

6-MTU: A RELIABLE BIOMARKER FOR DETECTING THIOURACIL ABUSE IN SHEEP ?

Audrey Poirier
Stéphanie Prévost
Nathan Dozias
Anaïs Dubourg
Gaud Dervilly
Bruno Le Bizec



Traces
Ghent, Belgium
June 3rd - 6th

SOMMAIRE

1

Context

2

Analytical procedure

3

Natural occurrence

4

Experiment

5

Conclusion

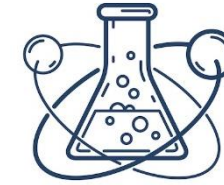
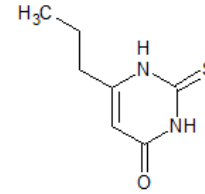
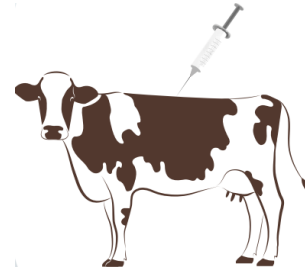
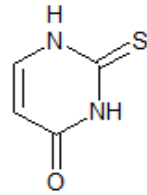


Context



Context

Why using thyrostats?



Inhibition of thyroid function

Objective of artificial weight gain in livestock by water retention in the tissues

Inexpensive, easy to obtain and suitable for oral administration

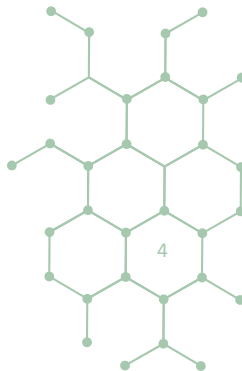


BUT

Decrease the meat quality (water instead of muscle)

Risk for health
IARC : 2B

Forbidden to be used within the EU
(A annexe 1 DIR 96/22/CE or A1b – REG 2021/1644)



What do we know about endogenous status?



Food Additives and Contaminants

Publication details, including instructions for authors and subscription information:
<http://www.tandfonline.com/loi/tfac19>

Evidence that urinary excretion of thiouracil in adult bovine submitted to a cruciferous diet can give erroneous indications of the possible illegal use of thyrostats in meat production

G. Pinel^a, S. Mathieu^a, N. Cesbron^b, D. Maume^a, H. F. De Brabander^c, F. Andre^a & B. Le Bizec^a

RAPID COMMUNICATIONS IN MASS SPECTROMETRY

Rapid Commun. Mass Spectrom. 2006; 20: 3183–3187

Published online in Wiley InterScience (www.interscience.wiley.com) DOI: 10.1002/rcm.2711

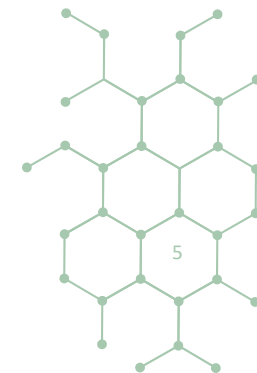
RCM

Unambiguous identification of thiouracil residue in urine collected in non-treated bovine by tandem and high-resolution mass spectrometry

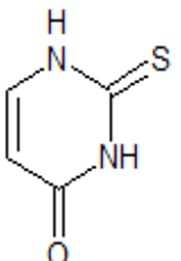
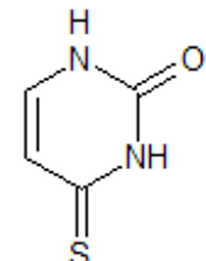
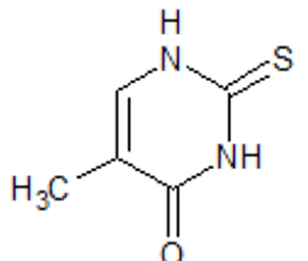
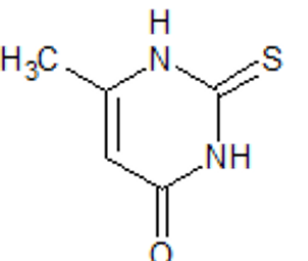
Gaud Pinel*, Daniel Maume, Yoann Deceuninck, François Andre and Bruno Le Bizec

Laboratoire d'Etude des Résidus et Contaminants dans les Aliments, Ecole Nationale Vétérinaire de Nantes, Route de Gachet, BP 50707, 44307 Nantes Cedex 3, France

Received 2 June 2006; Revised 22 August 2006; Accepted 23 August 2006



What do we know about endogenous status?

2-Thiouracile (2TU)	4-Thiouracile (4TU)	5-Méthyl-2-thiouracile (5MTU)	6-Méthyl-2-thiouracile (6MTU)
			

Endogenous




Exogenous



Metabolism investigations

Discrimination between the exogenous and endogenous origin of thiouracil in farm animals, the final chapter?

Marco H. Blokland , Frederike E. van Tricht, Maria J. Groot, Leendert A. Van Ginkel, and Saskia S. Sterk

Growth Promoters, Wageningen Food Safety Research (WFSR), Part of Wageningen University & Research, European Union Reference Laboratory for Residues, Wageningen, The Netherlands



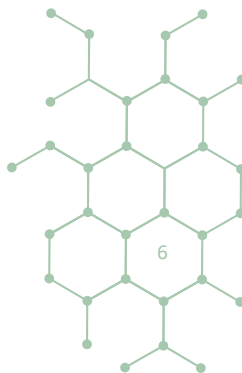
2022

EURL Reflection paper 2.0:

