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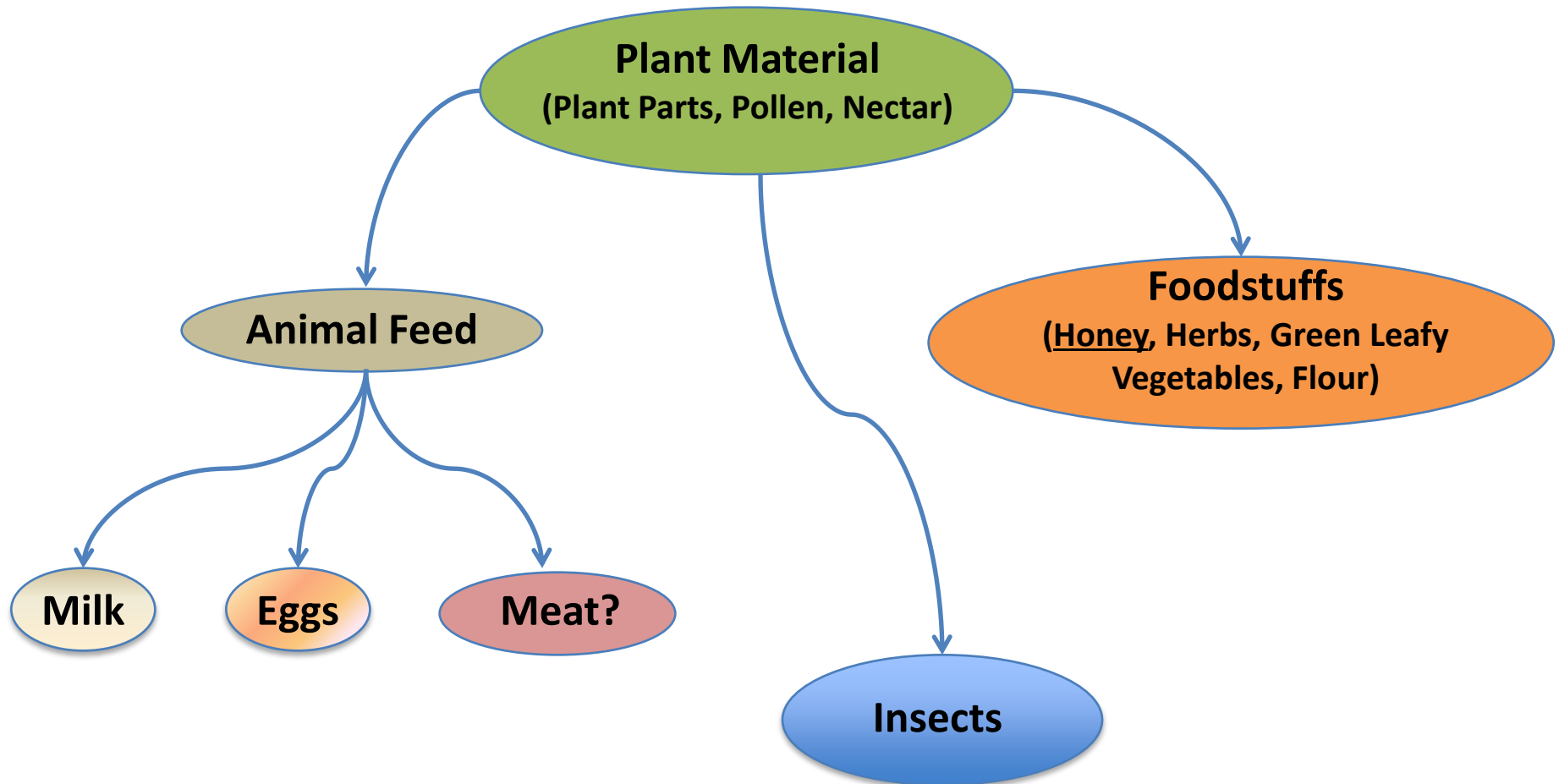
Pyrrolizidine Alkaloids, residues and GMO – Recent Developments

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)



Plants containing PAs used by Bees

*Senecio
madagascariensis*



© Quality Services International

*Eupatorium
macrocephala*



© Quality Services International

*Echium
plantagineum*

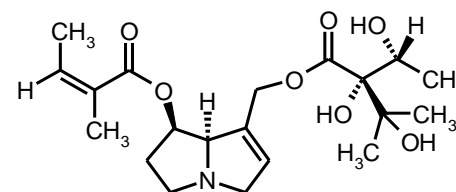


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Plants containing PAs used by Bees



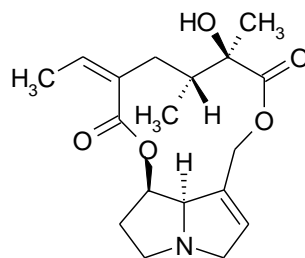
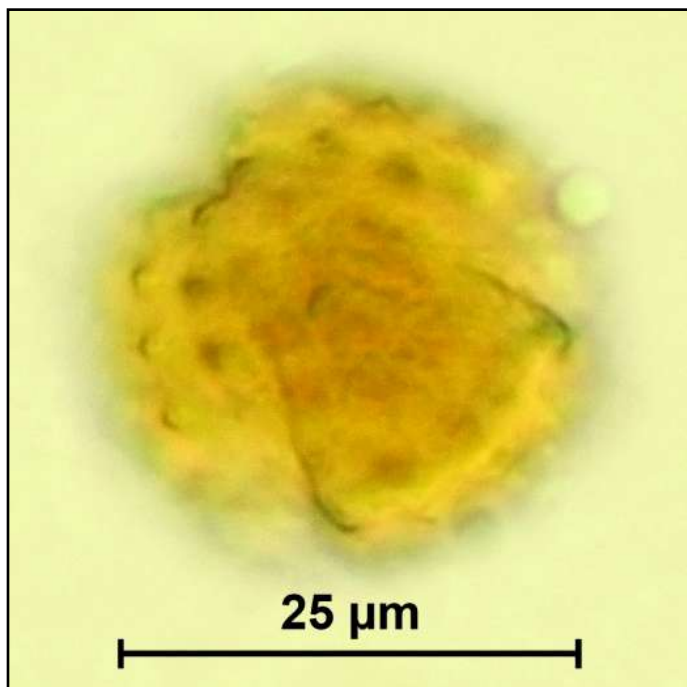
Blue Borage
(*Echium vulgare*)



Echimidin

Plants containing PAs used by Bees

Senecio bracteolatus



Senecionin



Plants containing PAs used by Bees

Eupatorium buniifolium



Chromolaena odorata



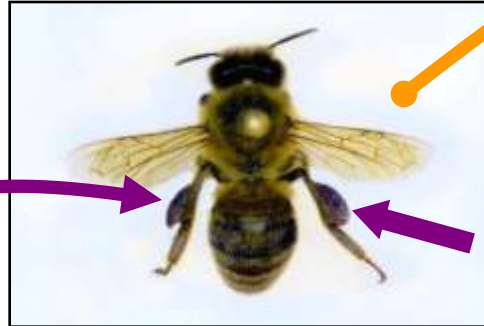
PA Transfer

*Echium
plantagineum*



© Quality Services International

Echium „Bee Pollen“



© Boppré et al. 2008



© Honigverband e.V.



