

BIOLOGICAL RAW MATERIAL CONTAMINANTS

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It's Not Just Heavy Metals and Solvents

- **In the 1980's companies trying to go to defined media for cell culture found pyrogens in final products.**
 - **Source was amino acids that were “wet.”**
- **Rabies was transmitted through a cornea transplant.**
- **CJD transmitted through growth hormone prepared from human pituitary glands, also via dura matter transplants.**

Materials on which “clearance studies” have been performed.

- **Monoclonal antibodies; Recombinant proteins; Tissue and Cell derived products; Human, Equine, Ovine and Bovine blood products; Human and Animal urine-derived products; Enzymes.**
- **Medical Devices (collagen derived products, tissue implants, coated-products).**

Transmissible Spongiform Encephalopathies (TSE, BSE)

- **First appeared as a possible contaminant in bovine serum for cell culture, then BSA, then blood from donors in England or who had lived in England.**
- **Eventually implicated all ovine and bovine source material, then material from any animal susceptible to TSE.**
- **Lots of weird ideas and claims in the media.**
 - **Mad squirrel disease? Could not destroy by ashing in a muffle furnace.**

EMA Note for Guidance (EMA/410/01 – Rev.4) (Has force of law in EU)

- **Applied to human and veterinary products sourced from animals susceptible to TSE.**
 - **Uses concept of risk minimisation via risk analysis.**
 - **There are food guidances. Nutraceuticals are not addressed directly, but substances such as chondroitin sulfate should be affected.**
 - **Methods used to kill animals are important.**
 - » **Non-penetrative stunning methods are preferred.**
 - » **Blood contamination with brain material has been demonstrated with penetrative stunning methods.**

EMEA Uses Geographic Sourcing

- **Based on World Organisation for Animal Health (OIE) classifications.**
 - **Category A: Countries or Regions with negligible risk:**
 - » Australia, Argentina, Finland, Iceland, New Zealand, Norway, Paraguay, Singapore, Sweden, & Uruguay.
 - **Category B: Countries or Regions with a controlled risk:**
 - » Canada, EU countries including UK, Switzerland, USA.
 - **Category C: Countries or Regions with an undetermined risk.**
 - » R.O.W.

EMEA Sourcing

- **Whenever possible animals should be sourced from category A countries.**
- **Collagen, gelatin and blood derivatives can be sourced from category B countries.**
 - **Provided that required controls (animal origins, prevention of cross contamination) are applied.**
- **Animals must not be sourced from category C countries.**
- *There is a good Western Blot assay for prions.*

EMEA Risk Analysis Is Based On:

- **Source of animals and geographic origin.**
 - **Bovine blood should be from category A countries.**
Category B acceptable with controls.
- **Nature of tissue source and procedures to prevent cross contamination from higher risk material.**
- **Production process, including a QA/QC system in place to ensure product consistency and traceability.**

Major Problem: Viruses and Mycoplasmas

- **They can be present in raw material.**
- **Operating personnel can contribute to the problem.**
 - **Watch out for University sourced material.**
- **Tissue and Cell sources can be a problem.**
 - **Endogenous viruses. Case of Marburg virus.**
- **Extremely low levels of contaminants can become a problem when you grow them up in your production system.**

Biological contaminants Have Been found in Manufacturing Material

- **Antibodies coupled to chromatography media.**
- **Cell culture media contained agents that infected clean cell lines.**
- **Enzymes used in manufacturing processes, such as porcine trypsin, have been the sources of problems.**
- **Amino acids in culture media have contained endotoxins, bacteria and mycoplasmas.**

Manufacturing Processes May Not Help

- **Hepatitis A and human parvovirus B19 are known to be particularly resistant to inactivation by conventional manufacturing processes.**
- **Non-enveloped viruses are resistant to solvent-detergent treatments.**
- **Problem with biologics is that processes that might kill biological agents may also kill the activity of the product.**

Commonly Used Cell Lines

- **CHO (hamster) – recombinant proteins.**
- **NS0 (mouse myeloma) – monoclonals.**
- **Mouse hybridoma – monoclonal antibodies.**
- **Vero (monkey) – viral vaccines.**
- **MRC-5 (human) – viral vaccines.**
- **293 (human) – adenoviral vectors for gene ther.**
- **PER.C6 (human) – gene therapy vectors.**
- **MDCK (canine) – viral vaccines (e.g. influenza)**
- **Various insect lines – recombinant proteins.**

Known Contaminants. I.