Natural growth promoting substances in biological samples



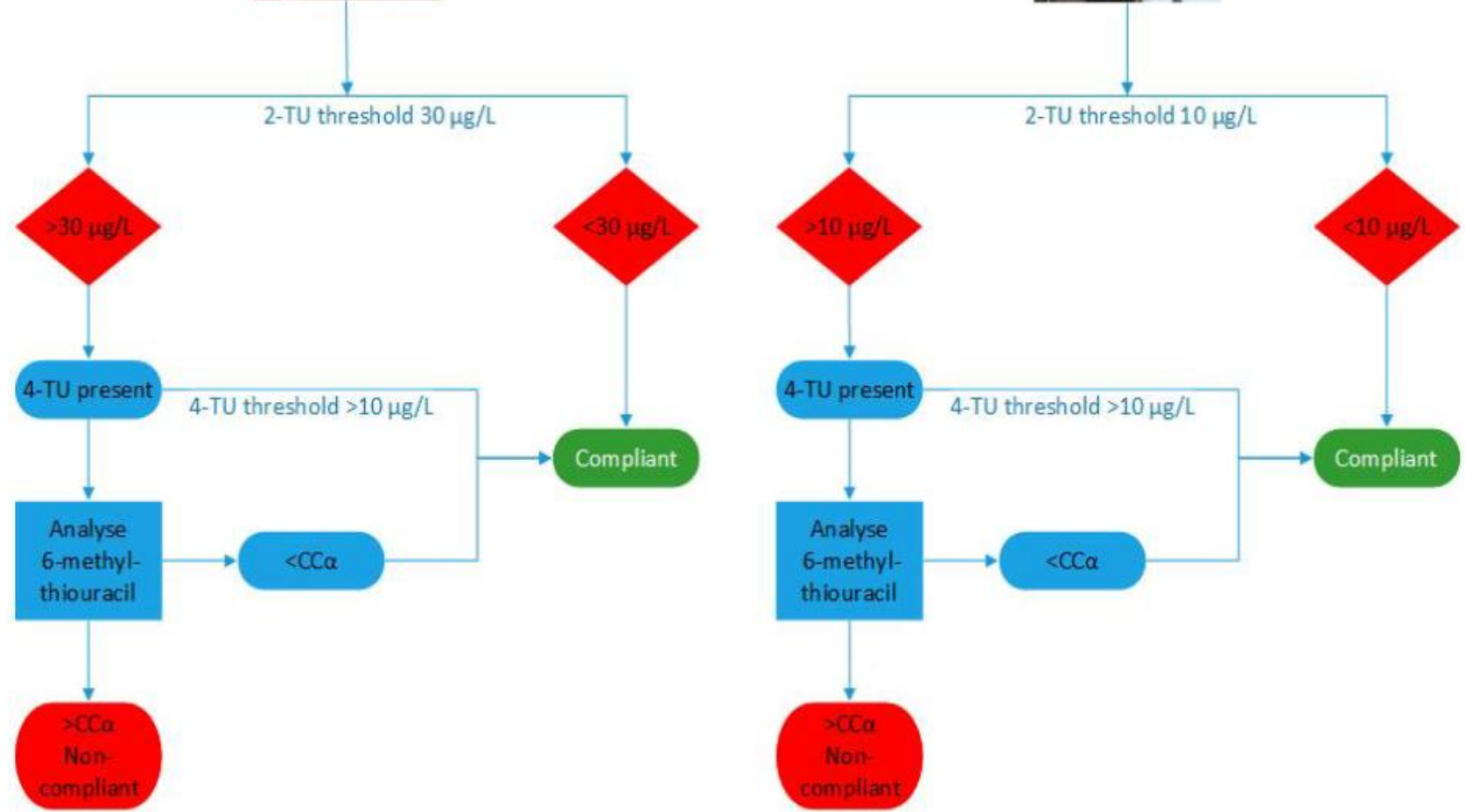
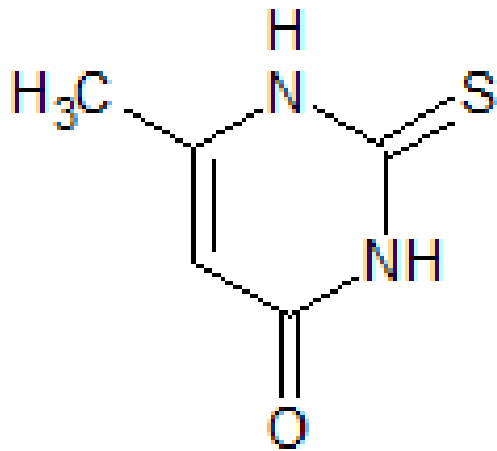
Criteria for bovine and porcine



What do we know

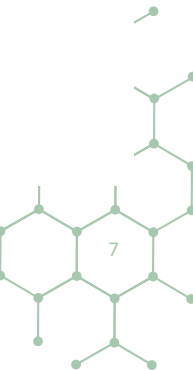


6-MTU : Marker of abuse



EURL Reflection paper 2.0:

Natural growth promoting substances in biological samples



Situation in France

National
Monitoring and
Surveillance Plans

→ Thyreostats

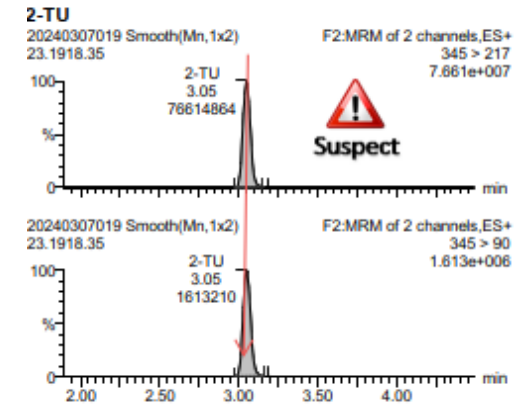


EURL's criteria

2-TU threshold
6-MTU marker of
administration



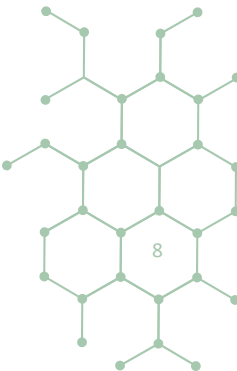
No data and no criteria
What conclusion when 2-TU identified?



