Cells and tissues - containment

- Cell line x from cell bank – no info or BSL2. What CL do I use?

- When can I move my human derived cells from CL2 to CL1?

Explore wider issues around tissue handling and cell culture

- Tissues, tissue and cell culture. Other biomaterials – excretions, secretions, extracts/lysates, fractions, proteins, nucleic acids etc. may need consideration

- Risks to human health – COSHH. Other compliances such as HTA, security, polio, SAPO, IAPO, PHO, (GM Regs) etc. may apply

What does COSHH say?

Duty to protect persons against risks to health arising from exposure to substances hazardous to human health – through risk assessment and control measures

‘Substance’ – various definitions covering mainly

Chemicals – toxic, harmful, corrosive, irritant etc

Biological agents – infection, toxicity, allergy

- Biological agent – any micro organism, cell culture or human endoparasite which may cause infection, allergy, toxicity or otherwise create a hazard to human health

- Cell culture – in vitro growth of cells derived from multicellular organisms

- Based on infectious risk, ACDP categorises these into hazard groups 1-4

- General duty to protect workers from infections at (to do with) work

Farmers, healthcare, post mortem, sewage, refuse etc.

- Laboratories (plus animal rooms and industrial processes) – Sch3 (ACOP) of COSHH stipulates control measures - Containment Level tables (correspond to HG of agent)

- Applies to biological agents or material that may contain them – deliberate or incidental work

- Cells themselves appear generally to present low infectious risk so HG1/CL1 (but may present risks in other ways)
- Cannot assume all are equally safe, consider tissue type (neurological?) and oncogenic status (aggressive tumour cell?)

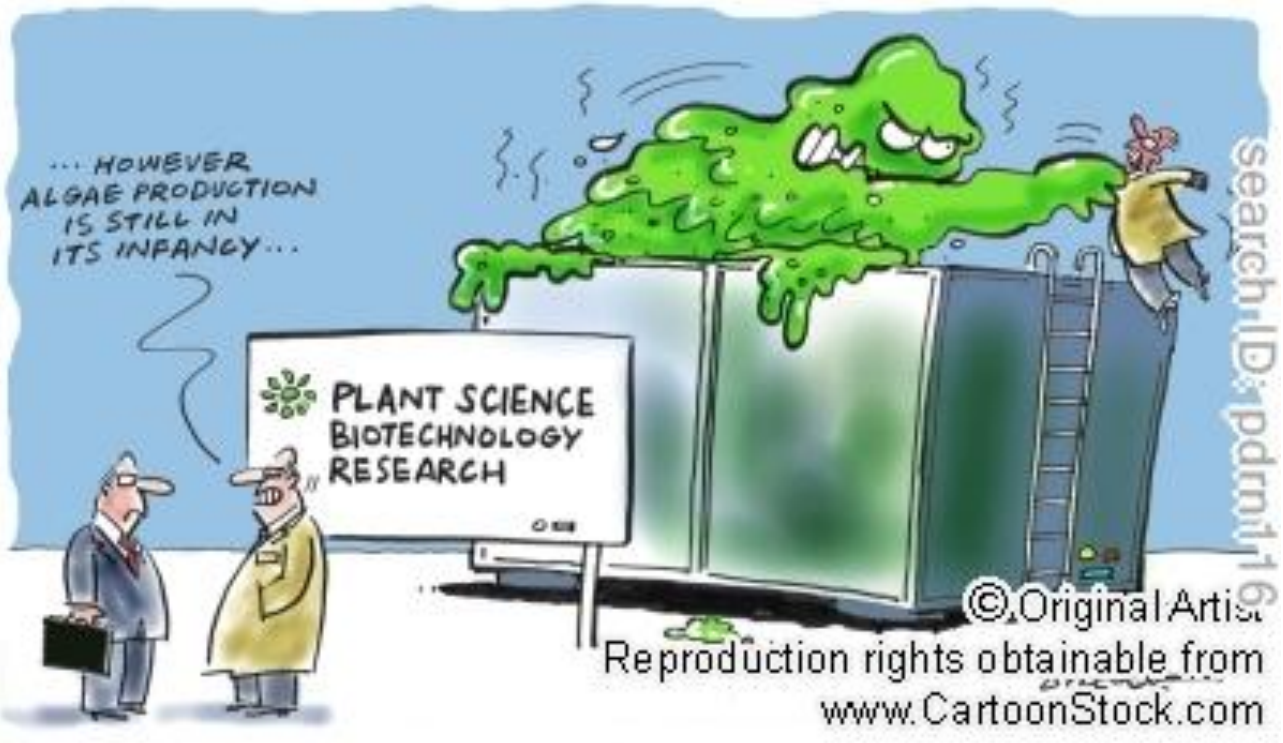
- So largely concerned with presence of adventitious agents capable of infecting humans

