by a very small amount (500 micrograms / size of a grain of salt).

- 1978 Ricin was (believed to be) the toxin used to assassinate Georgi Markov (Bulgarian defector) in London. While waiting for a bus, Markov was stuck with an umbrella gun that shot a platinum ball containing Ricin. Markov died three days later.

- 1991 Members of a Minnesota Patriot organization (Patriots Council) manufactured ricin in an attempt to kill a US Marshal. Their plans and the amount of toxin made could have killed more than a hundred people. They were unsuccessful.
History and Military Significance

• 1993 - Thomas Lavy (Neo Nazi) arrested in Canada in route to the US, was found to have enough ricin to kill 30,000 people, four guns, and 20,000 rounds of ammo.

• 1995 - Disneyland gets threat letter and a video of someone mixing chemicals possibly Ricin or Sarin.

• 1997 - Thomas Leahy arrested in a shooting. Raid on home finds ricin lab in basement along with nicotine sulfate and he was trying to grow C. botulinum.

• Some suspect that early Legionnaires Disease cases may have been a poorly done ricin release into a ventilation system.
History and Military Significance

• January 2003 – Seven men (4 Algerians) were arrested in a London apartment where they had manufactured ricin, a quantity of which could not be accounted for. Authorities refused to announce the nationality of the three other suspects.

• October 2003 – Ricin-containing letter found at a mail-processing facility in Greenville, SC. (Fallen Angel)

• February 2004 – Letter addressed to Senator Bill Frist was found to contain ricin.

• Al Qaeda caves in Afghanistan.

• January 2009 - 11 gay bars in Seattle
PRESS RELEASE

The Federal Bureau of Investigation (FBI), United States Postal Inspection Service, and the United States Department of Transportation (DOT), Office of Inspector General are offering a reward of up to $100,000 for information leading to the arrest and conviction of the individual(s) responsible for introducing a threatening letter addressed to DOT and containing the poison ricin into a U.S. Postal facility located in Greenville, South Carolina on October 15, 2003.

The author(s) of this typewritten letter claimed that he or she was a fleet owner of a tanker company and demanded that the present laws regarding truck driver hours of service regulations remain unchanged. On January 4, 2004, significant new federal regulations went into effect mandating more rest and orienting drivers toward a 24-hour work/rest cycle. A typewritten message on the exterior of the envelope indicated "caution RICIN POISON Enclosed in sealed container Do not open without proper protection." Inside the envelope was a small, metal vial which contained ricin, a white, granular, potentially deadly poison.

The author(s) of this letter claimed to have the ability to make large quantities of ricin and to use this poison if the new hours of service regulations were not repealed by January 4, 2004. The letter was signed "Fallen Angel".

Anyone with information concerning the identity of the individual(s) responsible for authoring this threatening letter is requested to contact the FBI toll free at 1-866-839-6241.
Ricin Structure

RIP = Ribosome-inactivating protein

Type 1
- Wheat
- Barley
- ~30 kDa

Type 2
- A chain
- B chain
- Ricin from castor beans
- ~32 kDa
- ~34 kDa
1. Ricin A/B bind to cell surface sugars (glycoproteins and glycolipids) and are internalized.
2. Vesicles are shuttled through the endosomes.
3. Some ricin is returned to the cell surface...
4. ...more is degraded in the lysosome...
5. ... and still more is sent “back” to the trans-Golgi.
6. Again, some is returned to the cell surface
7. Some escapes the Golgi into the cytoplasm
Mechanism of Action

• **A chain** attacks a single, highly conserved adenosine near position 4324 in eukaryotic 28S RNA (part of large ribosomal subunit).

• N-glycosidase activity removes the **adenosine base** (does not cleave the RNA chain). This is the adenine where **EF-2** binds. Prevents translocation step.

• This inactivates the ribosome.

• One molecule can inactivate 50,000 ribosomes (>1500 per minute).

