Challenges in the validation of emerging tools for monitoring the chemical quality of environmental waters

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BACKGROUND TO MONITORING

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Sampling and Chemical Analysis

- Measurement of any chemical in the environment is costly in time, transport, consumables, disposal, equipment costs
- Need to be sure what we need to measure and why.
- Then use method that gives us a measure that is reliable and of a quality that we can use, gives us the information that we need, and at as low a cost as possible

Why Measure Contaminants in Water?

- Comply with letter of law (compare levels of specific contaminants with Environmental Quality Standards) – annual average and maximum acceptable EQS
- Compliance testing
- Make risk assessments
- Improve quality of water (to enable us take remedial actions), and follow trends in quality
- Forensic applications (find source of pollution)

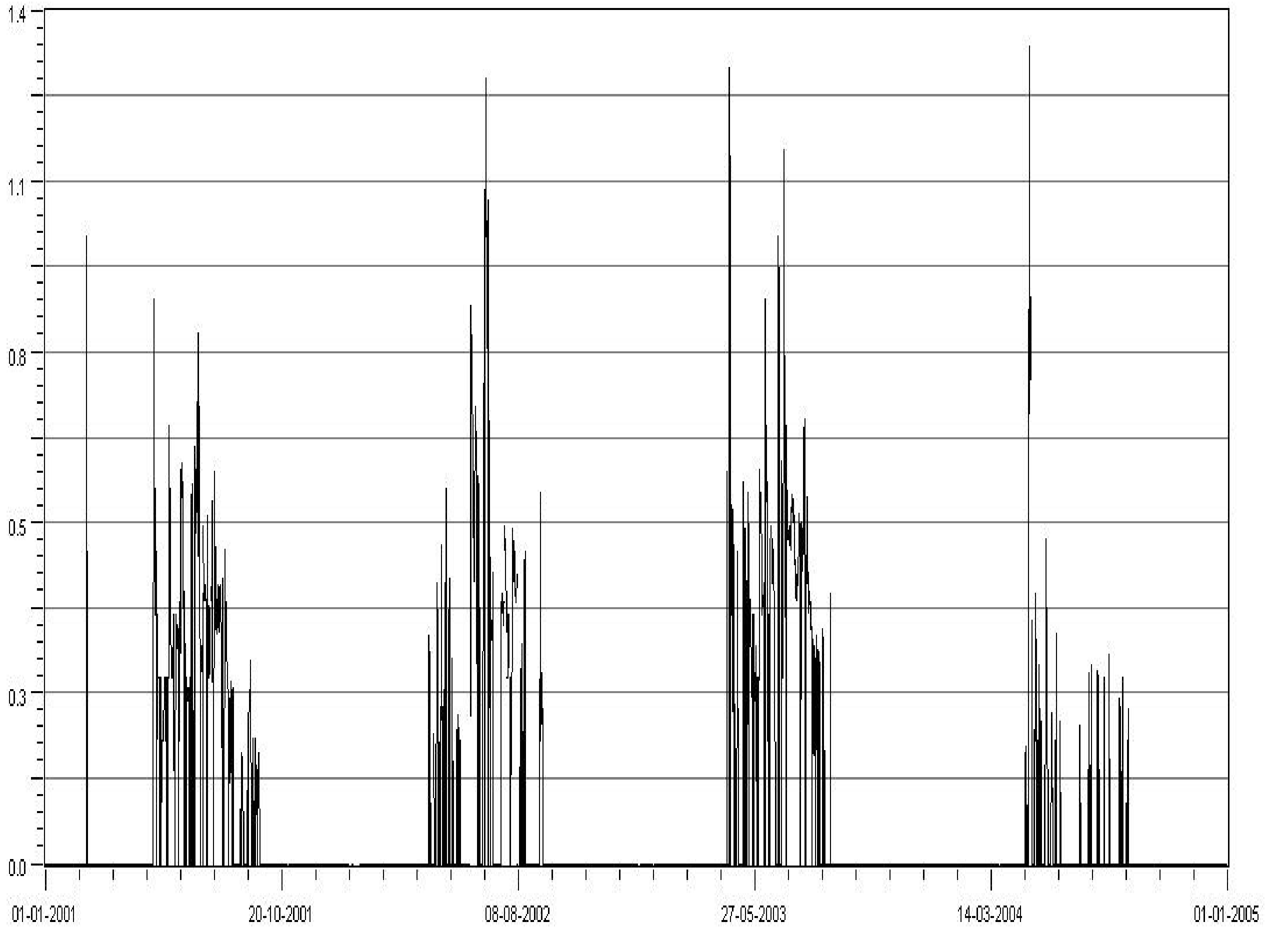
What Do We Need from Monitoring?