HSE guidance on cell cultures

Hazard	Cell type	Baseline containment level
Low	Well characterised or authenticated finite or continuous cell lines of human or primate origin with a low risk of endogenous infection with a biological agent presenting no apparent harm to laboratory workers and which have been tested for the most serious pathogens.	CL1
Medium	Finite or continuous cell lines/strains of human or primate origin not fully characterised or authenticated, except where there is a high risk of endogenous biological agents, eg blood-borne viruses.	CL2
High	Cell lines with endogenous biological agents or cells that have been deliberately infected.	Containment appropriate to the agent
	Primary cells from blood or lymphoid cells of human or simian origin.	Containment appropriate to the potential risk

- Likelihood/suspicion
- Characterisation

Plants/Algae & Fungi
Cells & Tissues

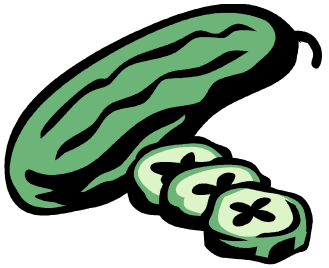


Plants/Algae/Fungi

Typically low risk to human health, but…

- **Allergens?**
  - Pollen
    - 1st reported case occupational asthma from Arabidopsis thaliana 2008
  - Fungal spores
  - Peanuts, latex & cross reactive proteins
- **Toxic?**
  - Irritant hairs/sap etc?
- **What controls?** … gmp? LEV? PPE?

Plants/Algae/Fungi



- **Contamination**

E.coli O157:H7 - Mung bean sprouts

Salmonella spp - Peanut seedlings, tomatoes, melons, peppers etc

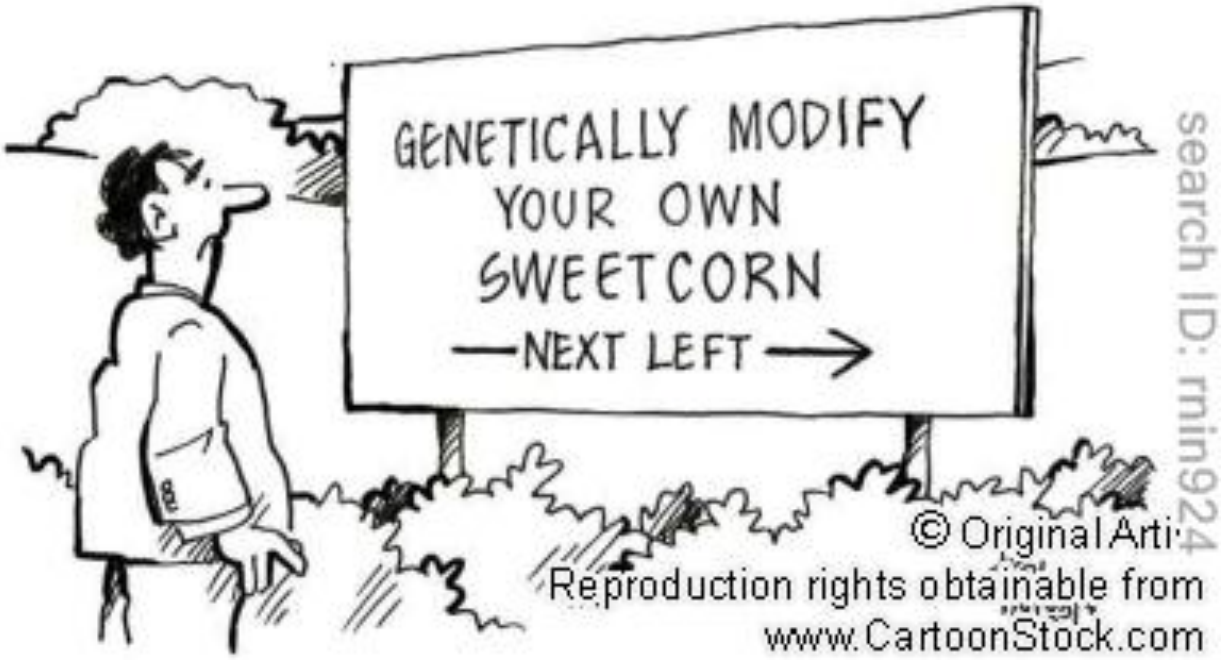
"The pathogens were in every major tissue, including the tissue that transports nutrients in plants" *Purdue University, 2011*