• Only inactivates eukaryotic ribosomes (prokaryotic ribosomes not affected).
Routes of Exposure

• There are three methods of ricin poisoning.
  – Intravenous - Introduced into a puncture or cut.
  – Inhalation - Aerosolized liquid or powder inhaled directly into the lungs. Latency 8-24 hours (dose dependent)
  – Ingestion - Entry through contaminated food or water into the stomach.

• Not easily absorbed through the skin. May cause redness and pain.

• Initial symptoms in <6 hours.

• Lethal Dose (injection or inhale) = 500 micrograms (size of a pin head)

• If death has not occurred in 3-5 days, the victim usually recovers.

• Signs and symptoms depend on the route of exposure.
Signs & Symptoms - Inhalation

• Symptoms within 8-12 hrs
  – Difficulty breathing
  – Fever
  – Cough
  – Nausea
  – Chest tightness
  – Muscle aches
  – Profuse sweating

• Signs
  – Inflamed airways
  – Excess fluid in lungs (pulmonary edema)
  – Cyanosis (blue skin)
  – Necrosis of airways
  – Respiratory failure – leads to death

Most toxic route of exposure!

• No human cases have been described.
• Data from animal models.
Signs & Symptoms - Ingestion

• Symptoms (latency <6 hrs)
  – Nausea/Vomiting
  – Severe Diarrhea (may be bloody)
  – Abdominal pain/cramping
• Lower toxicity because harder to adsorb and enzymatic digestion in the GI tract.
• Death on day 3 or later.

1985 Study
751 cases
14 deaths
1.9% death rate

• Signs
  – Severe Dehydration
  – Hypotension (low blood pressure)
  – GI bleeding
  – Hematuria (blood in urine)
  – Low urine output
  – Dilated pupils
  – Fever
  – Thirst
  – Sore Throat
  – Headache
  – Hallucinations
  – Seizures
  – Liver, Spleen, & Kidney failure
Signs & Symptoms - Injection

• Muscle and lymph node death (at the injection site)
• Liver, Spleen, and Kidney failure
• Massive bleeding from stomach and intestines
• Death – multiple organ failure
  – Usually 36-48 hours after exposure
Treatment

• No antidote exists for ricin (vaccine being developed).
• Prevent further exposure to ricin (decontaminate).
• Supportive treatment (depends on route of exposure)
  – Ventilation
  – Management of seizure and low blood pressure
  – Activated charcoal if the ricin was very recently ingested
  – Gastric lavage and cathartics (magnesium citrate)
  – Fluid resuscitation (IV fluids)
  – Flush eyes if irritated
• Death occurs within 36 to 72 hours of exposure.
• If death has not occurred in 3 to 5 days, recovery is likely.
Diagnosis and Vaccine

• Diagnosis
  – Lots of people in the same area get sick at the same time (fever, cough, fluid in lungs).
  – Can be confirmed by ELISA from nasal swab(<24 hours).
  – Direct analysis of tissue

• Vaccine
  – No vaccine or antitoxin available.
  – Promising vaccines currently being tested in animal models.
Therapeutic Applications

• Bind A chain to monoclonal antibodies (immunotoxins) that target unwanted cells (i.e. cancer, AIDS, bone marrow transplants).
• The monoclonal antibody targets the cancer cell.
• Ribosomes are inactivated.
• Cell dies.
• Clinical trials (phase I and II) have been successful in treating lymphoma and liver cancer.
Staphylococcal Enterotoxin B (SEB)
SEB: History

• Staphylococcus aureus toxins:
  – SEB (staphylococcal enterotoxin B)
  – Related exotoxins
    - SEA, SEC1-3, SED, SEE, SHE, TSST-1…

• Route of exposure dictates clinical syndrome
  – Inhalational (fever, respiratory symptoms, pulmonary edema, ARDS, septic shock, death)
  – Ingestion (food poisoning)
  – Absorption through mucous membranes (local and GI effects)

• Weaponized by the U.S./Significant morbidity with aerosol attack
SEB: Characteristics

