Work carried out in the Czech Republic

(version for CIPAC website)

Olga Nováková
1. Introduction
2. Steps of laboratory control of PPPs in CZ
3. Example of analysis of suspicious samples
4. Problems with analysis of suspicious samples
5. Suggestion to discussion
6. Conclusion
REORGANIZATION OF FORMER STATE PHYTOSANITARY ADMINISTRATION (SPA)

1997-2013: 2 institutes
• SPA (State Phytosanitary Administration)
  – Postregistration Control Division
    • Department of Laboratory Testing Pesticide (= NRL for PPP)
• CISTA (Central Institute for Supervising and Testing in Agriculture)
  – Division: National Reference Laboratory (9 laboratories)

From 1.1.2014: 1 institute
• SPA + CISTA = CISTA
  – Division: National Reference Laboratory (9+1 laboratories)
    • Department of Testing Plant Protection Products = former Laboratory Testing Pesticide
STRUCTURE OF CENTRAL INSTITUTE FOR SUPERVISING AND TESTING IN AGRICULTURE (CISTA)

General Director

Authority Office

Economic and Administration Section

Section of Protection against Harmful Organisms

Section of Plant Production

Section of Agricultural Inspection

Section of Agricultural Inputs

Division of Feed, Fertilisers and Soil

Division of Agricultural Inputs Control

Division of PPP

Coordination

Evaluation

Postregistration

National Reference Laboratory

Division of Audit and Quality Management
STRUCTURE OF NATIONAL REFERENCE LABORATORY

Director of NRL

Regional Departments
- Brno
- Opava
- Plzeň
- Praha
- Planá nad Lužnicí

Specialized Departments
- Brno
  - Department of Testing Plant Protection Products
- Brno
  - Department of Proficiency Testing Programmes
- Brno
  - Department of Microbiology and Biochemistry
- Lípa
  - Department of Special Plant and Feed Analyses
**SYSTEM OF POSTREGISTRATION CONTROL – responsibility**

**Post-registration Control Department - responsibility**
- annual plan with cooperation of laboratory
- sampling original packages - PPP samples are taken directly in the distributor stores
- labeling control
- control of sale, storage and use of PPP
- control of technical state of application machinery

**Laboratory- responsibility and competence**
- **Laboratory analysis**
  - laboratory check on physical and chemical properties of plant protection products and other plant protection preparations and chemical compositions
  - report of analysis (+results with evaluation)

**Post-registration Control Department – responsibility**
- reconvey the rest of samples
- evaluation of results from laboratory in accord with act (law)
- information of authorization holders
- fine
LABORATORY CONTROL OF PPPs

Planned samples
• postregistration control according to annual plan
• samples within the process of PPP approval

Unplanned samples
• unknown samples
• suspicious samples (e.g. counterfeit)

Proficiencies tests (AAPCO, AFSCA, ...)
**STEPS OF LABORATORY CONTROL**

- Registration of samples (LIMS)
- Laboratory sampling
  - Original sample package is higher 1L or 1kg
  - Original sample package is lower 1L or 1kg
    Partition of laboratory sample = Analytical sample
- Laboratory analysis
- Evaluation of laboratory analysis
  - Certificate of analysis (Agreement or disagreement with specification)
  - Detailed expert reports
LABORATORY ANALYSIS

Planned samples – postregistration control according to annual plan

FAO specification or existing national specification
  – Identity and content of active substances
  – Identity and content of relevant impurities
  – Physical, chemical and technical properties
  – Storage stability tests

Xylene in EC formulations

Samples from parallel import – chromatographic and FTIR comparison with reference sample.

Methods: CIPAC, OECD or equivalent and the validated methods of producers, which are submitted as part of registration dossiers.

Laboratory verifies all methods according to standard operation procedure (SOP-PP-08-01).
Samples within the process of PPP approval

Aim:

• Verify input data (technical specification)
• Verification or validation analytical methods in CZ conditions (different column...)
• Obtain knowledge of PPP
• Focus on chemical composition
• Time for discussion of laboratory analysis - analytical results with authorization holders
• Input data for postregistration control
• Future - Help with detection of counterfeit
Unplanned control PPPs samples

- Unknown samples (samples without label, confusion of active ingredient,...)
- Suspicious samples
LABORATORY ANALYSIS – SUSPICIOUS SAMPLES

• Identity and content of active ingredient
• Identity and content of relevant impurities
• Physical, chemical and technical properties
• Chemical composition of sample (co-formulants, impurities,...)
• Comparison with reference sample (GC, LC, FTIR)

