



**CRL-NRL Workshop**  
**Bilthoven, NL, 20 October 2006**  
**Boldenone and Nortestosterone issues**

October 2006.

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**Boldenone and nortestosterone: an anabolic steroids**





Testosterone



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**A bit of boldenone-history**

- 1996; 17alpha-boldenone detected in samples of bovine urine not treated with boldenone (first report on natural occurrence)
- Increasing number of animals tested positive for boldenone in several EN Member States
  - Several hypothesis to explain this phenomenon
- On 25 February 2003, during a meeting of a SANCO workingparty, it was decided that an expert report on the current state-of-the-art should be written as soon as possible.

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Food Additives and Contaminants, Vol. 21, No. 6 (June 2004), pp. 515-525

**ARODOChr. 22941** 

**Presence and metabolism of the anabolic steroid boldenone in various animal species: a review**

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<sup>2</sup>boldenone metabolites (free and conjugated forms) in certain matrices of animals is proposed as a marker for the illegal treatment with boldenone.

**Keywords:** boldenone, endogenous, illegal treatment, bovine, calves, anabolic steroids, phytosterol, metabolism

-Content of report published in Food additives and Contaminants, (2004, 21, 515-525) De Brabander et al.

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**30 September 2003**

- **Conclusions from an expert meeting in Brussels**
  - Sampling of urine should be done without faecal contamination.
  - The presence of beta-boldenone conjugates at any levels in urine, from veal calves is a proof of illegal treatment.
  - For regulatory purposes, boldenone residue findings must always be specified as free or conjugated beta-boldenone and/or alpha-boldenone results with the explicit identification of the animal species.
  - If only alpha-boldenone conjugates are found at levels of 2 ppb or higher in urine of veal calves, additional investigations, in order to prove abuse of boldenone, are strongly recommended. The further study of appropriate marker metabolites of boldenone is encouraged.
  - The recommended Minimum Required Performance Limit (MRPL) for the analytical methods for the detection of beta and alpha-boldenone in urine of veal calves is 1 µg/l.

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**13 July 2005: reformulated text**

- **17Beta Boldenone-conjugates**
  - On the basis of scientific information available, the experts of the member States agreed that the presence of 17beta-boldenone conjugates at any level in urine from veal calves is a proof of illegal treatment
- **17Beta Boldenone unconjugated**
  - In case the analytical procedure used included a hydrolysis step in order to remove the conjugate-part from the steroid, which results in the detection and identification of 17 beta-boldenone, sampling of urine must be done without faecal contamination
- **17Alpha Boldenone**
  - There is sufficient scientific knowledge to conclude that the presence of 17alpha-boldenone in urine and faeces of bovine animals can come from other sources than illegal treatment.
  - If only 17alpha-boldenone conjugates are found and if the levels are above 2 µg/kg in urine of veal calves, additional investigations would need to be carried out before concluding on illegal use of boldenone

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### Today's objective: an update from 2003 - 2006

- **Prof. dr Bruno Le Bizec**
  - Report on research activities in France
- **Drs. Marco Blokland**
  - Report on research activities in The Netherlands
- **Prof. Dr. Hubert de Brabander**
  - Report on research activities in Belgium
- **Dr. Glenn Kennedy**
  - 19NT in casualty animals in Northern Ireland
- **Dr. Giovanni Ferretti**
  - New boldenone hydroxyl metabolites in cattle urine
- **Draft conclusions and recommendations: to be circulated and agreed upon**

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### 13 December 2002: Meeting of experts with the European Commission

- The presence of **beta-boldenone** at any levels in bovine animals is an indication of **illegal treatment**
- The recommended Minimum Required Performance Limit (MRPL) for the analytical methods for the detection of beta and alpha-boldenone should be 1 µg/l
- When alpha-boldenone alone is found at levels of 2 ppb or higher in urine of bovines, a statistically significant number of additional samples of the same group of animals should be taken and analysed as suspect with the aim of detecting alpha and/or beta-boldenone (the latter at the lowest possible level of detection)
- If beta-boldenone is found in these circumstances and/or if the percentage of samples with a level of alpha-boldenone of 2 ppb or higher exceed 20% of the number of animals analysed, the whole group, is considered as having been illegally treated.
- For regulatory purposes, boldenone residue findings must always be specified as beta-boldenone and/or alpha-boldenone results with the explicit identification of the animal species.

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