Alkaloids in food and feed: an "*emerging risk*" ?

Hans van Egmond & Hester van den Top, 22 April 2010

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De Telegraaf

Paniek in Duitsland om rucola

HANNOVER - Duitse supermarkten halen rucola uit de schappen omdat tussen de bladeren het giftige klein kruiskruid kan zitten. De discounter Plus maakte woensdag bekend uit voorzorg de slasoort voorlopig nergens meer te verkopen. Plus reageerde op een waarschuwing van een Duitse wetenschapper.

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Likken van kop hallucinerende pad kan levensgevaarlijk zijn

In Leeuwarden ontstond vrijdag enige opwinding toen bleek dat drie Zuid-Amerikaanse padden uit een dierenwinkel gestolen waren. De padden zijn populair bij drugsverslaafde paddenlikkers omdat ze hallucinerende stoffen uitscheiden, maar kunnen dodelijk zijn.

Eigenaar Richard Mastenbroek zoekt de daders dan ook bij de naastgelegen dagopvang voor drugswerslaafden.

De gestolen dieren hebben op de kop klieren die een melkachtig slijm kunnen afscheiden, dat het giftige bufotoxine bevat.

De stof veroorzaakt bij mensen hallucinaties die zeven tot acht uur kunnen duren. Bij gebruikers treedt een effect op dat vergelijkbaar is met een lsd-trip. De padslijm simpelweg op te likken. Het slijm wordt echter ook wel gedroogd en vervolgens gerookt.

Gemakkelijk verkrijgbaar

De reuzenpad, Bufo marinus, is afkomstig uit de tropische gebieden van Zuid-Amerika en komt ook voor in het zuiden van Texas, Mexico en Midden-Amerika. De dieren zijn licht- tot donkerbruin en hebben een wrattige huid. De pad werd in veel tropische gebieden ingevoerd om plagen van insecten te bestrijden. De pad komt ook voor op de Nederlandse Antillen en is daarom in Nederland vrij gemakkelijk verkrijgbaar.

Hoewel directielid Peter Brouwer van daklozendagopvang in Leeuwarden niet uitsluit dat de

De Bufo marinus-pad.

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Alkaloids:

- Naturally occurring chemical compounds containing basic nitrogen atoms
- Produced by various organisms, mainly plants
- Often physiologically active and poisonous
- Over 3000 compounds known, including atropine, caffeine, solanine, nicotine, cocaine

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- EFSA opinions and EU legislation
- PA in food: the Afghanistan drama
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- EU - FP7 project CON*ff*IDENCE
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efsa European Food Safety Authority

Opinions and limits

- Ergot alkaloids
  - EFSA opinion for feed, 2005
  - EU limits: 0.05 % for ergot in intervention wheat; 0.1% for feedstuffs containing unground cereals
- Pyrrolizidine and tropane alkaloids
  - EFSA opinions for feed, 2007 and 2008
  - EU legislation in feed: ranges from 0.01-0.3 % for botanical impurities (weed seeds and unground and uncrushed fruits containing alkaloids)
  - EU limits: not (yet) developed

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Ergot alkaloids

- Mycotoxins, formed by *Clavicipitaceae*
- Ergot occurs in grasses, grains, sorghum
- Adverse effects: convulsions, gangrene
- Animal sensitivity: poisoning outbreaks in livestock
- Methods of analysis: LC-FLD and LC-MS/MS, but not (yet) interlaboratory validated
- Limited data on carry-over do not point at animal products as an important source of exposure

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Tropane alkaloids

- Plant toxins, formed primarily by *Solanaceae*
- Seeds may contaminate plants, e.g. soybean
- Humans: accidental exposure rare
- Animals: pigs very sensitive (*Datura* poisoning)
- Methods of analysis: HPLC, GC, RIA, CE-MS, LC-MS/MS, but not (yet) interlaboratory validated
- No info on carry-over, traces of scopolamine found in eggs, no further data on residues