Extrapolation of thresholds for
bovin and porcin



Threshold of 10 ppb for suspicion and 30 ppb
for non compliant

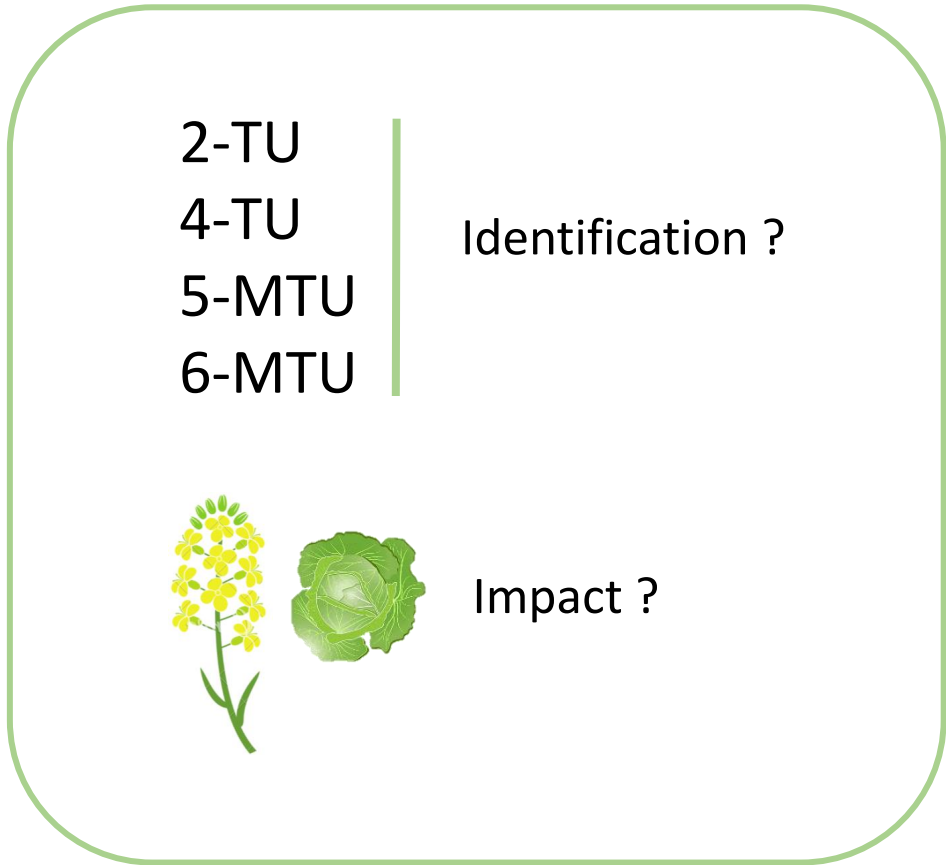


Study endogenous / exogenous

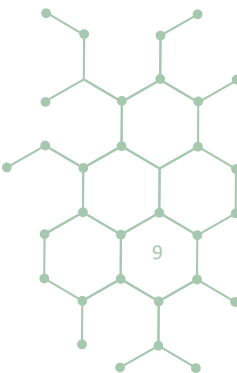
Part 1 : Natural occurrence



Data



Part 2 : Experiment





Analytical procedure

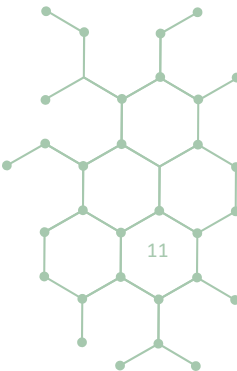
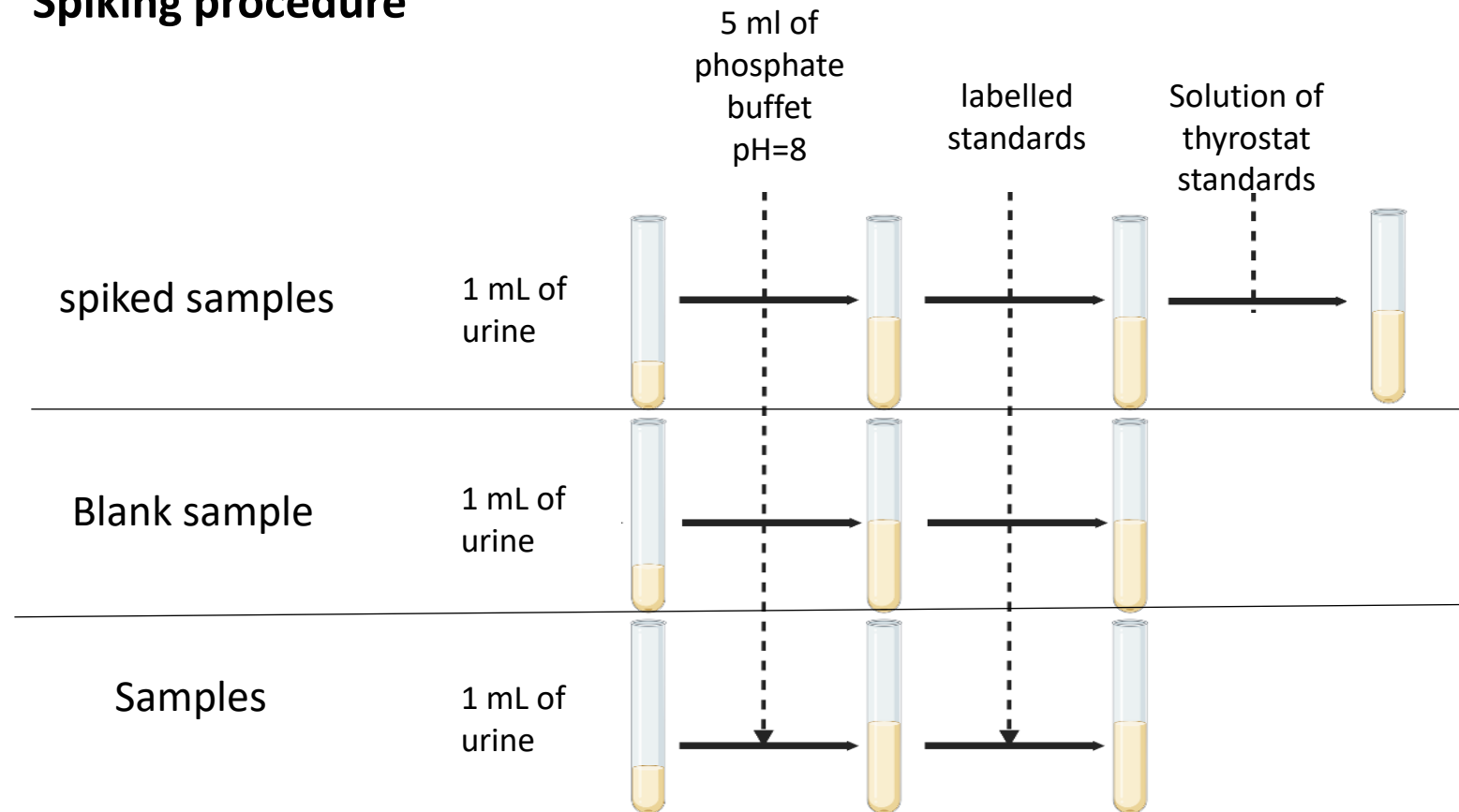


