LECTURE OBJECTIVES

- Understand the definition of “toxicology”
- Identify various aspects of chemical exposure
- Identify methods & common applications of toxicology in chemical-induced illness
- Understand routes & degree of exposure, absorption, & metabolism
- Understand exposure-dose response relationship, duration, frequency, distribution, affect on target organ, metabolism, & routes of excretion

DEFINITION

- The scientific study of the:
  - Mechanisms of action (adverse effects) caused by exogenous agents (chemical) to the study of molecular biology with assimilation of knowledge & techniques from chemistry, mathematics and physics and application of this discipline to safety evaluation and risk assessment.

TOXICOLOGY IDENTIFIES VARIOUS ASPECTS OF CHEMICAL EXPOSURE VIA...

- Assessment of Actual Health Damage
- Determination of Dose/Response Relationships
- Determination of Acceptable Exposure Limits

METHODS

- Controlled clinical studies
- Epidemiologic studies (e.g. cohort, case control, cross sectional, etc.)
- Animal/cell system testing (assumes underlying end target cells in either model is equivalent to humans)

Common Applications

- Controlled clinical studies
- Epidemiologic studies (e.g. cohort, case control, cross sectional, etc.)
- Animal/cell system testing (assumes underlying end target cells in either model is equivalent to humans)
Chemical-Induced Illness

Need practical understanding of a chemical's
- Exposure Routes
- Absorption
- Metabolism
- Distribution
- Excretion

Exposure Routes

• Lungs – inhalation
• GI tract – ingestion
• Skin – absorption & penetration (parenteral administration)
  - Subcutaneous
  - Intravenous
  - Intramuscular
  - Intraperitoneal

Exposure Routes

Potency and Response Rates in Decreasing Order
- Intravenous
- Inhalation – common occupational
- Intraperitoneal
- Intradermal
- Oral
- Dermal – common occupational

Dose Response Relationship

• Toxicity usually worsens with increasing dose
  - Often determined through animal models
  - Not always valid in diseases
    - e.g., hypersensitivity pneumonitis antigen
dose needed for symptoms may be small after initial sensitization

Exposure Duration

• Single Dose
• Short term ~ 1 week
• Subchronic ~ 3 months
• Long term ~ 2+ years - may influence types of effects seen, e.g., immediate effect vs. delayed effects

Exposure Frequency

• Smaller doses intermittently may allow for full or partial repair of damage
Absorption

- Process by which material passes through body membranes (defenses) & enters blood stream
- Administration affects
  - Rate of absorption
  - Concentration of toxicant at target tissues over time

Absorption - Lung

- Gases (H₂S, CO)
- Particulates (silica, asbestos)
  - Absorption is usually in alveoli (large surface area 50-100 sq meters)

Absorption - Skin

- Route of entry - substance must pass through either epidermal cells, sweat glands, sebaceous glands, or hair follicles
- Epidermal cells: most important as it constitutes the largest surface area

Absorption - Epidermis

- Must first pass through the stratum corneum (rate limiting membrane barrier)
  - Passive diffusion with different mechanisms for ionized vs non-ionized materials
- Differences in skin thickness - degree of permeability in various body regions, e.g., scrotum vs. abdomen vs. foot
- Compromised by injury (abrasion, burn, etc.)
Skin - Role of Water

- Normal skin - About 90 grams H₂O per gram of dry tissue
- With additional water contact get a 2-3x increase in permeability (wearing of work gloves & perspiration)
- Certain bipolar solvents, e.g., dimethyl sulfoxide (DMSO) can facilitate penetration of toxicants through skin

Absorption - GI Tract

- Important in
  - Food chain toxicants, e.g., mercury
  - Young children, e.g., lead
  - Accidental poisoning, e.g., workers eat/smoke with contaminated hands
- Less frequent than dermal or inhalation routes

Absorption - GI Tract

- Key determinants
  - Solubility (lipid)
  - Active transport mechanisms (GI membranes)
  - GI motility (ph & ionization state)
  - Physical properties of the toxin
  - Age
  - Nutritional state

Absorption - GI Tract

- Most toxicants cross body membranes by simple diffusion lipid solubility along with concentration gradient impact rates
- GI Membrane specialized transport systems impacts absorption rates of some toxicants
- Non-ionized molecules - more lipid soluble
- pH - Gastric juice or neutrality of GI contents may influence ionized or non-ionized states of materials

Absorption Routes of Administration

- Route of administration impacts:
  - Rate of absorption
  - Concentration of toxicant at organ/target tissues over time
- Intraperitoneal
- Subcutaneous
- Intramuscular
- Intravenous - direct to blood
Distribution to Target Organ