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Pyrrolizidine alkaloids

- Toxins formed in common plants, e.g. *Senecio* spp.
- These plants may contaminate food or feed
- Re-intro of certain species in nature may increase intoxications in wildlife and grazing animals
- Adverse effects in humans and livestock
- Methods of analysis: GC-MS, LC-MS/MS, but not (yet) interlaboratory validated
- Old data on carry-over point at low amounts of alkaloids in milk, significance unclear

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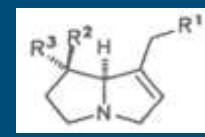
Pyrrolizidine alkaloids (PA)

- Increased interest for PA (EU workshop held)
- Occurring in 3% of world's plant species
  - protect plants against herbivores
- More than 350 different components:
  - liver toxic
  - liver carcinogenic
  - mutagenic
  - treatment not well defined



- Intake limit (RIVM): 30 ng/day (carc. riddelliine)
- Acute clinical symptoms (WHO): 1 mg/day

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Outbreak of liver disease in Hirat district

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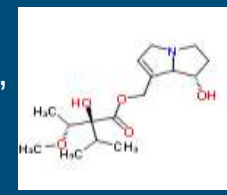
Hepatic veno-occlusive disease in history

- "Bread poisoning" in South Africa in 1920
- "Camel belly" in Uzbekistan, 1931 and 1945
- "Gulran disease" in Afghanistan, 1974 and 1999
- "Gulran disease" was attributed to consumption of bread, made from wheat contaminated with charmac
- Charmac *(Heliotropium* sp.*)* contains pyrrolizidine alkaloids (PA), primarily heliotrine



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Gulran district

- Approx. 150 km², approx. 110.000 inhabitants

- Remote villages, undulating hills, scanty vegetation, serving as pasture lands
- Inhabitants mostly wheat farmers who may keep sheep and goats

- Diet consists mainly of wheat bread, occasionally meat

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Outbreak of liver disease in Hirat district

- More than 270 people affected
- Approx. 50 people died
- WHO alarmed, and RIVM contracted to investigate the cause of the disease
- Hypothesis: PA poisoning
- Samples sent to RIVM
- Methodology: LC-MS/MS

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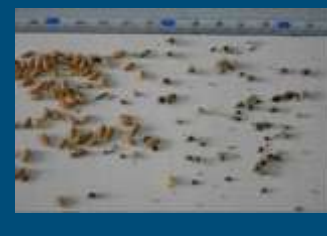
Suspect: local weed (charmac)



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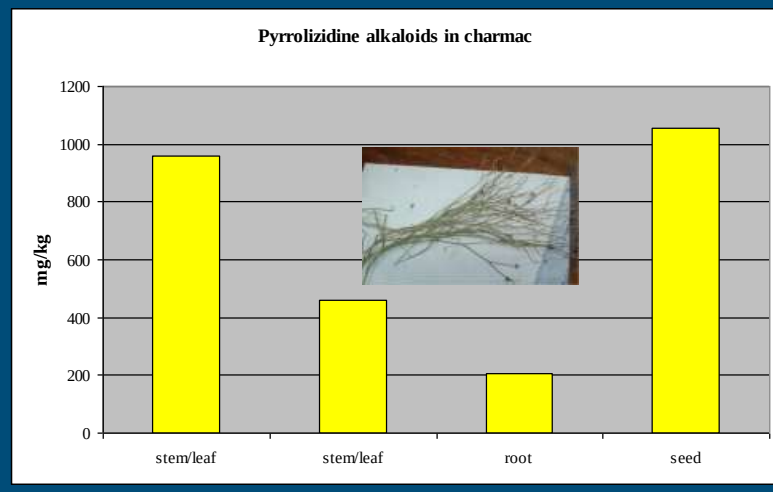
Results: charmac



