Nature, source and incidence of meat contaminants interfering with anti-doping tests

18th November 201, Saskia S. Sterk

Overview

- Introduction
- Compounds concerned
- Incidence
- Conclusion

Overview

- Introduction
- Compounds concerned
- Incidence
- Conclusion

PROHIBITED LIST JANUARY 2017

WORLD ANTI-DOPING AGENCY
play true

- **S0** Non approved substances
- **S1** Anabolic agents
  - 1. AAS: A. Exogenous
    B. Endogenous
  - 2. Other anabolic agents
    - Clenbuterol, SARMs, Zeranol, Zilpaterol
- **S2** Peptide Hormones etc.
  - 5. GHRPs
  - IGF-1
- **S3** Beta-2 Agonists
- **S4** Hormone and Metabolic Modulators, SERMs, AI, AE
- **S9** Glucocorticoids

European Legislation

23. 5. 96 EN Official Journal of the European Communities No L 125/3

COUNCIL DIRECTIVE 96/22/EC

of 29 April 1996

ARODOCnr. 16793

concerning the prohibition on the use in stockfarming of certain substances having a hormonal or thyrostatic action and of beta-agonists, and repealing Directives 81/602/EEC, 88/146/EEC and 88/299/EEC

14.10.2003 EN Official Journal of the European Union L 262/17

DIRECTIVE 2003/74/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL

of 22 September 2003

amending Council Directive 96/22/EC concerning the prohibition on the use in stockfarming of certain substances having a hormonal or thyrostatic action and of beta-agonists

European legislation (2)

ANNEX I

GROUP A — **Substances having anabolic effect and unauthorized substances**

(1) Stilbenes, stilbene derivatives, and their salts and esters

(2) Antithyroid agents

(3) Steroids

(4) Resorcylic acid lactones including zeranol

(5) Beta-agonists

(f) Others

European legislation (3)

1. Bovine animals

The minimum number of animals to be controlled each year for all kinds of residues and substances must at least equal 0,4 % of bovine animals slaughtered the previous year, with the following breakdown:

Group A: 0,25 % divided as follows:

— one half of the samples are to be taken from live animals on the holding;

(by derogation, 25 % of samples analysed for the research of Group A 5 substances can be taken from appropriate material (feedingstuffs, drinking water, etc.))

— one half of the samples are to be taken at the slaughterhouse.

Each sub-group in Group A must be checked each year using a minimum of 5 % of the total number of samples to be collected for Group A.

The balance must be allocated according to the experience and background information of the Member State.

European legislation (4)

- Porcine animals:

Group A: 0,02 %

In those Member States which carry out their sampling of animals at the slaughterhouse, in addition analysis of drinking water, feedingstuffs, faeces, or all other appropriate parameters must be undertaken at farm level. In that case, the minimum number of farms to be visited annually must represent at least one farm per 100 000 pigs slaughtered the previous year. .

European legislation (5)

- Matrices for residue control:
  - On farm: urine, hair, water, feed
  - Slaughtherhouse: urine, meat, liver, kidney

Outside EU

USA; use of T, E, P, MPA, Z, trenbolone, Zilaterol, Ractopamine, rBST allowed

Table 4. FY2016 NRP Residue Scheduled Samples -Number of Residue Samples Tested Per Chemical Method by Animal Class

Animal Class (# Samples Collected)	Number of Samples per Chemical Method									
	Aminoglycosides	Arsenic	Avermectins	βeta-Agonists	Carbadox	Hormones	Metals	MRM	Nitrofurans	Pesticides
Beef Cows (730)	725	397	392	289	--	357	114	730	--	286 (1)
Bob Veal (574)	571	326	323 (1)	216	--	294	118	574 (2)	--	211
Dairy Cows (739)	737	395	392	302	--	348	112	739 (2)	--	304 (1)
Heifers (526)	524	313	310	180	--	294 (2)	114	526	--	177
Steers (511)	510	306	303	175	--	276	107	511	--	175
Market Swine (800)	798	447	442	150	2	--	127	799	--	333
Roaster Swine (281)	280	65	64	--	215 (4)	--	17	281 (2)	--	--
Sows (769)	764	427	421	135	-	1	111	769	--	290 (1)
Mature Turkeys (93)	1	1	--	--	--	--	93	1	--	--
Young Chickens (760)	759	408	--	--	--	--	155	760	340	316
Young Turkeys (657)	656	371	1	--	--	--	154	657	275	141
Goats (337)	260	195	198 (7)	1	--	--	--	337	--	141
Mature Sheep (290)	200	155	153 (1)	1	--	--	--	290	--	131 (2)
Total (7,067)	**6,785**	**3,806**	**2,999**	**1,449**	**217**	**1,570**	**1,222**	**6,974**	**615**	**2,505**

