



istituto zooprofilattico sperimentale
del Piemonte, Liguria e Valle d'Aosta



CRReAA TORINO

Centro di Riferenza Nazionale per la
Sorveglianza e il Controllo degli Alimenti per gli Animali

GTH use and characterization of material

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Joint Workshop of EURL-TSE and AP, Rome 14th May 2024

ABPs

- Commission Regulation (EC) No 1069/2009
- ABPs classified in three categories, according to their related risk
- Article 8 Category 1
 - High risk by-products (dead bodies, products impounded for health reasons and specific risk materials) intended for incineration
- Article 9 Category 2
 - Moderate risk by-products, which can be used at technical purpose or as fertilizers, biogas and compost
- Article 10 Category 3
 - Low risk by-products (slaughterhouses), which can be used in pet food manufacturing or, after specific rendering processes, for PAP production





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END POINT

Reg. EC 1069/2009 Article 5

End point in the manufacturing chain

ABPS derived products which have reached the end point in the manufacturing chain are no longer subject to the requirements of ABP Regulation.

Reg. (EU) 142/2011 Article 3

End point in the manufacturing chain for certain derived products

Biodiesel

Petfood

Dogchew

Feathers/ down

Wool

Hides and skins

Fur

HFAA (EX FVO): HEALTH AND FOOD AUDITS AND ANALYSIS DIVISION

Feed safety and animal by-products

Policy area	Priority theme	Country status/ entity	Objectives 2021-2025	Controls 2024
Feed safety	Animal by-products and derived products	Member States	To verify Member States' compliance with the applicable EU legislation governing the handling, use and/or disposal of animal by-products (ABP) and derived products (DP) generated in the EU or placed on the EU market and the implementation of official controls thereon. ➤ In all the Member States	3 audits: Ireland, Italy and Poland
	Feed hygiene	Member States	To verify Member States' compliance with the applicable EU legislation governing feed hygiene (with a particular focus on approval and registration of establishments, contaminants, traceability and labelling and the implementation of official controls thereon. ➤ In all Member States (the project started in 2020)	5 audits: Austria, Croatia, Denmark, Estonia and Sweden
	Medicated feed	Member States	To verify Member States' compliance with the new EU requirements governing the production of medicated feedingstuffs, applicable from January 2022 (Regulation (EU) 2019/4). ➤ In all Member States, starting in 2023.	Addressed as part of audits on feed hygiene, starting in 2023.



