# Safe Handling of Acutely Toxic Chemicals, Mutagens, Teratogens and Reproductive Toxins

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By Scott Batcheller
R&D Manager
Milwaukee WI



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# Hazards Classes for Chemicals

- Flammables
  - *Risk of ignition in air when in contact with common energy sources*
- Corrosives
  - *Generally destructive to materials and tissues*
- Energetic and Reactive Materials
  - *Sudden release of destructive energy possible (*e.g. *fire, heat, pressure)*
- Toxic Substances
  - *Interaction with cells and organs may lead to tissue damage*
  - *Effects are typically not general to all tissues, but targeted to specific ones*
  - *Examples:*
    - *Cancers*
    - *Organ diseases*
    - *Inflammation, skin rashes*
    - *Debilitation from long-term accumulation with delayed emergence*

**Poison (ingestion)**

**Acute risk**

**Cancer, health or reproductive risk**

# Toxic Substances Are All Around Us

- Pollutants
  - Cigarette smoke
  - Automotive exhaust
- Common Chemicals
  - Pesticides
  - Fluorescent lights (mercury)
  - Asbestos insulation
  - BPA (Bisphenol A used in some plastics)

- Natural toxins
  - Venoms (snakes, spiders, bees, etc.)
  - Poison ivy
  - Botulinum toxin
  - Ricin
  - Radon gas
  - Arsenic and heavy metals in ground water

# Application at UNL

- Chemicals in Chemistry Labs
  - Chloroform
  - Formaldehyde
  - Acetonitrile
  - Benzene
  - Sodium azide
  - Osmium/arsenic/cadmium salts
- Chemicals in Biology Labs
  - Phenol
  - Ethidium bromide
  - Acrylamide
- Toxin-producing Microorganisms
  - Fungi
  - *Staphylococcus* species
  - Shiga-toxin from *E. coli*
- Select Agent Toxins (see register)
  - *Botulinum* neurotoxins
  - T-2 toxin
  - Tetrodotoxin
  - Diacetoxyscirpenol
- Radioactive Substances

  with toxic effects not related to radioactivity

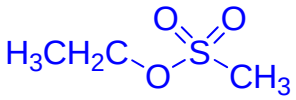
# Scope of This Talk

- Subject: Laboratory Safety With Highly Toxic Materials
- Topics
  - **Definitions**
  - **Resources for Determining Toxicity**
    - Known substances

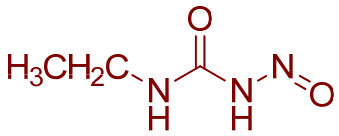
      Case History: 1997 dimethylmercury incident
    - Unknown substances
  - **Working With Toxic Materials**
    - Regulatory obligations
    - Biologically-derived toxins
    - PPE (respirators and gloves)
    - Lab hygiene
    - Waste disposal
  - **Case Study: Procedure Involving Reaction of a Carcinogen**

# Definitions

- **Mutagen** a substance that is suspected to cause mutation in DNA



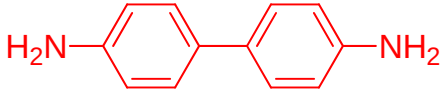

ethyl methanesulfonate
(EMS)



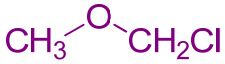

ethylnitrosourea
(ENU)

- **Carcinogen** a substance that is suspected to cause cancer

*A common initiation of cancer is a mutation in DNA.*

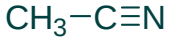



benzidine

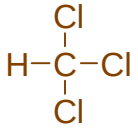



methyl chloromethyl ether

- **Teratogen** a substance that is suspected to impact fetal development




acetonitrile




chloroform

- **Reproductive Hazard**

a toxic substance that targets reproductive organs and cells

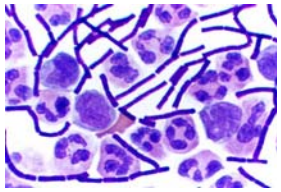

ethylene oxide

Pb

lead

# More Definitions

- **Biotoxin** a toxin with a biological origin; source can be microorganisms, insects, reptiles, fish, and amphibians