Additional tests for clarification of unregistered sample:
GC/MS, FTIR, Particle size distribution (CIPAC MT187),
Determination of Sulphated ash (CIPAC MT29), Pour and tap
bulk density (USP2/ASTM), Density, Viscosity (CIPAC MT192),
Surface tension (OECD 115), TGA method, DSC method
EXAMPLES OF ANALYSIS

Appearance:

Differences of shape granules between reference sample and suspicious sample

Differences in colour between reference sample and suspicious sample
EXAMPLES OF ANALYSIS

Density:

<table>
<thead>
<tr>
<th></th>
<th>Density [g/ml]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference PPP</td>
<td>1,170</td>
</tr>
<tr>
<td>Suspicious PPP</td>
<td>1,126</td>
</tr>
</tbody>
</table>

Result:

Suspicious PPP is probably diluted or has different chemical composition.
## EXAMPLES OF ANALYSIS

### Density:

<table>
<thead>
<tr>
<th></th>
<th>Density [g/ml]</th>
<th>Dean – Stark (CIPAC MT 30.2) Amount of water (% w/w)</th>
<th>Amount of active ingredient (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference PPP</td>
<td>1,170</td>
<td>44,6</td>
<td>30,4</td>
</tr>
<tr>
<td>Suspicious PPP</td>
<td>1,126</td>
<td>60,7</td>
<td>21,8</td>
</tr>
</tbody>
</table>

**Confirmation:**
- determination of water by Dean-Stark (CIPAC MT 30.2)
- determination of amount active ingredients

**Result:**
Suspicious sample is diluted.
### EXAMPLES OF ANALYSIS

**Sulphated ash (CIPAC MT 29):**

<table>
<thead>
<tr>
<th>Reference PPP (different batches)</th>
<th>Suspicious PPP</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>year</strong></td>
<td><strong>Year</strong></td>
</tr>
<tr>
<td>2001</td>
<td>2001</td>
</tr>
<tr>
<td>1,52</td>
<td>2003</td>
</tr>
<tr>
<td>1,30</td>
<td>2006</td>
</tr>
<tr>
<td>1,37</td>
<td>2008</td>
</tr>
<tr>
<td>1,22</td>
<td>2012</td>
</tr>
<tr>
<td>1,26</td>
<td>2013</td>
</tr>
<tr>
<td>1,05</td>
<td></td>
</tr>
<tr>
<td><strong>average</strong></td>
<td></td>
</tr>
</tbody>
</table>

**Result:** There are differences between inorganic ions in suspicious sample and reference sample - the chemical composition of suspicion sample is different from reference sample.
EXAMPLES OF ANALYSIS

Surface tension (OECD 115):

<table>
<thead>
<tr>
<th>PPP</th>
<th>Surface tension of 0,01% w/w solution (mN/m)</th>
<th>Surface tension of 0,5% w/w solution (mN/m)</th>
<th>Surface tension of 1,0% w/w solution (mN/m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference PPP</td>
<td>57,0</td>
<td>32,9</td>
<td>32,7</td>
</tr>
<tr>
<td>Suspicious PPP</td>
<td>59,4</td>
<td>69,9</td>
<td>71,8</td>
</tr>
</tbody>
</table>

**Result:**
Suspicious PPP probably does not contain surface active agent.
EXAMPLES OF ANALYSIS

Particle size distribution (CIPAC MT 187):

Reference PPP

Suspicious PPP
EXAMPLES OF ANALYSIS

Particle size distribution (CIPAC MT 187):

<table>
<thead>
<tr>
<th></th>
<th>Reference PPP</th>
<th>Suspicious PPP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diameter at 10%</td>
<td>0,68 μm</td>
<td>0,80 μm</td>
</tr>
<tr>
<td>Diameter at 50%</td>
<td>2,45 μm</td>
<td>3,77 μm</td>
</tr>
<tr>
<td>Diameter at 90%</td>
<td>6,07 μm</td>
<td>9,68 μm</td>
</tr>
<tr>
<td>Mean diameter</td>
<td>2,97 μm</td>
<td>4,56 μm</td>
</tr>
</tbody>
</table>

Result:
Particle size distribution of particles which disperse in water in suspicious PPP are different. It means that it can be different technology of production.
EXAMPLES OF ANALYSIS

HPLC screening by HPLC/UV:

Result:
Suspicious PPP 1 has probably identical chemical composition with reference PPP,
Suspicious PPP 2 has not identical chemical composition with reference PPP.

