PRIONS AS POTENTIAL ENVIRONMENTAL HAZARDS: ASSESSMENT AND MANAGEMENT OF RISK

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RISK ASSESSMENT: PROCEDURE

- **Problem formulation**
- **Hazard identification**
- **Hazard characterization**
- Exposure assessment

- Risk may be communicated at any phase

RISK ASSESSMENT

- **QUALITATIVE**
  - Implicit
  - Subjective
  - Limited data and resources
  - Narrow scope
- **QUANTITATIVE**
  - Explicit
  - Transparent
  - Large amounts of data and resources
  - Broad scope

Qualitative RA is an initial step toward quantitative RA.

OST focus: To monitor the state of prion science and to determine implications for EPA water programs.

PRIONS AS POTENTIAL HAZARDS

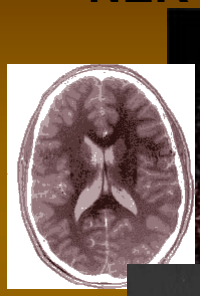
What are prions?[1]

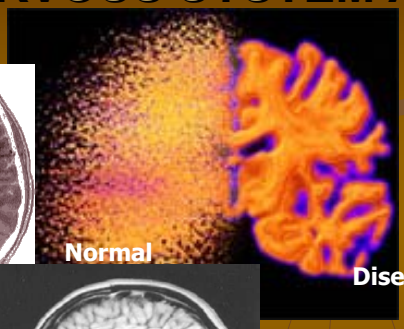
- Prions are…forms of a normal, chromosome-encoded glycoprotein [25-35 kilodaltons in size]
- Prions are…[associated with] transmissible spongiform encephalopathies characterized by fibril formation)
- Prions are cytoplasmic membrane components of certain cell types (e.g., neurones)[2]…
- Prion synthesis involves post-translational modification, [including] glycosylation…

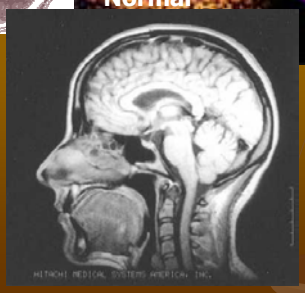
[1] *Dictionary of Microbiology and Molecular Biology*, 3rd ed., 2001

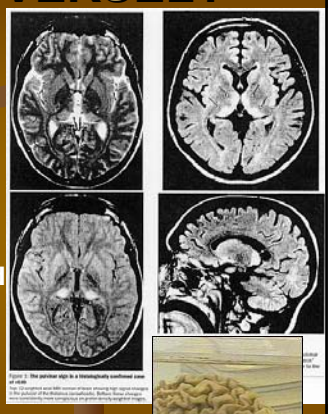
[2] Especially those of the central nervous system

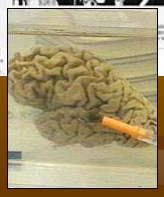
PRIONS AFFECT THE CENTRAL NERVOUS SYSTEM ADVERSELY











PRIONS AS POTENTIAL HAZARDS

Transmissible spongiform encephalopathies (TSE's) occur in many mammalian species; the diseases have distinct names

Examples:

- Sheep and goats (scrapie)
- Cattle (mad-cow disease, or BSE)
- Cervids -- deer, elk, moose, etc. (Chronic Wasting Disease, or CWD)
- **Humans** [variant Creutzfeld-Jakob disease, or vCJD; CJD; Fatal familial insomnia; Gerstmann-Straussler disease (GSD); kuru]

PRIONS AS POTENTIAL HAZARDS

- **Abnormal prion progeny can vary subtly**, and may represent a mixture of different strains with different tissue distributions
- These abnormal-prion **strains "compete"** within the host, at differing replication rates
- The normal ⇔ abnormal **conversion is reversible**; the forms are interconvertible
- Abnormal prion conformers are **more stable, thermodynamically**, than are normal prion forms and represent an equilibrium state.