- **Cytotoxic** a substance that is lethal to cells or limits their ability to divide

  *Many anti-cancer drugs are cytotoxic because of their ability to target cancer cells and kill them or stop them from growing.*

  *Cytotoxic drugs will likely destroy cells indiscriminately and only be beneficial if targeted to tumors and if dosed very carefully.*

- Other forms of toxicity—by targeted organs and cells

| | | |
|---|---|---|
| Neurotoxic | Hemotoxic | Myotoxic |
| Hepatotoxic | Cardiotoxic | etc. |

# Acute and Chronic Toxicity

- Duration and level of exposure are important elements in assessing a toxic risk
- Acute exposure risks
  - Substances that produce a toxic response almost immediately after exposure
  - Generally involves a tangible quantity of toxic agent to cause toxic response
  - Examples: snake bite, carbon monoxide poisoning
- Chronic exposure risks
  - Substances that accumulate over prolonged exposure to produce a delayed toxic response
  - Level of exposure at any time could be very small
  - Examples: asbestos, lead poisoning

Example: **Alcohol**
Acute risk—impairment, nausea, unconsciousness
Chronic risk—liver disease

# Determining Toxicity for Known Substances

- Container label
- MSDS
  - Hazard identification
  - First aid measures
  - Personal protection
  - Toxicological summary
  - Regulatory information

- What to look for:
  - Health hazard placard

  - Acute toxic effect
  - Chronic toxic effect
  - Target organs
  - Exposure level for effect
  - Recommended precautions
  - Applicable standards and regulations
  - Antidotes or countermeasures

**Precautionary Note:** Toxicology data is only as good as the testing that has been done! "No data" does not mean it is non-toxic.

# Toxicity Risks Are Not Always Obvious From the Documentation

- Read toxicity data in MSDS's and on labels carefully
- Look for "red flags"
  - Evidence of lethality by means other than ingestion
  - Substance listed or suspected as carcinogen, mutagen, teratogen, or "highly toxic"
  - Recommended use of unusual handling equipment or PPE
  - Specific and detailed regulated limitations and requirements
- Interpret what you read against your lab situation
- Don't take chances with your health

**Put away the rose-colored glasses when evaluating toxicity risks.**

# Case History: Dimethylmercury Exposure

- 1997 Incident involving ***48-year-old university professor***
- Wearing disposable ***latex exam gloves***
- Transferred ***dimethylmercury*** by pipet
- Some drops fell on her gloved hand
- She removed the gloves immediately and washed her hands
- She experienced no symptoms for five months
- Then, she reported neurological problems (tingling, difficulty speaking, etc.)
- She was diagnosed with ***mercury poisoning*** (five months after exposure)
- In spite of aggressive chelation treatment, she died of encephalopathy *after 10 months*

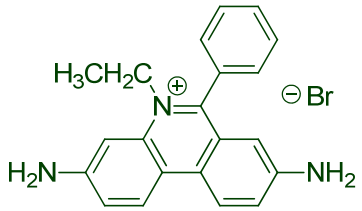
$H_3C\text{-}Hg\text{-}CH_3$

# Dimethylmercury Incident: What It Teaches Us

- It can happen to ***anyone***, even the most experienced scientists
- Gloves are not always an adequate defense
  - …and ***latex exam gloves offer no protection*** to chemicals at all
- Some substances can ***pass through your skin*** very quickly
- Some substances can ***cause great harm*** without obvious signs of exposure
- Toxicity effects may be significantly delayed
- Red flags that were missed
  - Heavy metal alkyl and liquid
  - "Highly toxic by skin absorption" (MSDS)
  - "Fatal if swallowed or in contact with skin." (MSDS)
  - "Latex, neoprene and butyl gloves do not provide suitable protection for direct contact with dimethylmercury." (Wikipedia)