PA-Limits – Recent Developments

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

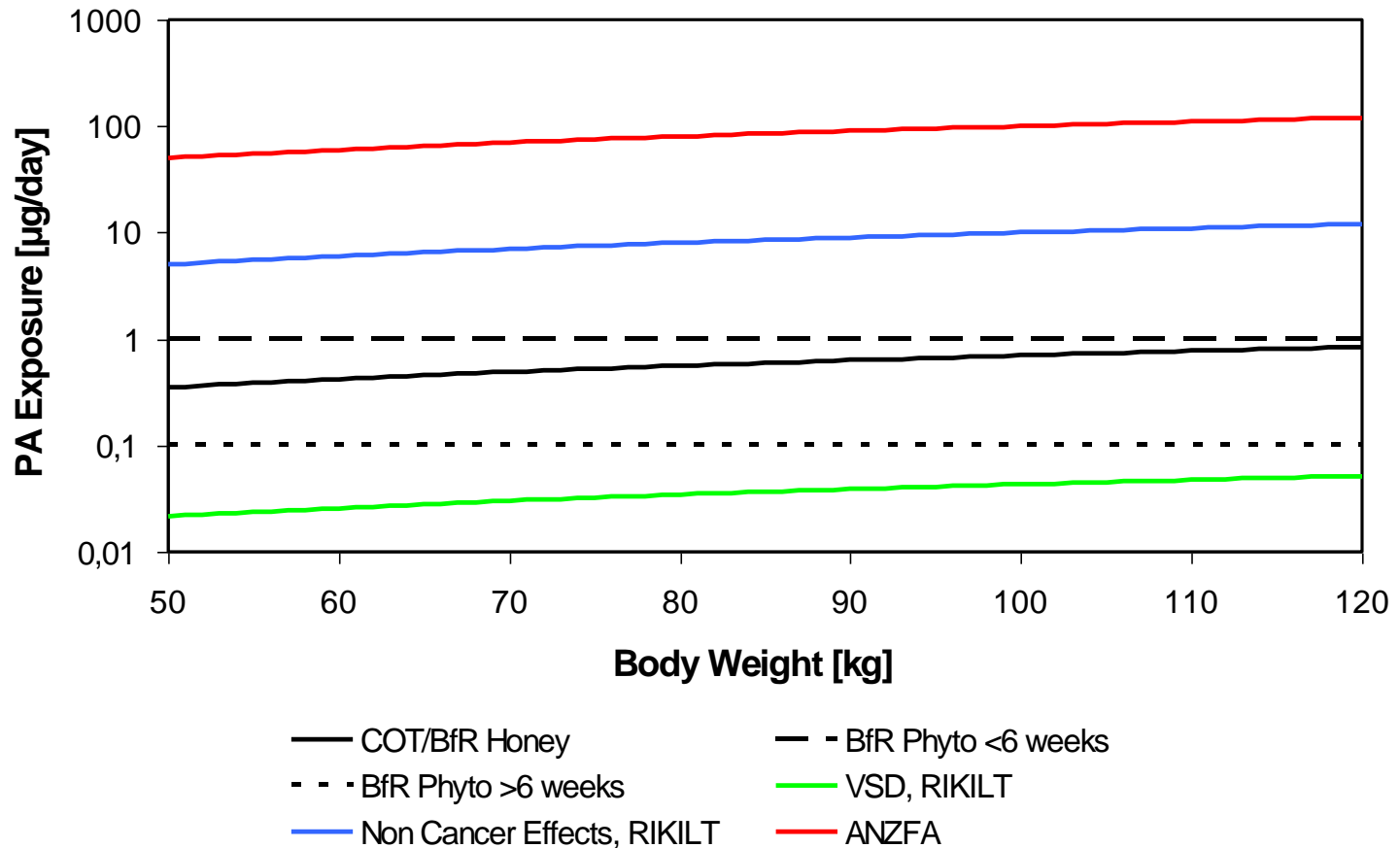
German Federal Institute for Risk Assessment (BfR) recommended a maximum daily intake of **0.007 µg PAs/kg** bodyweight.

→ 0.42 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





PA in Honigen aus Kuba – Übersicht

	Lycopsamin	Lycopsamin-N-Oxid und Lycopsamin-Isomere und deren N-Oxide
pos. Proben [%]	48	72
Ø Konz. PA-pos. Proben [µg/kg]	17	164
Ø Konz. alle Proben [µg/kg]	9	127



Empfehlung des BfR

Durchschnittliche PA-Konzentration in
kubanischem Honig:

127 µg/kg

eine Hotel-Portion (20 g) enthält somit

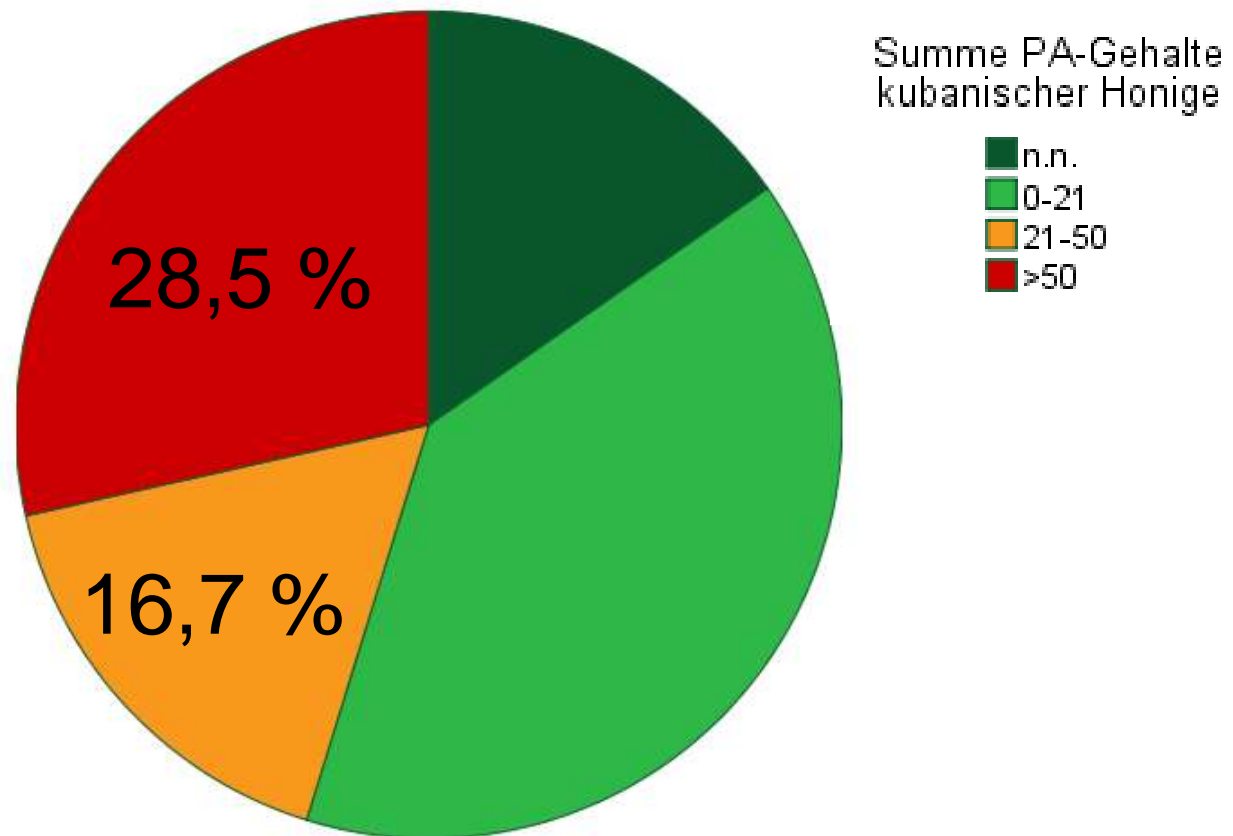
2,54 µg PA

**Empfohlene maximale PA-Aufnahme
pro Tag (60kg schwere Person) =**

0,42 µg PA

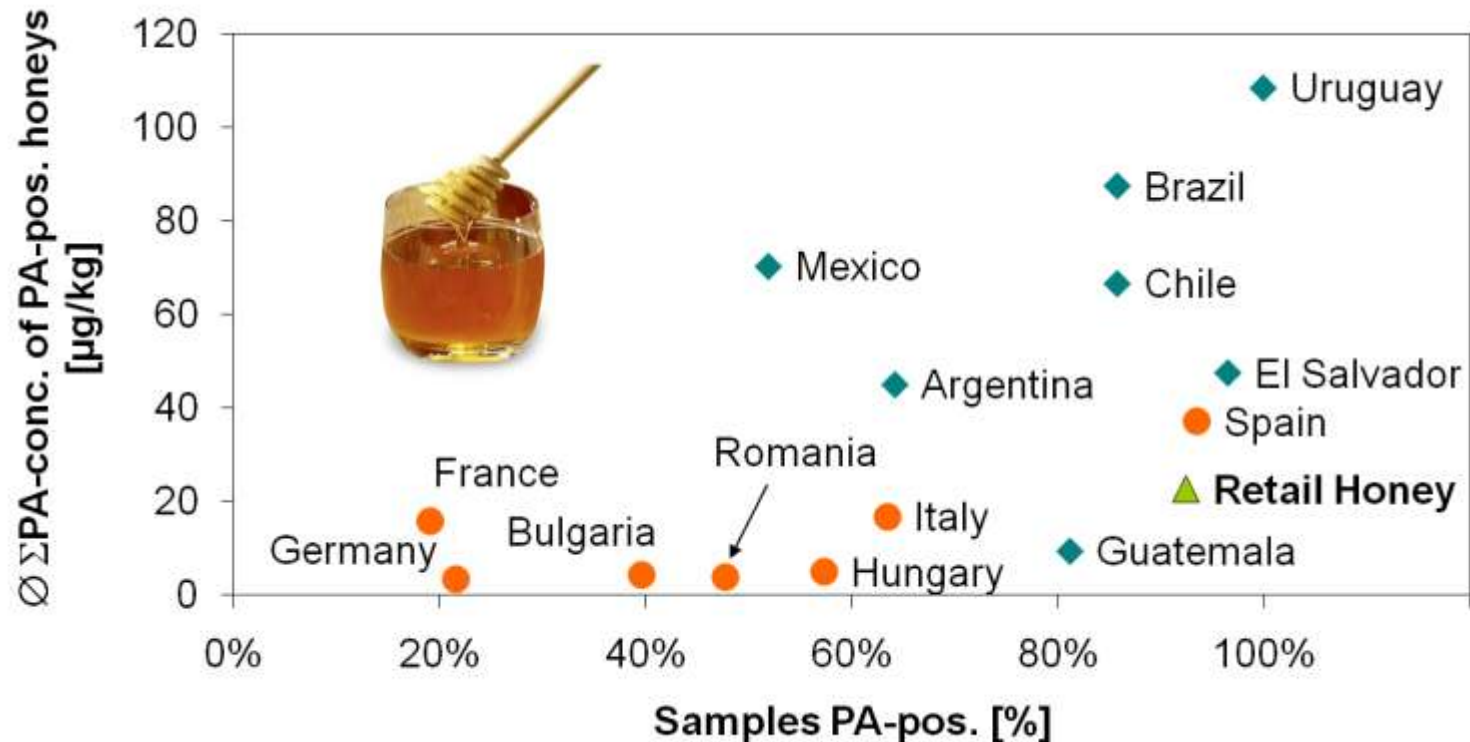
→ 6-fache Überschreitung der Empfehlung des BfR