PRIONS AS POTENTIAL HAZARDS

TSE's occur within several mammalian orders:

- *Artiodactyla* (goats, kudu, nyala, pigs, bison,…)
- *Carnivora* (housecats, ocelots, dogs (?), tigers, cheetahs,…)
- *Rodentia* (hamsters, mice, rats, ferrets, minks, otters,…)
- ***Primates*** (humans, orang-utans, gibbons, chimpanzees, lemurs,…)

Prion structures change as the agents pass between different species

PRIONS AS POTENTIAL HAZARDS
PROBLEM FORMULATION

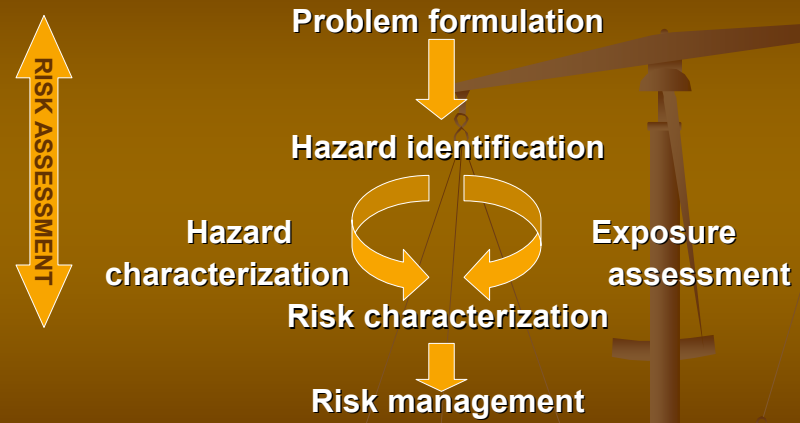
What risk is posed by the potential presence of prions in biosolids?

Biosolids are the solid component of treated sewage sludge

- 60% is used as agricultural fertilizer
- 27% is disposed to surface sites
- 13% is incinerated

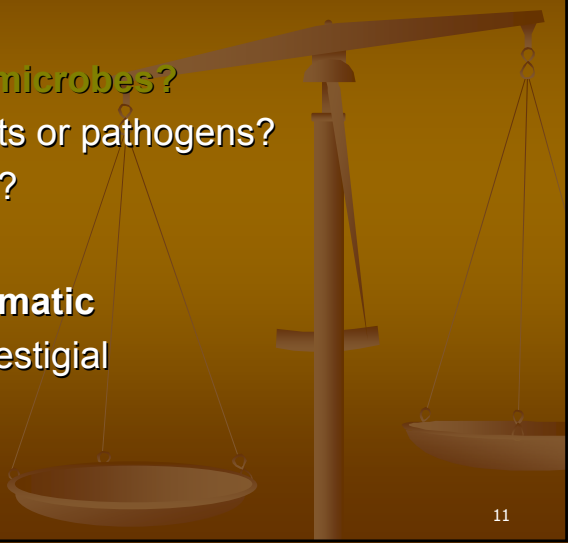
Human exposures to biosolids have occurred

PRIONS AS POTENTIAL HAZARDS
RISK ASSESSMENT PROCESS



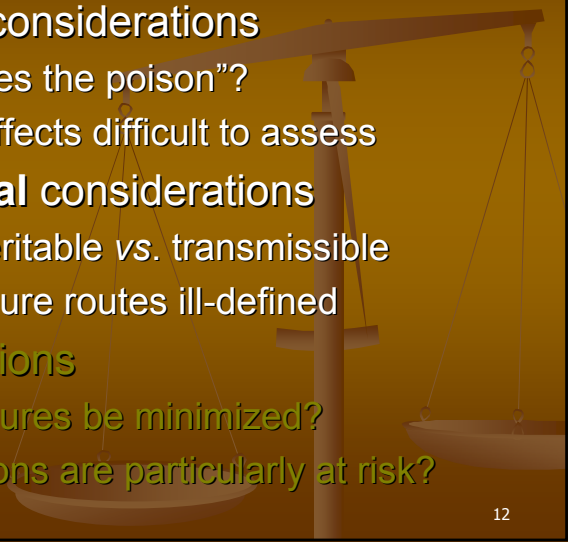
PRIONS AS POTENTIAL HAZARDS

- Characteristics
  - Chemicals or microbes?
  - Immunotoxicants or pathogens?
  - Overt or cryptic?
- Origins
  - **Genetic *vs*. somatic**
  - Emergent *vs*. vestigial