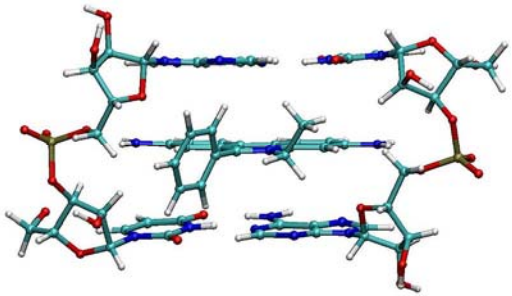
**In 1997, this information may not have been available.**

# Evaluating Potential Toxicity for Untested and Unknown Substances



ethidium bromide

- Known mutagens disrupt DNA through…
  - Alkylation — ex. Ethyl methanesulfonate, methyl iodide
  - Deamination — ex. Nitrosoureas, nitrosamines
  - Base substitution — ex. Bromodeoxyuridine
  - Intercalation — ex. Cationic planar polyaromatics



*Two A-T base pairs in DNA*

- Other common sources of high toxicity…
  - Compounds of heavy metals, especially organometallics
  - Bio-active compounds (like antibiotics and other microorganism-derived compounds)
  - Analogs of known toxic materials (such as an active drug derivative)

- *Handle all uncharacterized materials with care.*
- *If the material contains any "red flag" characteristics of a highly toxic material, treat it as highly toxic.*

# Biologically-Derived Toxins

## ➢ Definition:

- Poisonous substances produced as by-products of microorganisms, plants or animals
- Not living organisms
- Not contagious

## ➢ Examples in use at UNL:

- **Cholera toxin** (secreted by *Vibrio cholerae* bacteria)
- **Mycotoxins** (derived from fungi) (e.g. aflatoxin, ochratoxin, fumonisins)
- **Pertussis toxin** (*Bordetella pertussis*)
- **LPS** (Lipopolysaccharide – from cell membrane of gram-negative bacteria)
- **Diptheria toxin** (Corynebacterium diphtheriae)

# So You Need to Work With a Highly Toxic Material… What Next?

1. **Seek Alternatives**
   - *Do you <u>really</u> need to do this?*
2. **Are there regulations that apply?**
   - Occupational Safety and Health Administration, Nuclear Regulatory Commission, Department of Homeland Security, State regulations, UNL and Departmental policies
   - UNL Committee approval may be required if the work involves biotoxins, microorganisms, recombinant DNA, RAM?
     - For biologically-derived toxins (contact the UNL Biosafety Officer at 472-9554). Some are subject to strict Select Agent regulations and possession above a designated threshold requires registration.
     - An Institutional Biosafety Committee protocol may be required
   - Notification, monitoring, and reporting may be required
   - Area isolation and marking may be required
   - Strict procedures may be mandated

# So You Need to Work With a Highly Toxic Material… What Next?

3. **Confirm availability of antidotes in case of emergency**
4. **Characterize the hazard and potential routes of entry**
   - Biological or chemical
   - Respiratory (gas, vapor, fume or dust)
   - Skin contact (especially liquids and solutions)
5. **Choose work area that best isolates hazard from other lab personnel**
6. **Choose PPE to best protect the handlers**
7. **Determine how you will deactivate/destroy residues and excesses**
8. **Buddy system—have a trained backup ready to assist**

# Work With Biologically-Derived Toxins

- **Work Practices**
  - Most work with biological toxins should be conducted in compliance with BSL-2 containment guidelines

    http://ehs.unl.edu/sop/s-bio-containment_levels.pdf

    http://www.cdc.gov/biosafety/publications/bmbl5/index.htm
- **Exposure Routes**
  - Aerosol production (centrifuge, aspiration, etc)

    http://ehs.unl.edu/sop/s-bio-aerosol.PDF
  - Dermal exposure (e.g., T-2 mycotoxin)
  - Mucous membranes exposure
  - Ingestion
  - Parenteral (e.g., needlestick, bite)
  - Toxin work involving animals

# Work With Biologically-Derived Toxins

- **Storage and Security**
  - Maintain an accurate inventory of purified toxins
  - Toxins should be secured whenever unattended (e.g., locked room, locked freezer, locked box)
  - Possession of Select Agent toxins requires specific security and storage requirements
- **Disposal/Inactivation**
  - Use an appropriate disinfectant for the toxin. Many toxins are susceptible to 10% bleach, 2N NaOH
    - Work surfaces
    - Equipment
  - Procedures for inactivation of dry biological toxins or solution containing biological toxin, see http://ehs.unl.edu/sop/s-bio-disinfectants.pdf
  - Treat with 2N NaOH for at least 1 hr
  - Other proven effective inactivating agents