Analytical procedure

Criteria of
DEC 2002/657

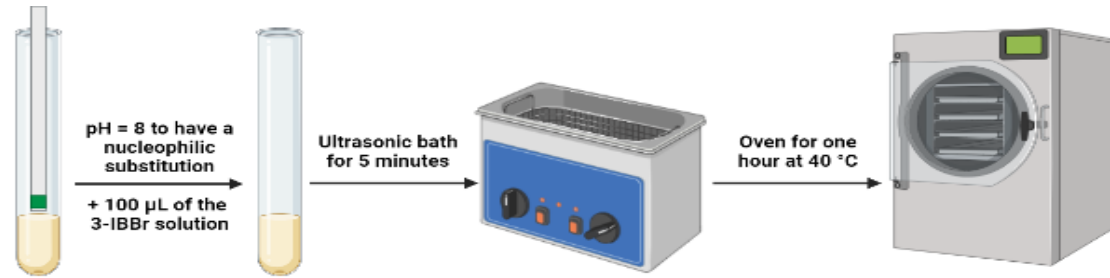


Spiking procedure

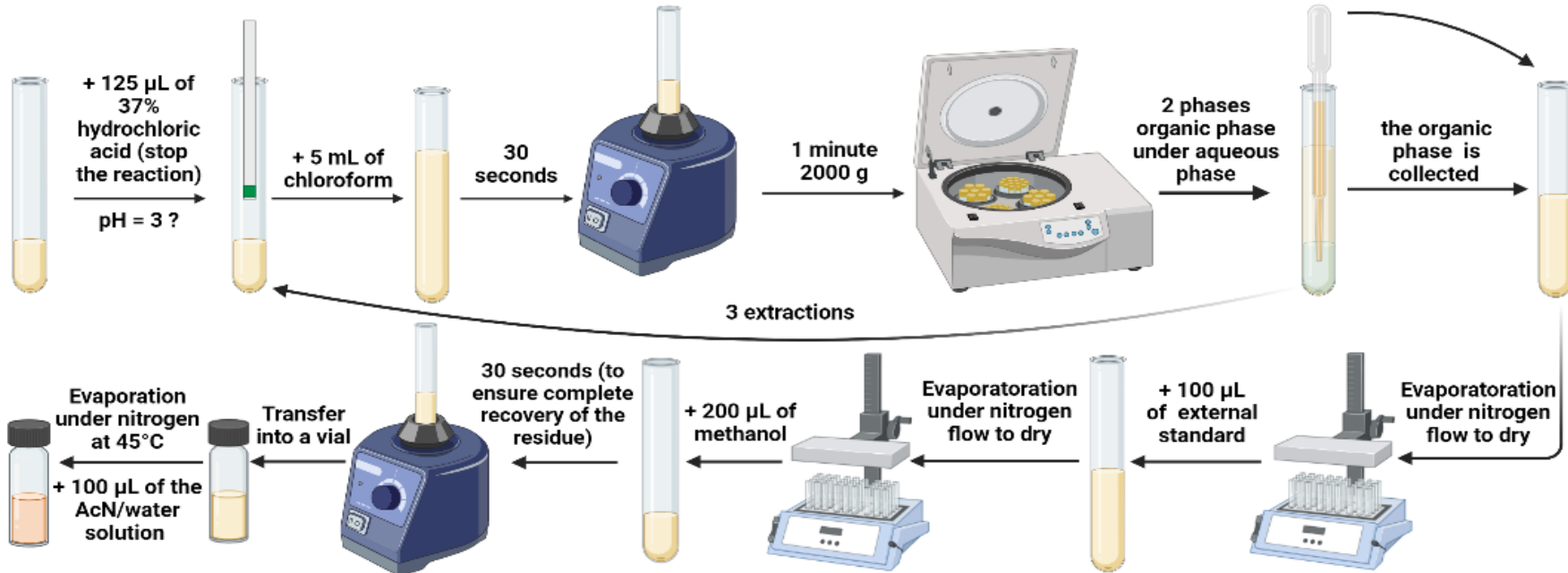


Analytical procedure

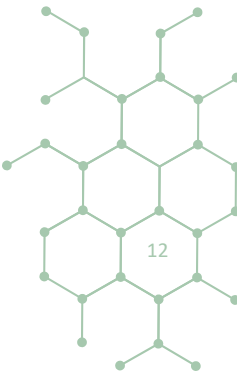
Derivatisation 3-IBBr

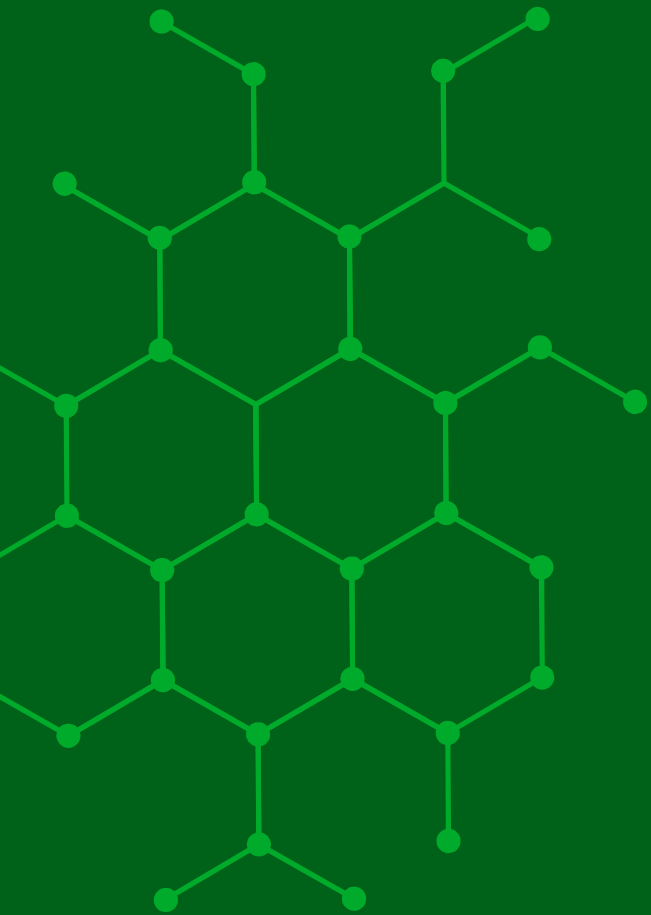


L/L extraction Centrifugation Evaporation



And analysis using LC-MS/MS (ESI+ after C18)





Natural occurrence

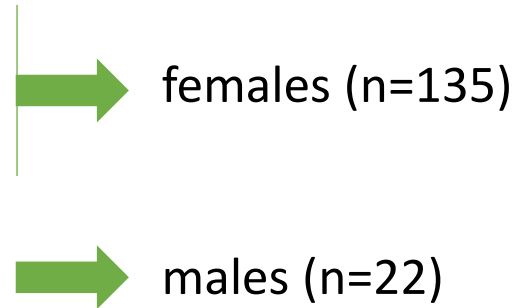


Part 1- Samples

Sheep urine samples taken from **surveillance and control plans** on the one hand, but also from **various research projects**. In this sense, they are not fully representative of national production

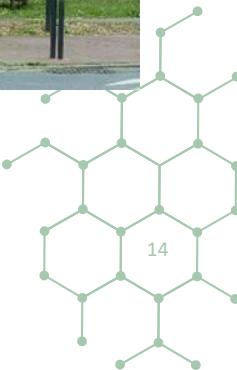
3 groups :