Outside Europe (2)

- **Use allowed in ao:**
- South America (ao Ractopamine, Zeranol)
- USA: (natural steroids, zeranol, trenbolne, melengestrol, rBSt, ractopamine)
- South Africa (zilpaterol, steroids, rBSt)
- Australia, New Zealand

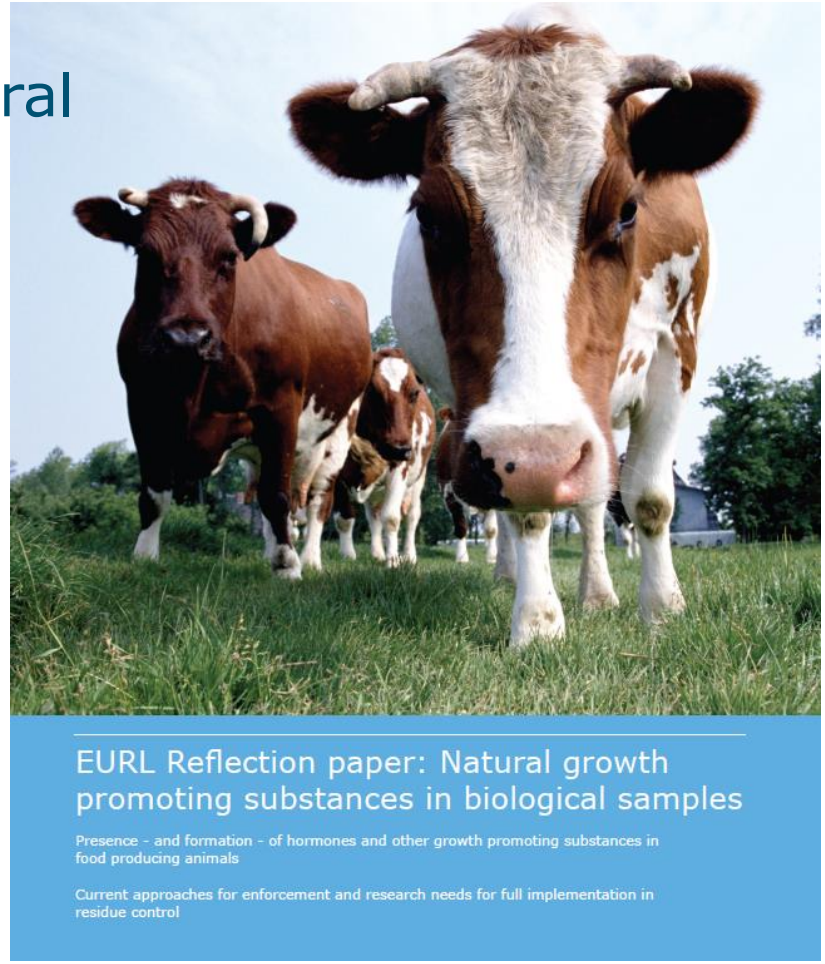
Overview

- Introduction
- Compounds concerned
  - Endogenous source
  - Exogenous source
- Incidence
- Conclusion

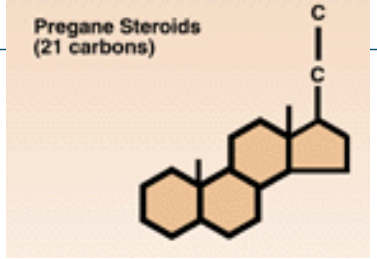
Compounds concerned
Compounds from endogenous source