PRIONS AS POTENTIAL HAZARDS

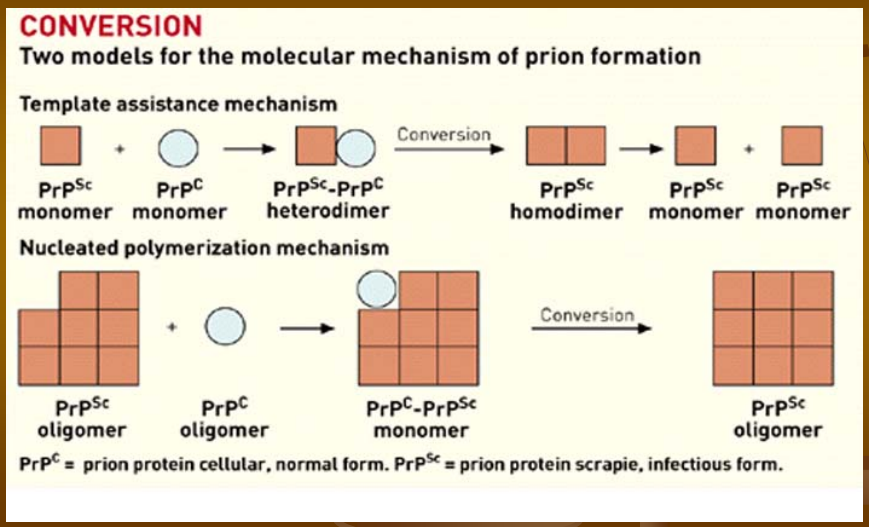
- **Toxicological** considerations
  - "The dose makes the poison"?
  - Physiological effects difficult to assess
- **Epidemiological** considerations
  - Sporadic *vs*. heritable *vs*. transmissible
  - "Natural" exposure routes ill-defined
- OST considerations
  - How can exposures be minimized?
  - Which populations are particularly at risk?



PRIONS AS POTENTIAL HAZARDS

- **Is dose related to response?**
- Is exposure route- or time-specific?
- Is disease progression related to host susceptibility?
  - Age
  - Life stage
  - Genetics
  - Immunocompetence

PRIONS AS POTENTIAL HAZARDS
DISEASE PROCESS

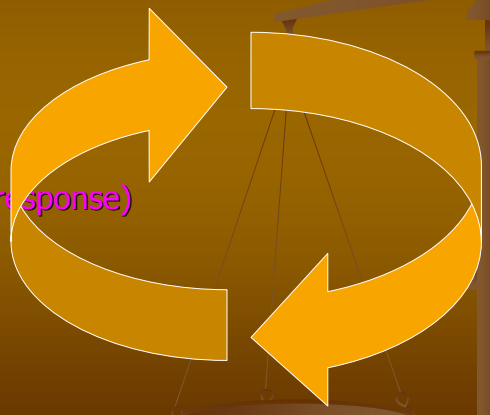


PRIONS AS POTENTIAL HAZARDS

DISEASE PROGRESSION REFLECTS SIX COMPETING ACTIVITIES

Host

Turnover
Clearing
(immune response)
Apoptosis



Prion

Recruitment
Conversion
Replication

PRIONS AS POTENTIAL HAZARDS

DISEASE PROGRESSION REFLECTS SIX SIMULTANEOUS ACTIVITIES

INCREASE

Recruitment
Conversion
Replication

Prion

(Abnormal prion concentration)

