Contaminants from natural and food processing sources

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Background

In the last few years an increased incidence of low levels of banned substances or their metabolites in samples taken in the framework of NRCP.

Following thorough investigations these findings could not be explained by illegal drug treatments.

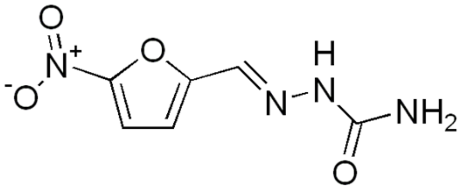
Information about research projects to investigate natural or process related occurrence of 3 substances will be presented as examples :

- Semicarbazide
- Chloramphenicol
- Thiouracil

Semicarbazide

Semicarbazide (SM) is a metabolite of the nitrofurane **Nitrofurazone** (5-nitro-2-furaldehyde-semicarbazone), used as indicator to detect the use of nitrofurazone in food-producing animals

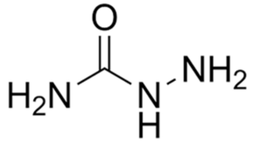
Parent compound



Nitrofurazone

Metabolite,

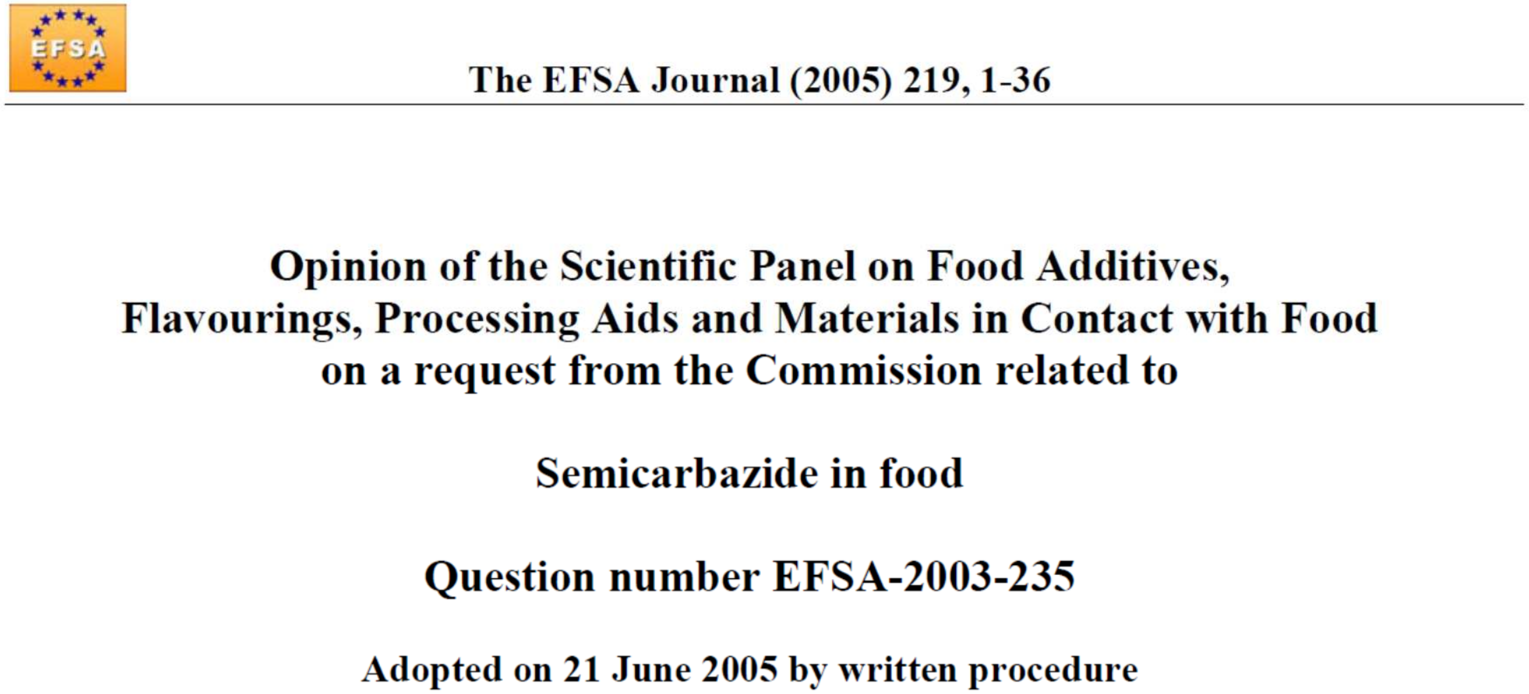
covalent bound to the matrix



Semicarbazide (SEM)