- 2014 Reflection paper on natural occuring growthpromoters

- Natural hormones
- Minor androgens
- Zeranol
- prednisolone

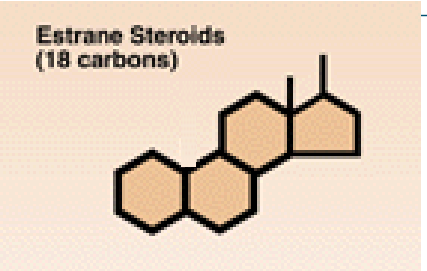


Progestagens



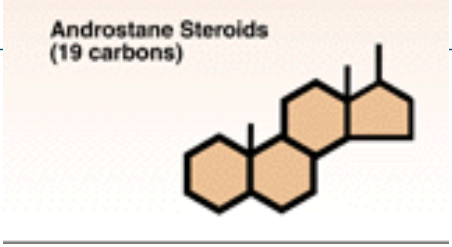
- Suppress the production of progesterone and other sex steroids such as the androgens (weak) and the estrogens (strong)
- Keep the animals relaxed (observation)
- Pro-hormone for other sex-hormones
- Slow the release of estradiol from compressed pellet implants
- Found in cocktails in combination with andogens

Estrogens



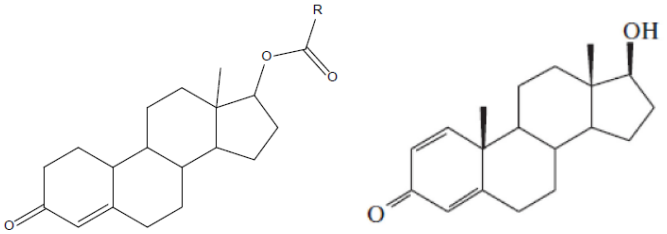
- Increase fat stores
- Stimulation the somatotropic axis to increase growth hormone and thus IGF-1
- Increases growth rate by 10%–20% in steers, lean meat content by 1%–3%, and feed efficiency by 5%–8%.
    - In steers effective
    - Some anabolic effects in heifers and veal calves.
    - In lambs in conjunction with androgens.
    - Not effective as an anabolic agent in pigs.

Androgens



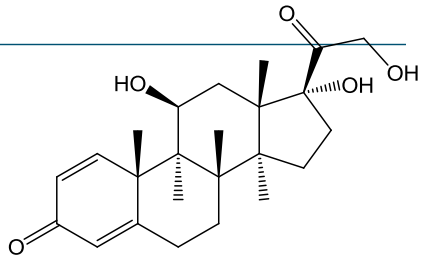
- It is the principal male sex hormone
- Increasing muscle mass
- Slow the release of estradiol from compressed pellet implants
- Found in cocktails as ester forms

Nandrolone and Boldenone



- NT Natural occurring in boars, pigs, casualty animal (bovine), pregnant cattle, male horse
- NT and BOL Not registered for growth promotion
- Bol naturally occurring in intact male animals (horse) free form artefact due to enzyme, temperature
- Bot found in cocktails containing different androgens

Prednisolone



- Prednisolone, a corticosteroid with glucocorticosteroid activity. Used for treatment of a wide range of inflammatory and auto-immune conditions.
- The maximum residue limit for therapeutic use of prednisolone in bovine animals is 4 µg/kg in muscle and fat, 10µg/kg in liver and kidney and 6 µg/kg in milk (EU Commission Regulation 37/2010).
- Corticosteroids can prolong the effect of growth-promoting substances, such as anabolic steroids and β-agonists, in the last weeks before slaughter. In addition low doses of glucocorticoids can result in improved feed intake, increased live weight gain, reduced feed conversion ratio, reduced nitrogen retention and increased water retention and fat content

Literature Conclusion Prednisolone

Concluding from literature:

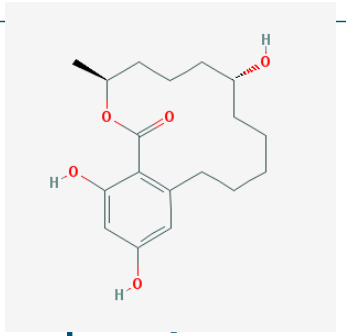
PRD seen in Bovine, different ages, beef and dairy cattle, porcine and equine

Relation with stress and Cortisol. Seen in some studies but not in all

Relation with faecal contamination, in-vitro proof

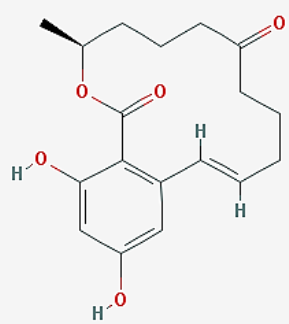
Proposed by bacterial transformation, bacteria from faeces or soil.