- **Human blood and blood products:**
 - **Hepatitis A, B, C, D, E and more; Human Immunodeficiency Virus (HIV), Human T-cell Lymphotropic Virus I & II (HTLV I & II in cellular portions); Human parvovirus B19.**
 - **In transplant patients: Adenoviruses and all herpes viruses are of concern.**
 - » **Human Herpes Viruses 6 – 8 (HHV 6, HHV 7 & HHV 8), Epstein-Barr Virus, and Cytomegalovirus (CMV).**
 - » **Transplant patients and others are often immunosuppressed and are susceptible to novel agents not previously known to cause disease..**

Known Contaminants. II.

- **Animal Cell Cultures have been known to be contaminated with:**
 - **Rodent cell lines harbor endogenous retroviruses.**
 - **Bovine serum used in cultures may contain bovine viral diarrhea (BVD) virus or bovine polyoma virus.**
 - **Porcine products such as trypsin used for cell culture may contain porcine parvovirus (PPV) and porcine circovirus.**
 - **Reovirus type 3 is a potential contaminant of hybridomas and recombinant cell lines.**

Known Contaminants. III.

Early viruses contaminants.

- **SV40 in poliovirus and adenovirus vaccines.**
- **Avian leukosis virus and hepatitis B in yellow fever vaccine.**
- **nvCJD (new variant) actually believed to be human form of BSE transmitted by poorly cooked beef.**
- **Murine minute virus (MMV) a mouse parvovirus from media components has been known to infect CHO cells.**

More Recent Known Events. I.

- **Cache Valley Virus (from bovine serum) infected CHO cells.**
- **Reo Virus (from bovine serum) infected BK/CHO cells.**
- **Non-cytopathic BVDV (from bovine serum) has infected several cell types.**
- **Nodavirus found in insect cell cultures.**
 - **Kills mice.**
- **Calicivirus 2117 found in CHO cells.**

More Recent Known Events. II.

- **Epizootic Haemorrhagic Disease Virus (normally found in deer) found in CHO cells.**
- **Human blood with SARS and West Nile Virus.**
- **Hepatitis E (from porcine source) outbreak in Italy.**
- **Human parvovirus 4 found in human plasma.**
- **Arenavirus infections in transplant patients.**
- **Human bocavirus and polyoma virus strains KI & WU found as respiratory pathogens.**
- **Rodent cells contain endogenous retrovirus particles (types A and C)**

More Recent Known Events. III.

- **Xenotropic murine retrovirus found in human prostates. Possibly associated with cancer.**
- **Ebola-Reston found in pig herds.**
- **Mycoplasmas found in several media additives including plant-derived material.**
 - **Spiroplasmas have been found in plant-derived material used for serum-free media.**
- *And then there is the issue of host cell DNA.*

Detection

- **Methods run anywhere from PCR to animal infectivity tests.**
- **PCR testing suffers from lack of valid primers and specificity problems.**
- **Animal and tissue culture testing suffers from problems with specificity.**
- **Most testing requires a combination of methods and the use of model organisms to validate the performance of the tests.**

Wonder where the problems come from????

- **... employees were observed wearing gloves and performing various unclean tasks, such as handling garbage, machinery, street clothing and touching their faces/noses, without cleaning hands or changing their gloves, between operations.**
- **On 2/19/09, none of the employees washed or sanitized hands after morning break.**
- **On 2/20/09, seven (7) out of nine (9) employees did not wash or sanitize their hands after the morning break.**

AND...

- **Six (6) rodent excreta pellets (REPs) and a dead roach observed along the north wall of the small room just outside of the walk-in cooler behind pieces of equipment, boxes and cleaning supplies;**
- **At least twenty-eight (28) REPs, live spider with webbing, live roach and a dead roach observed on and around a pallet stored with packaged jelly and hot sauce and within close proximity to a bag of dry roasted peanuts along the east wall approximately fifteen (15) feet north of the back screened door in the processing area.**

They're watching CSI.

- **In the same location as the forty-four (44) REPs, fluorescing stains, characteristic of rodent urine, were observed under a black light along the exposed cardboard boxes.**

Warning Letter

- **Sunshine Peanut Company**
- **7405 Temple Terrace Hwy**
 - **Suite A**
- **Tampa, Florida 33511**