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Investigated materials

Weed

Flour

Qurut

Milk

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Not all samples well-packed

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Not all samples well-packed

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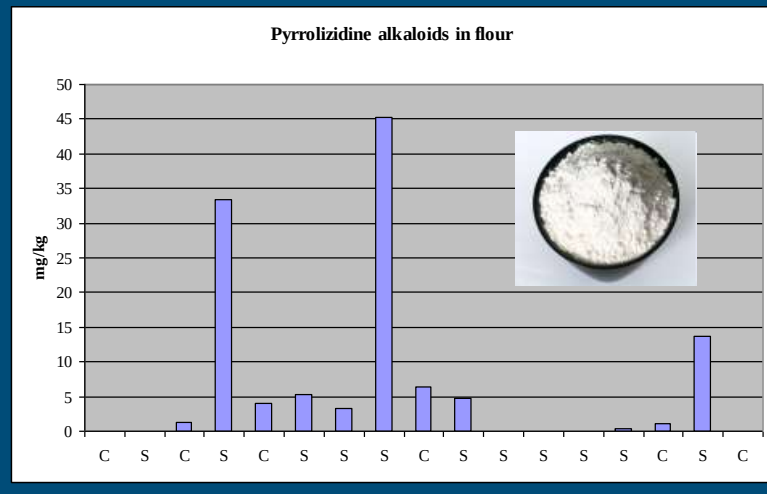
Results: wheat flour



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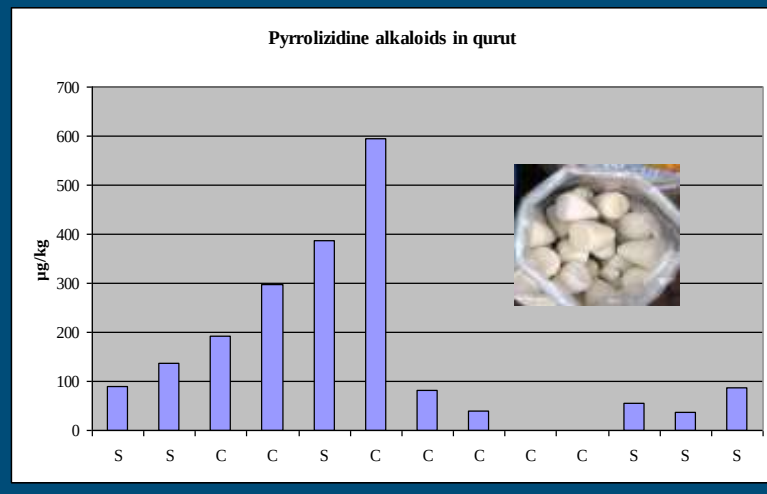
Results: qurut



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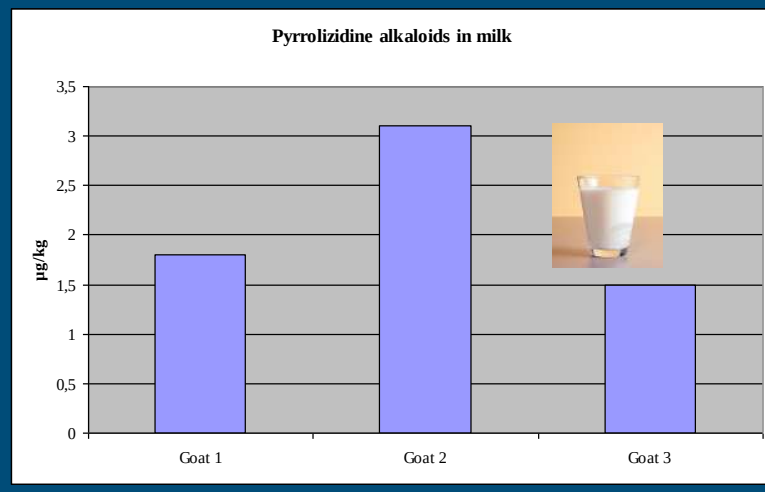
Results: milk



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Conclusions of the investigations

- High concentrations of PA found in charmac
- Same PA were found in wheat flour
- In dairy products PA were also found; qurut contained *trichodesmine*, not present in charmac
- Prolonged, regular exposure to contaminated wheat, in combination with low protein diet, likely to be the cause of the disease
- Publication about the Hirat poisoning episode in press in *Journal of Toxicology*

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Pilot carry-over study ragwort

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Background

- *Senecio jacobeae:* PA containing weed, occurring as impurities in hay
- Other possible contaminants: *S.vulgaris, S. inaequidens*
- Consumption by dairy cattle could lead to PA into milk
- Very limited data exist about carry-over → experiment