Zeranol

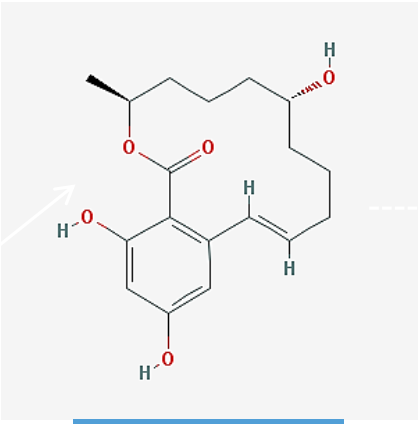


- Zeranol is approved for use as a growth promoter in livestock in USA and Canada
- Implantation of Zeranol in calves causes an improvement in mean liveweight gain
- Zeranol is banned within the EU
- Zeranol is prepared commercially from zearalenone, one of a number of structurally similar toxins produced by *Fusarium spp. fungi*
- CRL Guidance paper of December 2007 a recommended concentration was proposed of 1 µg/l or kg in urine and muscle and, 2 µg/kg in liver.
-

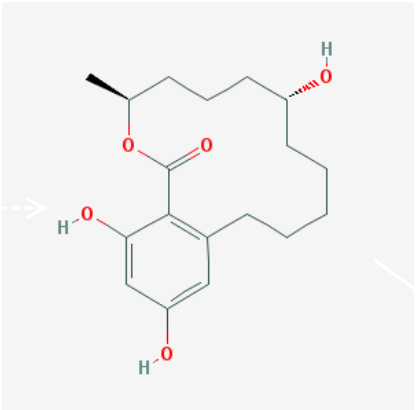
Structure *Fusarium* toxins and zeranol



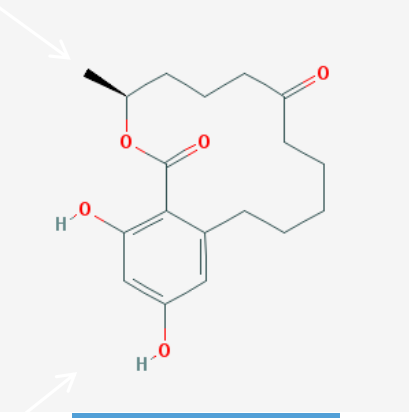
zearalenone



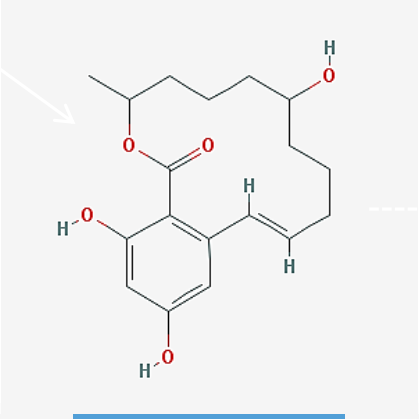
a-zearalenol



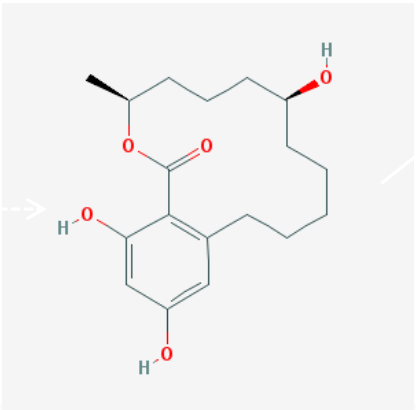
Zeranol (a-zearalanol)



zearalanone



ß-zearalenol



Taleranol (ß-zearalanol)

Exogenous Compounds Commercially available in US

Chronological sequence of FDA approval of growth promotants used in the U.S. beef cattle industry

Growth Promotant	Year of FDA Approval
Oral diethylstilbestrol (DES)	1954
DES implant	1956
Estradiol benzoate / progesterone (steers)	1956
Estradiol benzoate / testosterone propionate (heifers)	1958
Oral melengestrol acetate (heifers)	1968
Zeranol (36 mg) implants (cattle)	1969
Oral DES removed from market	1972
DES implants removed from market	1973
Silastic estradiol implant (cattle)	1982
Estradiol benzoate / progesterone (calves)	1984
Trenbolone acetate (TBA) implants (cattle)	1987
Estradiol (17-β) / TBA implants (steers)	1991
Bovine somatotropin (lactating dairy cows)	1993
Estradiol (17-β) / TBA implants (heifers)	1994
Zeranol (72 mg) implants (cattle)	1995
Estradiol (17-β) / TBA implants (stocker cattle)	1996
Ractopamine hydrochloride (cattle)	2003
Zilpaterol hydrochloride (cattle)	2006