- Via blood or reticuloendothelial system
- Target organ impact depends on chemical’s ability to pass through cell membranes & tissue affinity for substance
- Plasma bound chemicals may bind with protein (albumin) & not be significantly toxic until unbound chemicals saturate protein diffuse through capillaries

Distribution to Target Organ

- If one agent displaces a protein-bound toxicant may see significant impact, for example:
  - Sulfonamide drug given to patient taking oral hypoglycemic may displace oral hypoglycemic drug causing hypoglycemic coma

Distribution to Target Organ

- Lipid soluble molecules with a molecular weight of 50 or less readily permeate cell membranes
- Water soluble diffuse through aqueous channels or through active transport
  - May concentrate in some areas more than others

Distribution to Target Organ

- When material accumulates in toxicologically inactive sites (e.g., adipose tissue) may result in initial protective mechanism
- When excreted a delayed impact on target cells may be seen - e.g., inorganic lead symptoms may be seen some time after exposure due to slow release from bone & other areas

Target Organ - Examples

- Asbestos & radioactive dusts accumulate lungs
  - Lungs become target organ for toxic impact
- Liver & Kidney
  - Concentrate more toxicants due to high capacity to bond chemicals along with active transport & protein binding capabilities

Target Organ - Examples

- Brain - often less because:
  - Capillary endothelial cells tightly joined
  - Capillaries surrounded by astrocytes
  - CNS interstitial fluid has lower protein concentration than other body areas
- Materials that do enter the brain are generally:
  - Lipid soluble
  - Non-protein bound
  - Non-ionized
  - Small molecular size
**Target Organ - Examples**

- Fetus
  - Placenta less effective than CNS barriers
  - Simple diffusion most common mechanism for chemicals, viruses, etc.

**Metabolism**

- Enzymatic chemical transformations of compounds
  - Mainly in liver
  - Some in intestine, kidney, lung, brain & skin
- Biotransformation rates impacted by
  - Age
  - Sex
  - Underlying disease
  - Nutritional status
  - Presence of enzyme inducing or activating agents (e.g., phenobarbital P450 can rates of transformation of other substances)

**Metabolism**

- Types of metabolism
  - Catabolic
  - Oxidations (P-450 mono oxygenases)
  - Reduction
  - Hydrolysis
  - Synthetic conjugation reactions (joining parent material with another to form a third substance)
- Glucuronic acid conjugated to:
  - Aliphatic & aromatic alcohols
  - Mercaptans
  - Certain acids
  - 1º and 2º aliphatic and aromatic amines

**Excretion - Routes**

- Kidney (primary)
- Liver & biliary
- Lungs
- Sweat
- Tears
- Breast milk

**Excretion - Kidney Factors**

- Passive glomerular filtration
- Passive tubular diffusion
- Active tubular secretion
- Urine pH impacts ionic/non-ionic form of chemical
- Lipid solubility
- Particle size
- Protein binding
- Active transport

**Excretion - Liver Factors**

- GI tract
  - Blood passes through liver before reaching systemic circulation
- Liver
  - Can remove chemicals, biotransform chemicals, & lessen reabsorption after bile excretion to intestine
Excretion - Liver
Biotransformation

- Organic compounds transformed to polar metabolites make them less lipid soluble for GI reabsorption
- Protein bound chemicals become fully available for biliary excretion
  - Lead, arsenic, manganese - rapidly concentrated & excreted in bile
  - Zinc, iron, gold, chromium - poorly concentrated in bile.

Excretion - Lung

- Important for materials that are in the gas phase of body temperature or that have high vapor pressures

Excretion - Breast Milk

- Primarily lipid soluble compounds (e.g., DDT, polychlorinated & polybrominated biphenyls)

Excretion - Other

- Elimination through sweat, saliva, skin, hair, & nails
  - Quantitatively of minor importance
  - May play role in biologic monitoring

Toxicologic Testing Methods

- Influenced by Toxic Substances Control Act (TSCA) - 1976
  - EPA requires chemical data 90 days before production begins
    - Mutagenic effects
    - Carcinogenic effects
    - Teratogenic effects
    - Synergistic effects
    - Behavioral effects

Obtaining Toxicology Data

- Methods
  - Epidemiologic
  - In vitro assays
  - Laboratory animals
Animal Testing

- In general, if the absorption, distribution, metabolism, & excretion of a material is similar in humans & a particular animal species, test results in that species generally are predictive of human toxicity
  - Assumes that underlying end-target cells are equivalent

