• Introduction

• Stability Tests of Stock Solutions - Experiments by EURL-SRM

• Template for Submission of Compound Stability Data to EURL DataPool

• Testing the Stability of Pesticides in Stock Solutions by Quantitative NMR

• Summary
The stability of analytical pesticides-standards is a very “old issue”, but pesticide residue analysts have always to deal with it!

Reason:

One major source of errors → degradation of standards in

- stock solutions,
- working solutions (e.g. mixtures),
- matrix extracts.
• Start of project: 2008
• 137 compounds tested so far
• Procedure:

**Preparation of stock solutions (1 mg/ml)**

**Storage**

**Preparation of working solution (10 µg/ml; mixture)**

**Preparation of injection-solution (0.1 µg/ml)**

**Measurement by GC-MS/MS / LC-MS/MS**

**Data Evaluation and Storage**
Preparation of stock solutions (1 mg/ml)

- Concept for stability project
- **One person responsible** for project

- **New certified standards** were purchased for the **entire period of stability project**
  => same batch was used for stability experiments of compound

- Weight: 25 + X mg of pure standard for almost all compounds;
  expensive compounds: not less than 10 mg
- Corrected for purity of standard
Preparation of stock solutions (1 mg/ml)

- Dissolved in 25 + X ml acetonitrile / acetonitrile (+0.4 Vol% acetic acid) / other solvent (e.g. carbendazim in ACN:DMF = 1:1)

- Base-labile pesticides:
  50 + X mg standard + 50 + X ml acetonitrile: 25 ml were acidified with acetic acid

- 1.5 ml vessels: glass, brown transparent; plastic snap cap with seal; cap inner material: TEF

- Check for precipitation during storage: **one white glass** vessel was filled with solution
Storage

• Temperature: 4°C (refrigerator) / -20°C

• Period: 6 months, 1, 2 and 4 or 5 years

=> 9 vessels for each pesticide

Check for Solvent Loss

• Possible loss of solvent during the storage period was monitored for each vessel by weighing.

• The weight deviation must not be more than 1%. Otherwise vessel was discarded or result corrected for solvent loss.
Preparation of Working-Solution (10 µg/ml)

- As mixture

- Dilution to 10 µg/ml acetonitrile / acetonitrile (+0.4 Vol% acetic acid) / other solvent

Preparation of NEW Solutions

- NEW stock solution: 10 + X mg of pure standard of the same batch + solvent

- NEW working solution: mixture

Preparation of Injection-solutions

- Dilute working-standard solution to 0,1 µg/ml
Measurement by GC-MS/MS / LC-MS/MS

- Sequence (alternate injections):
  \( (\text{stored-sln (4°C)} - \text{stored-sln (-20°C)} - \text{reference-sln}) \times 5 \)

- 5 times injection of stored and new solutions
- ISTD: Chlorpyrifos-D10
Data Evaluation

• The response of the stored solution was compared with the response of the new solution of the same standard batch
  
  • Quotient X
    
    Quotient X = \frac{\text{Response of standard solution}}{\text{Response of ISTD}}
    
  • Average of quotients for stored and reference solutions
  
  • Difference of stored versus new solution
    
    \text{Difference in } \% = \left( \frac{\bar{X} \text{ of stored solution} \times 100}{\bar{X} \text{ of new solution}} \right) - 100\%

Data Storage

• Compound Stability database at www.eurl-pesticides-test.eu
• Template for Submission of Compound Stability Data
Welcome to our Tutorial Page!

Whether you are a new visitor or a registered user of our EURL DataPool-website, our Tutorial is here to help you get the most out of our database services.

<table>
<thead>
<tr>
<th>Category</th>
<th>Format</th>
<th>Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>Getting Started</td>
<td>Video</td>
<td>How to customize and filter the tables</td>
</tr>
<tr>
<td>Stability of Compounds</td>
<td>zip-file</td>
<td>Template for Data Submission: Submission of Compound Stability Data</td>
</tr>
<tr>
<td>Method Validation Data</td>
<td>Website</td>
<td>Template for Data Submission: Submission of Method Validation Results</td>
</tr>
</tbody>
</table>

Download the Template for Submission of Compound Stability Data
The preparation date of the stock solution is needed to calculate the storage duration.