- due to their carcinogenic properties the use of nitrofurans is prohibited within the EU in animal breeding
  (*Commission Regulation 37/2010*)
- to harmonise the detection of semicarbazide a Minimum Required Performance Limit (MRPL) of 1 µg/kg is set within the EU
  (*Commission Decision 2003/181*)

Semicarbazide



The EFSA Journal (2005) 219, 1-36

Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food on a request from the Commission related to

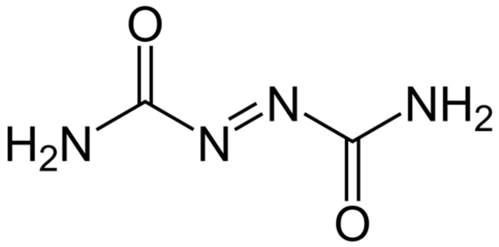
Semicarbazide in food

Question number EFSA-2003-235

Adopted on 21 June 2005 by written procedure

Semicarbazide

Semicarbazide as by-product of Azodicarbonamide



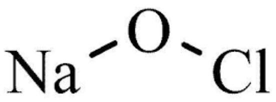
- Azodicarbonamide used for the production of foamed lining in seals
  (forbidden in the EU since August 2005)
  → migration of semicarbazide into food can occur (e.g. baby food)

- Azodicarbonamide may be used as an flour treatment agent (permitted in the US and Canada, banned in Australia and the EU!)

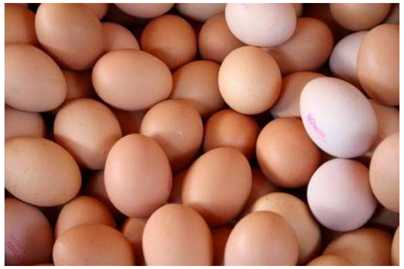
Semicarbazide

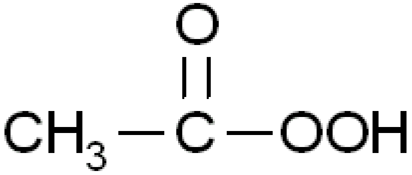
Semicarbazide as reaction product during food processing

- Food and food products subjected to hypochlorite treatment showed formation of SEM depending on the concentration of active chlorine; e.g. carrageenan, gelatine
- Heat treatment during the production of egg powder or whey can cause formation of SEM
- Hypochlorite or per-acetic acid used for disinfection of equipment and surfaces without sufficient subsequent rinsing



Sodium hypochlorite





$$CH_3-\overset{\overset{O}{\|}}{C}-OOH$$

Per-acetic acid

Semicarbazide

> “The Panel therefore concluded that the issue of carcinogenicity is not of concern for human health at the concentrations of SEM encountered in food.”

EFSA Journal (2005) 219, 1-36

➡ there is the need for another marker substance as is not an unambiguous marker for the misuse of nitrofurazone and it has no adverse effects in food

Semicarbazide

October 2010:

in a honey sample we found
1,38 µg/kg Semicarbazide

- ➡ 2 samples of foamed lining in seals analysed:

  135 µg/kg Semicarbazide
  3083 µg/kg Semicarbazide

- ➡ the cause of contamination was migration

Semicarbazide

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INVESTIGATION INTO THE POSSIBLE NATURAL OCCURRENCE OF SEMICARBAZIDE IN *MACROBRACHIUM ROSENBERGII* PRAWNS.

C. Van Poucke[1], C. Detavernier[1], M. Wille[2], J. Kwakman[3], P. Sorgeloos[2] and **C. Van Peteghem**[1*]

[1]: Ghent University, Department of Bioanalysis, Laboratory of Food Analysis, Ghent, Belgium.

