Pyrrolizidine Alkaloids, residues and GMO – Recent Developments
Pyrrolizidine Alkaloids (PAs)

- PAs are secondary plant metabolites
- PAs are formed by approx. more than 6000 plant species
- PAs are toxic to the liver
- PAs show cancerogenic properties
Pyrrolizidine Alkaloids (PAs)

Plant Material
(Plant Parts, Pollen, Nectar)

Animal Feed

Foodstuffs
(Honey, Herbs, Green Leafy Vegetables, Flour)

Insects

Milk

Eggs

Meat?
Plants containing PAs used by Bees

*Senecio madagascariensis*

*Eupatorium macrocephala*

*Echium plantagineum*
Plants containing PAs used by Bees

Blue Borage
(*Echium vulgare*)
Plants containing PAs used by Bees

*Senecio bracteolatus*

Senecionin
Plants containing PAs used by Bees

*Eupatorium buniifolium*  
*Chromolaena odorata*
PA Transfer

Echium plantagineum

Echium „Bee Pollen“

© Boppré et al. 2008

© Quality Services International

© Honigverband e.V.
Currently there are no official limits for PAs in honey!

EFSA Opinion – PAs in Food and Feed

Codex Alimentarius discussed PA-issue and will evaluate the possibility to develop a code of practice

German Federal Institute for Risk Assessment (BfR) recommended a maximum daily intake of $0.007 \text{ µg PAs/kg}$ bodyweight.

$\rightarrow 0.42 \text{ µg PAs for a person of 60 kg}$

Eq. to one hotel serving (20 g) of honey containing 21 µg/kg PAs.
Comparison of (suggested) PA-Limits in different countries

Body Weight [kg]

PA Exposure [µg/day]

- COT/BfR Honey
- BfR Phyto <6 weeks
- BfR Phyto >6 weeks
- VSD, RIKILT
- Non Cancer Effects, RIKILT
- ANZFA
### PA in Honigen aus Kuba – Übersicht

<table>
<thead>
<tr>
<th></th>
<th>Lycopsamin</th>
<th>Lycopsamin-N-Oxid und Lycopsamin-Isomere und deren N-Oxide</th>
</tr>
</thead>
<tbody>
<tr>
<td>pos. Proben [%]</td>
<td>48</td>
<td>72</td>
</tr>
<tr>
<td>Ø Konz. PA-pos. Proben [µg/kg]</td>
<td>17</td>
<td>164</td>
</tr>
<tr>
<td>Ø Konz. alle Proben [µg/kg]</td>
<td>9</td>
<td>127</td>
</tr>
</tbody>
</table>
Empfehlung des BfR

Durchschnittliche PA-Konzentration in kubanischem Honig: 127 µg/kg

eine Hotel-Portion (20 g) enthält somit 2,54 µg PA

Empfohlene maximale PA-Aufnahme pro Tag (60kg schwere Person) = 0,42 µg PA

→ 6-fache Überschreitung der Empfehlung des BfR
PA-Konzentrationsverteilung – Kuba

- 28,5 %
- 16,7 %
PAs in Honey Worldwide (approx. 8000 analyses)

- Cuba
PAs in Consumer Product

Consumer Product = Honey available in shops
Usually a mixture of EC/Non-EC Countries

In total 1726 samples were analysed
92% PA-positive
PA-Concentration ranges from 1 ppb to 267 ppb
Average PA-concentration is 22 ppb
Zusammenfassung

• Derzeit kein offizieller PA-Grenzwert
• Jedoch existieren z.T. Vorgaben durch den Handel
• Jüngere Risikoabschätzungen wurden vom Bundesinstitut für Risikobewertung (BfR) und der European Food Safety Authority (EFSA)
• Codex Alimentarius arbeitet an einem „Code of Practice“ mit dem Ziel, die PA-Belastung in Nahrungsmitteln niedrig zu halten
• Eine potentielle PA-Quelle in Kuba ist die Pflanze Chromolaena odorata (früher: Eupatorium odoratum)
• Imker sollten ihre Beuten möglichst nicht in die Nähe größerer Vorkommen von PA-Pflanzen aufstellen
Genetically Modified Organisms (GMO)
Actual Judgement of EU Court of Justice

Mr. Bablok sued State of Bavaria in 2005
(MON810 maize pollen from research crop in his honeys, 500 m distance)