- Eco pasture
- Urban pasture
- National plans



2 periods: 2022 & 2023

$\Sigma = 157$ sheep urine samples



Part 1- Results Natural occurrence



Compound	Number	Nb identified	Average of [] in ppb	Median of [] in ppb	Min	Max	P25	P75	P90
2-TU	157	60 %	0,98	0,19	0,05	30,65	0,05	0,79	2,14
4-TU	157	91 %	12,00	1,41	0,05	150,88	0,27	12,59	35,08
5-MTU	157	68 %	0,35	0,17	0,05	2,07	0,05	0,46	0,91

- 2-TU identified in 60 % of the samples
- 4-TU identified in 91 % of the samples
- 5-MTU identified in 68 % of the samples

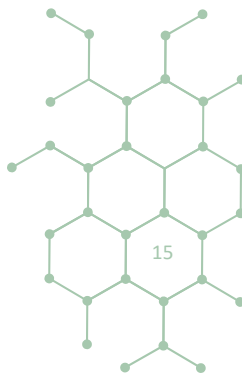
Criteria of
DEC 2002/657

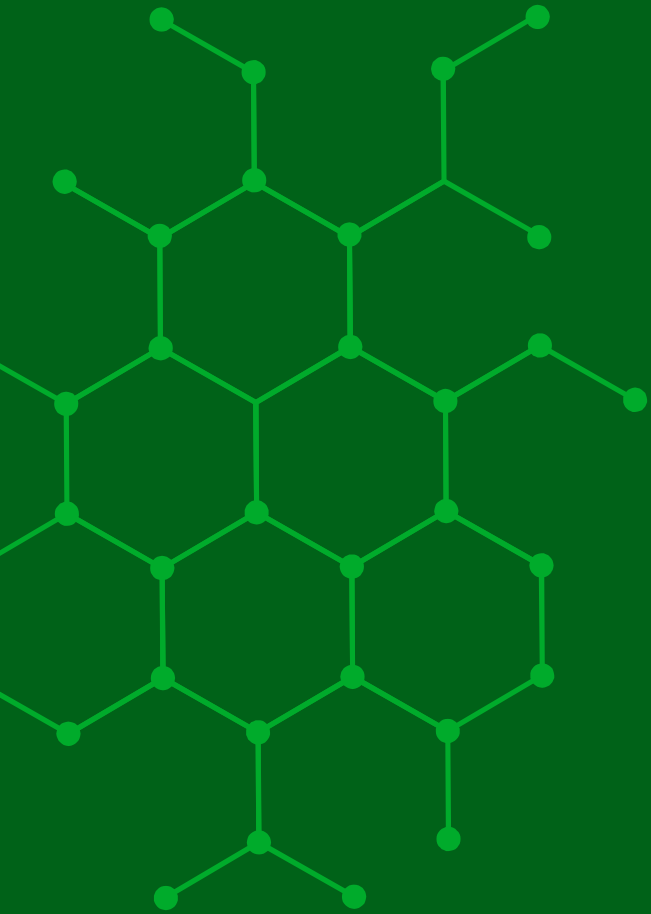
Confirm natural occurrence of 2-TU and metabolites 4-TU and 5-MTU

6-MTU not detected



Hypothese still valid





Experiment



Part 2 - Experiment

December 2023 : Conduct a specific experiment to investigate the criteria of 6-MTU in sheep