Toxicity Rating

<table>
<thead>
<tr>
<th>Class</th>
<th>Probably Oral Lethal Dose</th>
<th>70 Kg Person</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 - Super Toxic</td>
<td>Less than 5 mg Kg</td>
<td>Less than 7 drops</td>
</tr>
<tr>
<td>5 - Extremely Toxic</td>
<td>5-50 mg Kg</td>
<td>7 drops to 1 tsp.</td>
</tr>
<tr>
<td>4 - Very Toxic</td>
<td>50-500 mg Kg</td>
<td>1 tsp to 1 ounce</td>
</tr>
<tr>
<td>3 - Moderately Toxic</td>
<td>5-5 gm/Kg</td>
<td>1 ounce to 1 pint</td>
</tr>
<tr>
<td>2 - Slightly Toxic</td>
<td>5 - 15 gm/Kg</td>
<td>1 pint to 1 quart</td>
</tr>
<tr>
<td>1 - Practically Non Toxic</td>
<td>Over 15 gm/Kg</td>
<td>More than 1 quart</td>
</tr>
</tbody>
</table>

Acute Toxicology Studies

- Animal model
- One-time dose
  - Evaluate for effects over hours/days/weeks
  - Helps determine LD 50 (lethal dose 50%)
  - Inhalation studies use LC 50 (lethal concentration 50%)

Obtaining Toxicology Data - Subchronic Exposure

- Assess mutagenesis/teratogenesis effects on reproduction
- Daily administration for 3-4 months
- Use human exposure routes
- Usually uses 2 animal models
- Observe mortality, weight, oral food intake, blood, & chemistries
- Gross & microscopic evaluation at study end

Obtaining Toxicology Data - Bioassays (Evaluate Carcinogenicity)

- Usually rats & mice
  - Usually 3 groups (1 for each exposure; 50 animals each)
  - Control group of 50
  - Exposure length
    - Generally 30 months for rats
    - 24 months for mice
Obtaining Toxicology Data - Mouse Skin Bioassays

- Applied to shaved skin of mice
- 2-3X per week
- Population usually 50 male mice
- Single dosage level
- Assay for dermal tumors

Obtaining Toxicology Data - In Vitro Assays

- Use cell cultures & bacterial systems outside of animal
- Use to assess mutagenicity
- Ames assay uses Salmonella typhimurium

Tools & Resources For Addressing Potential Exposure / Effects

- MSDS
- Med line
- Tox line
- IARC Monographs
  - International Agency for Research on Cancer
- ACGIH TLV's (threshold limit values)

Tools & Resources

- ASTDR (Agency for Toxic Substances & Disease Registry)
- Toxnet
- TOMES

Example Areas for Board Review

- Occupational Renal, Liver, Lung, Cardiovascular, Skin disease
- Vibration effects
- Fluorocarbons
- Carbon disulfide
- Asbestos
- Nitroglycerine
- Enzymatic detergents
- Platinum salts
- Beryllium
- Acrylonitrile
- Mercury
- Lead
- Organophosphates
- Silica
- Aluminum
- Hydrocarbons
- Arsenic
- Isocyanates
- Nickel
- Cadmium
- Radiation
- Alcohol

CASE STUDY

- 56-year-old homemaker seen at your office has a 3-month history of chronic, nonproductive cough with chest pain associated with the cough. The cough has recently become unresponsive to OTC liquid cough suppressants. She denies:
  - Shortness of breath
  - Wheezing
  - Hemoptysis
  - Fever
  - Chills
  - Sore throat
  - Hoarseness
  - Postnasal drip
American Osteopathic College of Occupational and Preventive Medicine  
Basic Course in Occupational and Environmental Medicine, Part I  
March 14, 2012, St. Petersburg, Florida

**Cough**  
- Independent of time of day, physical activity, weather conditions, or exposure to dust or household cleaning agents  
- Daughter's cigarette smoke does not seem to aggravate the cough  
- Fatigue  
- Weight loss - lost 18 pounds over past 6 months without dieting

**Fatigue**  
**Weight loss** - lost 18 pounds over past 6 months without dieting

**PMH noncontributory**  
- Nonsmoker; nondrinker  
- No contact with known chemical substances or irritants other than typical household cleaning agents

**Family medical history**  
- Father - died age 65 of myocardial infarction  
- Mother - breast cancer at age 71  
- First husband died of CVA 3 years ago  
- Newly remarried to retired shipyard worker. She & current husband live with 28-year-old daughter & 9 y/o grandson in New Hampshire home  
- Has not been outside the New England area X5 years.