• SEB is both lethal and incapacitating
  – $LD_{50} = 1.7 \, \mu g$
  – $ED_{50} = 30 \, ng$
  – 0.0536 \, \mu g \, \text{aerosol exposure}$
    – 1/3 no disease, 1/3 mild disease, 1/3 incapacitating

• Extreme toxicity allows variable application
  – Open-air weapon

• Easily produced
SEB: Mechanism of Action

• Bacterial superantigen
  – Binds to MHC class II receptors on antigen presenting cells (APCs)
  – Stimulates significant amount of T-cell proliferation
    - Results in cytokine production (mediate toxic effects)
      - Tumor necrosis factor, interferon-gamma, interleukin-1

• Intense inflammatory response results in
  – Tissue injury
  – T-cell anergy
  – Apoptosis
SEB: Lab Exposures

• 1943-’69
  – Significant aerosol exposures at Fort Detrick)
    - Ruptured hose under pressure with crude filtrate
    - Residual of highly purified SEB from NHP facial fur
    - Pressurized tube leak
  – Main differential diagnosis was community-acquired pneumonia (high fever, chills, cough, dyspnea, chest discomfort, elevated WBC)

• 1989-’02
  – Various ocular exposures from rubbing eyes
SEB: Lab Work Conditions

- Primary hazards
  - Ingestion, inoculation, mucous membranes, droplet, aerosolization
- BSL-2 practices when handling SEB or potentially contaminated materials.
- BSL-3 high aerosol or droplet formation potential or large quantities
- Stable in environment with fomite-like particles
- BSCs, gloves, face masks, eye protection
SEB: Clinical Features

- Severely incapacitating illness dictated by route of exposure
- Rapid onset
  - 2-12 hours after aerosol exposure (range 1.5-24)
  - 1-6 hours after ingestion (range 1-12)
- Modest duration depending on dose and timeliness of care
  - 1-2 weeks
- Fever, chills, myalgia and headache
  - Fever of 103-106 F
  - Duration 1-3 days
SEB: Clinical Features (continued)

• Ingestion/GI symptoms
  – Acute with salivation, nausea, vomiting
  – Abdominal cramping and diarrhea

• Inhalation
  – Abrupt high fever (103-106 F) lasting 2-5 days
  – Chills, headache, fatigue, myalgias
  – Respiratory symptoms
    - Cough, dyspnea, retrosternal chest pain
    - May progress to ARDS

• Ocular
  – Conjunctivitis, periocular swelling, potential GI symptoms
SEB: Diagnosis

• Inhalational based on clinical and epi features
  – Symptoms plateau early

• Epidemiology

• Laboratory identification
  – Confirmed by ag-detection ELISA and ECL, gene amplification (not fully validated)
  – Persists at detectable levels for short time period in
    - Urine, blood, respiratory secretions, nasal swabs
  – Acute and convalescent sera – retrospective diagnosis
SEB: Medical Management

• Treatment limited to supportive care
• No specific antitoxin available for human use
• No post-exposure prophylaxis
• Asymptomatic
  – Observation for intoxication
  – Baseline serum
• Symptomatic
  – Oxygenation, ventilator support, hydration
  – 0-3 hours – nasal swab, respiratory secretions for toxin assays, baseline serum, serology
  – 2-6 hours – serum, urine, nasal swabs, respiratory secretions, baseline serum
  – 6 days or longer serum for IgM and IgG and convalescent research serology
SEB: Prophylaxis

• Vaccine not available for human use
• Vaccine candidate tested in monkeys
  – Recombinant SE vaccine
  – Pending transition to advanced development
Mycotoxins

- Toxins produced by fungi (mostly molds)
  - Aflotoxins
  - Rubratoxins
  - Ochratoxins
  - Fumonisins
  - Trichothecenes

- [http://www.themoldsource.com/clinical/tri.html](http://www.themoldsource.com/clinical/tri.html)
- [http://nbc-med.org/sitecontent/medref/onloneref/fieldmanuals/medman/mycotoxins.htm](http://nbc-med.org/sitecontent/medref/onloneref/fieldmanuals/medman/mycotoxins.htm)
Trichothecenes