Sheep A



Administration of 2-TU

Sheep B



0.2 g/100 kg body weight

Low dose

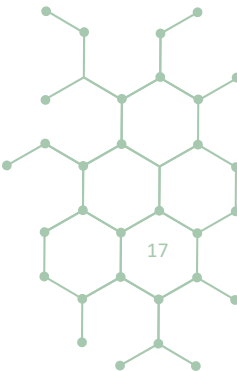


Sheep C



2 g/100 kg body weight

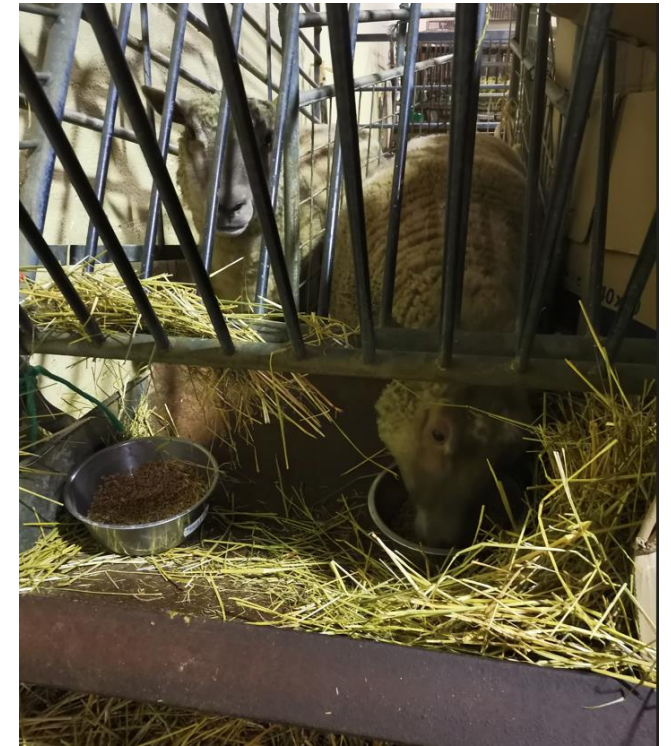
High dose



Few pictures

Sheep A

Animal control and his friend



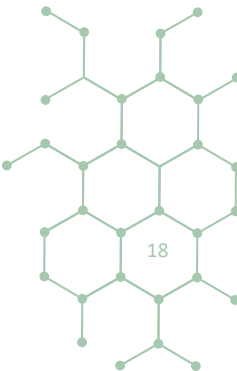
Animals with traitment

Sheep B

Sheep C



Powder of 2-thiouracile

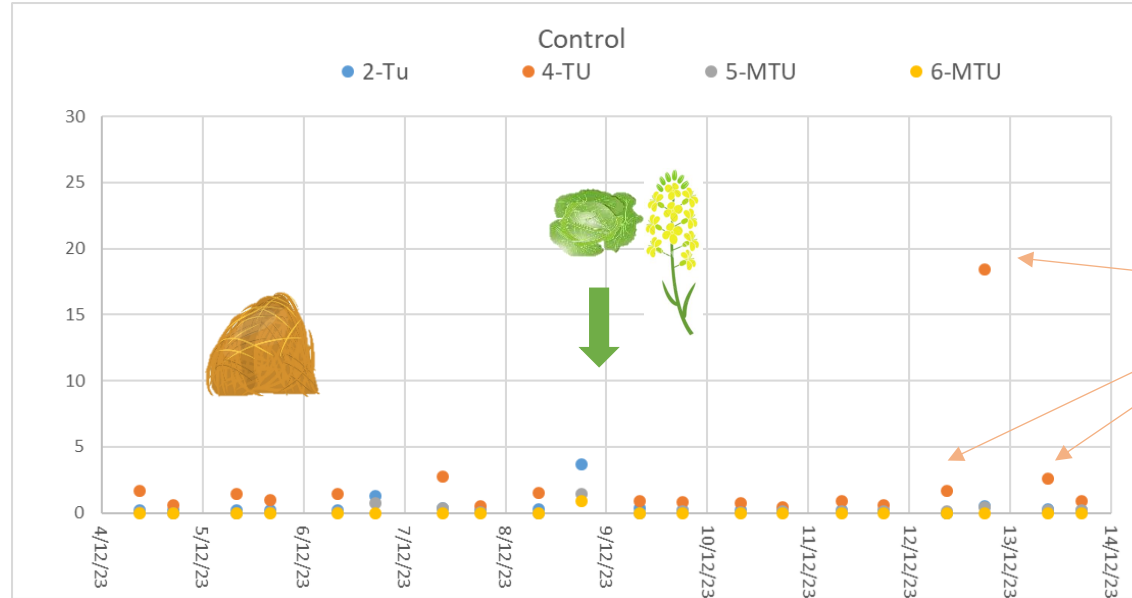


Part 2- Results on control sheep

Sheep A



Characterisation of 2-TU, 4-TU, 5-MTU and 6-MTU in the control sheep urine



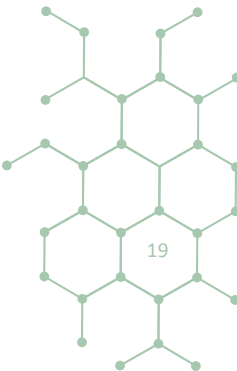
Slow increase of 4-TU at J+2 after administration of crucifers



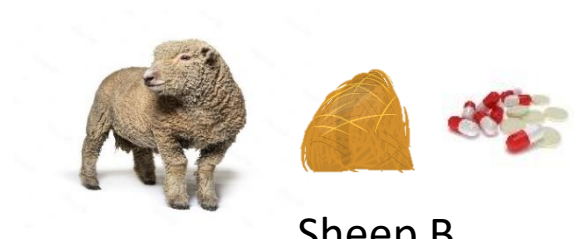
- Concentration of 2-TU and 5-MTU < 2 ppb
- Concentration of 4-TU < 3 ppb except at J2 and J9
- 6-MTU not detected



➤ Crucifers and rapeseed in feed :
no effect on 6-MTU

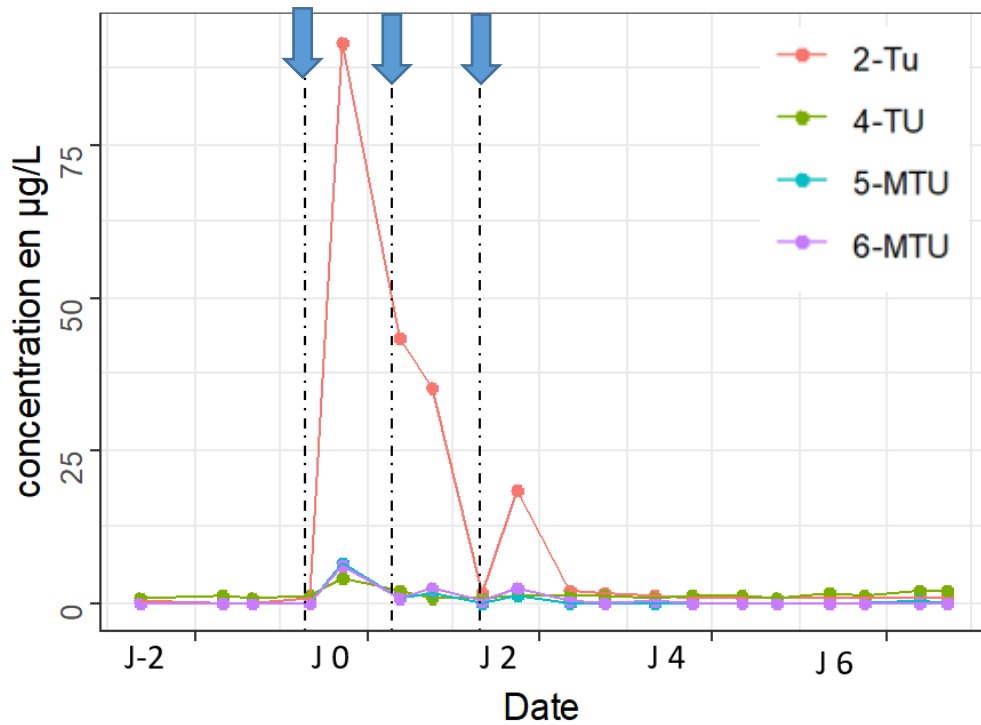


Part 2- Results on sheep treated (Sheep B)



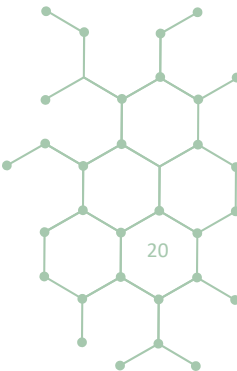
Sheep B
Low dose

Characterisation of 2-TU, 4-TU, 5-MTU et 6-MTU in sheep B



- Compounds detected as early as 8 h after administration
- Decrease rapidly after administration : 2 or 3 days
- **6-MTU detected few hours after injection**

↓ administration of 2-TU
(0.2 g/100kg)

