# Take-home messages/reminders for the next ICAW

**Standards**

- Negative and positive standards are useful, and may even be essential, especially in biomonitoring.
- They allow identification of anomalous experiments (quality control).
- They also allow normalisation of results.
- Reference standard cells; prepare a batch, and freeze as aliquots.
- Internal reference standards would be very useful – based on cells with different genome size.

**Controls**

What is the correct term for the population group that is used to compare with the exposed or diseased group?

‘Control group’ rather than ‘negative control’ – or should we avoid the word ‘control’ altogether?

**Appropriate samples for human biomonitoring:**

- Lymphocytes from fresh blood are most commonly used, but there are alternatives:
  - Whole blood
  - White cells from frozen whole blood
  - Buccal epithelial cells
  - Cells from saliva
  - Cells from tears
  - Biopsies

- Different cell types should be compared; if there are poor or no correlations, what should we do?

**The ComNet project**

Aim is to collect as many human biomonitoring comet assay results as possible, to carry out a pooled analysis and answer basic questions such as effect of smoking, age, sex, occupational exposure, nutrition etc.
Secondary aim is to validate and publish standard protocols

- >100 participants; tens of thousands of individual results promised
- Waiting for outcome of application for COST project

## Automating scoring

High throughput assays are very useful, but lead to a scoring bottleneck.

- automated scoring should be available at a reasonable cost

## Alternative test systems

- In vitro skin model – very promising results so far
- In vitro comet assay – hoping for OECD approval and guidelines
- FPG and aphidicolin could be incorporated in a general in vitro protocol to increase sensitivity/specificity

## Measuring cytotoxicity

- Trypan blue and other ‘leakage’ assays
- Metabolic assays
- Relative growth activity (proliferation)
- Plating efficiency (colony forming ability)

Should we stick to the rule of testing for genotoxicity at non-cytotoxic concentrations?

## Calibration

- X-irradiation is the best agent to use
- Ring studies have shown general agreement

- ➢ It is difficult to be confident about the estimated initial damage level, because repair can occur very quickly
- ➢ It is important to minimise opportunity for cells to repair damage, by irradiating cells after embedding and getting to lysis as soon as possible.

**Aphidicolin:**

- Useful in the in vitro repair assay to suppress non-specific nuclease activity
- Because aphidicolin blocks repair synthesis, it causes cells to accumulate incomplete repair sites as DNA breaks, and this will increase sensitivity of the comet assay in genotoxicity testing
- Pulsing with aphidicolin during an incubation of cells undergoing NER allows monitoring of the kinetics of repair

## Electrophoresis

- Listen to Gunnar!
- Voltage gradient is critical, current is not
- Recirculating of electrophoresis solution reduces experimental variability

**Hedgehogs, ghosts and clouds**

- How to recognise them
- How to deal with them
- How to explain them

## Gender balance

- In this workshop, there were equal numbers of male and female speakers.