[2]: Ghent University, Laboratory of Aquaculture & Artemia Reference Center, Ghent, Belgium

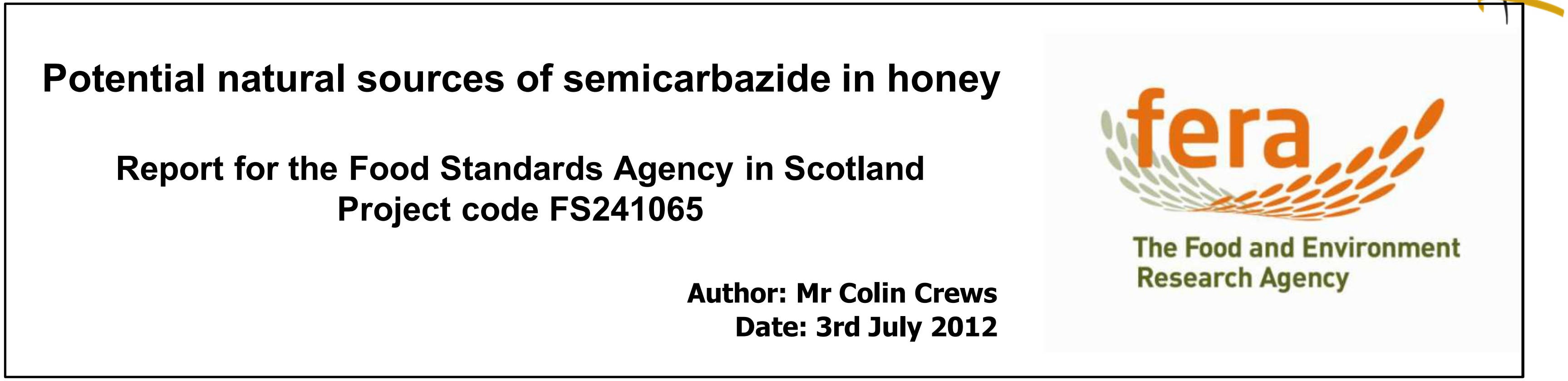
[3]: Seafood Importers & Producers Alliance (SIPA), Kontich, Belgium

Presented at the 6th International symposium on Hormone and Veterinary Drug Residue Analysis, Ghent, Belgium, 1 – 4 June 2010

CONCLUSIONS:

- **SEM naturally occurs in *M. rosenbergii* prawns but at the current MRPL no tissue bound SEM in meat of non treated animals**
- **30 ppb tissue bound SEM in the shell of non treated prawns**
- After treatment with nitrofurazone the concentration of tissue bound SEM in the shell significantly increases
- Higher tissue bound SEM concentrations in meat than in shell after treatment with nitrofurazone
- Also in the shell of other crustacean species SEM could be detected above the MRPL. However because of the commercial origin of these samples no conclusions on the natural occurrence of SEM in the shell of these species can yet be made.

Semicarbazide



Potential natural sources of semicarbazide in honey

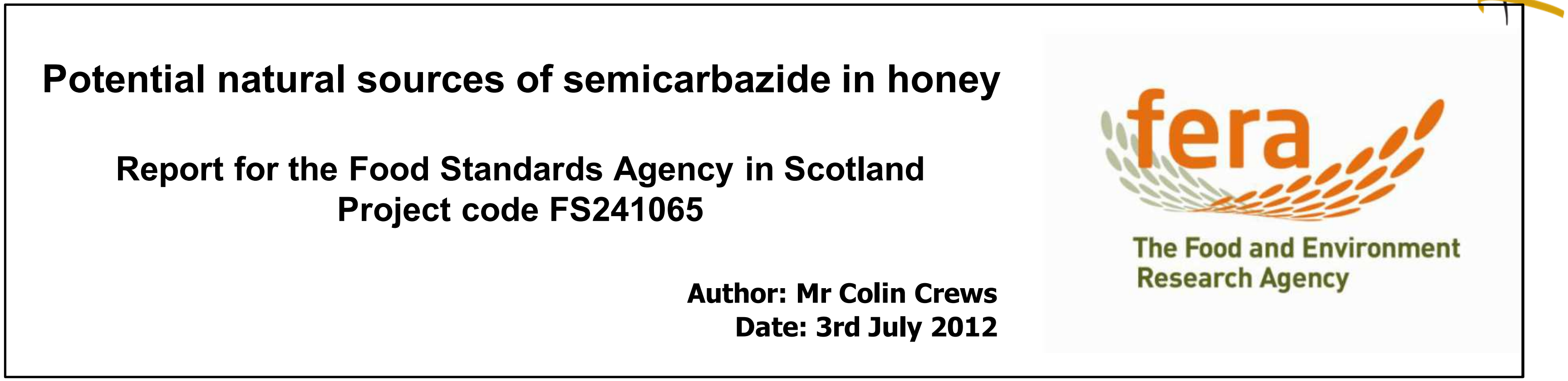
Report for the Food Standards Agency in Scotland
Project code FS241065