From Smith&Johnson, USADA 2017

Illegally available

New emerging compounds in veterinary field

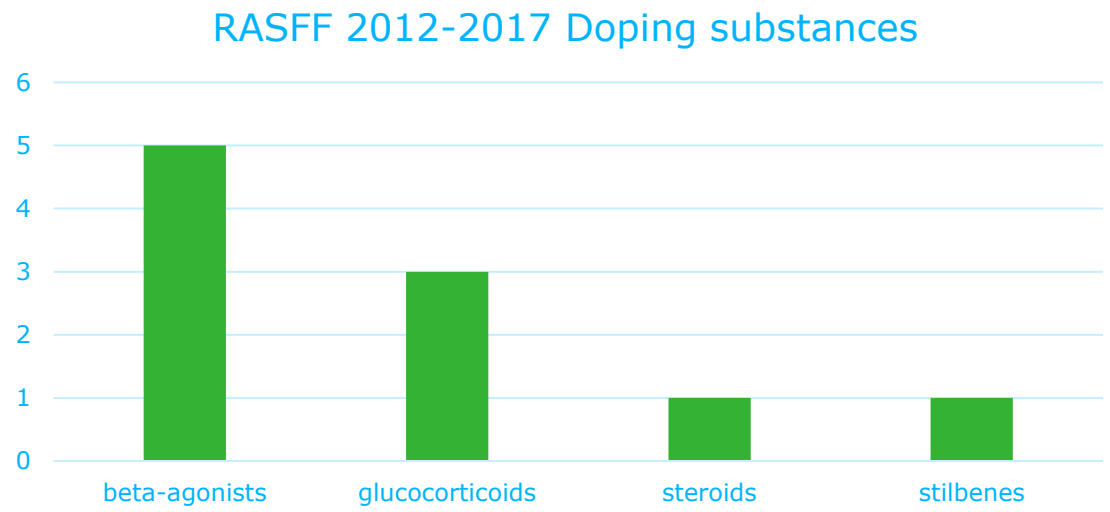
- SARMs and SERMs
- AI, AE
- IGF-1
- GHRP
- rBST
- PST
- Myostatine inhibitors
- ……..