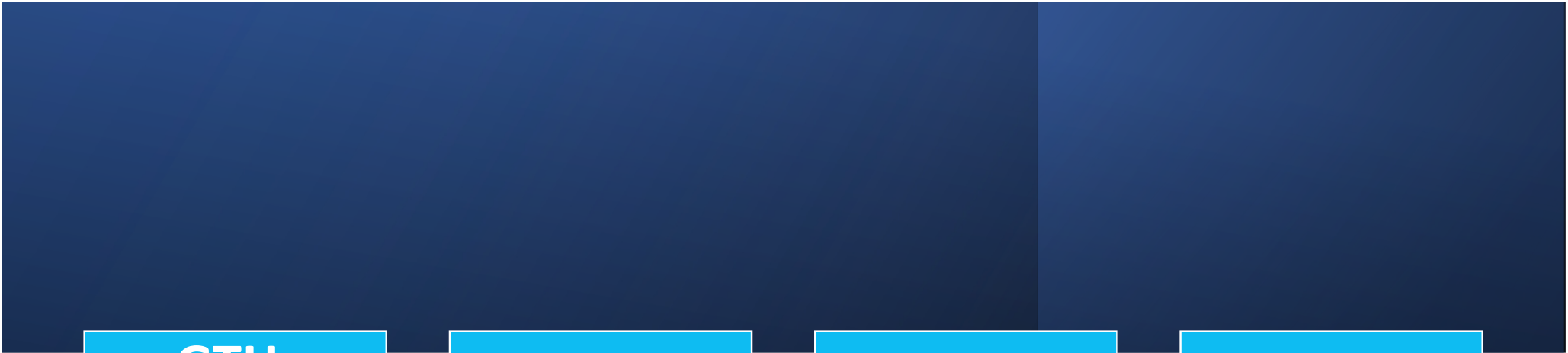
Commission Regulation (EC)
No142/2011



**MBM and fat from category 1
and 2 ABPs are required to be
permanently marked with
glyceroltriheptanoate (GTH)**

Min conc 250 mg/kg in fat
Homogeneously distributed





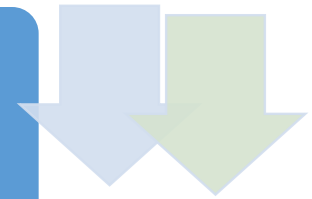
GTH:
synthetic
fat with
three n-
heptanoic
acids
esterified
with
glycerol