Reference PPP (black),
Suspicious PPP 1 (blue)
Suspicious PPP 2 (green)
EXAMPLES OF ANALYSIS

GC/FID – identification of co-formulant:

Reference PPP (blue)
Suspicious PPP (red)
EXAMPLES OF ANALYSIS

GC/FID – identification of co-formulant:

Overlay chromatograms of reference PPP and suspicious PPP and Surface-active agent, extract 10-18min

Result:
Suspicious PPP does not contain surface-active agent which is present in reference PPP.
EXAMPLES OF ANALYSIS

GC/MSD identification of co-formulants:
GC/MSD chromatographic profile of PPP (split 100:1, extract 2-4min)

Reference PPP (green), Suspicious PPP (black)
**EXAMPLES OF ANALYSIS**

**GC/MSD identification of co-formulants:**

GC/MSD chromatographic profile of PPP (split **10:1**, extract **4-8min**)

Reference PPP (**green**), Suspicious PPP (**black**)

![MSD spectrum of naphthalene](image)
EXAMPLES OF ANALYSIS

GC/MSD identification of co-formulants:

GC/MSD chromatographic profile of PPP (split 10:1, extract 8-11.6 min)

Reference PPP (green), Suspicious PPP (black)

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CIPAC Symposium 24th June 2014, Liege, Belgium
EXAMPLES OF ANALYSIS

GC/MSD identification of co-formulants:

GC/MSD chromatographic profile of EW formulation PPP - active ingredient (extract 11.6-12.6 min)

Reference PPP (green), Suspicious PPP (black)
EXAMPLES OF ANALYSIS

GC/MSD identification of co-formulants:

Summary:

<table>
<thead>
<tr>
<th>Identified components</th>
<th>Reference PPP</th>
<th>Suspicious PPP</th>
<th>Content % w/w</th>
</tr>
</thead>
<tbody>
<tr>
<td>N,N-dimethylformamide</td>
<td>No</td>
<td>Yes</td>
<td>16.78</td>
</tr>
<tr>
<td>N-octyl-2-pyrrolidone</td>
<td>No</td>
<td>Yes</td>
<td>0.306</td>
</tr>
<tr>
<td>Ethylene glycol</td>
<td>No</td>
<td>Yes</td>
<td>1.525</td>
</tr>
<tr>
<td>Naphthalene</td>
<td>No</td>
<td>Yes</td>
<td>0.018</td>
</tr>
</tbody>
</table>

Result:
The chemical composition of suspicious PPP are different from reference sample.
UV-VIS spectroscopy:

VIS spectrum:

<table>
<thead>
<tr>
<th>Sample</th>
<th>λ max v DI water [ nm]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference sample</td>
<td>628 nm</td>
</tr>
<tr>
<td>Suspicious sample</td>
<td>620 and 657 nm</td>
</tr>
</tbody>
</table>

Result:
The suspicious PPP does not contain the same dye as reference sample.
EXAMPLES OF ANALYSIS

FTIR spectroscopy:

Reference PPP (blue), Suspicious PPP (red)
EXAMPLES OF ANALYSIS

FTIR spectroscopy:

FTIR spectrum of Insoluble residues of PPP in CH$_2$Cl$_2$

Reference PPP (blue), Suspicious PPP (red)
EXAMPLES OF ANALYSIS

FTIR spectroscopy:

FTIR spectrum of Insoluble residues of suspicious PPP in CH₂Cl₂ and kaolin CAS No. 1332-58-7

Result:
The suspicious PPP contains kaolin = the chemical composition is different from reference sample.
EXAMPLES OF ANALYSIS

Differential Scanning Calorimetry (DSC):

Reference PPP

Suspicious PPP

Result:
The chemical composition of suspicious sample is different from reference sample.
EXAMPLES OF ANALYSIS

Thermogravimetric analysis (TGA):

Reference PPP

Result:
Different chemical composition of volatile compounds present in PPP or/and other compounds in PPP.

Suspicious PPP
CONCLUSION

• Active ingredients and relevant impurities in suspicious PPP mostly agree with specification.

• We usually find differences in chromatography profile and/or in FTIR spectra between reference and suspicious samples:
  – we use the other technics and methods for identification.

• Necessity of cooperation between laboratories of national authority and producers (in interpretation of raw data, support of analytical method, standards...)

• Necessity of clear legislation.
THANK YOU FOR THE ATTENTION!

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