- Overall representative, reliable measure of chemical quality based on pollutants of concern [on the basis of potential harm to the health of humans and the environment]

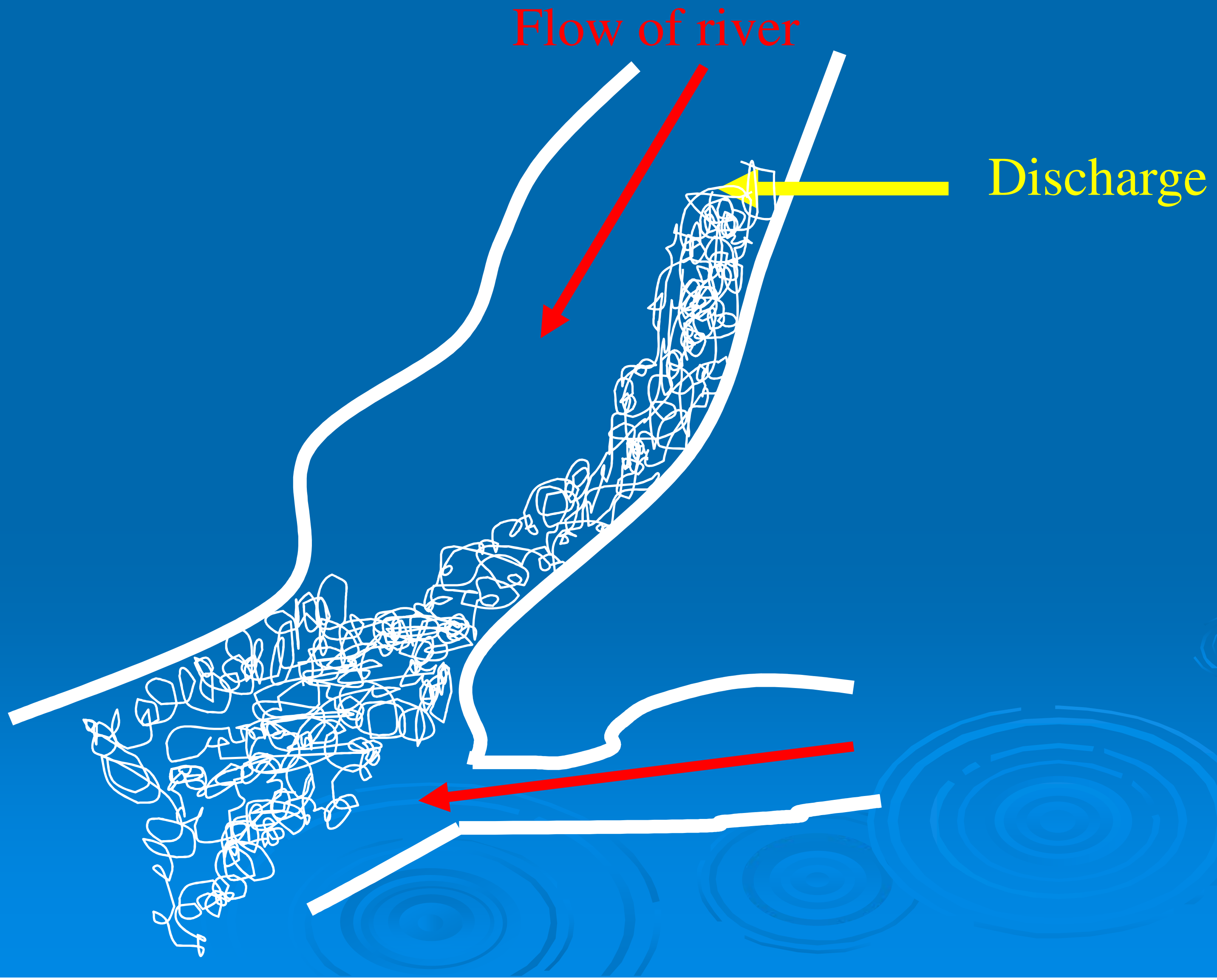
Monitoring Tool Box

- No one method or technology will deliver all the information needed to support current legislation
- A tool box is needed from which those involved in monitoring can select the most appropriate and cost effective methods for a particular requirement
- All of the tools should be reliable, of established quality, and should have associated validated standard operating procedures
- The tools should operate at low cost

Diuron concentration (µg L^{-1}) continuously monitored in the Meuse River - RIZA's Eijsden station (2001-2005).