- Low molecular weight (250-500 Da)
- Produced by:
  - *Fusarium*
  - *Myrotrichum*
  - *Trichoderma*
  - *Cephalosporium*
  - *Verticimonosporium*
  - *Stachybotrys*
- Naturally found in moldy grains, cereals, and other agricultural products.
Trichothecenes (continued)

- The structure of approximately 150 described in the literature.
- Insoluble in water.
- Highly soluble in ethanol, methanol, and propylene glycol.
- Very stable to heat and UV light.
  - Inactivated by heating at 900°F for 10 min or 500°C for 30 min.
  - Inactivated by 3-5% sodium hypochlorite (bleach) or 0.1 M NaOH.
Mechanism of Action

- Cytotoxic to most eukaryotic cells
- Affects rapidly proliferating cells
  - Bone marrow, skin, mucosal epithelia, germ cells
- Inhibit protein synthesis
  - 60S ribosomal subunit (peptidyl transferase)
- Inhibit RNA and DNA synthesis
  - Secondary affect of protein synthesis inhibition
- Multiple cell membrane effects
  - Permeability
  - Phospholipid turnover
  - Lipid peroxidation
- Mitochondria effects
History and Military Significance

• T2 Toxin

• Soviet Union (1974-1981)
  – Afghanistan (3042 deaths)
  – Laos (6310 deaths)
  – Cambodia (981 deaths)

• Iran/Iraq War (1983-1984)
  – Used in combination with mustard

• “Yellow Rain”

• Primarily considered a blister agent
Delivery

- Dust
- Droplets
- Aerosols
- Smoke

- Aircraft
- Rockets
- Missiles
- Artillery
- Mines
- Portable sprayers

Aerosol exposure is more (10-50 fold) lethal than when injected parenterally.

\[ \text{LD}_{50} \text{ (dermal)} = 2-12 \text{ mg/kg} \]
Clinical Features

- In a BW attack the toxin could stick to and penetrate the skin, be inhaled, or ingested.
- Enter body through skin and digestive or respiratory epithelium.
- Main effects are on rapidly proliferating tissues (bone marrow, skin, mucosal epithelia, germ cells).
Early Symptoms

- Begin in minutes
- Skin = burning pain, red, tender, blister
- Nasal = itching and pain, sneezing, bloody and runny nose (epistaxis and rhinorrhea)
- Mouth and throat = pain and blood tinged saliva
- Pulmonary = difficulty breathing, wheezing, and cough
- GI = anorexia, nausea, vomiting, abdominal cramping, and bloody diarrhea
- Eyes = pain, tearing, redness, and blurred vision
Additional Symptoms

• Systemic toxicity
  – Weakness
  – Prostration
  – Dizziness
  – Ataxia
  – Loss of Coordination

• Fatal symptoms = tachycardia, hypothermia, and hypotension

• Death may occur in minutes, hours, or days.
Diagnosis

• No rapid diagnostic test currently available.
• T2 poisoning should be considered when multiple patients present similar symptoms consistent with T2 exposure and droplets of yellow fluid are seen on clothing.
• Clinical samples (nasal or throat swabs, urine, blood, environmental samples) should be collected.
  – GLC-MS (gas liquid chromatography-mass spectrometry)
  – Immunoassay
Treatment

• Chemical protective mask and clothing will protect against T2 aerosol attack.
• No antidote or therapeutic regimen available.
• No vaccine available.
• Remove outer clothing and decontaminate with 5% sodium hypochlorite for 6-10 hours.
• Wash exposed skin with soap and water.
• Super-activated charcoal should be given orally if toxin is swallowed.
• Wash exposed eyes with water or saline.
• Supportive care as needed.
Toxins, Chemicals and Reproductive Risks

• Many women of child bearing age work in laboratories
• May not know they are trying to become pregnant
• May not know when they are pregnant
• Should not need restrictions if exposures are controlled
• Many choose not to conduct hazardous work or work around anesthetics
• Workplace accommodations