DECREASE

Turnover
Immunological clearing
Apoptosis

Host

PRIONS AS POTENTIAL HAZARDS
RISK ASSESSMENT DEVELOPMENT

- **Toxicant?**
  - Defined as material that causes harm to host
  - Exposure to agent markedly increases the likelihood of a unique and rare response
- **Pathogen?**
  - Defined as parasite that causes harm to host
  - Disease progression is related to the disease agent's multiplication within the host

The effect of a toxicant or pathogen is seen **at the level of the organ or system**, not at the level of the cell or tissue affected

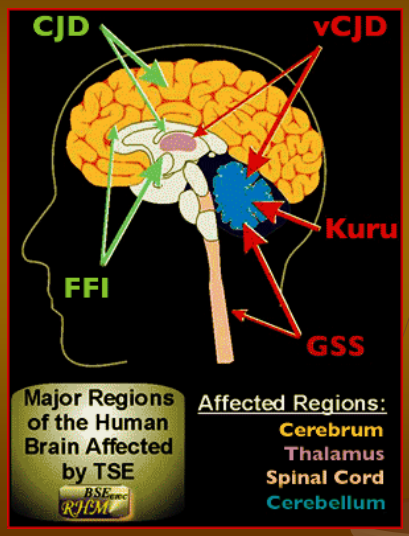
PRIONS AS POTENTIAL HAZARDS

PRION-DISEASE SYNDROME MANIFESTS AS SOME COMBINATION OF THE FOLLOWING

- Depression
- Cognitive deficit
- Muteness
- Insomnia
- Amnesia
- Unsteady gait
- Personality change
- Inability to move
- Myoclonus (jerking)
- Psychiatric problems

Different symptoms reflect prion proliferation in different areas of the brain.

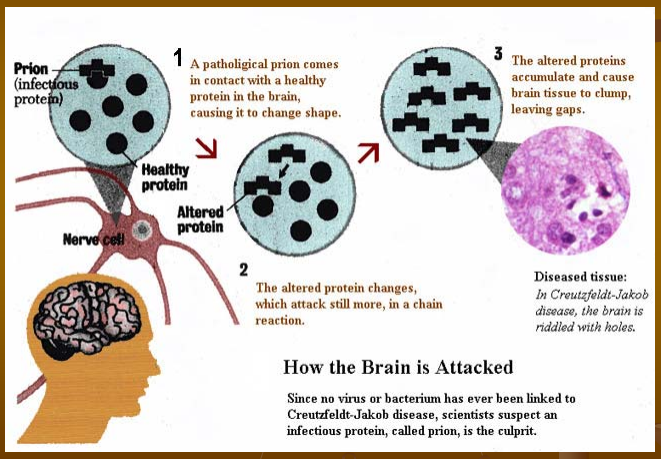
PRION TISSUE DISTRIBUTION IN HUMANS



Prions distribute to other tissues (e.g., skeletal muscle or rectal mucosa) in other mammals

PRIONS AS POTENTIAL HAZARDS

DISEASE MECHANISM



WHAT IS THE ROLE OF GENETICS IN PRION-ASSOCIATED DISEASE?

Susceptible populations have been identified

- All cases of vCJD so far have occurred in persons homozygous for the amino acid methionine (i.e., met:met) at prion codon 129
  - This genotype is shared by 40% of the US population
- Persons heterozygous at codon 129 (i.e., met:val) are thought to be asymptomatic carriers and/or to have subclinical disease
  - Methionine codon = ATG; Valine codon = GTG

WHAT IS THE ROLE OF BIOCHEMISTRY IN PRION-ASSOCIATED DISEASE?

- The valine ⇒ methionine substitution in humans introduces sulfur, a nucleophilic atom that alters prion behavior and tissue distribution
  - Normal homozygous (val:val) prion would be less polar than abnormal heterozygous (met:val)
  - Abnormal homozygous (met:met) would be most polar of all
- This change in prion conformation influences tissue distribution
  - Nonpolar (hydrophobic) associates with lipid membrane; polar (hydrophilic), with biological fluids

WHAT IS THE ROLE OF TOXICOLOGY IN PRION-ASSOCIATED DISEASE?