Spatial variation



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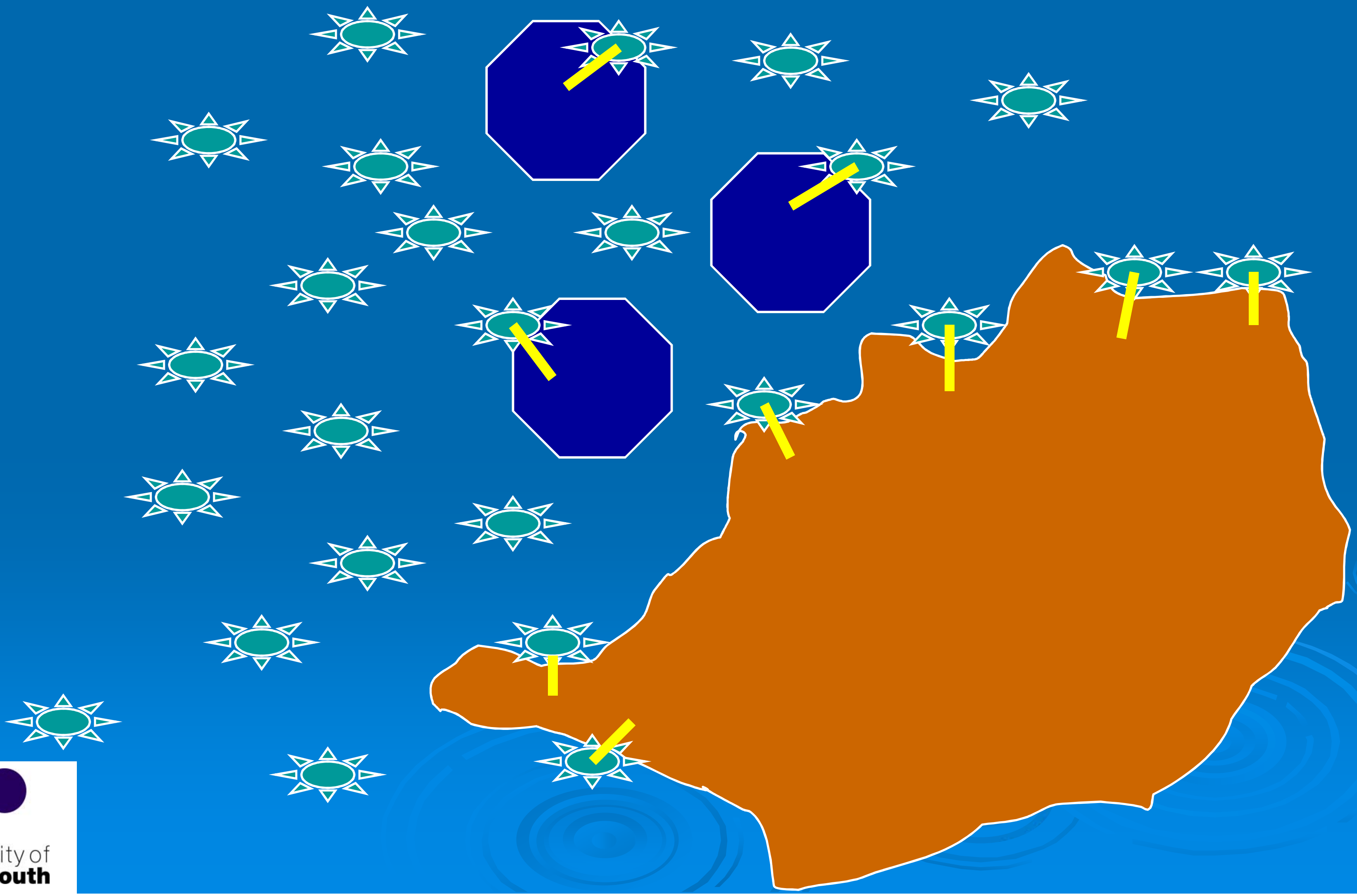
Variation in Water Quality