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Carry-over experiment: collection of material

- Ragwort (*Senecio jacobaea*)
  - Full grown plants near Wageningen
  - Period: June-July 2008
  - Complete plants air-dried and homogenised
  - 12.5 kg
- Narrow-leaved ragwort (*Senecio inaequidens*)
  - Ditto, Ede (3 places)
  - 2.5 kg
- Materials of both samples mixed

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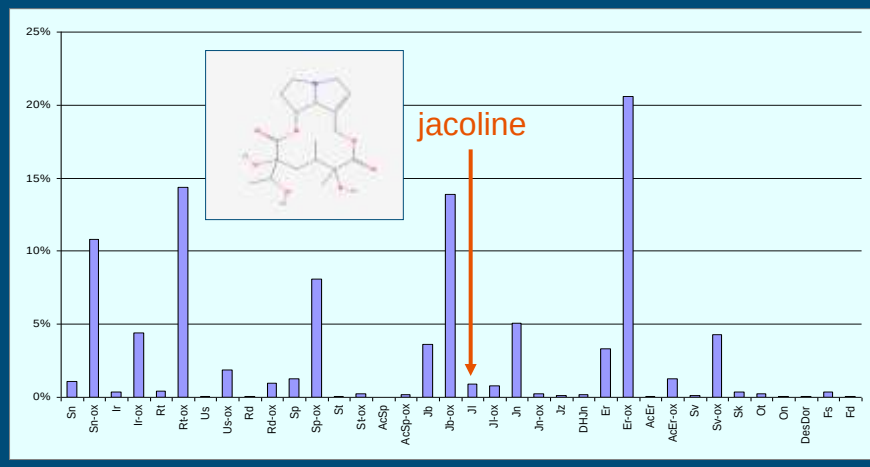
Composition feeding material (RIKILT)



Total: 2.34 mg/g (82% N-oxides; 17% tertiary bases)

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Pilot carry-over study: experimental set-up

- Waaiboer farm (ASG, Lelystad)
  - 3 cows (age: > 2 jr), weight: 600-700 kg
  - exposure: September-October 2008
  - dosing of ragwort via fistula, directly after milking
- Exposure periode: 5 weeks
  - Week 1: clean feed
  - Week 2: 25 g, twice daily
  - Week 3: 50 g, twice daily
  - Week 4: 100 g, twice daily
  - Week 5: clean feed (“wash out”)
- Sampling scheme: milk, twice daily, 2 x 40 ml

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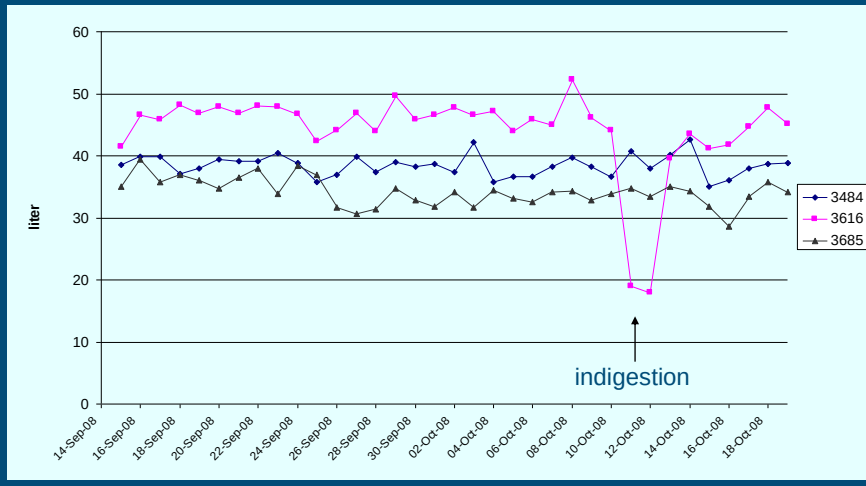
Results: daily milk production (ASG)