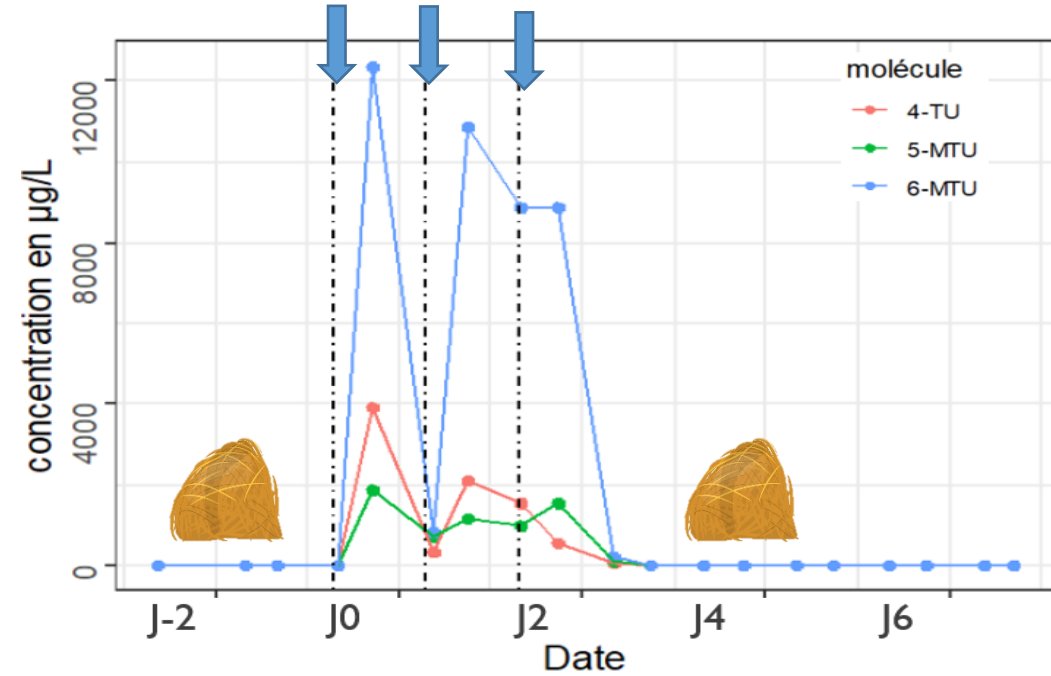
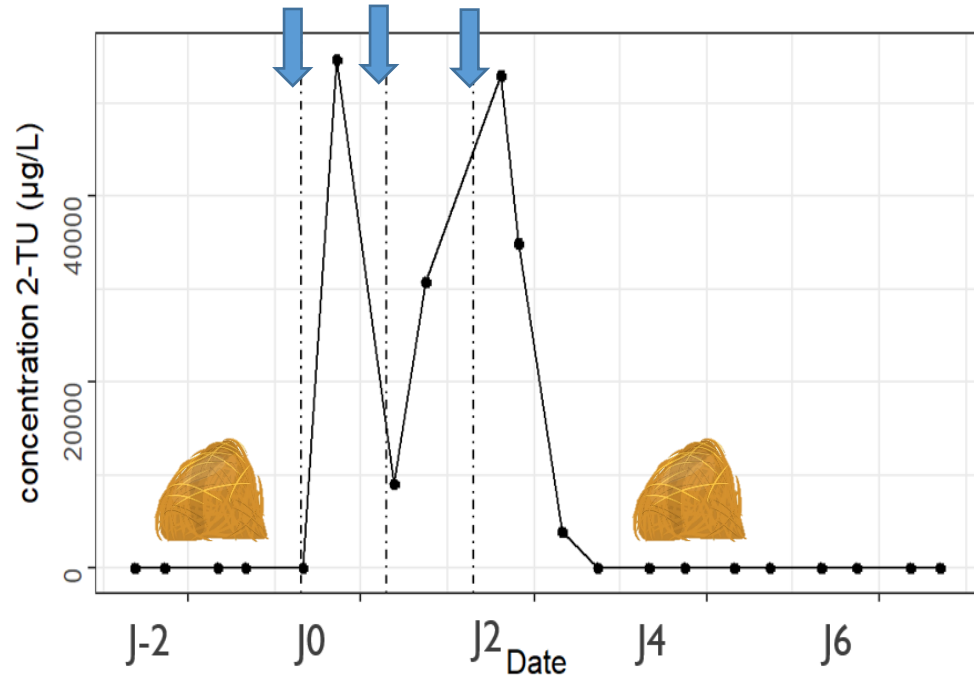


Part 2- Results on sheep treated (Sheep C)



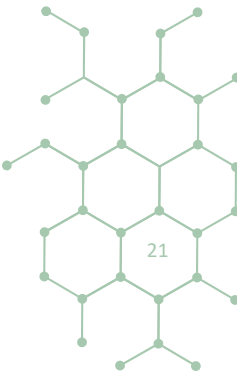
Characterisation of 2-TU (at left) and 4-TU, 5-MTU et 6-MTU (at right)
in sheep C

administration of 2-TU
(2 g/100kg)



- Compounds detected as early as 8 h after administration
- Decrease rapidly after administration : 2 or 3 days
- **6-MTU detected few hours after injection**