- Levels of major components
- Hardness
- pH
- Suspended matter
- Dissolved organic material

Distribution of Pollutants in Water Sample



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Properties of DOM

- Properties of dissolved organic matter (DOM) vary depending on source and age of the material
  - Mature DOM (e.g. from peat deposits) has different properties from new DOM (e.g. from urban waste water discharges)
  - The former has a greater mitigating effect on bioavailability than the latter (Tusseau-Vuillemin et al., 2007)

What is Being Measured?

- For some metals, and for organic compounds that are very non-polar then measuring the concentration in filtered samples will quantify the freely dissolved fraction as well as that bound to DOM
  - Methods depending on spot sampling (e.g. some hand-held sensors, spectroscopic methods, test kits and immunoassays) will be affected by these factors
  - Passive samplers will sample only the freely dissolved fraction, and that bound to DOM that dissociates in the time scale of diffusion across the diffusion limiting layer

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Operationally Defined Results

- In many cases the various methods of sampling, transport, storage, and sample preparation and analysis will give different estimates of concentrations of a chemical in the same sample
- The outcome of the analysis is operationally defined
- This is why standard operating procedures are important

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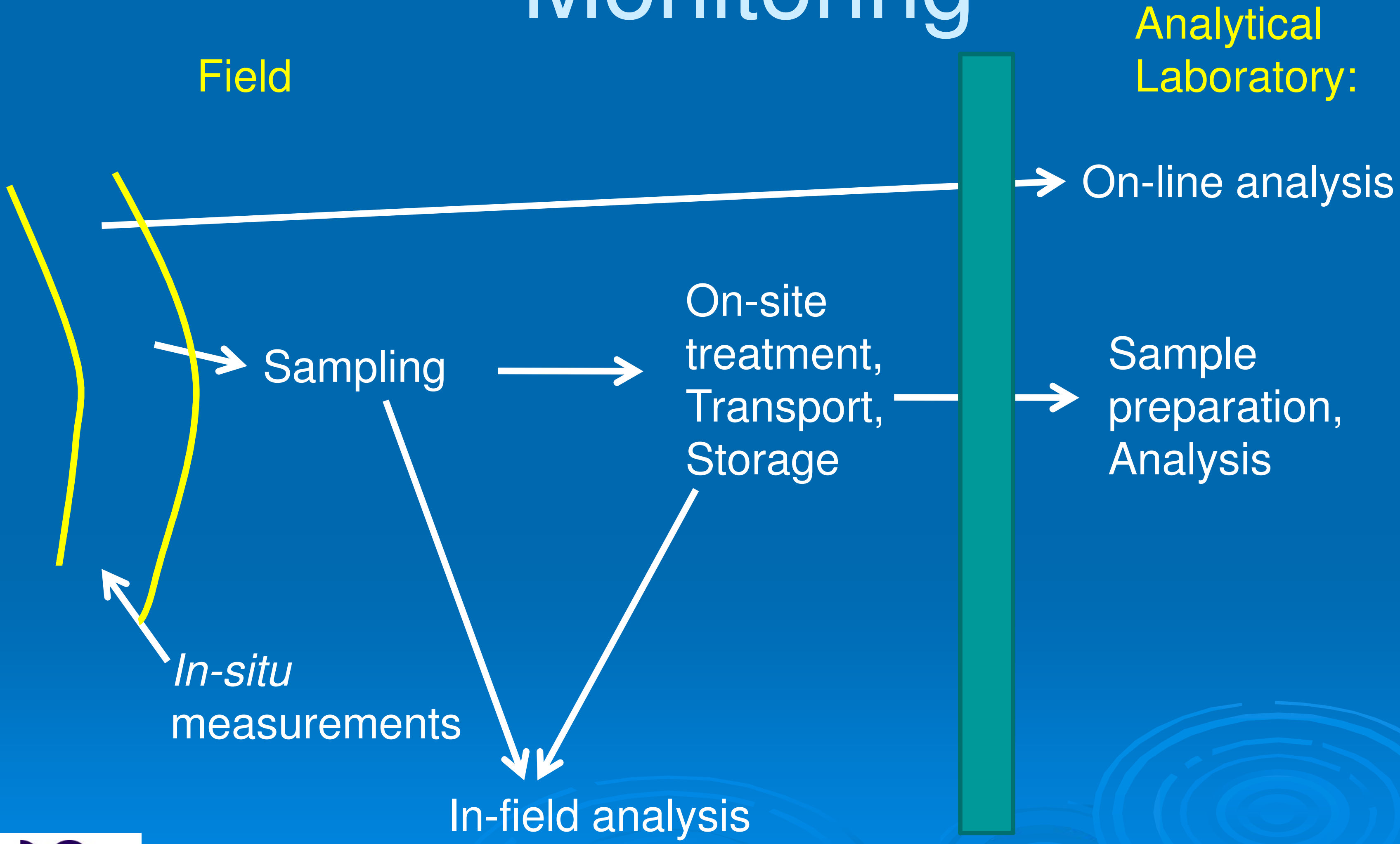
Processes in Environmental Monitoring