**JRC-IRMM
Belgium**

**The most
appropriate
marker for
fat and
MBM**

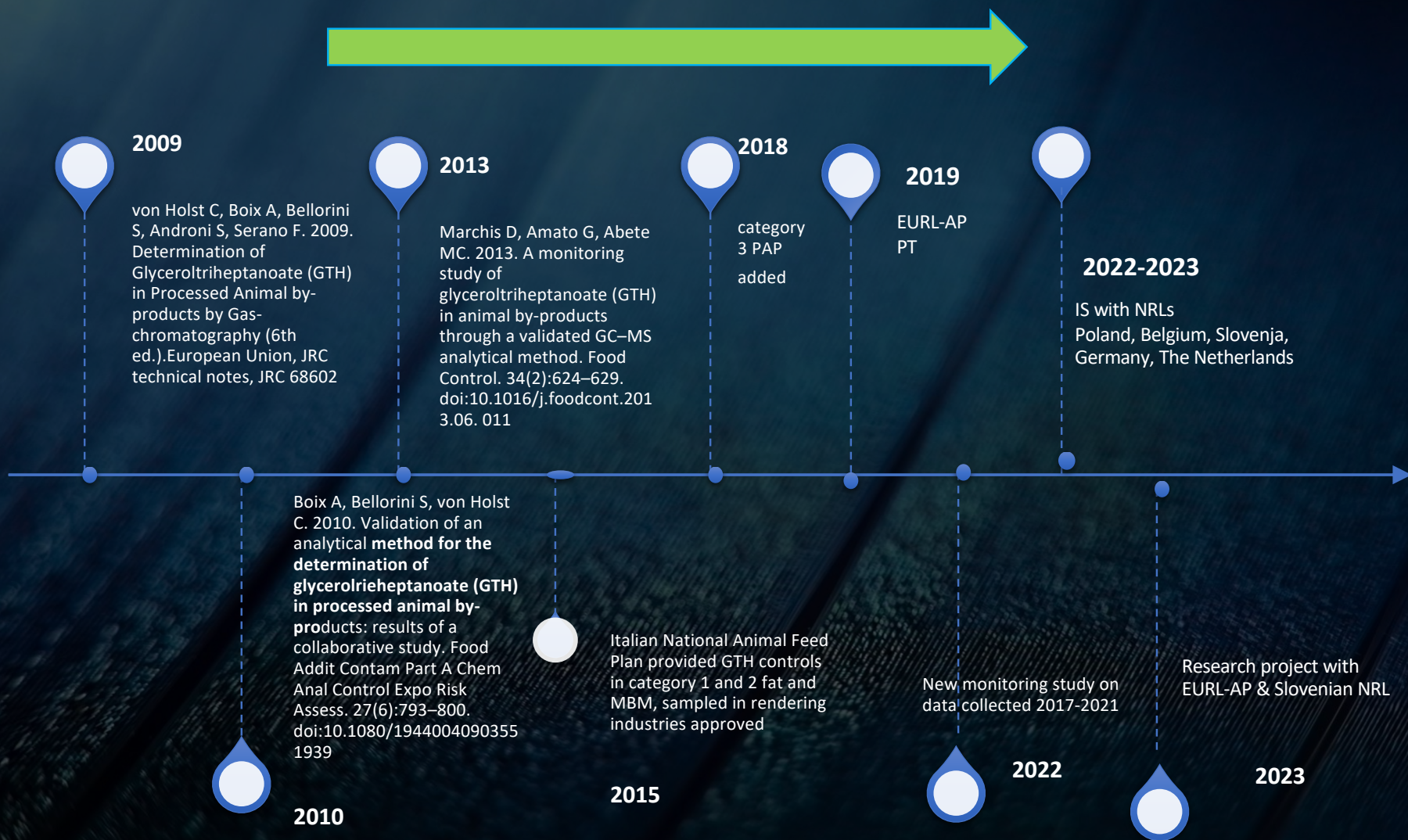
**GTH cannot
be removed**

Some ABPs cannot be GTH marked, as they do not contain (a fair amount of) fat:



Blood meal

Feather meal



GTH detection method

- **No official/ISO/ methods available**
- *In-house* GC-MS validated method based on the JRC-IRMM
- Fats and MBM cat. 1 and 2
- Fats and PAP cat. 3

MBM extraction step

100 mL Petroleum ether+10 g (MBM category 1 and 2) or 15 g (category 3 PAP).

Supernatant filtration and collection in a 500 mL round-bottom flask. Repeat twice.

Rotary evaporator ($50^{\circ}\text{C} \pm 5^{\circ}\text{C}$; 300–150 mBar).

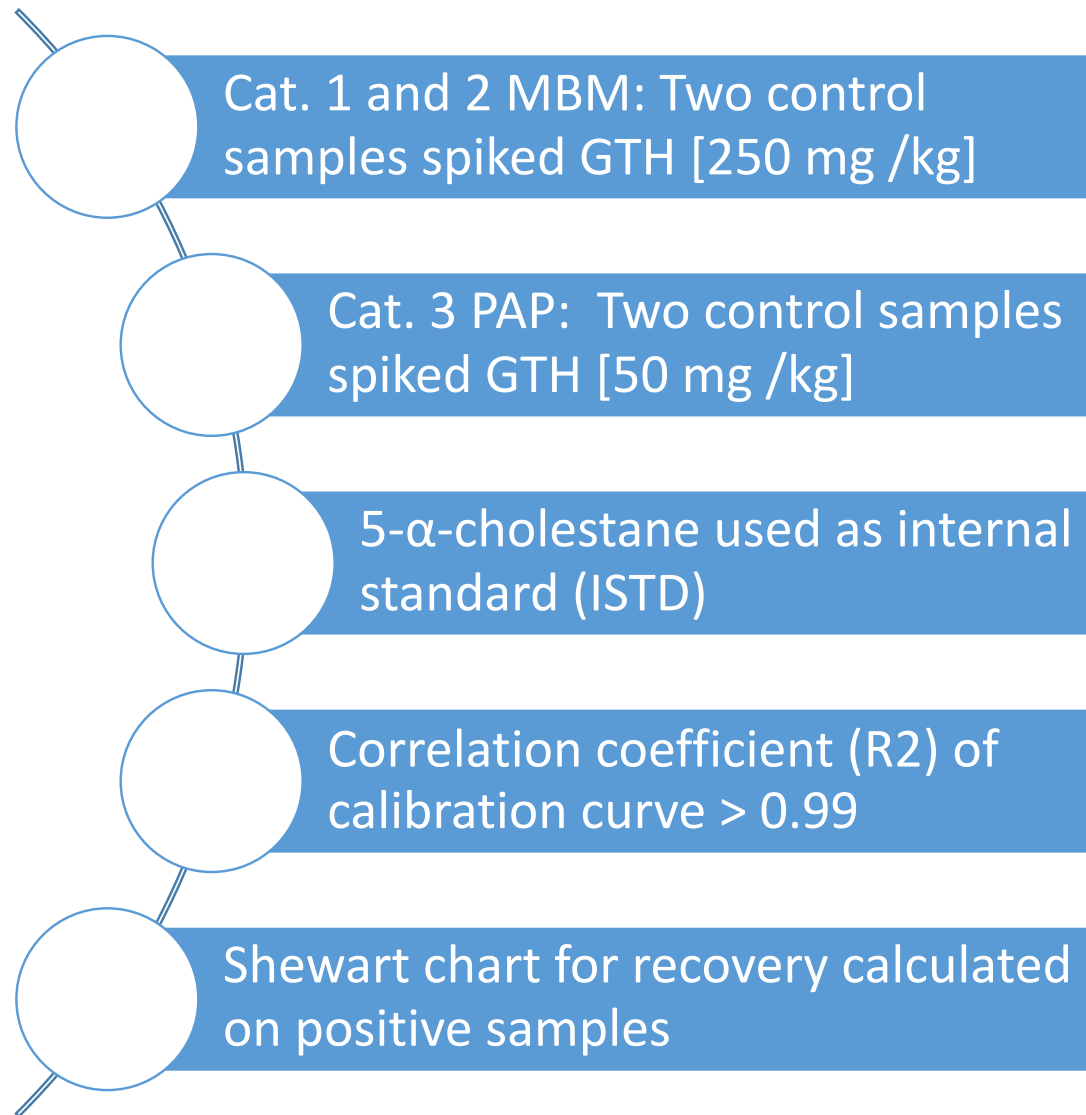
0.5 g residual fat dissolved in 5 mL of n-hexane.