Author: Mr Colin Crews
Date: 3rd July 2012

in 2010 in samples of Scottish heather honey of good quality semicarbazide has been detected, with the confidence that it had not arisen from use of nitrofurazone

- only samples of heather honey showed the presence of SEM, with levels between 0,6 and 1,8 µg/kg
- other honeys, produced from the same hives at different times and/or locations, did not show SEM
- the consistent pattern of the occurrence of SEM in heather honey suggests possible botanical peculiarities with heather nectar

Semicarbazide



Potential natural sources of semicarbazide in honey

Report for the Food Standards Agency in Scotland
Project code FS241065

Author: Mr Colin Crews
Date: 3rd July 2012

The Food and Environment Research Agency

CONCLUSIONS:

- Arginine seems to be the most likely precursor for SEM
  - that assumption is supported by the fact that high levels of arginine occur in certain shellfish that have been shown to contain SEM naturally
  - the arginine content (free and protein-bound) of some plants increases rapidly at certain times of the year
  - bees probably digest proteins containing arginine
  - formation of SEM might be promoted by the higher temperature in the hives
- plants could be contaminated with sheep urine, containing purine derivatives urea and creatinine, known precursors of SEM
- other sources, e.g. using nitrofurans against foul brood or bleach like hypochlorite for disinfection, would be very unlike sources of SEM

Semicarbazide

Semicarbazide in milk powder

2010: Milk powder, produced in Austria, rejected by a baby food producer in Germany due to positive detection of Semicarbazide below MRPL (MRPL: 1 µg/kg)

➡ 131 samples of different stages of production analysed

Results:

samples	% SEM detected
whey	41,2%
whey (conc.)	82,1%
whey powder	89,4%

Semicarbazide

Semicarbazide in milk powder

Studies to elucidate the origin of SEM:

- analyses of
  milk from animals hold under controlled conditions, milked by hand
  without previous disinfection of the udder and
  milk milked with a milking machine
- experimental production of milk powder in cooperation with the University of Natural Resources and Life Sciences, Vienna

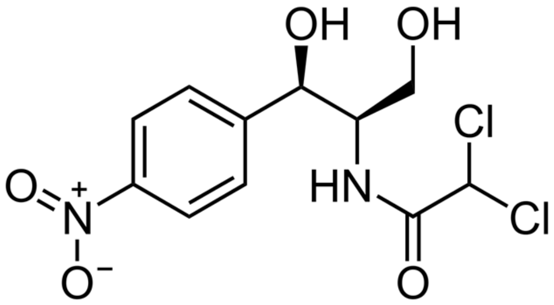
➡ independent of the way of milking concentrations of SEM < LOQ (0,01-0,04 µg/kg) detected in milk

➡ no evidence that disinfection with hypochlorite is a source of SEM

➡ higher levels of SEM in milk powder correspond with the factor of concentration

Chloramphenicol

Chloramphenicol (CAP) is a very effective a broad-spectrum antibiotic, historical veterinary uses in all major food-producing animals.
CAP can be biosynthesized by the soil organism *Streptomyces venezuelae*



- causes aplastic anemia and is suspected to be carcinogen and genotoxic, for that reason it is banned for use in food producing animals in the EU, USA, Canada, Australia, Japan, China and many other countries
(*Commission Regulation 37/2010*)
- to harmonise the detection a minimum required performance limit (MRPL) of 0,3 µg/kg is set within the EU
(*Commission Decision 2003/181*)