Field

Analytical Laboratory:

On-line analysis

Sampling

On-site treatment, Transport, Storage

Sample preparation, Analysis

In-situ measurements

In-field analysis

Available Monitoring Methods

- Equipment for measuring physical and physicochemical variables
  - dissolved organic carbon (DOC)
  - pH
  - temperature
  - turbidity
- Biological assessment techniques
  - biomarkers
  - bioassays
  - biosensors
  - biological early warning systems (BEWS)
- Chemical analytical methods
  - Sensors
  - Test kits
  - Passive sampling devices
  - Immunoassays
  - Spectroscopic methods

Tools for Chemical Monitoring

- Sampling
    - Spot sampling
    - Composite sampling (time or flow weighted)
    - On-line sampling
    - Passive sampling
- Analysis
    - Chromatography linked to appropriate detector
    - Immunoassays
    - Spectroscopic methods
    - Sensors
    - Test kits

Developing On-line and Laboratory Based Methods

- Screen-printed, disposable biosensors – enzymatic systems to detect and identify specific pollutants
- Automated optical immunosensors with multi-analyte determination of selected compounds, depending on monitoring situation (Automated Water Analyser Computer Supported System (AWACSS); RIANA; FIAA)

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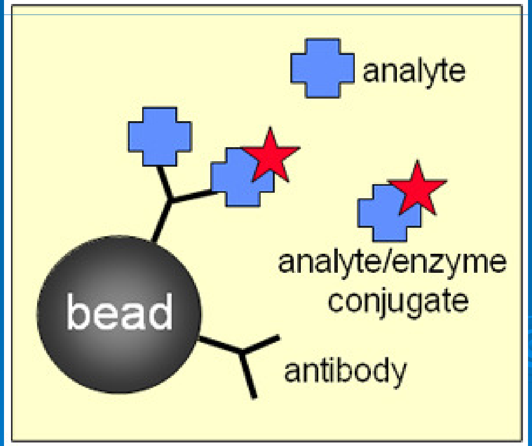
Immunochemical Methods

- Use antibodies as selective recognition element
- Used to analyse spot samples
- Sensitive
- Specific
- Available for a wide range of organic analytes
- Can be automated
- On-line and/on site applications



Immunochemical Methods

- For parallel measurement of many samples use ELISA (microtitre plate, tubes, magnetic beads)
- Immunosensor for on-site measurements
- Automated control at monitoring station use flow injection immunoaffinity analysis
- For selective sample preparation use affinity columns

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Test Kits

- A commercially packaged system of the principal components of an analytical method
- Used to determine specific analytes in defined matrices
- Include directions for use
- May be self contained or require additional supplies and equipment
- Key components are often proprietary elements or reagents that can be prepared readily by only producer of kit
- Some adapted for use in field
- Use spot samples

Hand Held Sensors

- A range of hand held sensors with screen printed electrodes is now available for
  - heavy metals
  - polyaromatic hydrocarbons
  - some pesticides
- Some used *in situ*, but some depend on spot sampling with associated errors
- For some difficult to standardise operational conditions (e.g. speed of stirring) during *in situ* use