# PPE for Chemical Toxins

- Three primary considerations for selection of PPE
  - State of hazard (gas, liquid, solution, solid, fume or dust)
    - This tells you what kind of exposure you need to guard against (inhalation, skin, etc.)
  - Chemical compatibility and resistance to penetration
  - Severity of effect of exposure
- Gases, vapors, fumes and dusts
  - Inhalation hazard
  - Review MSDS for toxic effects of inhalation
  - Review MSDS for toxic effects of skin or eye contact
  - Consider respirator
  - May require full body isolation
    - Supplied breathing air
    - "Moon suit"

NOTE: Researchers should contact EHS **prior to** assigning work or working in atmospheres that may require respiratory protection. UNL has a Respiratory Protection Program for workers required to use respirators.

# PPE for Toxic Liquids and Solutions

- Liquids may be inhalation hazards
- Liquids also may be able to penetrate protective equipment material
- Some liquids can be absorbed through skin
- Review EHS guides for specific chemicals and PPE recommendations
- Review MSDS for toxic effects of inhalation
- Review MSDS for toxic effects of skin or eye contact
- Consider inhalation and full body protection
- Gloves
  - Review for breakthrough rate to liquid to be used (link)
    - It is just a matter of time before a liquid will penetrate
  - Consider potential for cuts, needle sticks, and abrasions
  - Multiple gloving can protect against multiple hazards, increase penetration time
    - Example: Silver shield inner gloves
    - Evaluate mobility, flexibility, dexterity

Z529559 (sm)
Z529567 (med)
Z529575 (lg)

# Laboratory Hygiene and Control

- Trained persons only should be allowed to handle highly toxic materials
- Isolate hazard from co-workers
  - Regulated zone—no unauthorized personnel allowed while materials in use
- Handler must return all toxic materials to a stable, safe condition before leaving control zone
- Work area must be thoroughly decontaminated immediately after toxic materials are stowed
  - Decontamination is necessary even if no spill is obvious
  - All exposed equipment should be decontaminated in the regulated zone before being taken out for routine cleaning
  - Consider the appropriate decontamination procedure *before* handling the toxic material
    - Cleaning solvent or solution
    - Chemical destruction
    - Sterilization, etc.

# Laboratory Hygiene: Quantities and Storage

- Highly toxic materials must be stored separately from routine-use chemicals to prevent unprepared or untrained persons from contact with them
- Preferably, highly toxic materials won't need to be stored at all
  - The best plan is to acquire just what is required
  - Use it all up immediately
  - Destroy or dispose any excesses following guidelines available within EHS Safe Operating Procedures or consult EHS
- Toxic materials retained for long periods may require reporting with regulatory bodies and UNL-Environmental Health & Safety.

NOTE: The Department of Homeland Security requires reporting of possession or intent to possess ANY "chemical of concern (COC)" above the established threshold quantity.

# Waste Disposal

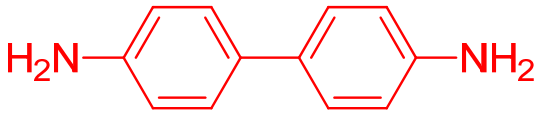
- Waste streams containing highly toxic agents should be separated from general waste
  - Protects lab workers from toxic exposure
  - Allows separate disposal protocol

    ***Ethidium Bromide*** *Disposal SOP*

    *Disposal of* ***Chloroform Contaminated Materials*** *SOP*
- Waste Disposal at UNL is handled by Environmental Health and Safety
  - EHS can address specific collection considerations.
- Waste containers must be clearly labeled and tagged following the protocol within the SOP ***Hazardous/Radioactive Materials Collection Procedures***
- Disposal of toxic waste is much more expensive than routine lab waste
  - It pays to keep volumes to a minimum and not mix with other wastes

Waste Disposal at UNL is handled by a third-party waste disposal contractor.