Loading of 0.2 mL on SPE cartridge, elution 8 mL of n-hexane/diethyl ether (85:15, v/v), and collection in a tube containing 0.100 mL ISTD (0.3 mg mL^{-1} in isooctane).

to dryness under a gentle stream of nitrogen ($50^{\circ}\text{C} \pm 5^{\circ}\text{C}$)

In 5 mL of isooctane





Quality controls



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GC-MS analytical method

DSQ™ single quadrupole mass spectrometer, FOCUS™ GC gas Chromatograph and AS 3000 autosampler

Capillary column

Programmed temperature ramp

GTH

Quantification ion:
113 m/z

Qualification ions
285 and 299 m/z

ISTD

Quantification ion:
217 m/z

Qualification ions
357 and 372 m/z

Table 2. Validation results sorted by category.

	Category 3 PAP and fat	Category 1 and 2 MBM			
Linearity	25–125 mg kg ⁻¹	75–625 mg kg ⁻¹			
LOQ	50 mg kg ⁻¹	125 mg kg ⁻¹			
LOD	16.6 mg kg ⁻¹	41.6 mg kg ⁻¹			
Ruggedness	Yes	Yes			
Specificity	Yes	Yes			
Working range	50–125 mg kg ⁻¹	125–500 mg kg ⁻¹			
	Concentration levels				
	50 mg kg ⁻¹	125 mg kg ⁻¹	250 mg kg ⁻¹	500 mg kg ⁻¹	
Repeatability (RSD%)	7.43	3.36	5.09	7.87	
Recovery (%)	111	88.0	89.0	85.0	
Repeatability limit (r)	13.5	52.9	46.2	143	
Uncertainty	25%	–	14%	–	

PNAA 2024-2025-2026

PIANO NAZIONALE DI CONTROLLO UFFICIALE SULL'ALIMENTAZIONE DEGLI ANIMALI

Ministero della Salute

PNAA 2024/2026

Ricerca di Triptanoato di glicerina (GHT)

Il triptanoato di glicerina è un marcatore dei prodotti derivati di categoria 1 e 2, previsto dall'allegato 8, capo V del Regolamento (CE) n.142/2011.

Al fine di garantire che i prodotti derivati di categoria 1 e 2 non entrino nella catena dei mangimi, stante anche l'alta percentuale di non conformità rilevate, si mantiene la ricerca del GHT in tali materiali.

La ricerca mira a evidenziare l'assenza di tale marcatore nei prodotti derivati di categoria 3 oppure la giusta quantità prevista dalla norma nei prodotti derivati di categoria 1 e 2.