ERRORS AND VALIDATION

Error and Uncertainty

- Statistical error is the difference between an estimate and some true value of the measured variable (measurand)
- Uncertainty in this context characterises the dispersion of values that could reasonably be associated with the measurand

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Error

Random error
- This can be measured readily by using statistical methods
- In method validation this is assessed in precision experiments

Systematic error (bias)
- This is more difficult to detect unless care is taken to ensure sound experimental design
- It is important to check for sources of bias in any validation procedure

Processes Contributing to Overall Error in Assessment of Endpoint

- Sampling
- Preparation for field sampling
- Transport to site
- Deployment
- Retrieval
- Transport from site
- Preparation for analysis
- Analysis/test/assay

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Sampling and Analytical Methods

- Difficulty in validating many methods is in part due to variability in properties of environment and matrix effects that affect each compound to different extents
  - Suspended matter
  - Dissolved organic matter
  - Micro-organisms present
  - pH
  - Hardness
- Even harder for field based methods where operating conditions (e.g. temperature) can vary widely

Analytical methods

- Laboratory based methods used under well controlled conditions are relatively easy to validate and can use reference materials and inter-laboratory trials
- Some methods are more affected by matrix effects than others
- Field based methods (including *in situ* methods) can be validated in laboratory but need to be validated in range of conditions found in field

Bottle Sampling

- Changes occur
  - as soon as sample taken and there can be further changes during transport and storage (e.g. by binding to the bottle walls)
  - during preparation (e.g. by filtration) for analysis
- These cause systematic errors (bias) rather than random errors, and these errors can be large compared with those associated with the analytical stages

On-Line Methods

- Changes can occur in samples between the intake from the bulk water of the environment, and the sample arriving at the detection system [e.g. spectrophotometer, chromatograph linked to mass spectrometer]
- Usually sedimentation and filtration processes are used
- These introduce systematic error (bias)

Passive Sampling

- Affected by temperature turbulence and biofouling
- Calibrated in laboratory
- Calibrations for some compounds (e.g. very non-polar organic compounds) affected by matrix effects that can change during a calibration run
- Can use performance reference compounds where kinetics of off-loading and uptake are isotropic
- PRCs can also be used with solid phase micr-extraction techniques

Spot sampling and classical analysis as benchmark?

- Difficult to compare passive sampling with spot sampling since can measure different fractions, and measures time weighted average concentration not instantaneous concentration – would need frequent spot sampling
- On-line sampling processes (pumping, transport through tubes, settlement to remove suspended solids, and filtration) can lead to the fraction being measured being different from that measured in spot samples or passive samplers