PA-Konzentrationsverteilung – Kuba



PAs in Honey Worldwide (approx. 8000 analyses)

● Cuba





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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**



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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 (06th September 2011)

- honey is not a product out of/with genetically modified plants **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)

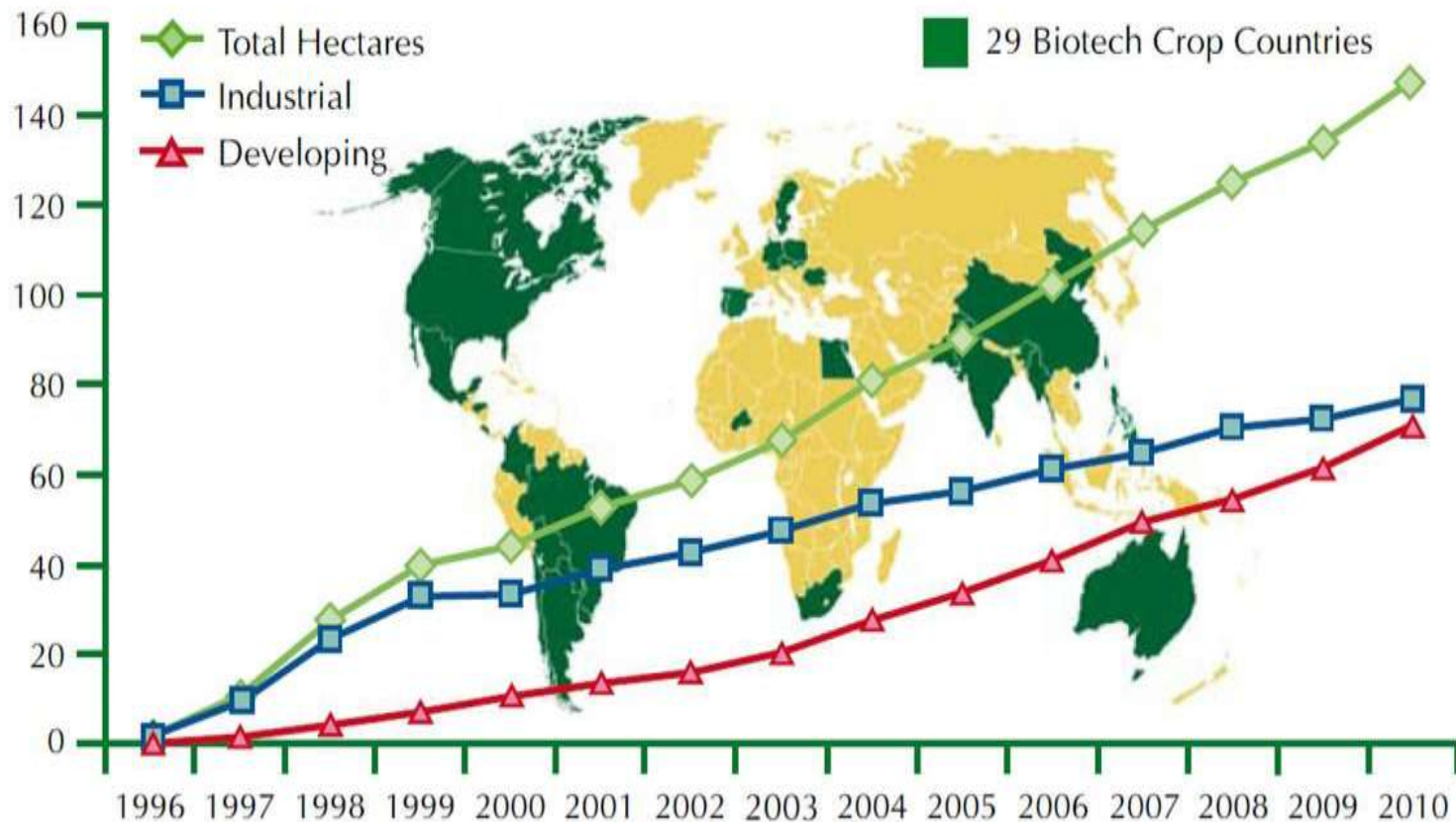
» Aim

- tolerance of plants against pests (insects, viruses...)
- immunity against the usage of total herbicides (RoundUp Ready)
- tailor made products (flavr savr tomato)



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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

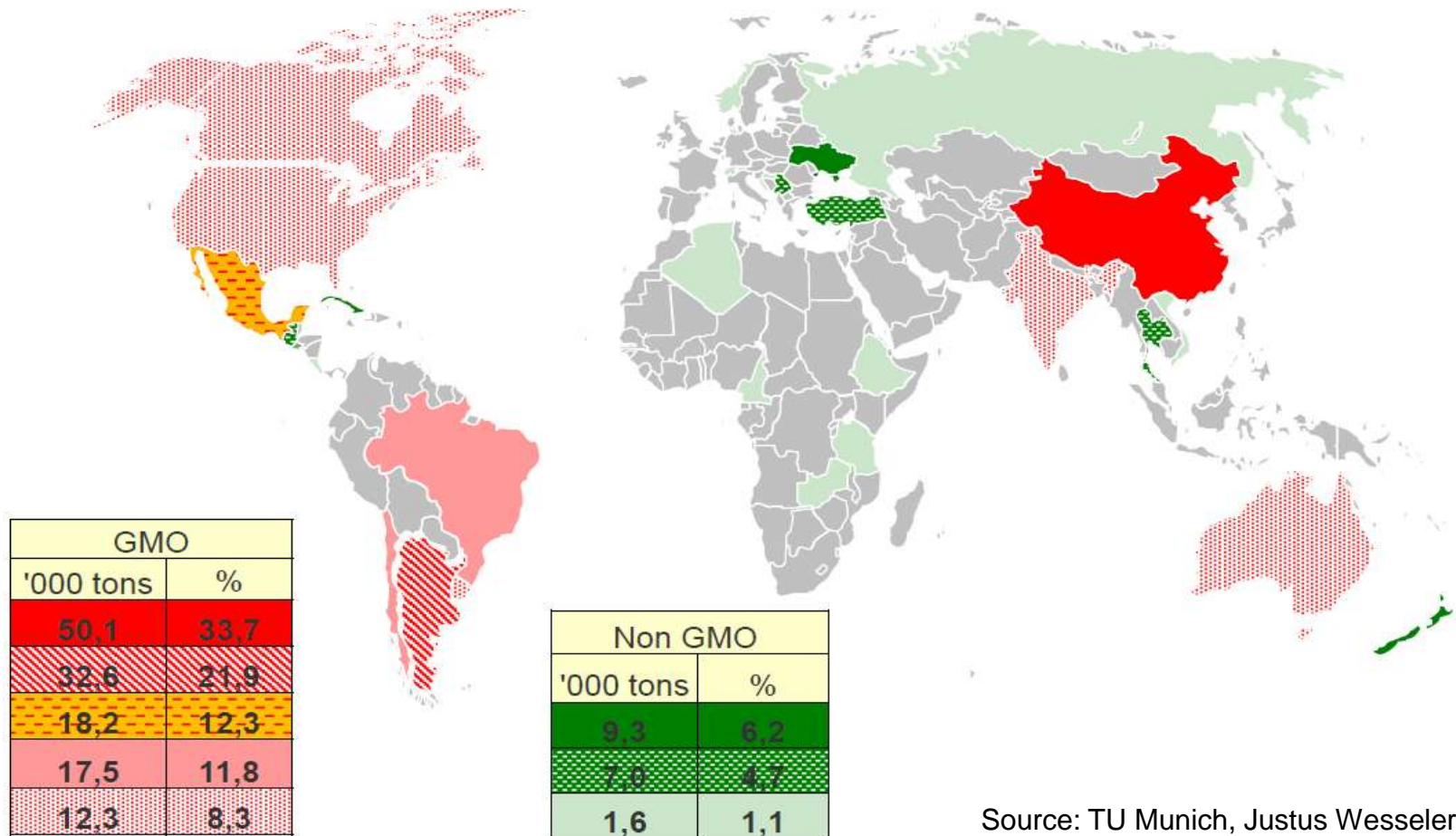
- e.g. → Canola: RT73 (Roundup Ready) canola in the
USA, Canada
→ Soybean: MON40-3-2 soybean in Argentina,
Brazil, Chile
→ Cotton: MON531 in India, Argentina, Brazil

Corn pollen is less attractive for bees, thus honey contaminated with gm corn isn't frequently found



