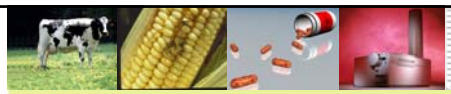

Marco Blokland | 20-11-2006

BOLDENONE IN CATTLE URINE (CONTINUED)

- Correlation between fecal marker and boldenone
- 6-Hydroxy-boldenone marker to discriminate endogenous vs. illegal

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Overview

- Facts and Hypothesis
- Experimental setup
- Analytical method
- Results for free α/ß-boldenone
- Results for conjugated α/ß-boldenone
- Conclusion

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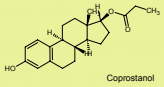
Facts

- After oral and intramuscular administration of ß-boldenone(-ester) one of the metabolites formed is 6ß-hydroxy-17α-boldenone
- Feces can contain endogenous free α/ß-boldenone
- As a fecal marker in waste water coprostanol is used



Coprostanol

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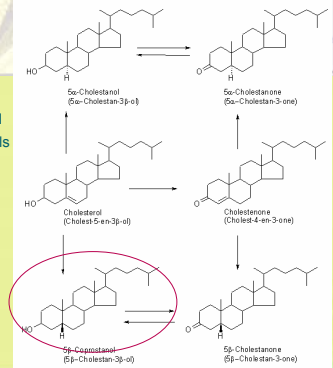
Origin of coprostanol

Coprostanol is formed from the biohydrogenation of cholesterol in the gut of most higher animals and birds



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Hypothesis

- By applying an analytical method which can discriminate between free boldenone, its glucuronide and sulphate conjugates, 6ß-hydroxy-17α/ß-boldenone and coprostanol, it should be possible to distinguish abuse from endogenous boldenone

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Experimental setup

- The method was applied to all suspected boldenone samples within the Dutch National Plan of 2004-2005.
- In total approximately 10.000 urine samples were screened (LC-MS) by the VWA east, from which 280 samples were suspected to contain boldenone.
- These 280 samples were all analysed for their conjugation state, 6ß-hydroxy-17α/ß-boldenone and for the presence of coprostanol.

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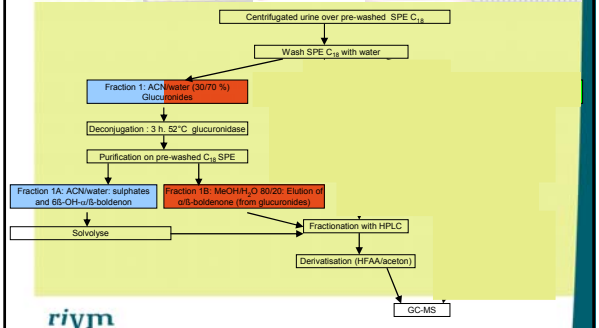
Analytical method



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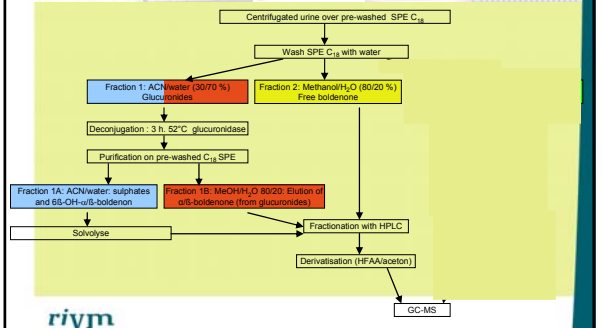
Analytical method



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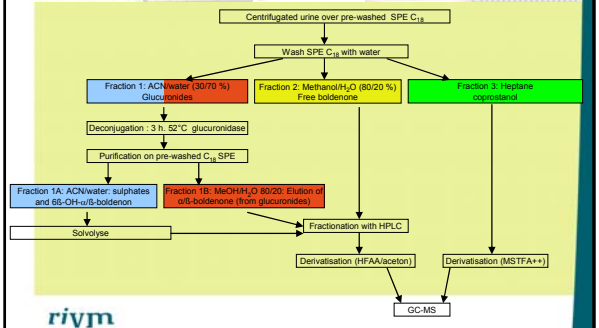
Analytical method



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Validation characteristics

	α-boldenone (µg/l)	β-boldenone (µg/l)	β-boldenone-glucuronide (expressed as free β-boldenone) (µg/l)
CCα	0.07	0.03	0.13
CCβ	0.12	0.05	0.22
Uncertainty of measurement (at 1 µg/l)	0.26	0.15	0.40

- For ß-boldenone-sulphate, 6ß-hydroxy-α/ß-boldenone and coprostanol a limited validation was performed by spiking 20 blank urines at 10 ng/ml, in all cases all the compounds were detected.