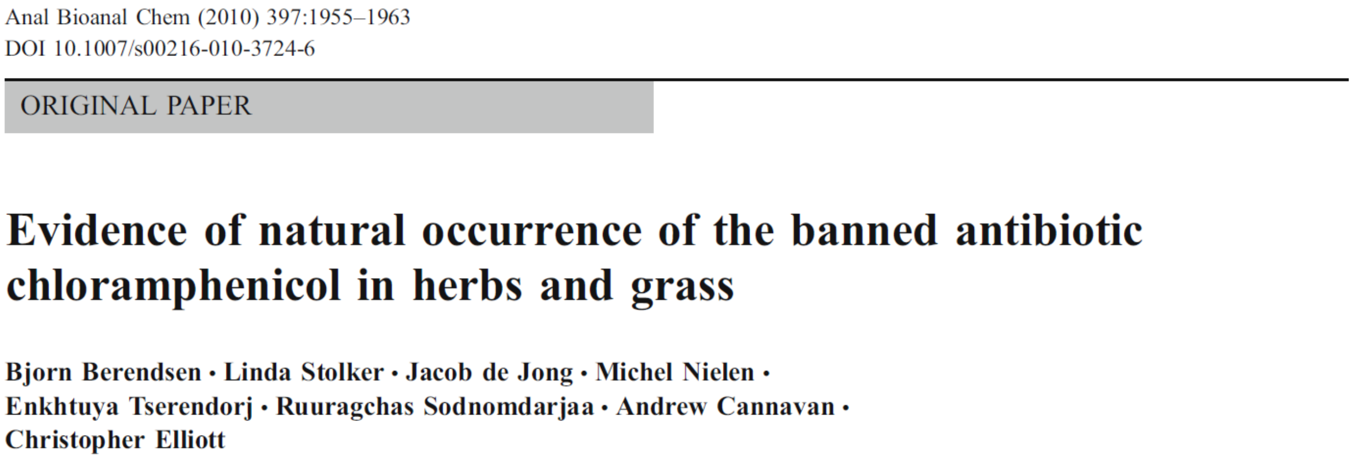
Chloramphenicol

In 2003 and 2006 in sheep casings exported from Mongolia to EU CAP was detected

- no intensive animal husbandry in Mongolia
- no evidence that CAP was applied
- some bitter tasting pasture plants like *Artemisia sp.* and *Thalictrum frigida* were analysed for CAP
- CAP was detected by ELISA and confirmed by LC-MS/MS

- Project together with RIKILT, NL (Linda Stolker and her team) within the framework of the Joint FAO/IAEA Programme for nuclear techniques in Food and Agriculture

Chloramphenicol



Anal Bioanal Chem (2010) 397:1955–1963
DOI 10.1007/s00216-010-3724-6

ORIGINAL PAPER

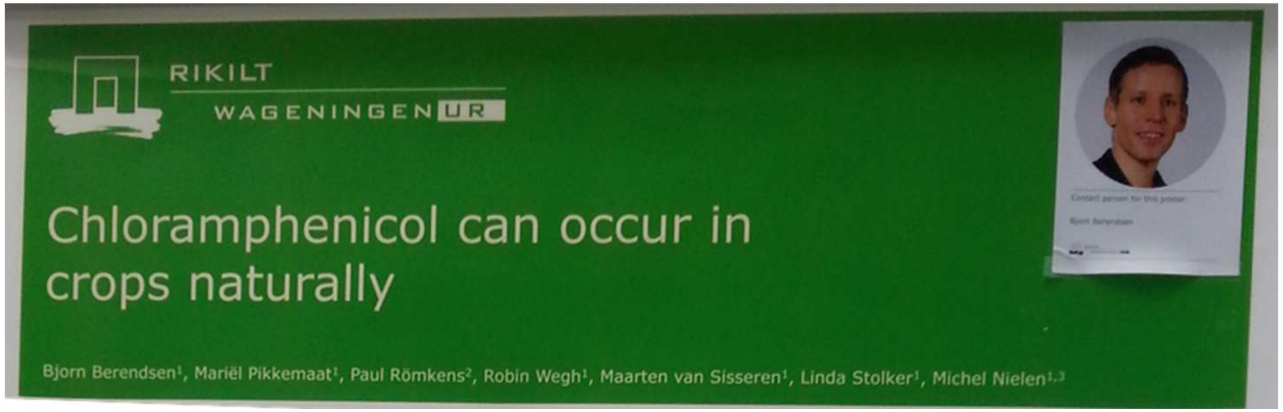
Evidence of natural occurrence of the banned antibiotic chloramphenicol in herbs and grass