Nel triennio 2021-2023 sono state rilevate 6 non conformità per GTH. La prevalenza di non conformità per GTH è pari a 12.5% (IC 95% 4.7 – 25.2%).

Ripartizione dei campioni

I campioni sono stati distribuiti per ciascuna Regione o Provincia Autonoma, in base al numero di stabilimenti che trasformano materiali di categoria 1, 2 e 3, riconosciuti ai sensi del Regolamento (CE) n. 1069/2009, presenti nell'elenco pubblicato sul sito del Ministero della Salute e sono riportati in tabella 5-2.

Campionamento

Devono essere prelevati esclusivamente campioni di prodotti derivati di categoria 1, 2 e 3 presso gli impianti di trasformazione e per i prodotti derivati di categorie 3 anche presso i produttori di mangimi composti che facciano uso di tali materie prime (PAT).

Composizione del campione

Il campione è ufficiale e deve essere composto da 4 CF di circa 500 grammi.

Sample Type	2017	2018	2019	2020	2021	2022	2023	2024	NC
								4 months	
MBM cat 1	16	14	13	1	10	6	11	7	6
MBM cat 2	5	6	7	18	16	4	7	8	4
PAPs cat 3	3	14	21	17	9	12	10	10	0
Fat cat 3	0	1	1	2	3	4	1	0	0
Total <i>per</i> year	24	34	42	38	38	26	29	25	10 out of 256 3,9%



Interlaboratory study on the detection of glycerol triheptanoate (GTH) in fats by GC-MS

June 2020

P. VEYS and O. FUMIERE

ANNEX III

Country	Laboratory name
Austria	AGES - Group for Contaminant and Special Analysis
Belgium	Servaco Food Control
Belgium	FLVVT
Finland	Finnish Food Authority - Chemistry Unit
France	Service Commun des laboratoires ; Etablissement de RENNES SCL L35
France	Inovalys
Germany	Chemisches und Veterinäruntersuchungsamt (CVUA) Sigmaringen
Germany	Chemisches und Veterinäruntersuchungsamt Westfalen (CVUA Westfalen), Anstalt des öffentlichen Rechts, Standort Arnsberg
Germany	Federal Institute for Risk Assessment (BfR), Standort Jungfernheide
Greece	General Chemical State Lab - A Chemical Service of Athens - section B
Hungary	Analytical National Reference Laboratory, NÉBIH - National Food Chain Safety Office
Italy	Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta
Netherlands	NutriControl
Poland	National Veterinary Research Institute - Department of Feedingstuffs Hygiene
Poland	Wojewódzki Inspektorat Weterynarii w Kielcach
Romania	Institutul de Igienă și Sănătate Publică Veterinară Institute for Hygiene and Veterinary Public Health
Slovakia	State veterinary and food institute Dolný Kubín
Slovenia	University of Ljubljana, Veterinary Faculty, National Veterinary Institute
Switzerland	Federal Department of Economic Affairs, Education and Research EAER, Agroscope, Division Method Development and Analytics

	GTH 25	GTH 50	GTH 100	GTH 250	Cat 2 Fat
Assigned value					
value :	22.77	45.55	91.10	227.70	737.46
	z score	z' score			

LAB CODE

L1	0.7	-0.3	-0.9	-0.8	-0.6
L2	0.3	-0.2	-0.4	0.2	0.1
L3	1.1	0.5	-0.6	-0.7	-0.5
L4	10.0	7.8	1.8	-2.1	0.7
L5	0.3	-0.1	-0.8	-1.0	-0.2
L6	0.2	1.3	0.2	0.0	-0.1
L7	7.6	4.1	0.6	1.8	2.0
L8	0.3	0.6	0.1	-0.1	0.2
L9	1.3	0.9	0.7	1.1	0.2
L10	3.1	-1.1	0.6	2.8	2.0
L11	1.4	0.5	-0.7	-0.3	-0.2
L12	1.5	-2.3	0.4	2.1	-0.3
L13	-1.3	-1.8	-0.1	5.0	-0.7
L14	2.4	1.1	0.8	0.4	0.2
L15	1.1	0.2	-0.2	0.7	0.2
L16	3.1	2.7	1.4	1.4	1.7
L17	2.7	-1.8	-2.5	-1.9	-1.3
L19	0.3	1.5	0.1	-0.7	-0.4
L20	0.4	-1.4	-0.3	-0.1	-1.6