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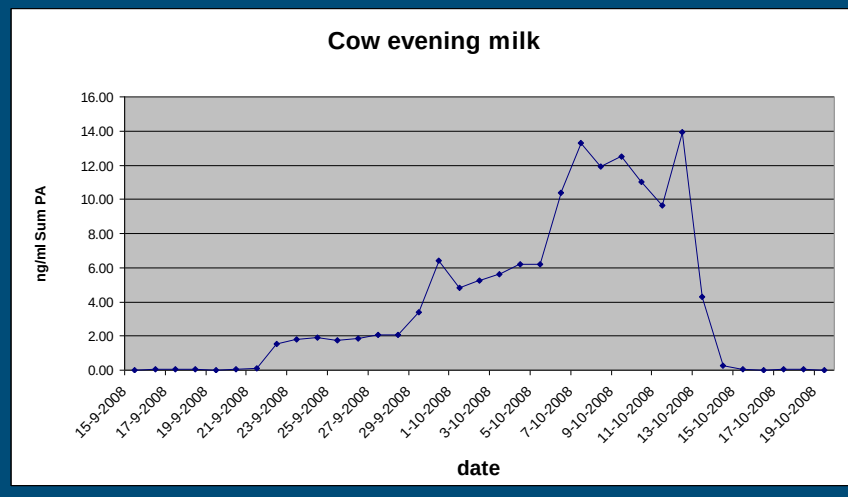
Results: total PA in milk (RIVM)



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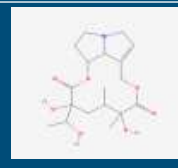
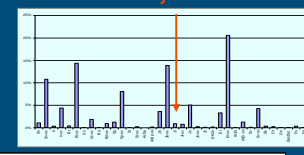
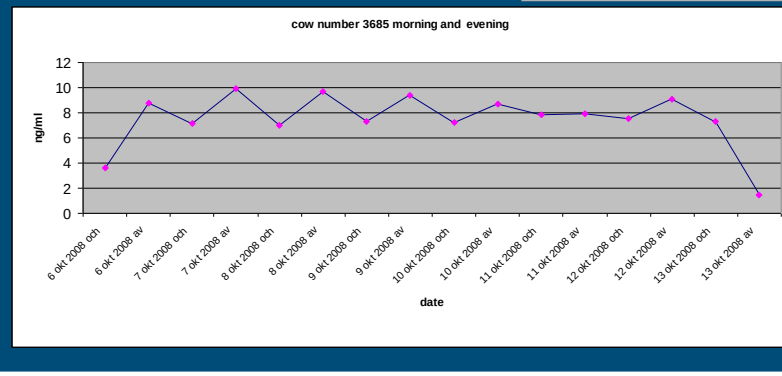
Jacoline in milk (RIVM)







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Conclusions carry-over experiment

- Succesful transfer experiment
- Milk: only tertiairy bases found, no N-oxides
- Senkirkine, jacoline, otosenine, jaconine, florosenine and jacobine found
- Jacoline: 81 % of PA in milk, only 1% in weed!
- Jacoline transfer: 6-8 % of ingested mount

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Results: risk assessment (RIVM)

- Human intake limit: 30 ng/day
- Daily milk consumption: 1 l
- Allowed milk concentration: 0.03 ng/ml
- Jacoline →milk transfer factor: 0.0021
- Allowed jacoline intake of cow: 14.3 µg
- Jacoline concentration in weed: 21.8 µg/g
- Allowed daily weed intake of cow: 0.66 g/day
- Assumptions:
  - ridelliine intake limit applies to jacoline as well
  - jacoline is stable upon pasteurisation

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Further investigations

- Second carry-over study to confirm findings
- Better insight in mass balance needed
- Production of several dairy products planned
- Distribution and stability of PA in dairy products
- Analysis of commercial milk products planned
- Surveys of animal feeds recommended

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About CON*ff*IDENCE

- *LCP* Food, Agriculture and Fisheries, and Biotechnology, 2008-2012
- Simple, fast, multi-analyte, multi-class detection
- Training workshops and educating modules
- Includes WP Biotoxins
  - alkaloids (pyrrolizidine, tropane, ergot)
  - *Fusarium* mycotoxins
  - phycotoxins