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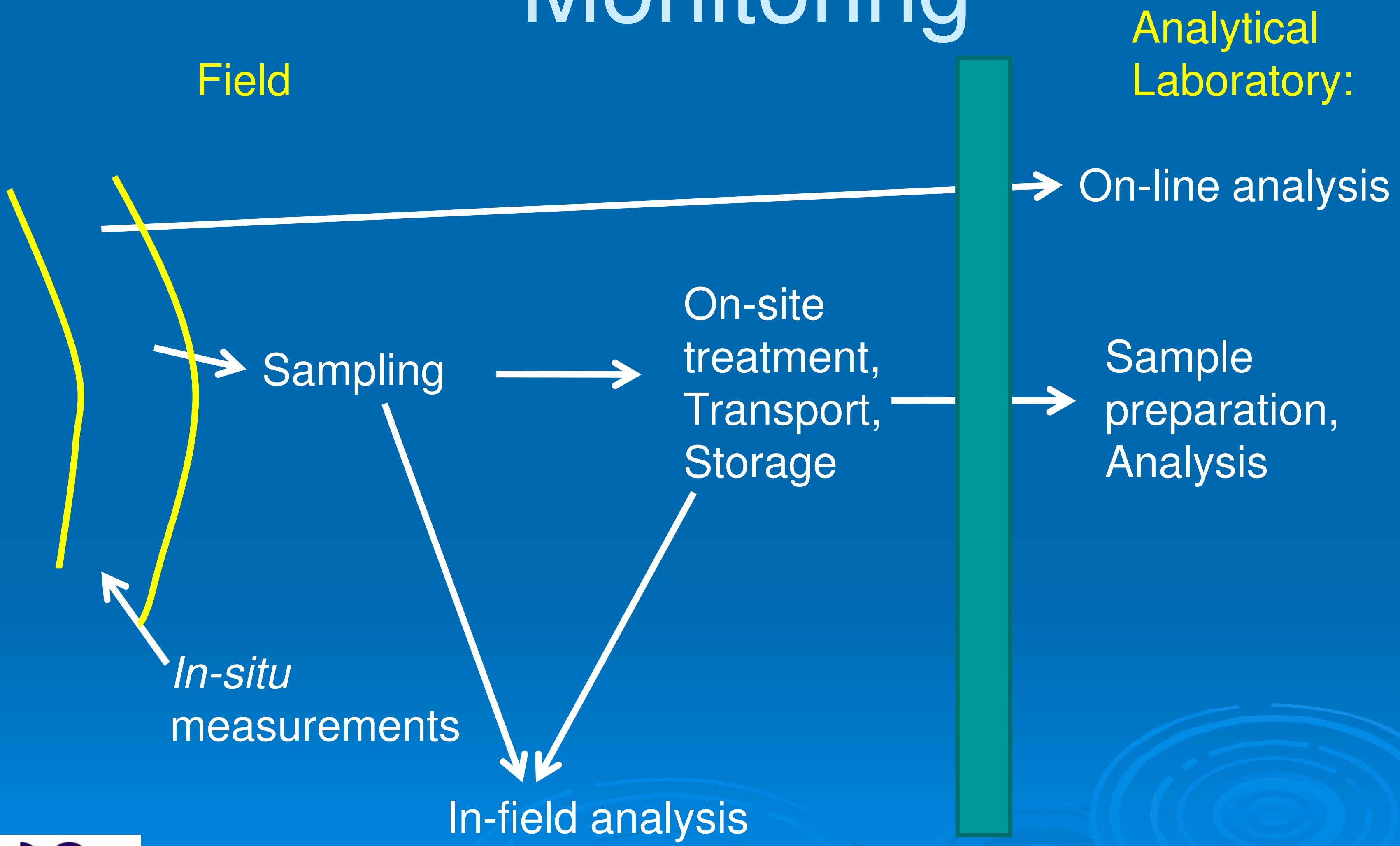
Location of Measurements

- The site at which measurements or qualitative assessments are made has an important effect on the number and type of sources of variation that can contribute to the overall error
- *in-situ* or next to the sampling site
- laboratory based
  - on-line
  - bottle samples transported to the laboratory

Processes in Environmental Monitoring



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In Situ Methods

- Methods that can be used *in situ* (e.g., in-river) decrease errors due to sampling, and sample handling.
- It is difficult to validate them in the laboratory since it is not straightforward to produce consistent, reliable simulations of environmental conditions
- Necessary to use wide range of conditions