Prions are not typical toxicants

- A dose-response relationship may not exist
  - Biomarkers must be identified
  - Detection technologies must be developed
- But **mutagenic toxicants** may induce abnormal prion formation
  - Prion-associated disease is neoplastic; **carcinogenic toxicants** may enhance fibril formation
  - **Ionizing radiation** (cosmic rays and sunshine) may induce mutation of genes that code for normal prions

WHAT IS THE ROLE OF MICROBIOLOGY IN PRION-ASSOCIATED DISEASE?

Prions are not truly microorganisms

- They are acellular, and thus are not life forms in the usual sense
- However, viruses and viroids are considered to be microbes
  - Either agent can be described as a nucleic acid-based macromolecular assemblages
  - Prions are protein based macromolecules
  - These novel agents together challenge the definition of “life”

WHAT IS THE ROLE OF EPIDEMIOLOGY IN PRION-ASSOCIATED DISEASE?

EPIDEMIOLOGICAL PARADIGMS ARE USEFUL

- Prion diseases are **usually sporadic** (= rare)
  - One (1) person per 100,000 is "infected" at any time; one (1) per 10,000 is "infected" at time of death)[3]
- **But what causes prion-associated disease?**
  - **85-90% of cases are idiopathic** (= of unknown cause; mutation?)
  - **5-10% of cases are familial** (genetic cause, or peripartum)
  - The balance of cases occur via transmission
    - Acquired (ingestion; other routes?)

[3] World Health Organization

HOW IS PRION-ASSOCIATED DISEASE TRANSMITTED?

- **Congenital**
  - Not genetic; *in utero*, like certain viruses)
- **Acquired**
  - Iatrogenic (surgical or medical intervention)
  - Via vehicle (food/air/water)
    - Via fomites (handled objects; infected carcasses)
    - Via vectors (unlikely in the case of biosolids)

Prophylaxis?

- Prions not affected by sterilizing agents
- Prion decontaminants have been developed

HOW IS PRION-ASSOCIATED DISEASE TRANSMITTED?

- **Prion-associated diseases are zoönoses**
  - Humans "catch" these diseases by eating animal products
  - But even herbivorous mammals can be "infected"
- The **environmental reservoir** for abnormal prions is unknown
- **Methods for detecting and quantifying** prions are needed
- There is **no current treatment** for prion-associated diseases
- **Healthy immune functioning is critical**

PRIONS AS POTENTIAL HAZARDS
HOW CAN PRIONS BE INACTIVATED?

By **simultaneous treatment** with at least two of the following:

- Surfactant
- Hydrolysis (certain enzymes, or acid)
- Alkali
- Heat
- Oxidizing agent?

PRIONS AS POTENTIAL HAZARDS

RISK ASSESSMENT REQUIRES IMPROVED UNDERSTANDING

- **Hazard-target profile**
  - Host(s) and prion(s)
  - Effects
    - Chronic
    - Acute
    - Subclinical
- **Exposure profile**
  - Occurrence
  - Exposure method
    - Inherited
    - Sporadic
    - Acquired

PRIONS AS POTENTIAL HAZARDS CONCLUSIONS

RISK = [ADVERSE EFFECT * PROBABILITY OF EXPOSURE]

THIS NOVEL HAZARD REQUIRES A NOVEL RISK ASSESSMENT APPROACH ENCOMPASSING…

PRION
Plasticity
Biophysical chemistry
Non-organismal nature

HOST
Species "barrier"
Symptomology
Susceptibility
(genetic and developmental)

DISEASE PROCESS
Tissue invasion
Non-enzymatic kinetics
Apoptosis vs. killing

…THE EXPOSURE ASSESSMENT COMES LATER