<table>
<thead>
<tr>
<th>Compound Name</th>
<th>Preparation Date of Stored Solution</th>
<th>Pesticide concentration in solution during storage (μg/mL)</th>
<th>DETAILS on pesticide (e.g., employed as salt...)</th>
<th>Pesticide stored individually or in mixture? (if in mixture, please provide an ID for pesticides in same snl)</th>
<th>Name of company where certified standard was purchased</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbofuran</td>
<td>01.07.2009</td>
<td>1000</td>
<td></td>
<td></td>
<td>Dr. Ehrenstorfer</td>
</tr>
<tr>
<td>2,4,5-T-Methylster</td>
<td>02.07.2009</td>
<td>10</td>
<td></td>
<td></td>
<td>Dr. Ehrenstorfer</td>
</tr>
<tr>
<td>Acrinathrin (sum isomers)</td>
<td>01.07.2012</td>
<td>10</td>
<td></td>
<td></td>
<td>Bristol-Myers-Squibb</td>
</tr>
<tr>
<td>Captan</td>
<td>01.07.2012</td>
<td>10</td>
<td></td>
<td></td>
<td>Dr. Ehrenstorfer</td>
</tr>
<tr>
<td>Chlorothalonil</td>
<td>01.07.2012</td>
<td>10</td>
<td></td>
<td></td>
<td>Dr. Ehrenstorfer</td>
</tr>
<tr>
<td>Dioxin</td>
<td>01.07.2012</td>
<td>10</td>
<td></td>
<td></td>
<td>Medco</td>
</tr>
<tr>
<td>Tetramethrin</td>
<td>01.07.2012</td>
<td>10</td>
<td></td>
<td></td>
<td>Dr. Ehrenstorfer</td>
</tr>
<tr>
<td>Trifluoracetyl</td>
<td>01.07.2012</td>
<td>10</td>
<td></td>
<td></td>
<td>Fluka</td>
</tr>
<tr>
<td>Captan</td>
<td>01.07.2012</td>
<td>10</td>
<td></td>
<td></td>
<td>Fidia Haen</td>
</tr>
<tr>
<td>Chlorothalonil</td>
<td>01.07.2012</td>
<td>10</td>
<td></td>
<td></td>
<td>Mitsu Chemicals, Inc.</td>
</tr>
<tr>
<td>Fiprosid</td>
<td>01.07.2012</td>
<td>10</td>
<td></td>
<td></td>
<td>Mitsu Chemicals, Inc.</td>
</tr>
<tr>
<td>Tetramethrin</td>
<td>01.07.2012</td>
<td>10</td>
<td></td>
<td></td>
<td>Mitsu Chemicals, Inc.</td>
</tr>
<tr>
<td>Trifluoracetyl</td>
<td>02.07.2012</td>
<td>1000,0</td>
<td>as Ca-salt</td>
<td></td>
<td>Sigma-Aldrich</td>
</tr>
<tr>
<td>Prohexadione</td>
<td>02.07.2012</td>
<td>1000,0</td>
<td>as Ca-salt</td>
<td></td>
<td>Sigma-Aldrich</td>
</tr>
</tbody>
</table>

Either choose a compound from the drop downlist or enter the compound name into this column.

Enter a unique identifier for all those compounds that were stored in the same solution (→ mixture).
This section describes the solvent that was used to store the compound.

If a matrix extract was used for the storage experiment, enter the matrix and the extraction as well as the cleanup procedure that were used to prepare the extract.
Details on the storage conditions (e.g. storage temperature) are used to compare the stability results.