**Physical examination (including HEENT & chest)** is normal  
- No cyanosis  
- No clubbing of extremities X4  
- No palpable lymph nodes

**Diagnostic tests**  
- CBC - normal  
- Chemistry panel - normal with exception of a total serum calcium level of 12.7 mg/dL (normal range: 9.2 - 11.0 mg/dL)  
- Chest radiograph - a non-calcified, non-cavitary 3.5 centimeter (cm) mass within parenchyma adjacent to right hilum. No other radiographic abnormalities appear  
- Urinalysis results are normal  
- Purified protein derivative (PPD) skin test for tuberculosis negative.

**INITIAL CHECK QUESTIONS** (Choose the one best answer)

**Given the clinical findings to this point, which of the following is most likely part of the differential diagnosis?**

A. Chronic obstructive pulmonary disease (COPD)  
B. Angina  
C. Pulmonary tuberculosis  
D. Primary pulmonary malignancy

**The differential diagnosis for the patient's radiographic solitary pulmonary nodule would include:**

- Primary pulmonary malignancy  
- Metastatic malignancy  
- Granulomatous disease (e.g., tuberculosis, coccidioidomycosis, histoplasmosis, nocardiosis)  
- Arteriovenous (AV) malformation  
- Pulmonary hamartoma  
- Bronchial adenoma  
- Pulmonary abscess  
- Pseudonodule (e.g., nipple shadow, superficial skin lesion)  
- Sarcoidosis

**The following increase the likelihood of a pulmonary malignancy:**

- Radiographic appearance of the lesion (size & lack of calcification)  
- Age  
- Sex (current or former women smokers are at higher risk)  
- Symptoms of cough & weight loss  
- Hypercalcaemia  
- Absence of residence in or travel to an area endemic for coccidioidomycosis (southwest USA) or histoplasmosis (Ohio/Mississippi Valley)  
- Absence of fever or evidence of infectious disease  
- Negative PPD skin test - does not rule out TB but makes less likely
What further testing might you order?
A. Search for previous chest radiographs for comparison
B. Low-dose, computerized tomography (LDCT) scan of the lungs
C. Lateral chest X-ray
D. Sputum studies for cytology and cultures (standard pathogens, fungus, acid-fast bacilli)
E. All of the above

NOTE
At this point, referral to a specialist such as a pulmonologist with expertise and clinical experience diagnosing, treating, and managing lung disease would be reasonable.

Additional testing and care based on the specialist’s assessment and recommended treatment plan may include further testing with additional referral (depending on the findings) to an oncologist, a chest surgeon, or both.

Initially, one or more of the following tests might be appropriate:
• Search for previous chest radiographs for comparison
• Sputum studies for cytology & cultures (standard pathogens, fungus, acid-fast bacilli)
• Low-dose lung CT scan
• Fiber optic bronchoscopy with bronchial brushings & specimens for cytology/culture.
• If primary lung cancer is detected metastatic work-up (scans of the brain, liver, adrenals, and bones) might be indicated. Again, this would be guided by specialist care and recommendations.

Which environmental causes have been associated with this patient’s probable disorder?
A. Daughter’s smoking.
B. Exposure to increased levels of radon gas
C. Persistent organic pollutants
D. Exposure to pesticides from the home’s foundation
E. Toxic products in the patient’s drinking water

Environmental causes of lung cancer may include:
• Arsenic see http://www.atsdr.cdc.gov/csem/arsenic/
• Asbestos
• Chloromethyl ethers
• Chromium
• Ionizing radiation (alpha, beta, gamma, or x-radiation)
• Nickel
• Polycyclic aromatic hydrocarbons
• Radon
• Tobacco smoke

NOTE
As previously mentioned, referral to & consultation with a specialist with expertise & experience diagnosing, treating, & managing lung disease should guide treatment options. Referral options might include recommendations for any additional referrals to an oncologist, a chest surgeon, or both. Depending on histological type, local extension into adjacent anatomical structures, presence of metastases, & the general health of the patient, treatment options might include surgical excision, radiation therapy, chemotherapy, & possibly immunotherapy. Again, specialist care and a recommended treatment plan should guide the choice of options.
Which route of exposure would most likely have been associated with this patient’s disorder?
A. Intravenous
B. Inhalation
C. Intraperitoneal
D. Intradermal
E. Oral
F. Dermal

RADON
Radon is a radioactive element. Two of its isotopes (radon-220 & radon-222) are progeny in two decay chains that begin with naturally occurring thorium and uranium, respectively, in rock, soil, water, and air.

Radon is a noble gas. It is colorless, odorless, tasteless, & imperceptible to the senses. Radon gas moves freely through the air, groundwater, & surface water. The main source of indoor radon gas infiltration is from soil into buildings. The growing popularity of CT scans and nuclear medicine in medical radiation have replaced radon as the primary source of ionizing radiation exposure.

TIP OF THE ICEBERG