# Case Study: Reaction Using Benzidine




benzidine

- Lab team decides to make a compound using benzidine as a starting material.
- Review of the product information and MSDS of benzidine
  - Carcinogen (known, human)
  - Regulated (OSHA, several states' registers)
- Handling of OSHA carcinogens is covered by 29 CFR 1910.1003, which mandates…
  - Full body PPE
  - Respirator (fit tested)
  - Cordoned "regulated zone"
  - Strict washing protocol
  - Ventilated work area
  - Medical surveillance
  - Warning signs
  - Formal, documented training



**CANCER-SUSPECT AGENT**
**AUTHORIZED PERSONNEL ONLY**

# Case Study: Preparation to Use Benzidine

- Reaction is thoroughly planned
  - Quantities
  - Procedural step by step
  - Wastes, hazard content, disposal plan (clean with solvent, water)
- Work area and equipment are identified
  - Secure containment of hazard
  - Safe for handlers (adherence to regulation)
- Operators are identified
  - Formal training provided in carcinogens and 29 CFR 1910.1003
  - Respirator fit test for all operators
  - PPE selected and acquired
  - Medical surveillance conducted (exam and blood test)
- Chemical is acquired
  - Minimum quantity to perform reaction

# Case Study: Set Up to Use Benzidine

- Set up apparatus according to plan
  - "Clean" period—no benzidine yet in area
  - Normal procedures
  - Bring all needed materials into area while it is unrestricted
  - Bring in materials required for waste handling (destruction and containment)
    - Large drum or bucket for used PPE and other large items
- Set up "regulated zone"
  - Put up zone limit boundaries and warning signs
  - Handler(s) suit up
  - Handler(s) enter regulated zone
  - Strict requirements in order to leave zone
    - Carcinogen must be sealed, stabilized
    - Handler must decontaminate
    - Decontaminated PPE must be taken off at zone boundary and left in regulated zone (for hazardous waste disposal)
    - Handler must immediately wash hands, forearms, face, and neck (after leaving zone)

# Case Study: Using Benzidine

- Aim: to complete all carcinogen handling in one sequence without leaving zone
  - Open container
  - Charge (weigh)
  - Close and decontaminate (dispose) spent container
  - Run reaction
  - Decontaminate used equipment
  - Verify everything is stable and contained
- The “regulated zone” must remain in force as long as the carcinogen is present above mandated levels outside of a permanent sealed container
  - The limit for benzidine is 0.1% by weight
  - Anything suspected of being above this level must be sealed before the regulated zone may be taken down
    - Sealed, labeled reagent bottle for excess unspent reagent
    - Sealed, labeled waste containers for items to be disposed

# Case Study: Finishing the Benzidine Reaction

- When the reaction is complete and all benzidine-containing vessels are sealed and labeled
  - Remove sealed chemicals from zone
    - Reaction mixtures or products for work up (benzidine < 0.1%)
    - Excess reagents
    - Chemical wastes
  - Decontaminate (or dispose) used apparatus
  - Wash down work area
  - Collect washes for hazardous waste disposal
  - Remove everything (clean) not bolted down from zone
  - Remove PPE and dispose as hazardous waste
  - Take down zone boundaries and warning signs
  - Wash hands, forearms, face, and neck (or take a shower)
- Follow up
  - Medical surveillance (exam and blood test)

# Summary

- Toxic substances are common in…
  - the environment
  - consumer products
  - the laboratory (especially)
- Look for "red flags" that you may encountering a highly toxic material
  - Information resources (MSDS, websites)
  - Your own awareness (biological origin, chemical structure)
- First line of defense: ***seek alternatives***
- Educate yourself on applicable regulations
- Consult with EHS on what needs to be done
- Plan all aspects of handling in advance
  - Quantities needed
  - Area control
  - Labeling
  - PPE
  - Destruction of residues
  - Waste handling
  - Health monitoring
  - Washing
  - Antidotes/emergency actions

# Contacts

- **Environmental Health and Safety**
  402-472-4925 or ehs@unl.edu