Bjorn Berendsen · Linda Stolker · Jacob de Jong · Michel Nielen · Enkhtuya Tserendorj · Ruuragchas Sodnomdarjaa · Andrew Cannavan · Christopher Elliott

- CAP was detected in plant materials from different origin (plant material from Mongolian pastures, herb mixtures from local stores in NL, herb sample from Utah, USA)
- concentrations varied from non-detectable up to 450 µg/kg
- plants belonging to different families (*Artemisia, Thalictrum*, grass) can contain CAP

➡ **Hypothesis:** CAP is biosynthesized in soil by S. venezuelae and the plants absorb it through their root system

Chloramphenicol



RIKILT
WAGENINGEN UR

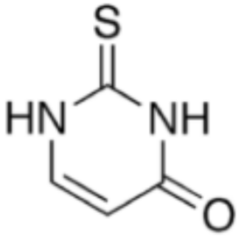
Chloramphenicol can occur in crops naturally

Bjorn Berendsen[1], Mariël Pikkemaat[1], Paul Römkens[2], Robin Wegh[1], Maarten van Sisseren[1], Linda Stolker[1], Michel Nielen[1,3]

- over 100 µg/kg CAP can be produced by *S. venezuelae* within a single day
- production and degradation of CAP in soil occur simultaneously, both processes depend on many environmental parameters
- low-ppb concentrations of CAP as well as high concentrations observed in crops can be explained by the natural production of CAP
- if crops containing CAP are fed to animals CAP residues may occur in products of animal origin

Thiouracil

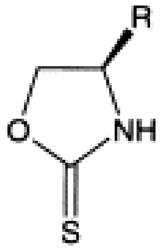
Thiouracil (TU) belongs to the thyreostats, a group of orally active drugs used to increase weight of cattle prior slaughter



- leads to a weight gain caused by the increased filling of the gastro-intestinal tract and retention of water in edible tissue by inhibition of the thyroid hormone production
- thyreostats have been classified as potential carcinogenic and/or teratogenic, therefor thyreostats have been banned in EU since 1981
  [*Council Directive 81/602/EEC*] ➡ [*Council Directive 96/22/EC*]
- to harmonise of the detection a recommended concentration of 10 µg/l in urine respectively 10 µg/kg in thyroid gland was proposed by EURL

Thiouracil

Natural occurring thyreostats



oxazolidine-2-thiones (OZTs)

$R-S-C\equiv N$

thiocyanates

originate from glycosinolates, secondary plant metabolites, present in plants of *Brassicaceae*
(e.g. cauliflower, broccoli, mustard and rapeseed)

Thiouracil

in recent years thiouracil has been detected occasionally in urine of livestock at concentrations between CCα and 10 µg/l, sometimes the values even exceeded the 10 µg/l-threshold

Pinet et al. (2006) investigated the hypothesis that the consumption of *Brassicaceae* (e.g. rapeseed) could be responsible for the presence of thiouracil in bovine urine

- ➡ could demonstrated the correlation between the consumption of rapeseed and the occurrence of thiouracil
- ➡ concentrations in urine did not exceed 10 µg/l
- ➡ no thiouracil could be detected in the feed

Thiouracil

Vanden Bussche (2011, PhD-thesis) elucidated the possible natural origin for the low levels of thiouracil in urine of various animals

- **myrosinase** is crucial to detect thiouracil in samples derived from *Brassicaceae*
- thiouracil seems to follow a similar pathway of synthesis as the known natural occuring thyreostats oxazolidine-2-thiones and thiocyanates
- due to the fact that thiouracil is unstable upon storage at room temperature as well as upon freezing at -70°C) a elaborated sample pre-treatment (pH=1, EDTA) is recommended