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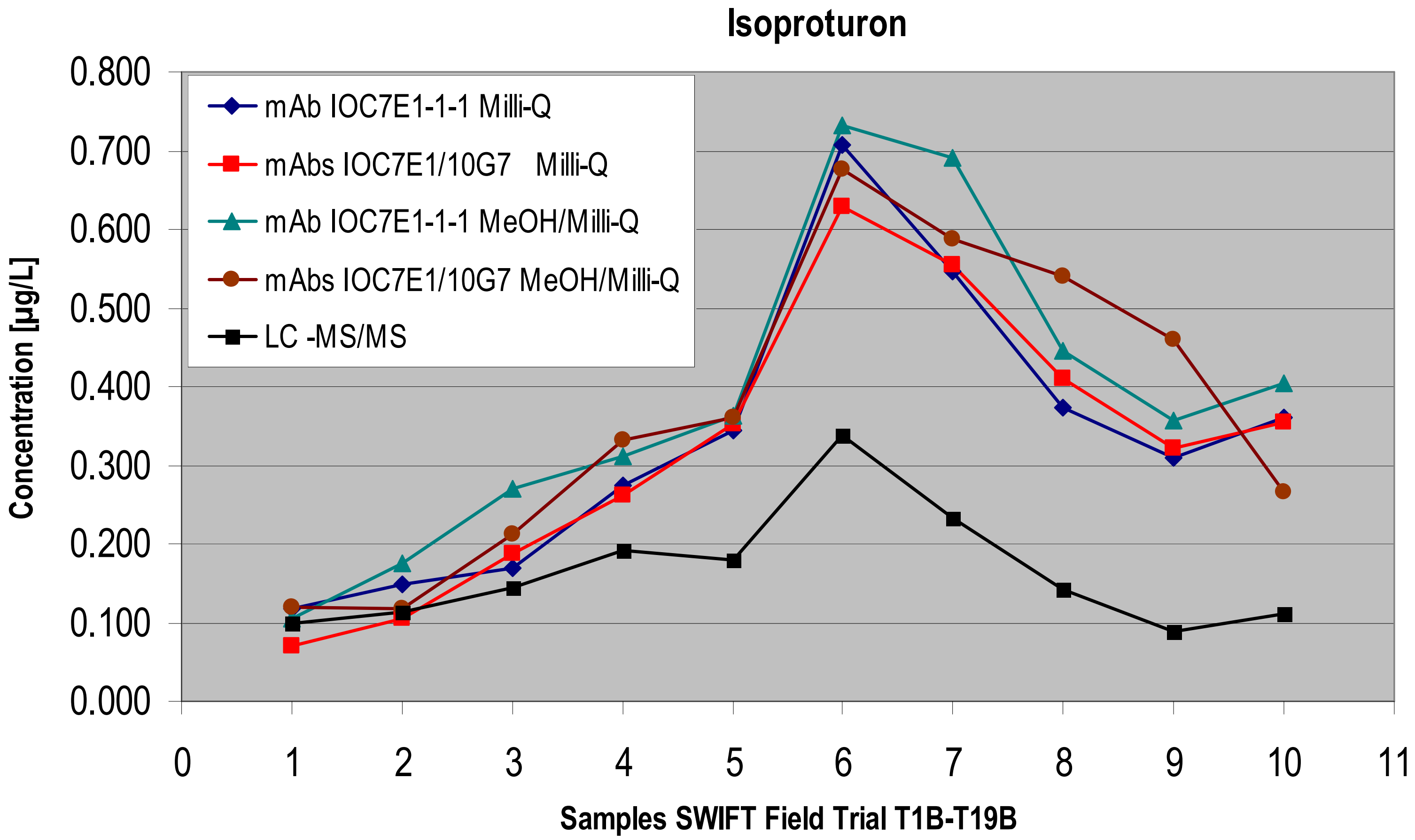
Errors Associated with Emerging Alternative Analytical Methods

- Some have specific sources of error associated with them
  - In immunoassays there can be
    - variability between batches of antibodies
    - in some cases cross reaction with related (interfering) compounds
  - With test kits similar problems may be encountered, and where test kits are used in the field it is not possible to control conditions (e.g. temperature) as tightly as in the laboratory

Matrix Effects

- Affect most analytical methods
- Should be thoroughly evaluated during validation
- Can lead to large errors if not taken into account

Tank Experiments – Analysis of Isoproturon



Part of the data in: P.M. Krämer et al., Anal. Bioanal. Chem. (2006)

Challenges to Validation

- Wide range of
  - environmental conditions affecting sampling [and analysis where effected *in situ* or in-field]
  - matrix composition, and potential effects
- Lack of inexpensive, appropriate reference materials [large volumes needed for instance in passive sampling]
- Need for long term validation trials for some technologies
- High cost of validation

Validation

- The fact that it is difficult to validate any method should not deter us
- It is, however, important to acknowledge the difficulties, and not to depend on reassurance from a statistical analysis on its own
- We need to understand the nature of the measurement we obtain, and it biological significance

Possible Ways Forward

- Reproduction of range of controlled, representative environmental conditions
  - Development of novel reference materials that can be produced in large volumes and in a range of matrix compositions [e.g. dispersible tablet formulations, and standard humic mixtures]
  - In-field tank studies
  - Use of controlled reference sites

Conclusions

- If decisions are to be based on chemical data it is essential that sources of error are controlled, and that associated uncertainties are characterised

Conclusions

Metrology

- Without clear rules for demonstrating the accuracy (hence the comparability) of data, the system cannot deliver a sound basis for proper decisions

(Professor Philippe Quevauviller)

Conclusions

- It may be possible to use a method with a large uncertainty associated with the results, providing that the uncertainty is defined and that the measurement is fit for the purpose for which it is used

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