Judgement (06\textsuperscript{th} September 2011)

• honey is not a product out of/with genetically modified plants \textbf{but}
pollen is a product of GMO

• pollen in honey = ingredient acc. to 1829/2003 (added by beekeeper during centrifugation)

→ honey with gm pollen falls under EC regulation 1829/2003
Genetically Modified Organisms (GMO)

- Since 1996 commercial cultivation of genetically modified plants (GM plants)
- Commercial use mainly focused on GM soybean, corn, cotton and rape (canola)

Aim
- tolerance of plants against pests (insects, viruses…)
- immunity against the usage of total herbicides (RoundUp Ready)
- tailor made products (flavr savr tomato)
GLOBAL AREA OF BIOTECH CROPS
Million Hectares (1996-2010)

Source: Clive James, 2010
Genetically Modified Organisms (GMO)

- Countries with largest areas of cultivated GM-plants:
  USA, Argentina, Brazil, Canada, China

- Up to now the global area of biotech crops reached more than 160 million hectares
GM Plants in Honey

Bees can contaminate honey with pollen of GM plants

e.g. → Canola: RT73 (Roundup Ready) canola in the USA, Canada
    → Soybean: MON40-3-2 soybean in Argentina, Brazil, Chile
    → Cotton: MON531 in India, Argentina, Brazil

Corn pollen is less attractive for bees, thus honey contaminated with gm corn isn‘t frequently found
Countries (GMO and Non GMO) Supplying EU with Honey, 2010

<table>
<thead>
<tr>
<th>GMO</th>
<th>'000 tons</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>50.1</td>
<td>33.7</td>
<td></td>
</tr>
<tr>
<td>32.8</td>
<td>21.9</td>
<td></td>
</tr>
<tr>
<td>18.2</td>
<td>12.3</td>
<td></td>
</tr>
<tr>
<td>17.5</td>
<td>11.8</td>
<td></td>
</tr>
<tr>
<td>12.3</td>
<td>8.3</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Non GMO</th>
<th>'000 tons</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.3</td>
<td>6.2</td>
<td></td>
</tr>
<tr>
<td>7.0</td>
<td>4.7</td>
<td></td>
</tr>
<tr>
<td>1.6</td>
<td>1.1</td>
<td></td>
</tr>
</tbody>
</table>

Source: TU Munich, Justus Wesseler
Detection of GMO in Honey

- Original Honey Sample
- Dilution
- Amplification of GM-pollen DNA using Real Time PCR
- Visualisation of Analysis results
- Diluted Honey Sample
- Centrifugation
- Extraction of total pollen DNA from honey sediment
- Pollen Sediment
Detection of GMO in Honey

With Real Time PCR, DNA sequences of a GMO can be amplified, resulting in several billion copies.

→ Even detection of very small amounts of GMO sequences initially being present in a honey is possible.
Detection of GMO in Honey

GMO Screening

→ Pollen of honey sample is tested for DNA sequences that are present in nearly 95% of all GMO worldwide
- Negative result indicates that sample contains no GMO
- Positive result indicates that sample almost certainly contains GMO (no proof!)
  → But: it does not say which specific gm plant was detected
  → Subsequent identification of GMO has to be performed by event-specific detection
Detection of GMO in Honey

GMO event-specific detection

→ to identify a specific GMO (e.g. Roundup Ready soybean, MON 810 corn, RT 73 canola, MON 531 cotton etc.)

→ DNA sequences that are only present in the specific GMO are multiplied by Real Time PCR
Strategy for Determination/Identification

- screening results
- Info from beekeeper/country or region of origin
- pollen analysis
- plant screening

Triple Screening

- positive
  - possible GMO?
- negative
  - no GMO

marketable honey
Strategy for Determination/Identification

Triple Screening

possible GMO?