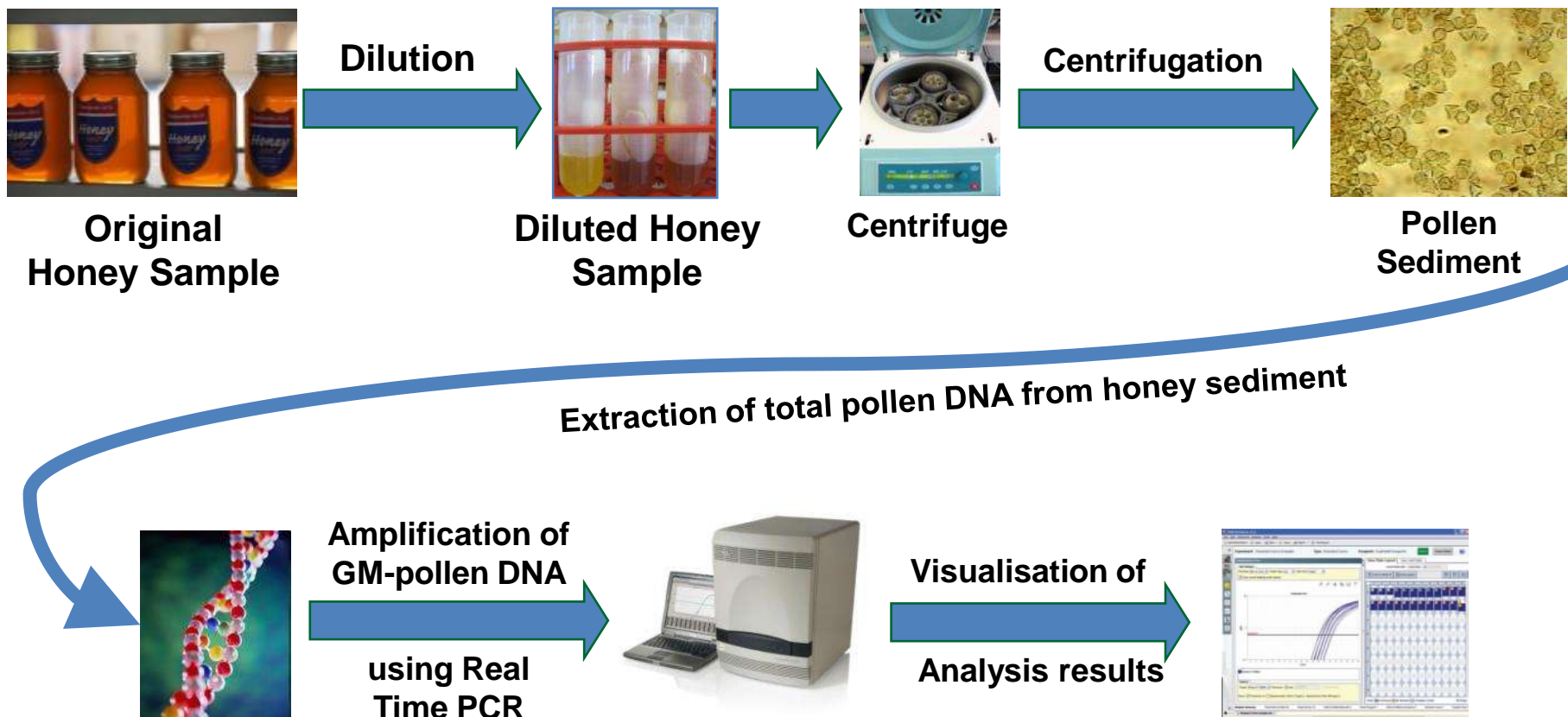
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Countries (GMO and Non GMO) Supplying EU with Honey, 2010



Source: TU Munich, Justus Wesseler

Detection of GMO in Honey





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

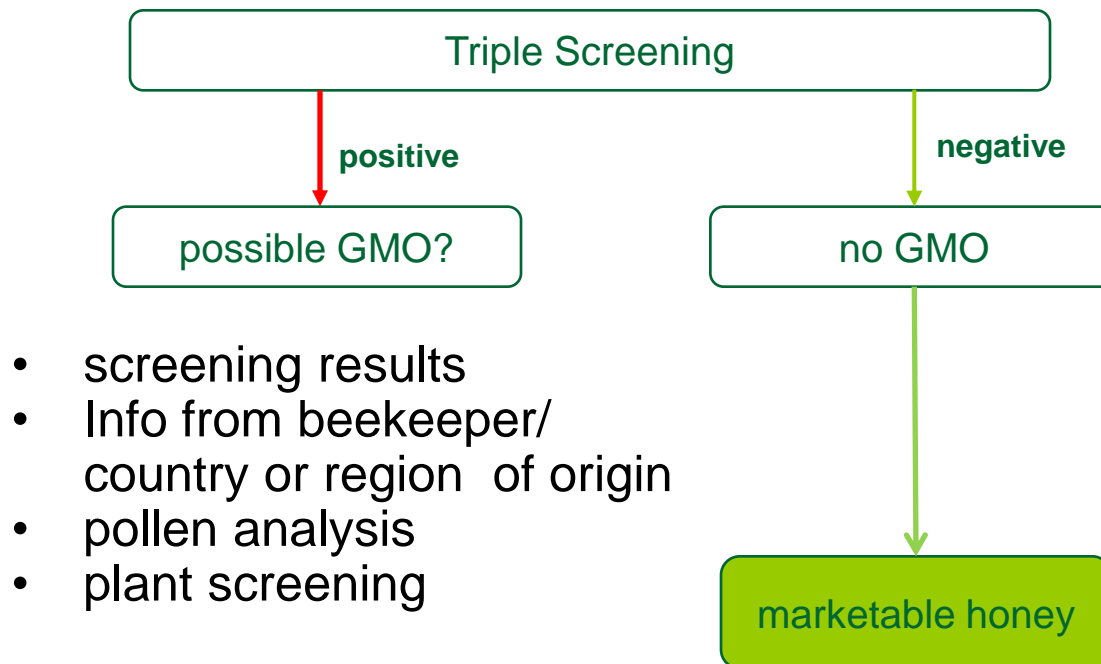


Detection of GMO in Honey

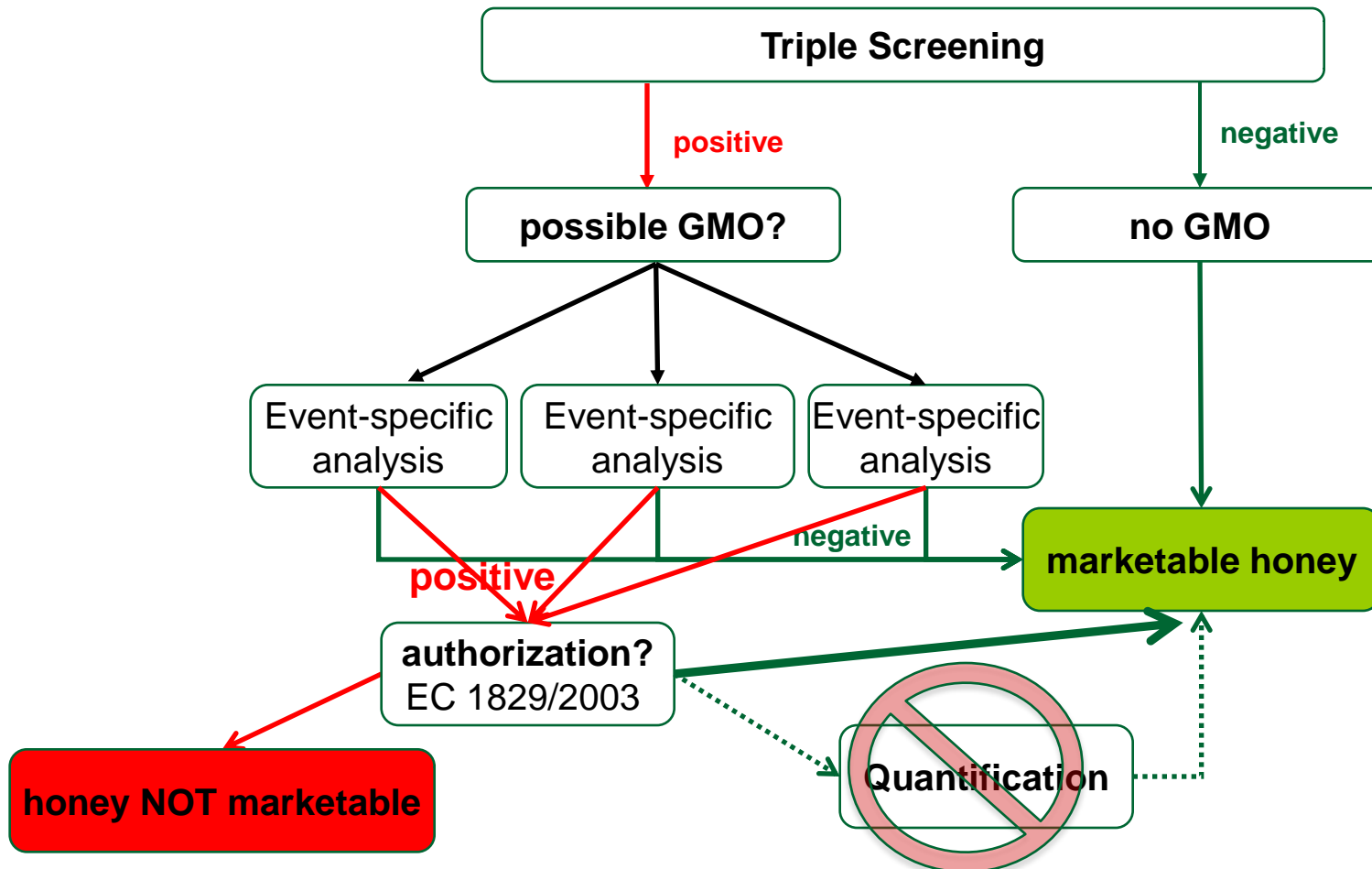
GMO event-specific detection

- 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



Strategy for Determination/Identification





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





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Bee Pharmaceuticals



Bee Pharmaceuticals

Problem

- Synthetic acaricides in beeswax, propolis and honey

Source of contamination

- Varroa control with synthetic acaricides



Varroa mite

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:

Problem

Substance

Bee Pharmaceuticals

licensed products, non-licensed products

Bee repellents

Phenol, Butyric Acid

Moth repellents

Dichlorobenzene, Naphtalene

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

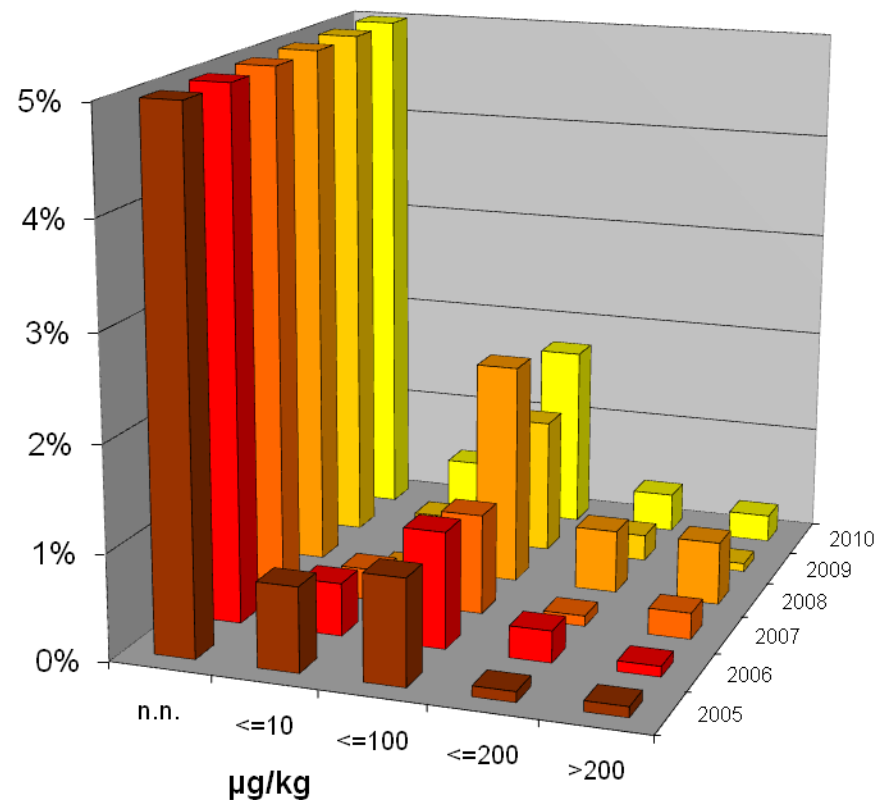


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Antibiotics – Statistics

Overview 2005 to 2010

Streptomycine - all origins

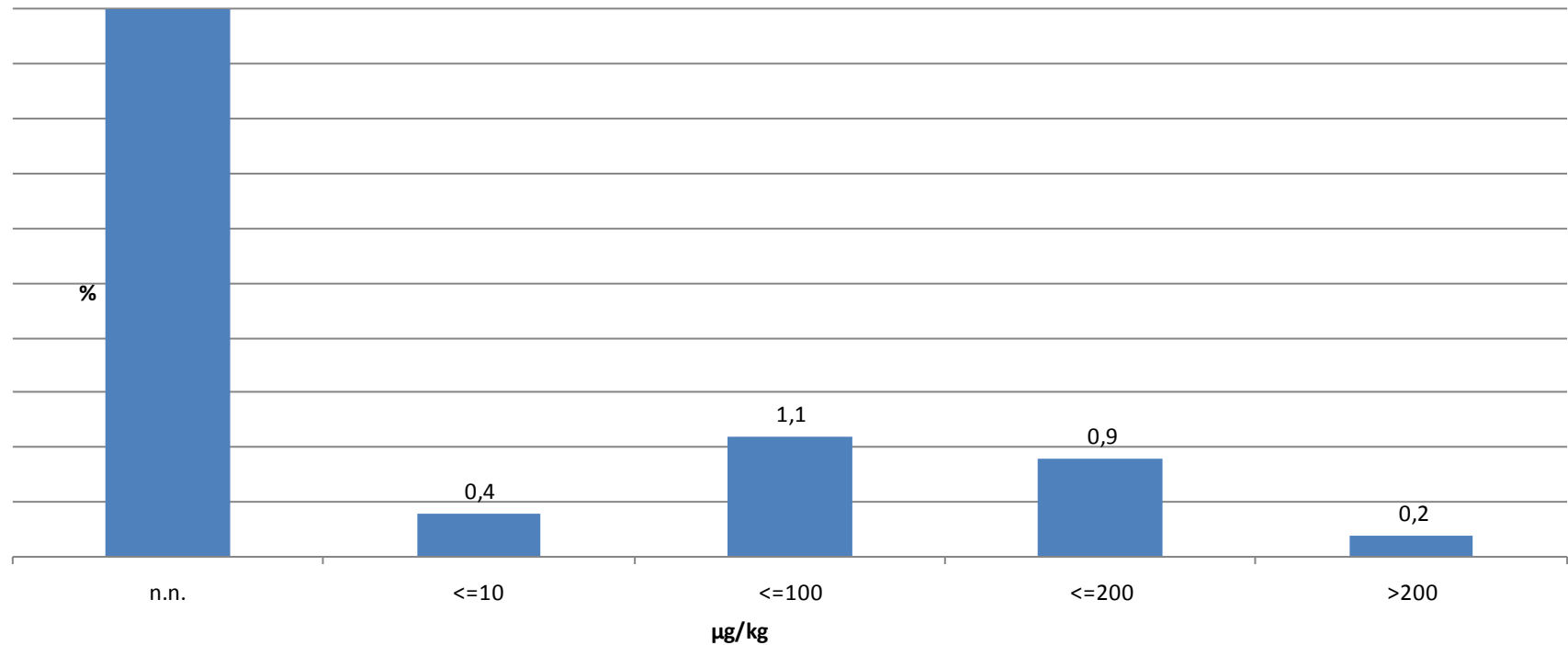




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Antibiotics – Statistics

Streptomycine - Cuba (2011 to 3/2012)