<table>
<thead>
<tr>
<th>Compound Name</th>
<th>Preparation Date of Stored Solution</th>
<th>Storage Temperature in °C</th>
<th>Stored in Darkness?</th>
<th>Vessel Material</th>
<th>Vessel-Cap Type</th>
<th>Vessel Cap Inner Surface Material (surface in contact w. solution)</th>
<th>Vessel Optical Properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbazin</td>
<td>11.09.2010</td>
<td>-20</td>
<td>Yes</td>
<td>Glass</td>
<td>Metal crimp cap w. seal/septum</td>
<td>PTFE (Teflon)</td>
<td>Brown transparent</td>
</tr>
<tr>
<td>2,4,5-T-Methylster</td>
<td>15.05.2010</td>
<td>-20</td>
<td>Yes</td>
<td>Glass</td>
<td>Metal crimp cap w. seal/septum</td>
<td>PTFE (Teflon)</td>
<td>Brown transparent</td>
</tr>
<tr>
<td>Acrinathrin (sum isomers)</td>
<td>01.07.2012</td>
<td>4</td>
<td>Yes</td>
<td>Glass</td>
<td>Metal crimp cap w. seal/septum</td>
<td>PTFE (Teflon)</td>
<td>Brown transparent</td>
</tr>
<tr>
<td>Captan</td>
<td>01.07.2012</td>
<td>4</td>
<td>Yes</td>
<td>Glass</td>
<td>Metal crimp cap w. seal/septum</td>
<td>PTFE (Teflon)</td>
<td>Brown transparent</td>
</tr>
<tr>
<td>Chlorothalonil</td>
<td>01.07.2012</td>
<td>4</td>
<td>Yes</td>
<td>Glass</td>
<td>Metal crimp cap w. seal/septum</td>
<td>PTFE (Teflon)</td>
<td>Brown transparent</td>
</tr>
<tr>
<td>Folpet</td>
<td>01.07.2012</td>
<td>4</td>
<td>Yes</td>
<td>Glass</td>
<td>Metal crimp cap w. seal/septum</td>
<td>PTFE (Teflon)</td>
<td>Brown transparent</td>
</tr>
<tr>
<td>Tetramethrin</td>
<td>01.07.2012</td>
<td>4</td>
<td>Yes</td>
<td>Glass</td>
<td>Metal crimp cap w. seal/septum</td>
<td>PTFE (Teflon)</td>
<td>Brown transparent</td>
</tr>
<tr>
<td>Trinexapac-ethyl</td>
<td>01.07.2012</td>
<td>4</td>
<td>Yes</td>
<td>Glass</td>
<td>Metal crimp cap w. seal/septum</td>
<td>PTFE (Teflon)</td>
<td>Brown transparent</td>
</tr>
<tr>
<td>Captan</td>
<td>01.07.2012</td>
<td>4</td>
<td>Yes</td>
<td>Glass</td>
<td>Metal crimp cap w. seal/septum</td>
<td>PTFE (Teflon)</td>
<td>Brown transparent</td>
</tr>
<tr>
<td>Chlorothalonil</td>
<td>01.07.2012</td>
<td>4</td>
<td>Yes</td>
<td>Glass</td>
<td>Metal crimp cap w. seal/septum</td>
<td>PTFE (Teflon)</td>
<td>Brown transparent</td>
</tr>
<tr>
<td>Folpet</td>
<td>01.07.2012</td>
<td>4</td>
<td>Yes</td>
<td>Glass</td>
<td>Metal crimp cap w. seal/septum</td>
<td>PTFE (Teflon)</td>
<td>Brown transparent</td>
</tr>
<tr>
<td>Tetramethrin</td>
<td>01.07.2012</td>
<td>4</td>
<td>Yes</td>
<td>Glass</td>
<td>Metal crimp cap w. seal/septum</td>
<td>PTFE (Teflon)</td>
<td>Brown transparent</td>
</tr>
<tr>
<td>Trinexapac-ethyl</td>
<td>02.10.2012</td>
<td>-20</td>
<td>Yes</td>
<td>Glass</td>
<td>Metal crimp cap w. seal/septum</td>
<td>PTFE (Teflon)</td>
<td>Brown transparent</td>
</tr>
<tr>
<td>Prohexadione</td>
<td>02.10.2012</td>
<td>4</td>
<td>Yes</td>
<td>Glass</td>
<td>Metal crimp cap w. seal/septum</td>
<td>PTFE (Teflon)</td>
<td>Brown transparent</td>
</tr>
<tr>
<td>Flonicamid</td>
<td>03.09.2014</td>
<td>23</td>
<td>Yes</td>
<td>Glass</td>
<td>Metal crimp cap w. seal/septum</td>
<td>PTFE (Teflon)</td>
<td>Brown transparent</td>
</tr>
<tr>
<td>Formothion</td>
<td>03.09.2014</td>
<td>23</td>
<td>Yes</td>
<td>Glass</td>
<td>Metal crimp cap w. seal/septum</td>
<td>PTFE (Teflon)</td>
<td>Brown transparent</td>
</tr>
<tr>
<td>Promecarb</td>
<td>03.09.2014</td>
<td>23</td>
<td>Yes</td>
<td>Glass</td>
<td>Metal crimp cap w. seal/septum</td>
<td>PTFE (Teflon)</td>
<td>Brown transparent</td>
</tr>
<tr>
<td>Fenarimol</td>
<td>03.09.2014</td>
<td>23</td>
<td>Yes</td>
<td>Glass</td>
<td>Metal crimp cap w. seal/septum</td>
<td>PTFE (Teflon)</td>
<td>Brown transparent</td>
</tr>
</tbody>
</table>
The storage duration is computed by calculating the difference between the measurement and preparation date.