E. coli 104:H4 - Fenugreek sprouts: Germany 2011 46 Dead, 3,900 sick

- **Soil/Manure/Water** – Others - Tetanus, Polio etc?

Plants/Algae/Fungi

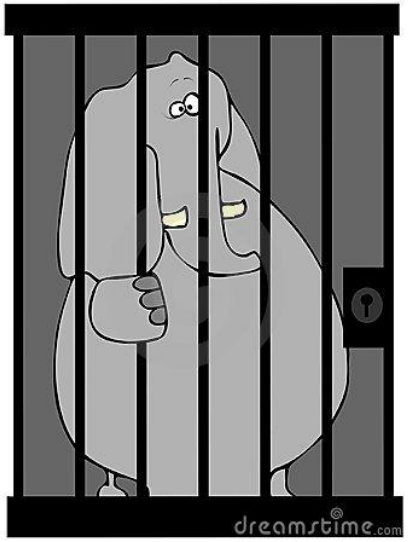
- **Environmental Risks?** (GM & Non-Indigenous sp)



Animal Cells & Tissues

Animal Cells/Tissues

- **Diverse use**
  - Research/Clinical/Diagnostic/Teaching
- **Research animals e.g.**
  - Mammals – Rats, Mice, Rabbits, Guineas Pigs, NHPs etc
  - Insects: Cockroaches, Locusts, Drosophila, Mosquitoes, Bees
  - Birds
  - Fish, Reptiles & Amphibians
  - Snails and many others
- **Wild animals** – e.g. Elephant tissues, bat blood
- **Farm animals** – Sheep, Cows, Goats Horses etc
- **Domestic pets** - Cats, Dogs, Horses, Ferrets, exotics etc



Animal Cells/Tissues

What are the key risks?

- **Infection**
  - Bacteria/Viruses/Parasites/Fungi
  - Not just humans! (SAPO)
- **Allergy**

What should we consider when assessing animal tissues?...

Animals

- **Origin of animal/tissue?**
  - UK Labs, Native, Exotic etc (overseas fieldwork?)
- **Health Status**
  - Known/suspected/unknown?
  - Research animals: SPF? Deliberately infected?
  - Zoonoses – rabies, avian influenza, anthrax, lepto., toxo..
  - Specified Animal Pathogens (SAPO)?
  - Vaccinated? (worker too?)
  - Screened/Tested?

Animals

- **Sources of Infection?**
  - Direct skin contact
  - Blood/Body Fluids/Body Parts
  - Excreta: Faeces/Urine/Vomit
  - Respiratory secretions / excretions

Animals

ALLERGY

- **Sources of Animal Allergens**
  - Secretions/excretions: Saliva, urine, faeces
  - Hair/fur/feathers
  - Dander
  - Insect frass, scales, hairs, chitin
  - (Food & Bedding too)

Animals

- **Exposure** to pathogens and allergens - How?
  - Inhalation
  - Mucus membranes
  - Needlestick/Sharps
  - Bites (Animals & Vectors)
- **Vectors** (direct / indirect) e.g.?
  - Fleas: Tapeworms, Cat-Scratch Fever, Typhus, Plague
  - Ticks - Lyme disease
  - Mosquitoes: Dissection of infected insect tissue
  - Snails: Schistosomiasis

Humans

Humans

- Wild population sources only
- Tissue type – associated pathogens
- All primary material generally CL2
- Higher/lower risk sources – geographical, lifestyle, age, clinical presentation, donors
- Types of handling risk – e.g. aerosol, sharps
- Known vs suspected

- Human material into animals – CL?

Tissue Handling/Processing

Tissue Handling/Processing

Examples of typical tissue handling/processing steps?
Can these affect containment?