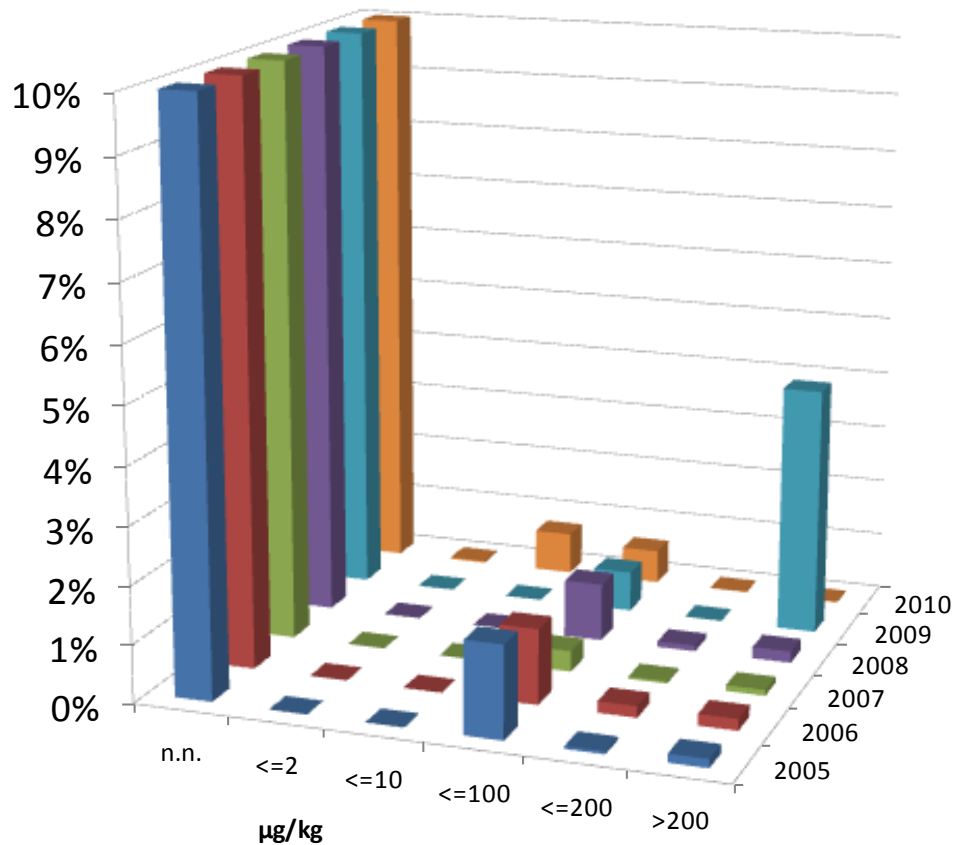


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Antibiotics – Statistics

Overview 2005 to 2010

Tetracycline - all origins

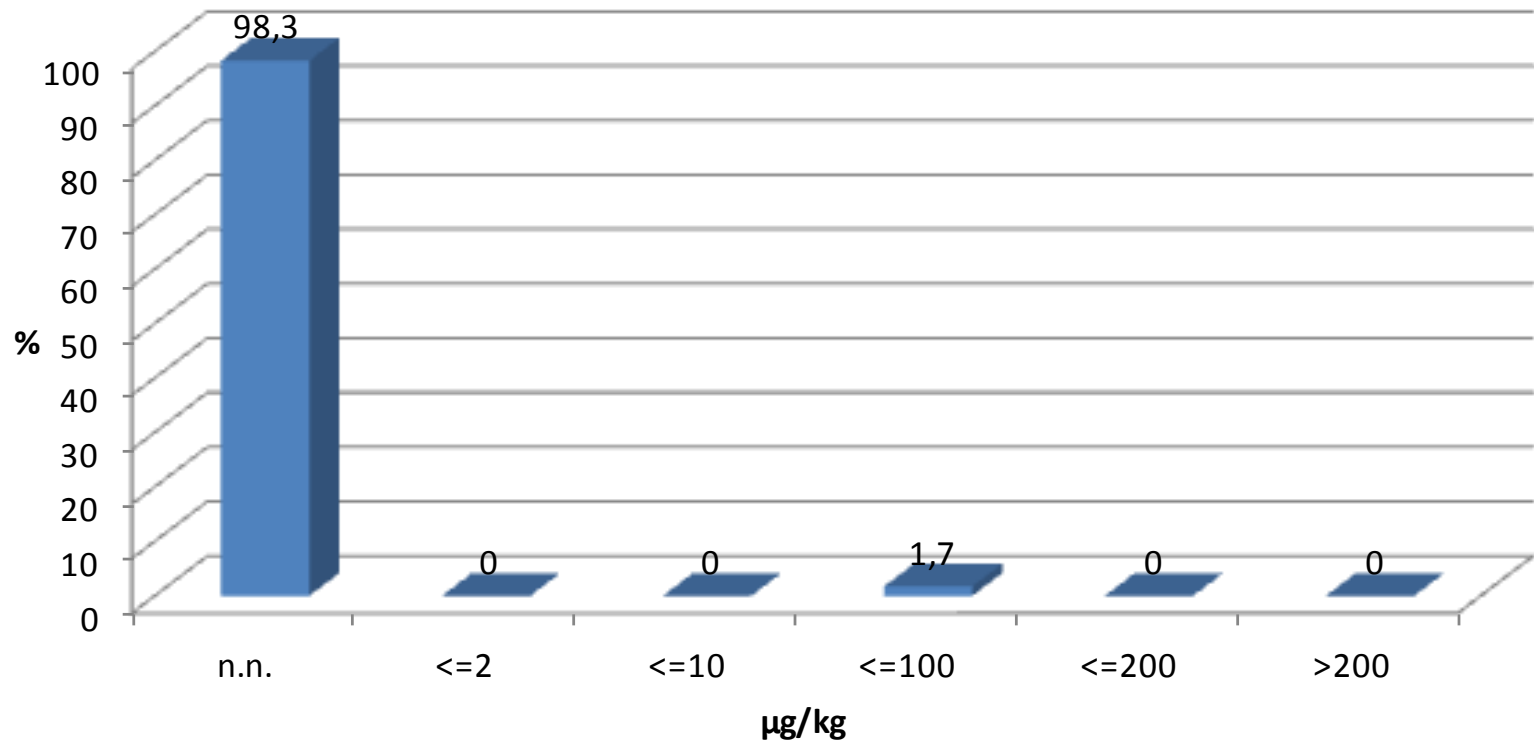




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Antibiotics – Statistics

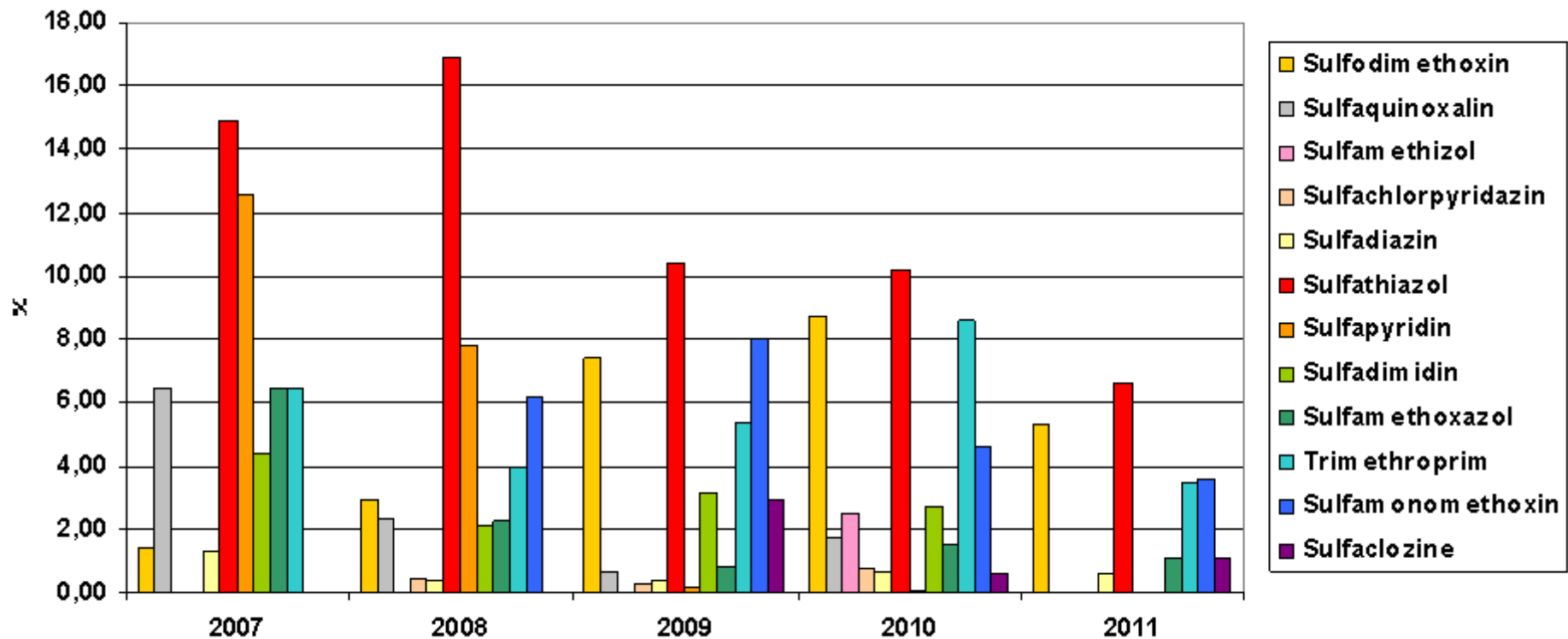
Tetracycline - Cuba (2011 to 3/2012)





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Sulfonamides - Distribution of positive findings