Thiouracil

2012: 91 urine samples were analysed for thyreostatic drugs for the NRCP in Austria

samples	n	$n_{detected}$	%	range
bovine	40	8	20,0%	11,0 – 37,9 µg/l
porcine	43	14	32,6%	10,1 - 40,0 µg/l

11 follow-up samples taken on a farm (porcine urine: 22,7 µg/l thiouracil)

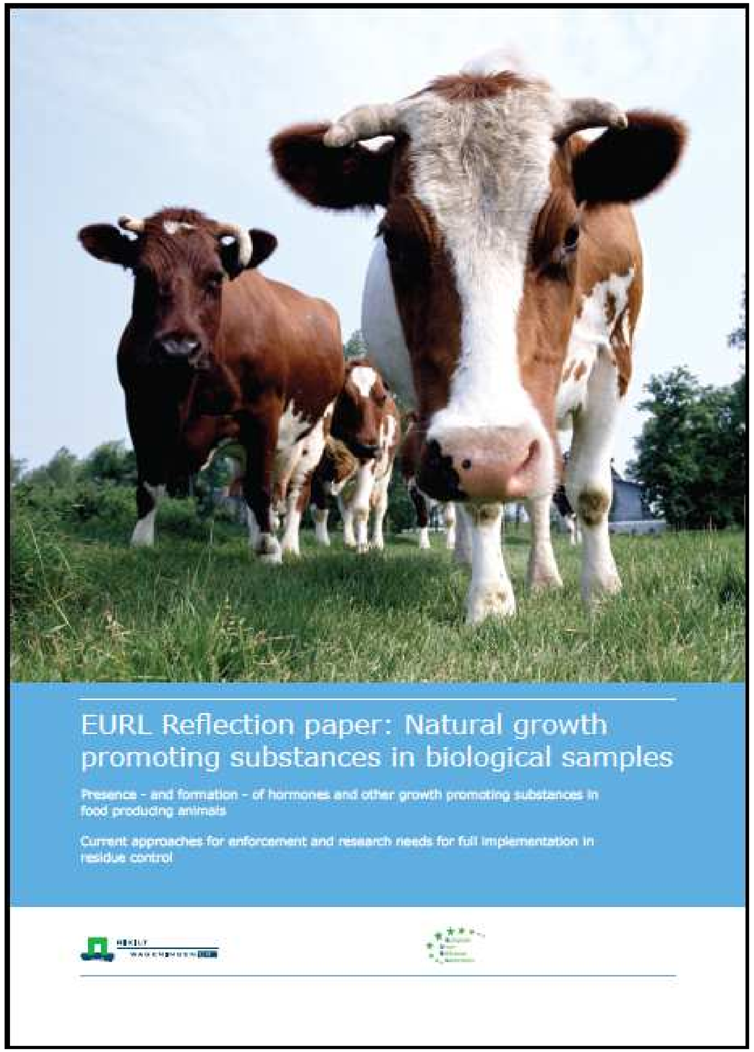
- 10,1 – 40,0 µg/l thiouracil
- no evidence of misuse of thyreostatic drugs
- kept in stable with no direct access to green fodder
- pigs are feed with whey (protein, minerals, etc.)
- **18,3 µg/l thiouracil** in the whey

- analysis of 27 milk and whey samples:
  - in 4 samples **2,9 – 7,5 µg/l thiouracil**

Thiouracil

May 2014

- The responsible EURL recommended to the European Commission to change the "recommended concentration" for thoiuracil from 10 µg/l to 30 µg/l.



EURL Reflection paper: Natural growth promoting substances in biological samples

Presence - and formation - of hormones and other growth promoting substances in food producing animals

Current approaches for enforcement and research needs for full implementation in residue control

Conclusions

- using more sensitive methods (factor 100 – 1000 in about 20 years) substances known as xenobiotic “become” natural
- classical approach focussing on detection of suspect samples containing banned substances with a screening procedure followed by a confirmatory step to identify the analyte unambiguously cannot be applied any more
- significant implications for the application of legislation or at least in the interpretation of analytical results and in the follow-up actions and penalties
- re-evaluation of the substances in question in the light of their natural occurrence by the risk-assessors (e.g. EMA, JECFA)

Acknowledgements

AGES – Department for Veterinary drugs, Hormones and Contaminants

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- Dr. Martin BRANDTNER