Nb of lab	19	19	19	19	19
Satisfactory	13	15	18	15	19
Questionable	2	2	1	3	0
Unsatisfactory	4	2	0	1	0
% satisfactory z or z'	68%	79%	95%	79%	100%



2022: GTH Interlaborator y study

- NRL-AP Belgium
- NRL-AP The Netherlands
- NRL-AP Germany
- NRL-AP Italy
- NRL-AP Poland
- NRL-AP Slovenija



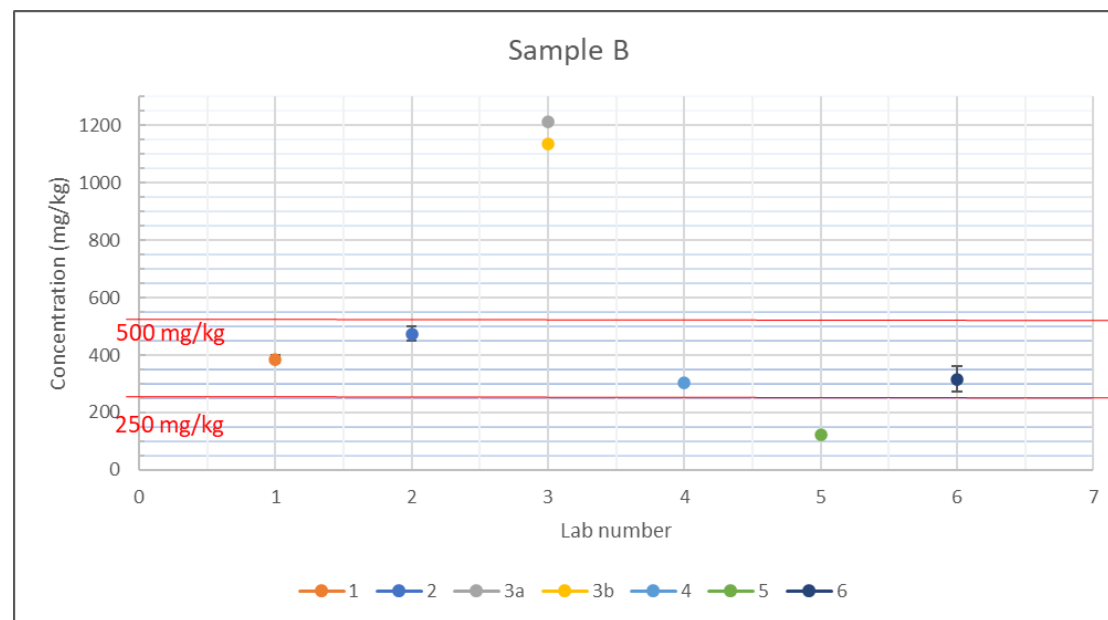
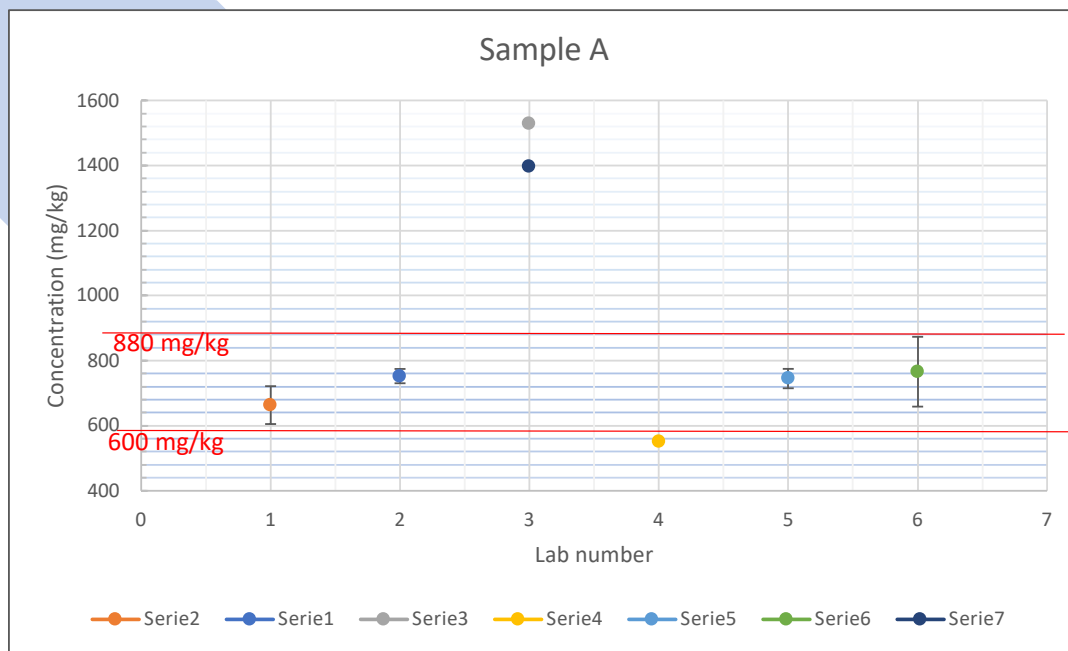
TWO Category 2 MBM samples