Event-specific analysis

Event-specific analysis

Event-specific analysis

positive

negative

marketable honey

honey NOT marketable

Quantification

EC 1829/2003
Situation on Cuba

• 235 samples

• all negativ
Recommendations to beekeepers

• contact your government for a gm-crop site map
• avoid these crops (especially for export to EU/organic honey)
• test your honey before export
• keep informed about recent developments
Bee Pharmaceuticals
Bee Pharmaceuticals

Problem
• Synthetic acaricides in beeswax, propolis and honey

Source of contamination
• Varroa control with synthetic acaricides

When treatments are not according to prescriptions i.e. during honey flow and more frequent use, synthetic acaricide residues in wax and honey will be elevated.
## List of Contaminants in Honey and Related Quality Problems

### BEEKEEPING:

<table>
<thead>
<tr>
<th>Problem</th>
<th>Substance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bee Pharmaceuticals</td>
<td>licensed products, non-licensed products</td>
</tr>
<tr>
<td>Bee repellents</td>
<td>Phenol, Butyric Acid</td>
</tr>
<tr>
<td>Moth repellents</td>
<td>Dichlorobenzene, Naphtalene</td>
</tr>
</tbody>
</table>
Antibiotics

Problem
Preventive Pharmaceuticals

Source of contamination
Control of bacterial diseases with antibiotics (AFB, EFB, Nosema) with substances like Tetracyclines, Streptomycine, Sulphonamides etc.
Antibiotics

Since many years the following substances are monitored:

- Streptomycine
- Tetracyclines
- Sulfonamides
- Chloramphenicol

Since several years analysis methodologies are implemented also for:

- Nitrofurane metabolites
Antibiotics – Statistics

Overview 2005 to 2010

Streptomycine - all origins
Antibiotics – Statistics

Streptomycine - Cuba (2011 to 3/2012)

µg/kg

- n.n.
- <=10
- <=100
- <=200
- >200

%
Antibiotics – Statistics

Overview 2005 to 2010

Tetracycline - all origins

µg/kg
Antibiotics – Statistics

Tetracycline - Cuba (2011 to 3/2012)
Sulfonamides -
Distribution of positive findings

- Sulfodim ethoxin
- Sulfachlorpyridazin
- Sulfam ethizol
- Sulfadiazin
- Sulfathiazol
- Sulfapyridin
- Sulfadim idin
- Trim ethoproprim
- Sulfam onom ethoxazol
- Sulfaclozine

4th Cuban Beekeeping Congress ©QSI 2012
Antibiotics – Statistics

Overview 2005 to 2010

Sulfathiazol - all origins

µg/kg

- n.n.
- <=2
- <=5
- <=10
- <=100
- <=200
- >200

2005
2006
2007
2008
2009
2010
Antibiotics – Statistics

Sulfonamides - Cuba (2011 to 3/2012)
No positive Chloramphenicol samples from Cuba for 2011 to 3/2012
Overview 2005 to 2010

Antibiotics – Statistics

Nitrofuran-Semicarbazid - all origins

No positive Nitrofuran samples from Cuba for 2011 to 3/2012
New Antibiotics

However:

During the last years, more and more antibiotics were found in honey samples besides the classic ones

These are e.g.:

- Tylosin (permitted in Canada, but also found in Honey from other origin, e.g. USA)
- Dapson and Trimethoprim
- Fluoroquinolone like e.g. Enrofloxacin, Norfloxacin
- Groups like Macrolide e.g.: Erythromycin, Lincosamide, β-Lactame antibiotics and Penicillines
- Nitroimidazole e.g.: Metronidazol
Antibiotics – Statistics: Tylosin

Tylosin - Cuba (2011 to 3/2012)

- 0.5% positive

n.n.  positive
Fluoroquinolone - Distribution of positive findings
New Antibiotics

• As well as „classical“ and „new“ antibiotics exist and are used there will be „newer“ ones.

• The only measure to have pure and uncontaminated honey is to improve and control the processes and the product.
Bee Repellents

Problem

• Smoker substances and bee repellents like phenolic compounds, Phenol and Butyric acid

Source of contamination

• Use of synthetic repellents during honey harvest
Bee Repellents

Too much smoke cause increased amounts of Phenol will change of honey taste and lead to an unpleasant flavour.

Use of brush and/or water
Chemical Control of Wax Moth

Use of para-dichlorobenzene (PDCB) and naphthaline against the wax moth leads to contamination of wax and honey.

Alternativen gegen Varao → Thymol bzw. organische Säuren, etherisches Öl, Bee repellents
Fazit

• Kein Problem mit Antibiotika  
  ➔ jedoch weiterhin ständige Überwachung

• Kein Problem mit GV-Pflanzen

• Problem: PA-Pflanzen  ➔ Risikoorientiertes Aufstellen von  
  Bienenstöcken
Thank you for your attention!

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