Behaviour similar for the
both sheep treated



Part 2- Results

Sheep B

0.2 g/100 kg body weight

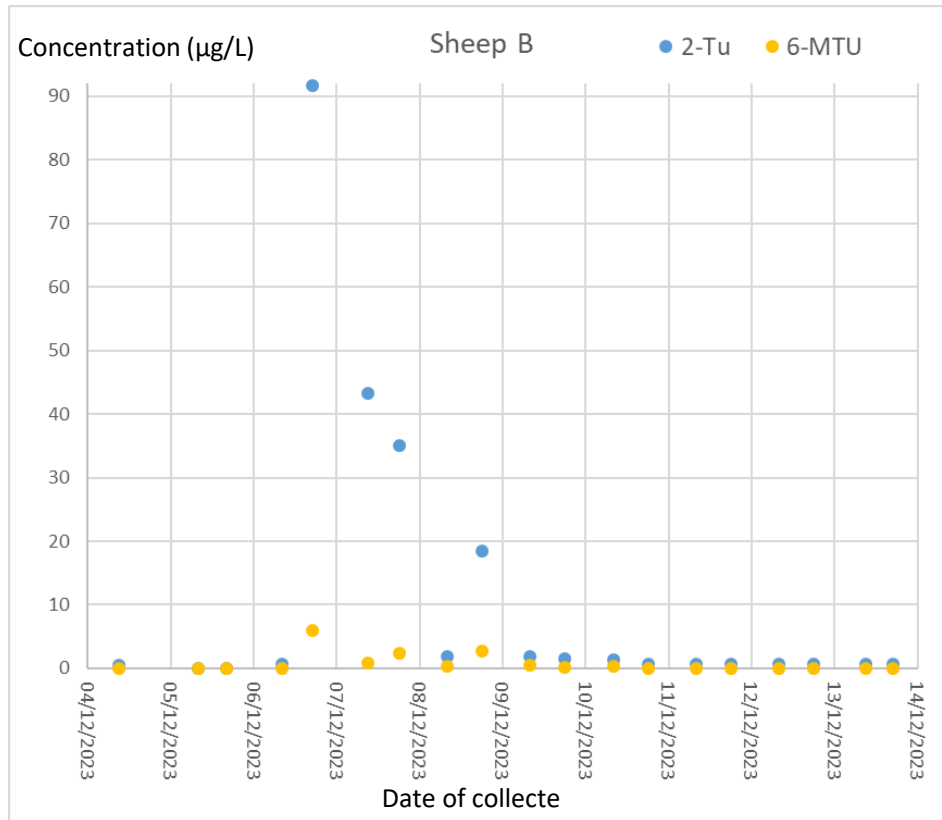


x 10

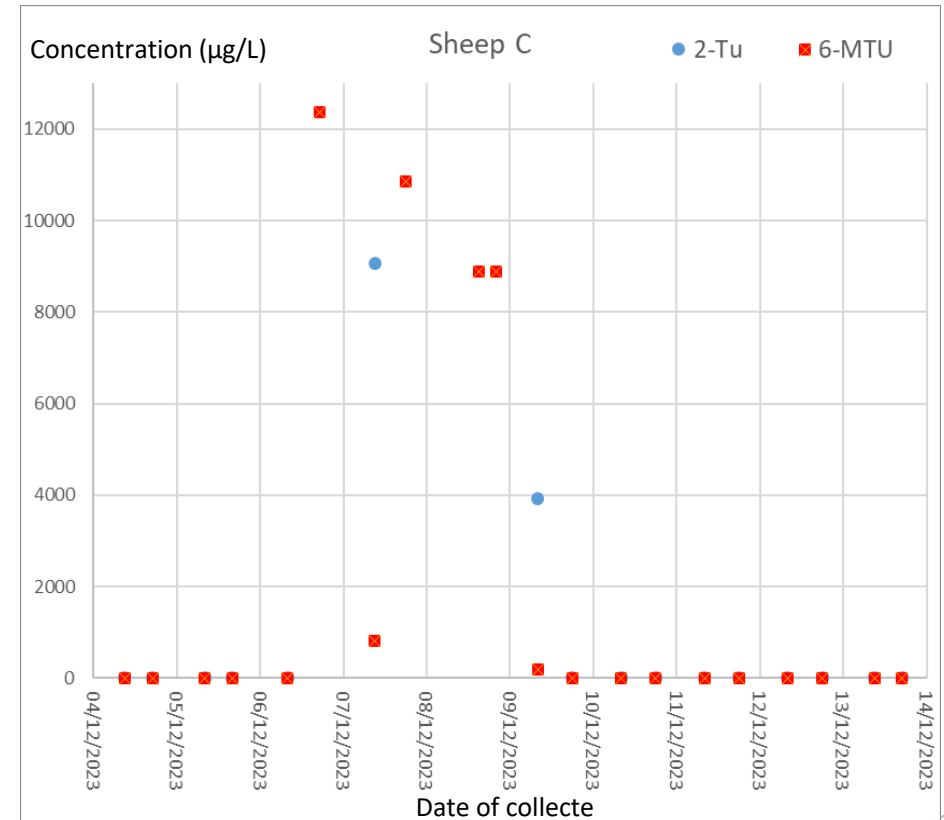
Oral dose

Sheep C

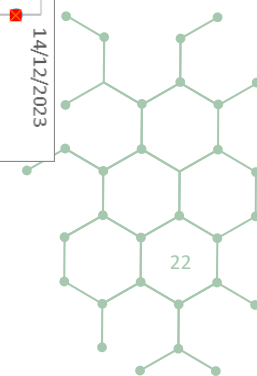
2 g/100 kg body weight

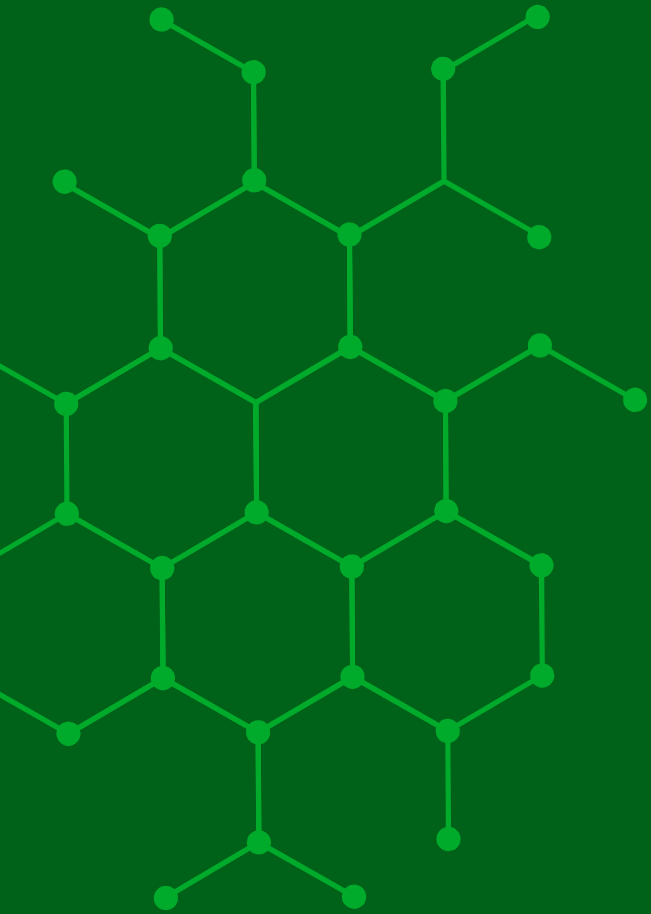


> X 500



No relationship : saturation of the concentrations / difficulties to eat feed for one sheep





Conclusion

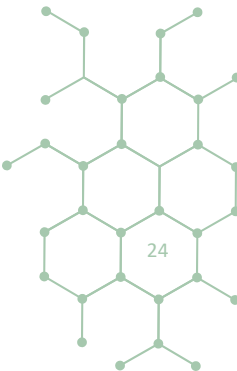


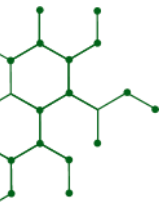
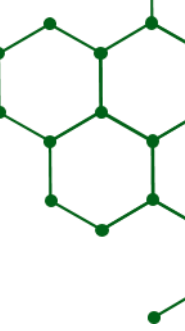
Conclusion

- 5-MTU, 4-TU and 2-TU found “naturally” in urine
- Administration of 2-TU induces presence of 6-MTU in urine
- 5-MTU & 4-TU also affected, but to a much lesser extent



6-MTU : a good candidate as a biomarker of thiouracil administration in sheep as bovin and porcin





Thank you for your attention