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Results depending on origin of samples

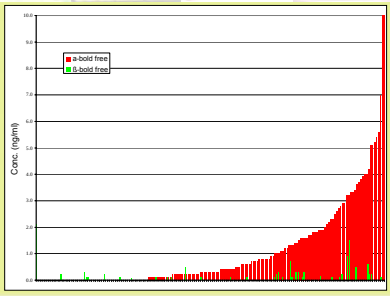
Percentages of samples positive for free and conjugated a/ß-boldenone

	α-bold free	α-bold conj	ß-bold free	ß-bold conj
Farm (n=254)	53	51	14	0
Slaughter (n=26)	4	33	8	0

Conclusion: there is a relation between origin and free α-boldenone

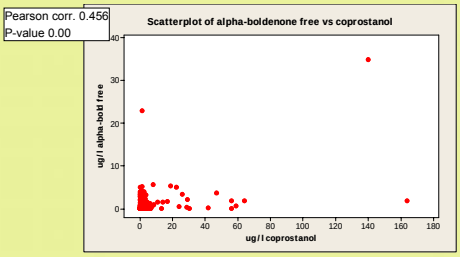
Free boldenone

- Results for free α- and ß-boldenone in the 280 suspected samples



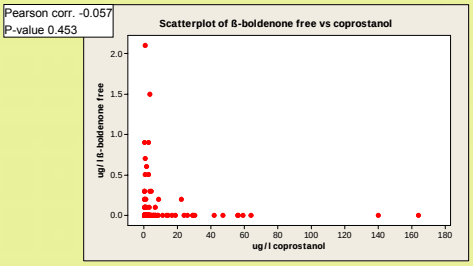
Free α-boldenone

- Pearson correlation between coprostanol and free α-boldenone



Free ß-boldenone

- Pearson correlation between coprostanol and free ß-boldenone



Conclusion on free boldenone and coprostanol

- There is a weak correlation between the fecal marker coprostanol and free α-boldenone
- In the study of Pompa, ß-boldenone was found in feces, but at much lower concentrations. This can explain the lack of correlation between the fecal marker coprostanol and free ß-boldenone.

Pompa G, Arioli F, et. al. *Neoformation of boldenone and related steroids in faeces of veal calves.* Food Addit Contam. 23(2), 2006, 126-32

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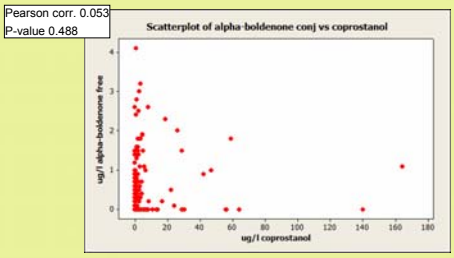
Conjugated α/ß-boldenone

Pearson correlation between coprostanol and conjugated α-boldenone



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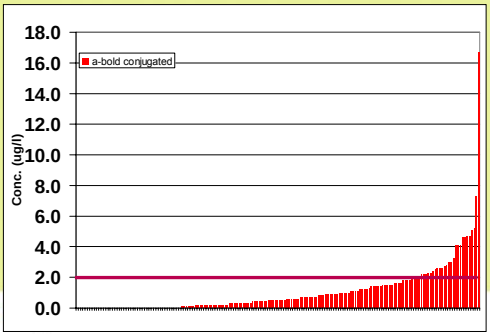
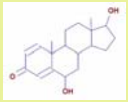
Conjugated α/ß-boldenone

- Overview of the results of the determination of conjugated α-boldenone in 280 urine samples suspected for boldenone.



Conjugated α/ß-boldenone

- Current EC guidelines advice further studies in case samples of urine from veal calves contain more than 2 µg/l conjugated α-boldenone
- 28 samples needed further testing for the presence of 6ß-hydroxy-17α/ß-boldenone



- In none of these samples this metabolite was found

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Conclusion

- Using coprostanol, it was confirmed that the presence of α-boldenone can be a result of fecal contamination.
- For free ß-boldenone and conjugated α-boldenone no such correlation was obtained.
- Approximately 10 % of the suspected samples contained more then 2 ug/L conjugated α-boldenone
- None of the samples tested contained residues of the metabolites 6ß-hydroxy-17α/ß-boldenone which confirms the endogenous origin of conjugated α-boldenone
- Free ß-boldenone and conjugated α-boldenone are no indicators for boldenone abuse.
- The current approach of conjugated 17-β-boldenone as indicator for abuse still is valid.

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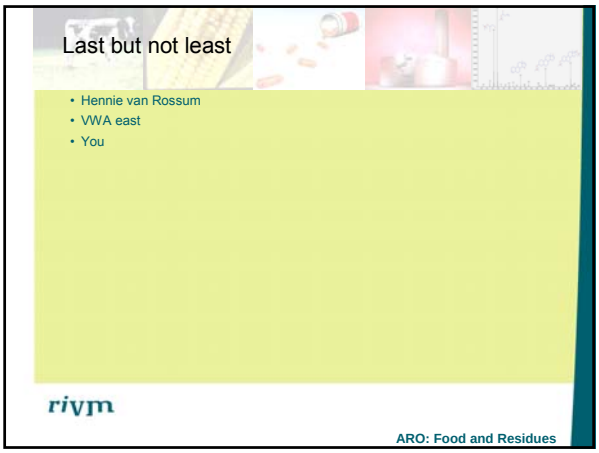
Last but not least
• Hennie van Rossum
• VWA east
• You
rivm
ARO: Food and Residues