- Sample collection - biopsy/swabs/needles etc
- Washing
- Snap freezing
- Dissecting
- Homogenisation/Blending
- Enzymatic treatments
- Sectioning
- Culturing ‘lumps’
- Passaging
- Sorting (Cytometry)
- Chemical treatments - chaotropic agents

Tissue Handling/Processing

- **Exposure to aerosols may include…**
  - Opening tubes
  - Pipetting
  - Centrifugation
  - Homogenisation
  - Pouring
  - Shaking
  - Mixing
  - Dropping tubes/flasks
  - Sorting

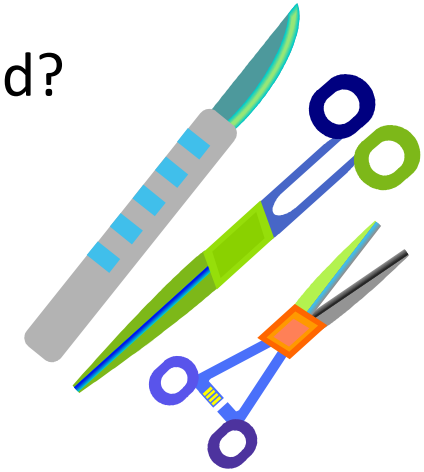
Tissue Handling/Processing

- **Sharps**

Cells on coverslips lifted with needles/sharps

Ouch!

- What did you know about the cells beforehand?
- Why worry about it AFTER a needlestick?



Tissue Handling/Processing

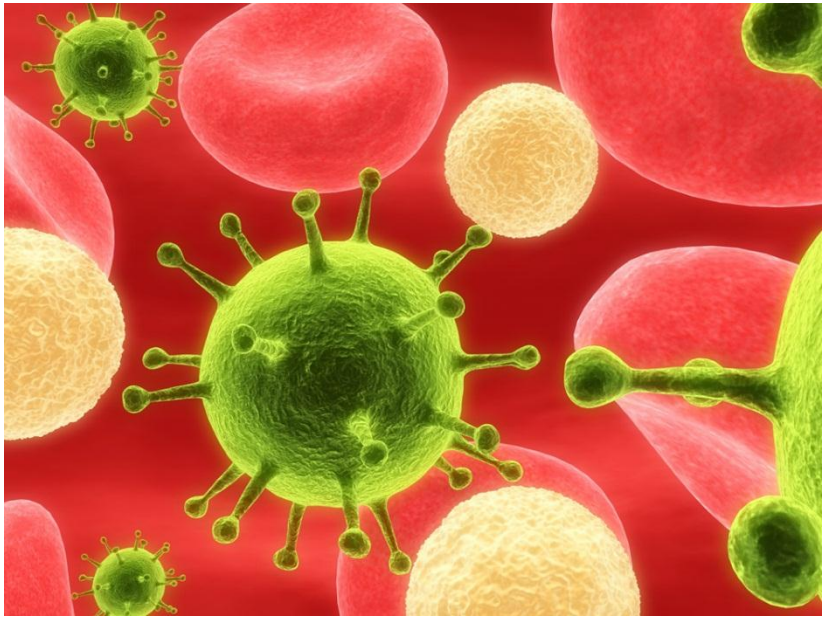
What do we need to consider?

- **Good micro practice** + appropriate containment
- Immunocompromised workers?
- **Engineered controls** for aerosols & allergens?
  - LEV (HEPA) MSC?
  - Downflow tables?
  - Lids
- **PPE**
  - e.g. allergen control - FFP2/3

Tissue Handling/Processing

- **Time in culture**
  - 100hr rule?
  - Multi-passage
- **Cross contamination**
  - **LN2 Storage:** Liquid/Gas phase?
- **Fury animal cadavers**
  - Damp down

Testing/Screening



Testing/screening

- What for? By who?
- How, how far?
- Residual risk – theoretical?
- Practicality
- Ethics

- Transplant material
- GMP manufacture

What’s the Answer?

What's the answer?

- Theoretical risk? = unlikely to cause harm (HG1)?
- Why move? Work to the higher CL if not sure?
- More CL2 space?
- Mixed working – dedicated MSCs?
- Policy/Guidance? Default CL? Lab design?
- HSE? (SRF?)