Please consider the paragraphs F8-F11 of the AQC Guidelines for the measurement of the stored and reference solution.
Our results are available at www.eurl-pesticides-test.eu

- Stability data for 321 pesticides submitted by 3 labs
- Storage of data about the stability of pesticide standards in
  - stock solutions
  - working solutions (-> mixtures)
  - sample extracts
  - (pure standards)
- Template for Submission of Stability Data = standardized data format
• Comparison of compound stability
  • in different **solvents**
  • at different **storage conditions**
• Could be used to derive compound stability criteria

**Stability tests = high costs and lots of labor**

⇒ many laboratories are unable to conduct these experiments!

**Cooperation and coordination by the NRLs may help?**
Testing the Stability of Pesticides in Stock Solutions by Quantitative NMR
Spin Quantum Number $I$

- Atomic nuclei have a *spin quantum number* $I$.
- If $I$ differs from zero, the nucleus possesses a *magnetic moment* ($\mu$) that may interact with an external magnetic field.

![Diagram of nuclear magnetic resonance principles](image)
Principles of Nuclear Magnetic Resonance

1H spectrum of Ethanol:
3 types of protons
$\text{CH}_3$, $\text{CH}_2$, $\text{OH}$
• NMR is generally used for compound identification and structural elucidation

• Signal intensity in NMR-spectrum is directly proportional to the number of nuclei responsible for that specific resonance*

=> simultaneous access to both qualitative and quantitative information

• Quantitative inaccuracy of qNMR has been reported to be less than 2.0%**

Main characteristics of qNMR and chromatographic techniques*

<table>
<thead>
<tr>
<th>Quantitative NMR</th>
<th>LC-MS/MS GC-MS(/MS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight/dilution</td>
<td>Sample preparation</td>
</tr>
<tr>
<td>Non-destructive analysis</td>
<td></td>
</tr>
<tr>
<td>Structural properties</td>
<td>Detection</td>
</tr>
<tr>
<td>(restriction: e.g. $^1$H)</td>
<td></td>
</tr>
<tr>
<td>Certified reference material</td>
<td>Calibrant Quantitation</td>
</tr>
<tr>
<td>(one universal calibrant)</td>
<td></td>
</tr>
<tr>
<td>Internal/external standard</td>
<td></td>
</tr>
<tr>
<td>(with/without calibration curve)</td>
<td>(with/without calibration curve)</td>
</tr>
<tr>
<td>Low µM</td>
<td>Sensitivity</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Resonance overlapping</td>
<td>Selectivity &amp; Specificity</td>
</tr>
<tr>
<td>(with signals of solvent, metabolites, ...)</td>
<td></td>
</tr>
<tr>
<td>Instrument independent</td>
<td>Reproducibility</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Possible</td>
<td>Identification &amp; Quantification of Impurities</td>
</tr>
</tbody>
</table>

* For details see: Simmler Ch, Napolitano JG, McAlpine JB; Universal quantitative NMR analysis of complex natural samples; Current Opinion in Biotechnology 2014, 25:51-59
1st NMR Experiments: Mixtures of Model Compounds

- Simulation of the degradation of parathion-methyl to paraoxon-methyl: mixtures of compounds prepared at different concentrations (1 mg/ml -> 0 mg/ml)
- $^1$H-NMR spectra at 400 MHz (Bruker Avance 400)
- Solvent suppression technique: suppress acetonitrile-signal
- External calibrant: 1,2,4,5-tetrachloro-3-nitrobenzene
\( ^1 \text{H-NMR-Spectrum of Parathion-Methyl} \)

- Suppressed acetonitrile-signal
- \( ^{13}\text{C-Satellites of Acetonitrile} \)
- Water
- Tetramethylsilane (TMS)
- Acetone
Mixtures of Parathion-Methyl and Paraoxon-Methyl

Resonance overlapping
Mixtures of Parathion-Methyl and Paraoxon-Methyl
Mixtures of Parathion-Methyl and Paraoxon-Methyl

4 mmol/L

0.08 mmol/L
Simulation of Parathion-Methyl Degradation

Mixtures of Parathion-Methyl and Paraoxon-Methyl quantified by qNMR

Theoretical concentration [mmol/L]

Measured concentration [mmol/L]

Parathion-Methyl
Paraoxon-Methyl
% of Parathion-Methyl in mixture

Slide 29
NMR-spectra were recorded after 0 day (black) / 3 days (red) / 7 days (green) of storage
• EURL-SRM: Stability of pesticides in stock solutions by qNMR for 50 compounds

• Results our stability experiments are available at www.eurl-pesticides-test.eu

• Any contribution to compound stability database is highly appreciated!
Thank You for Your Attention

www.eurl-pesticides.eu