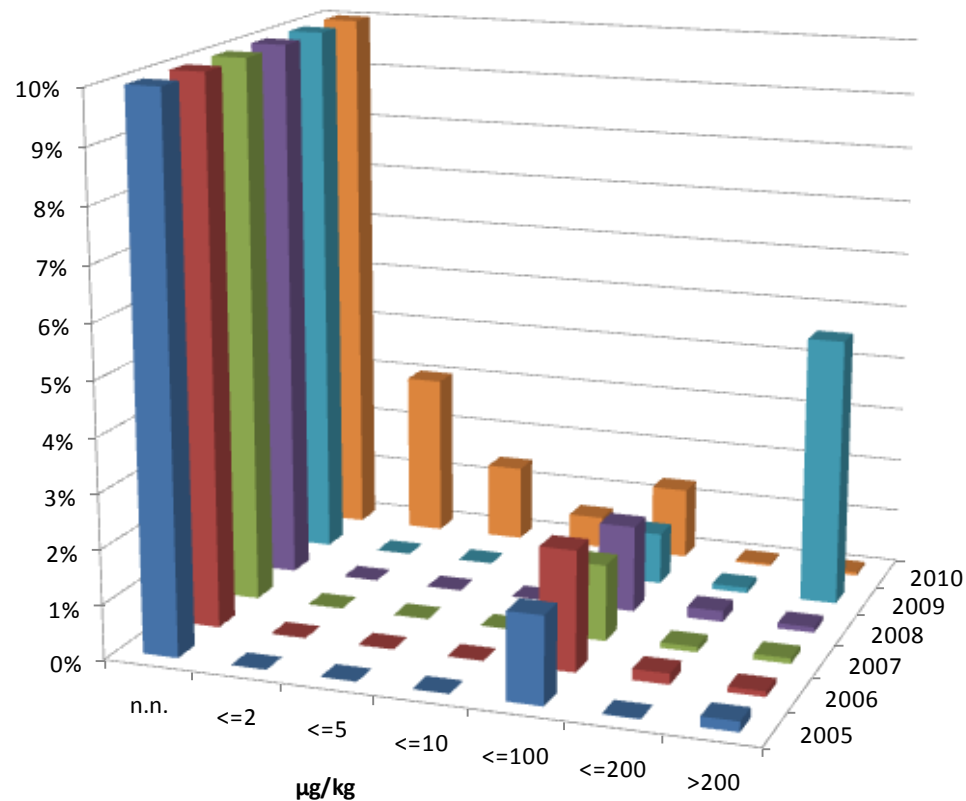


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Antibiotics – Statistics

Overview 2005 to 2010

Sulfathiazol - all origins

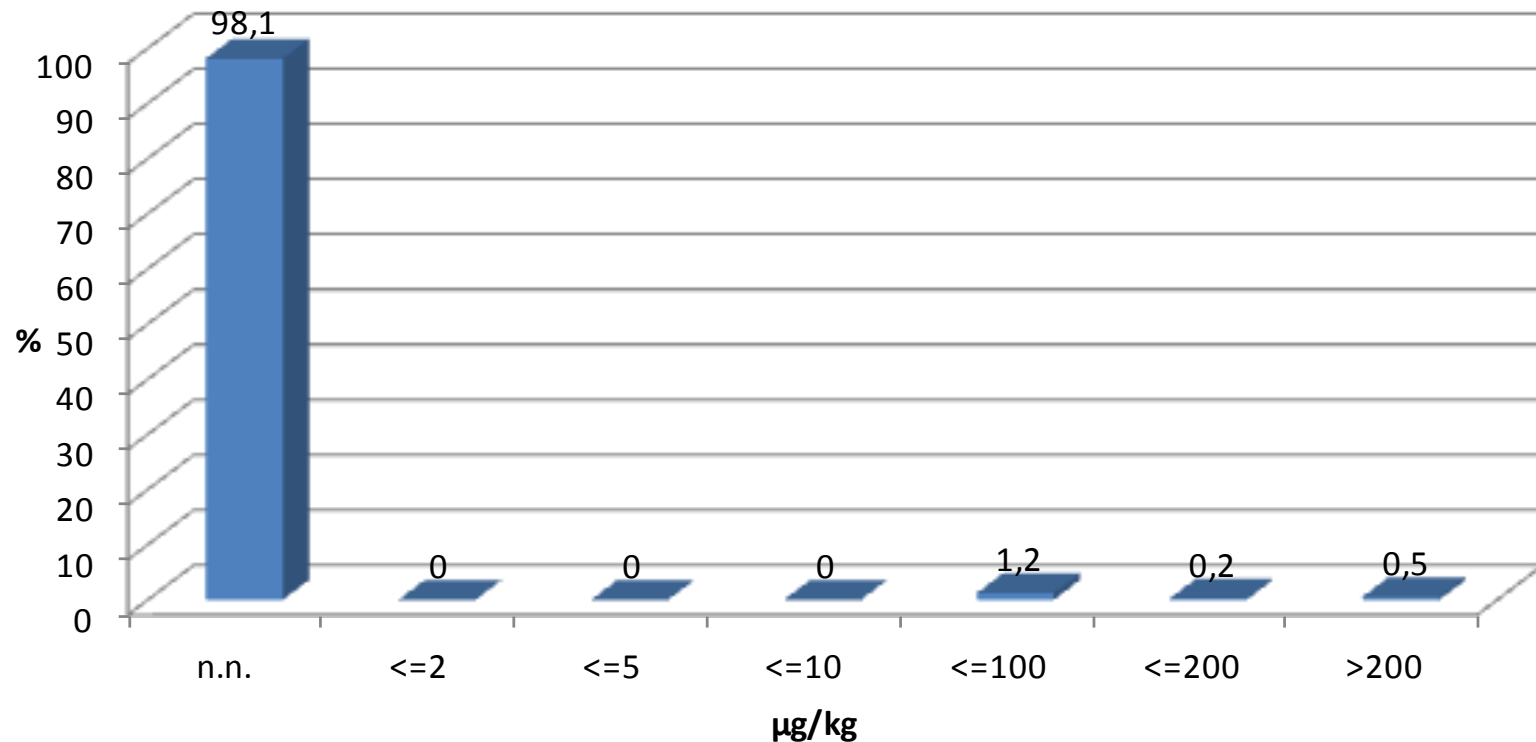




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Antibiotics – Statistics

Sulfonamides - Cuba (2011 to 3/2012)



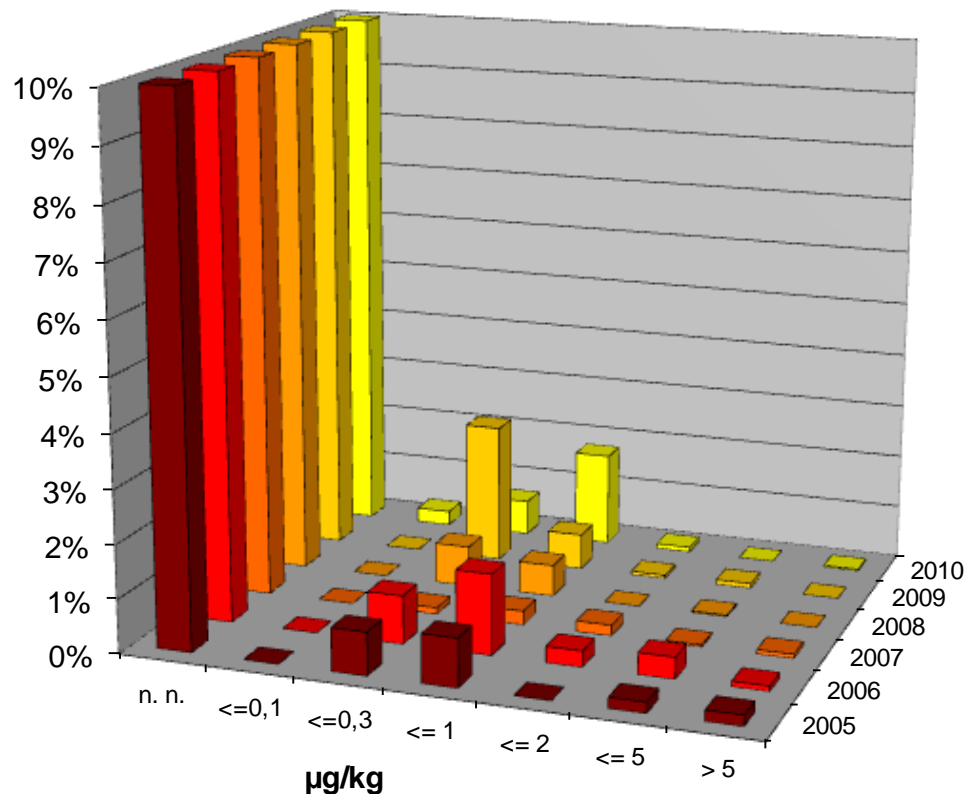


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Antibiotics – Statistics

Overview 2005 to 2010

Chloramphenicol - all origins



No positive
Chloramphenicol
samples from
Cuba for 2011 to
3/2012

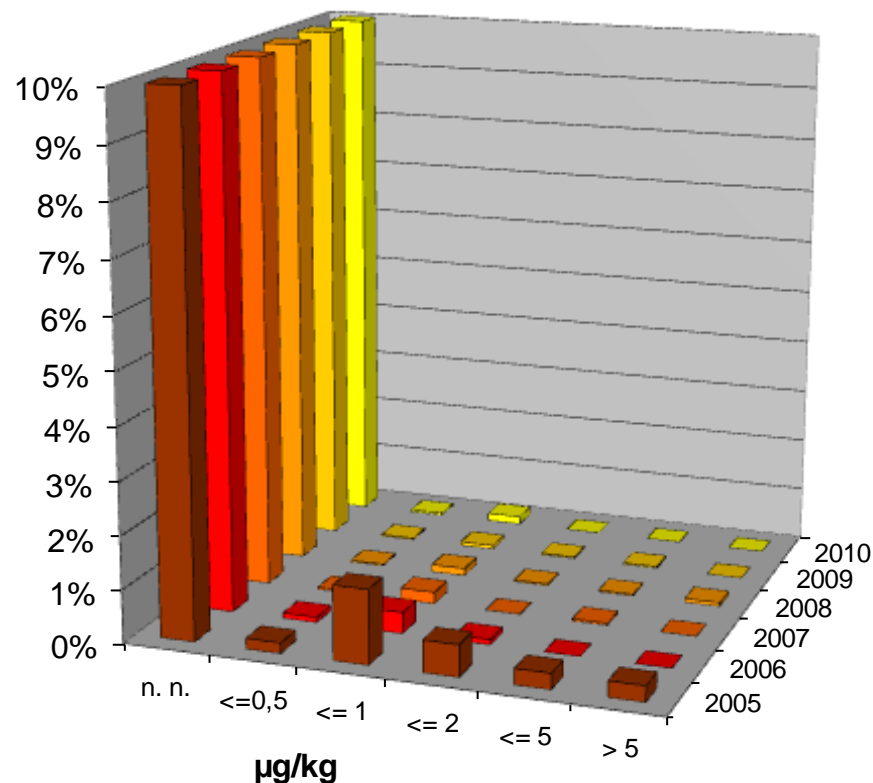


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Antibiotics – Statistics

Overview 2005 to 2010

Nitrofuran-Semicarbazid - all origins



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



New Antibiotics

However:

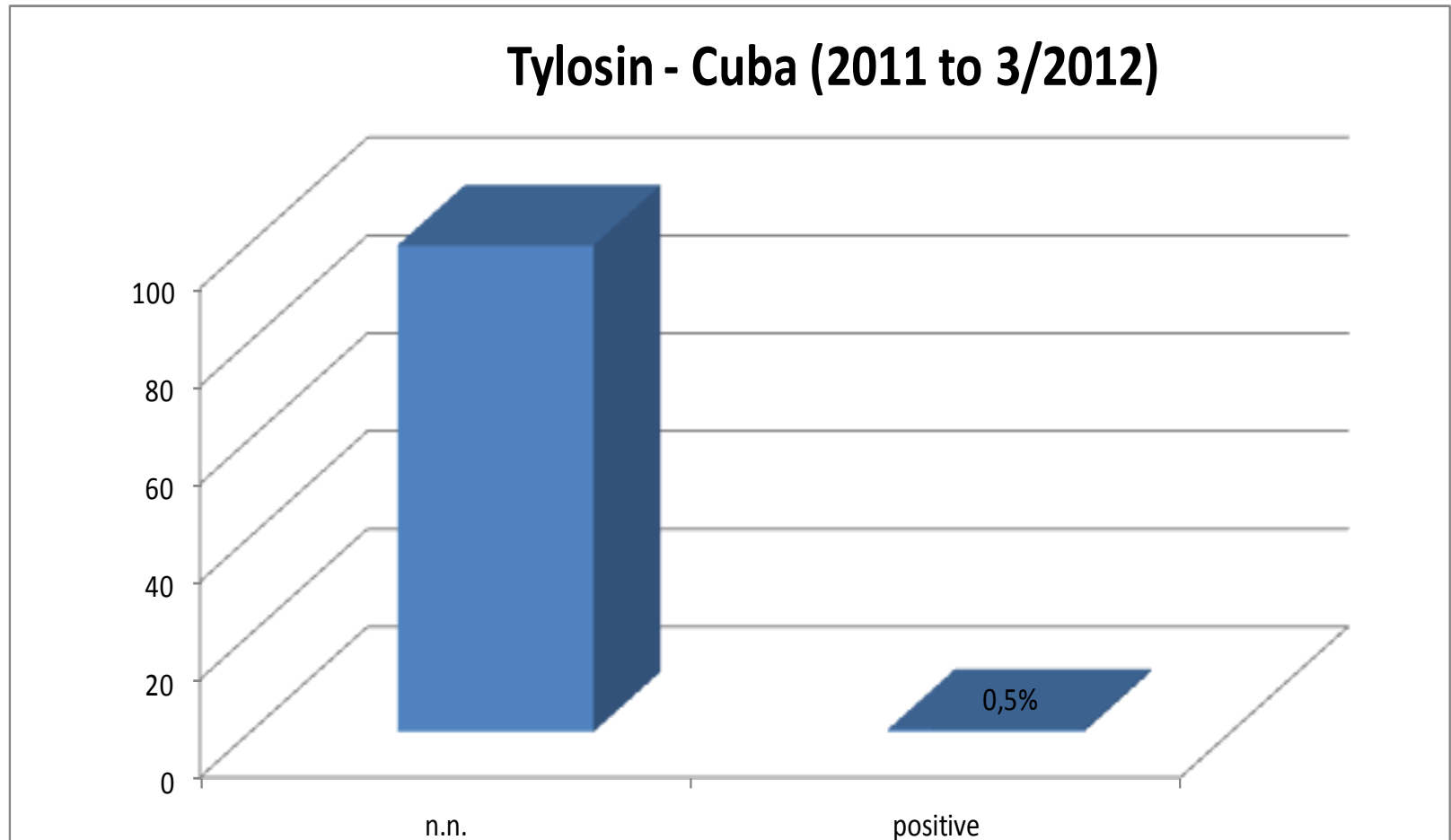
During the last years, more and more antibiotics were found in honey samples besides the classic ones

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 -Lactam antibiotics and Penicillins
- Nitroimidazole e.g.: Metronidazole



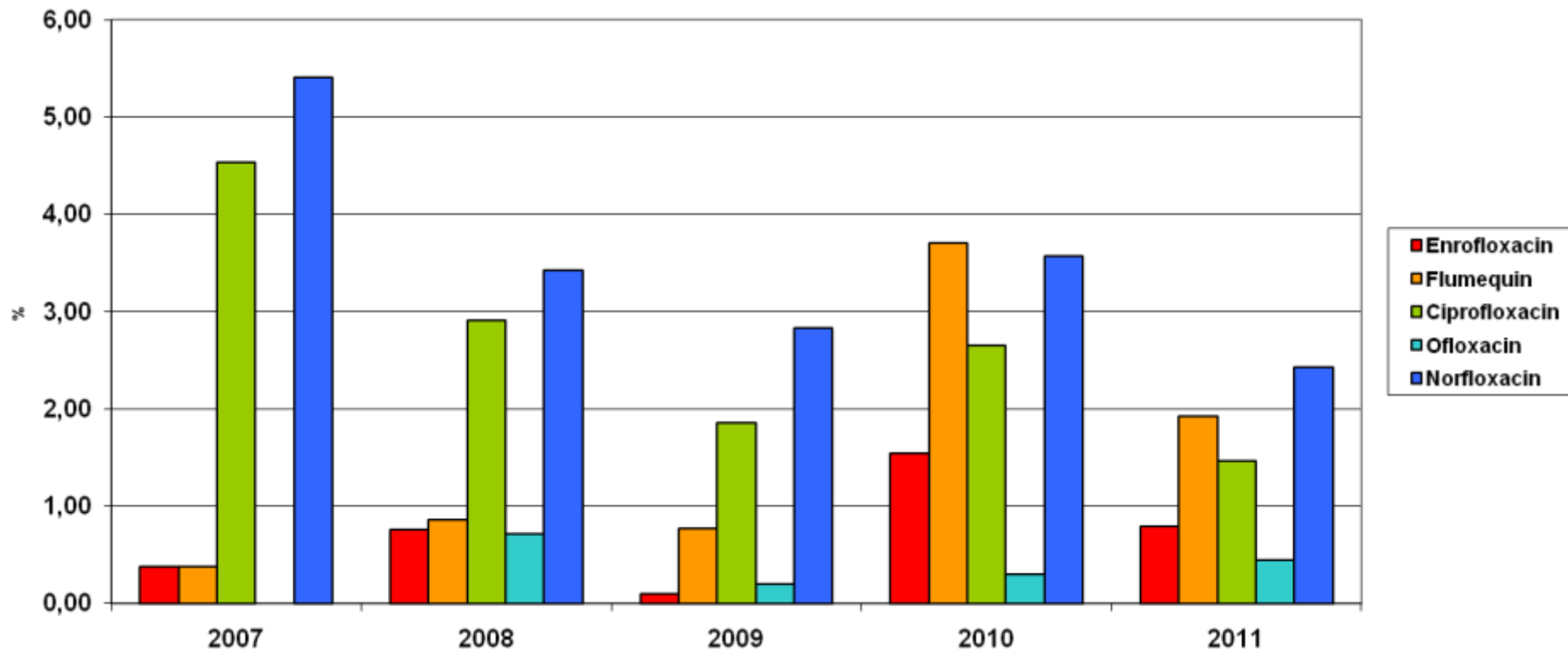
Antibiotics – Statistics: Tylosin





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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





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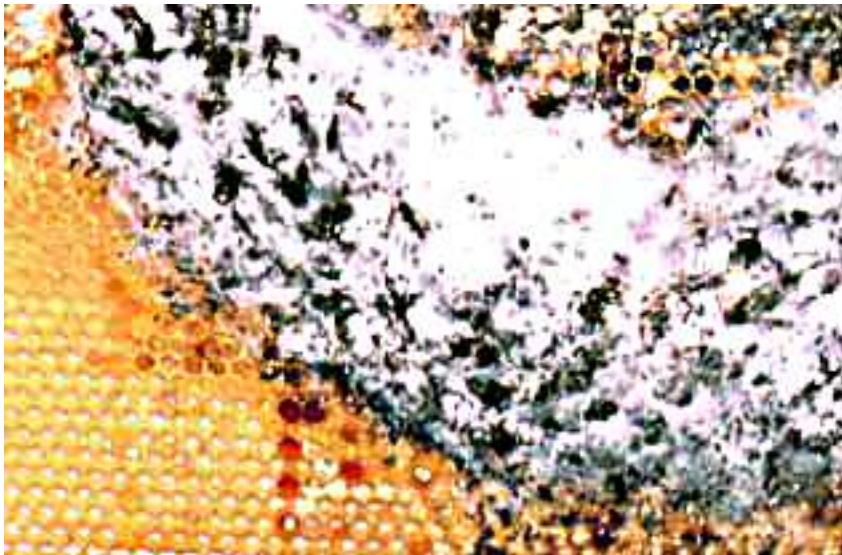
Bee Repellents

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



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Thank you for your attention!

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