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.