



## Opinion 08-2023 of the Scientific Committee established at the FASFC on a update of opinion 07-2013 - Endogenous presence of anabolic and/or prohibited substances in food producing animals

### Context & Questions

In 2013, the Scientific Committee issued an opinion (07-2013) on the presence of anabolic and/or prohibited substances of endogenous origin in food-producing animals. An amendment to this opinion regarding the presence of prednisolone residues of endogenous origin in porcine urine was published in 2014. The Scientific Committee also adopted an opinion (05-2020) on the re-evaluation of the action limit for thiouracil in bovine urine in 2020.

The Scientific Committee is now requested to update opinion 07-2013. The aim is to assess whether new scientific data in the last decade have demonstrated a natural origin for certain anabolic substances found in animal matrices, and to identify the thresholds and/or metabolites that allow to distinguish a natural origin from an exogenous administration.

To this end, the Scientific Committee is asked the following questions:

- Which of the substances examined by the FASFC are likely to have a natural origin (endogenous, animal feed, etc.) in a matrix of animal origin?
- In which matrices and for which animal species (categories, sex) can the natural presence of the substances possibly be determined?
- Can a residue concentration be determined that allows to distinguish between natural presence and illegal treatment for substance/matrix/animal species combinations?
- What alternative analysis, if any, can be used for substances whose natural origin cannot be excluded to determine whether the origin is natural or the result of illegal treatment (search for metabolites, serial or multiple sampling, etc.)?

### Method

This opinion was drafted based on expert opinion and available and relevant data from the scientific literature.

### Conclusions

To answer question 1, the Scientific Committee classified the substances in the opinion request into three groups:

- **Group 1:** substances of known or suspected natural origin
  - ✓ Group 1a: substances whose presence in a matrix of animal origin is known or suspected to have an endogenous origin via metabolism
  - ✓ Group 1b: substances whose presence in a matrix of animal origin is known or suspected to have a natural origin coming from animal feed
- **Group 2:** substances whose presence in a matrix of animal origin can be associated with accidental or environmental contamination;
- **Group 3:** substances for which there is no reason to suspect an endogenous origin.

Group 1 substances were studied in more detail to identify control strategies to distinguish endogenous origin (via metabolism or feed) from exogenous treatment. The table below summarises the available approaches for each molecule.

Substances	Species	Situations where an endogenous or natural origin can be suspected (Details in annex 1)	Control strategy or decision threshold as proposed by the EURL or NRL	Matrix
17 $\alpha$ -nortestosteron	Cattle	Endogenous: <ul style="list-style-type: none"> <li>✓ Neonatal calves</li> <li>✓ Pregnant female cattle</li> <li>✓ After acute injury in male cattle (fractures, paralysis, severe limping,...)</li> </ul>	Decision tree based on: <ul style="list-style-type: none"> <li>✓ Condition of the animal</li> <li>✓ 5<math>\alpha</math>-estraan-3<math>\beta</math>,17<math>\alpha</math>-diol</li> <li>✓ Confirmation by GC-C-IRMS</li> </ul>	Urine
17 $\alpha$ -nortestosteron	Sheep/ Goat	<ul style="list-style-type: none"> <li>✓ Endogenous in females - low concentrations</li> </ul>	Decision tree based on: <ul style="list-style-type: none"> <li>✓ Threshold concentration</li> <li>✓ Gender of animal</li> </ul>	Urine
17 $\beta$ -nortestosteron	Poultry	<ul style="list-style-type: none"> <li>✓ Endogenous</li> </ul>	Decision tree based on: <ul style="list-style-type: none"> <li>✓ Threshold concentration</li> <li>✓ Confirmation</li> </ul>	Liver
17 $\beta$ -nortestosteron	Pig	Endogenous at <ul style="list-style-type: none"> <li>✓ Boars</li> <li>✓ Sows/barrows - Low concentrations</li> </ul>	Decision tree based on: <ul style="list-style-type: none"> <li>✓ Gender of animal</li> <li>✓ Ratio of norepiandrosteron/norandrostenedion</li> <li>✓ Steroid profile</li> </ul> Or: confirmation via identification of esters in hair	Urine
Progesteron	Cattle	<ul style="list-style-type: none"> <li>✓ Endogenous</li> </ul>	<ul style="list-style-type: none"> <li>✓ Threshold value: 4,2 <math>\mu</math>g/L</li> </ul>	Urine
Cortisone	Cattle	<ul style="list-style-type: none"> <li>✓ Endogenous</li> </ul>	<ul style="list-style-type: none"> <li>✓ Threshold value: 2 <math>\mu</math>g/L</li> </ul>	Urine
Prednisolon	Cattle /Pig	<ul style="list-style-type: none"> <li>✓ Endogenous</li> </ul>	<ul style="list-style-type: none"> <li>✓ Threshold value: 5 <math>\mu</math>g/L</li> </ul>	Urine
Boldenon	Calves	<ul style="list-style-type: none"> <li>✓ Endogenous</li> </ul>	<ul style="list-style-type: none"> <li>✓ 17<math>\alpha</math>-Bol conjugate &gt; 2 ng/ml - Suspected illegal use</li> <li>✓ Presence of conjugated 17<math>\beta</math>-Bol in any concentration - Confirmation of illegal use</li> </ul>	Urine
Thiouracil	Cattle /Pig	<ul style="list-style-type: none"> <li>✓ Natural through feed</li> </ul>	Decision tree based on: <ul style="list-style-type: none"> <li>✓ Threshold value 2-thiouracil</li> <li>✓ 4-thiouracil</li> </ul>	Urine

			✓	6-methyl-thiouracil	
Zeranol/Taleranol	Cattle/Pig	✓	Natural through feed	Statistical model based on the quantitative profile of the metabolites of Resorcylic acid lactones (RALs)	Urine

## Recommendations

- The Scientific Committee recommends applying the control strategies presented in the summary table in the context of routine analyses for:
  - thiouracil in the urine of cattle and pigs: decision tree based on setting a threshold value (more than 30 µg/L in pigs and more than 10 µg/L in cattle) and the presence of metabolites (4-thiouracil and 6-methyl-thiouracil);
  - zeranol and taleranol in the urine of cattle and pigs: statistical model based on the quantitative profile of zeranol and taleranol with zearalenone and its two main metabolites (α- and β-zearalenol) in the urine of cattle and pigs to distinguish between the feed and treatment origin;
  - progesterone in bovine urine: threshold value of 4.2 µg/L;
  - prednisolone in the urine of cattle and pigs: threshold value of 5 µg/L;
  - cortisone in bovine urine: threshold value of 2 µg/L
- The Scientific Committee recommends the development of analytical methods (e.g. GC-C-IRMS), the search for biomarkers or the determination of concentration ratios between substances that allow to distinguish between an endogenous and an exogenous origin for substances for which no clear control strategy exists. In addition, the Scientific Committee recommends establishing guidelines with performance indicators for non-targeted methods in order to facilitate their accreditation in accordance with ISO 17025 and thus their application in official control programmes. Currently, the guidelines provide criteria for classical physicochemical methods such as LC/MS-MS or immunological methods such as ELISA. As the approach to non-targeted methods is different, it is important to establish specific minimum performance criteria to meet the requirements of ISO17025.
- The Scientific Committee recommends caution in interpreting results in case of detection of Group 1 substances for which no control strategy, which can reliably distinguish between natural origin and treatment, is yet available in routine analyses.
- The Scientific Committee recommends further investigations to confirm the endogenous origin of 11-deoxycorticosterone in bovine urine.

The full text is available on this website in dutch and in french.