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The alkaloids work package

- Alkaloids
  - PA in honey and feed
  - TA in feed
  - EA in feed and cereals
- Methods
  - Multiplex dipsticks
  - NIR (in line)
- Status
  - Prototypes in development

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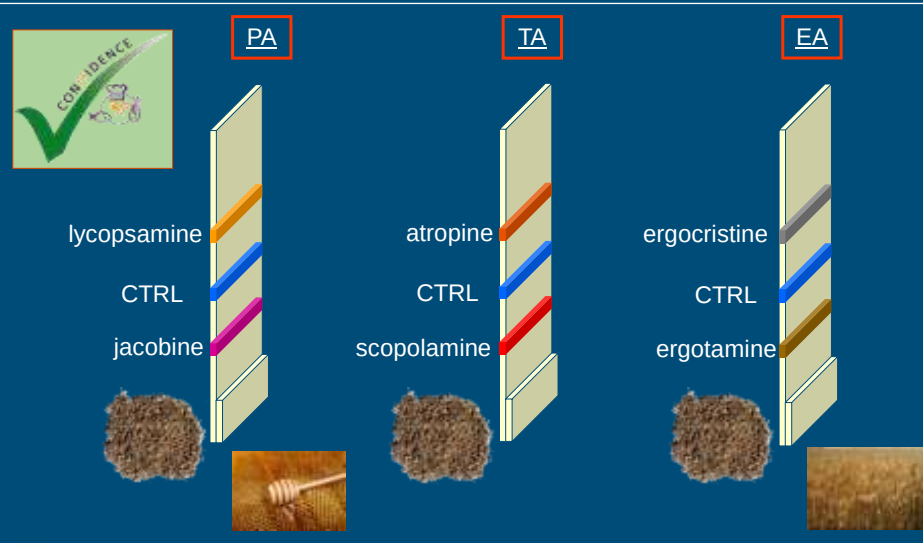
Multiplex dipsticks for PA, TA and EA alkaloids



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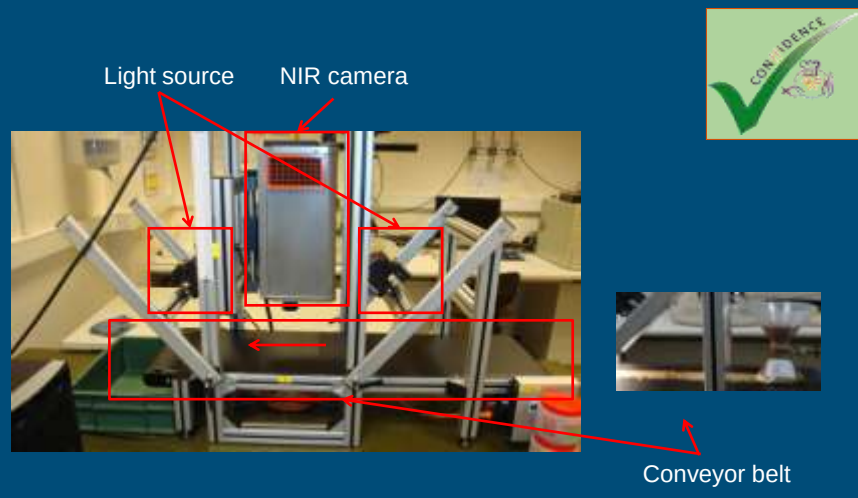
NIR imaging method to detect ergot



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Summary and conclusions

- Many alkaloids exist, particularly in plants
- Alkaloid poisonings: recurring events
- EFSA opinions: limits better set for toxins; methods of analysis to be validated; RMs to be developed; data on carry-over to animal products scarce
- Small amounts of PA in feed could lead to unacceptable amounts in milk; needs confirmation
- CON*ff*IDENCE: a project contributing to the development of rapid tests for alkaloids

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Alkaloids in food and feed:

an "*emerging risk*"

With thanks to Ron Hoogenboom, Patrick Mulder, Gerrit Remmelink, Ronald Schothorst, Marco Zeilmaker and .... the audience

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