Information required :

- Accredited method
- Internal standard use
- Limit of quantification (LOQ)
- Range of measurement
- Uncertainty approach
- Certified standard use ISO 17034
- Sample amount
- Extraction solvent
- Drying step
- Clean-up technique
- Calibration curve
- QC samples
- Analytical technique
- m/z qualifier and quantifier

Results information:

- **GTH concentration**
- **Uncertainty (%)**
- **Recovery**
- **Concentration corrected for the recovery**
- **Repeatability**





2-3 FAT SAMPLES

COULD BE SENT
NEXT JULY

DEAD LINE
BY THE END OF
SEPTEMBER

2024: a new
GTH
Interlaboratory
study
is going to be
set up

**Enrolments
are open**

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Progetto presentato da:

ISTITUTO ZOOPROFILATTICO SPERIMENTALE
DEL PIEMONTE, LIGURIA E VALLE D'AOSTA

Area tematica: Sicurezza Alimentare - SIC

Titolo del progetto: I sottoprodotti di origine animale e il gliceroltriptanoato (GTH): garantire la tracciabilità e contrastare l'uso improprio nella produzione di mangimi, pet food e fertilizzanti-GTHTRACK

Research project

ABPs and GTH: Traceability
and prevention of misuse in
animal feed, pet food and
fertilisers

Granted by the Italian
Ministry of Health

8) Unità Operative impegnate nella ricerca:

N. identificativo	Ente appartenenza	Responsabile Scientifico
UO 1 IMS	IZS PLV - CreAA	Avolio Rosa
UO 2 IMS	IZS Mezzogiorno	Gallo Pasquale
UO 3 IMS	IZSLER	Faggionato Elena
UO 4 EMS	EURL-AP	Fumiere Olivier
UO 5 EMS	NRL-AP sloveno- Università di Lubiana	Ujčič Vrhovnik Igor

Partners:

- Italian NRL-AP
- IZSME (Naples, Italy)
- IZSLER (Brescia, Italy)
- EURL-AP
- Slovenian NRL-AP

A scenic landscape photograph of a hillside in Tuscany, Italy. In the foreground, there are terraced vineyards and olive groves. A small village with a prominent tower is visible on a hill in the middle ground. The background shows rolling hills under a warm, golden sunset sky. The text "Thank you very much for your attention" is overlaid in the center in a white, sans-serif font.

Thank you very much for your
attention