Overview

- Introduction
- Compounds concerned
- Incidence
- Conclusion

Incidence RASFF

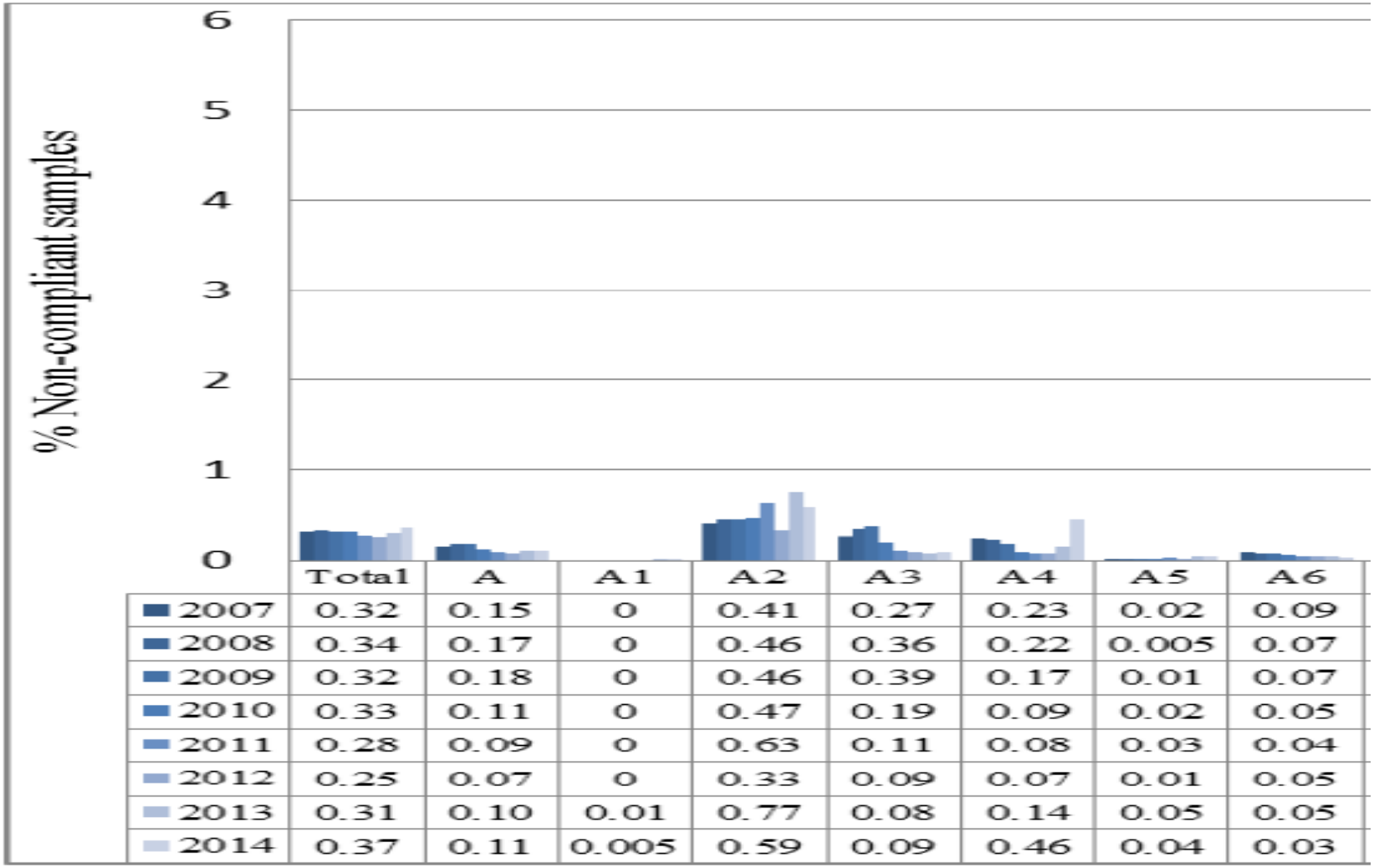
- Rapid Alert System for Food and Feed from EU
- 1-1-2012 till 12-9-2017
  - 377 notifications on veterinary medicinal products
  - 10 out of 377 concerning doping related substances



Incidence RASFF (2)

Compound	Matrix	Species	Origin	Conc.
clenbuterol	?	horse	NL	4.2 ppb
zilpaterol isoxuprine	meat	horse	Mexico	
zilpaterol Isoxuprine	liver	horse	Mexico	62 ppt 6 ppt
clenbuterol	?	horse	Canada	2.3 ppb
ractopamine	liver	bovine	Canada	0.6 ppb
dexamethasone	?	calf	Spain	3 ppb
dexamethasone	liver	calf	Italy	155 ppb
prednisolone	liver	horse	Poland	0.6-1.7 ppb
boldenone	meat	bovine	Italy	
DES	?	porcine	Denmark	1.3 ppb

Incidence EFSA data (3)



	Total	A	A1	A2	A3	A4	A5	A6
2007	0.32	0.15	0	0.41	0.27	0.23	0.02	0.09
2008	0.34	0.17	0	0.46	0.36	0.22	0.005	0.07
2009	0.32	0.18	0	0.46	0.39	0.17	0.01	0.07
2010	0.33	0.11	0	0.47	0.19	0.09	0.02	0.05
2011	0.28	0.09	0	0.63	0.11	0.08	0.03	0.04
2012	0.25	0.07	0	0.33	0.09	0.07	0.01	0.05
2013	0.31	0.10	0.01	0.77	0.08	0.14	0.05	0.05
2014	0.37	0.11	0.005	0.59	0.09	0.46	0.04	0.03

Incidence EURL data
NC results 2012-2015

Substance groups	NC 2012	NC 2013	NC 2014	NCs* 2015
A1 Stilbenes	0	3	1	0
A2 Antithyroid agents	36	68	54	62
A3 Steroids	40	49	59	33
B2f Corticosteroids	68	33	47	20
A4 RALs	16	34	68	71
Total	**160**	**187**	**229**	**186**

Incidence EURL data (2)
NC Results ANPs 2015 (B + P)

Substance groups	Non compliant findings	Non compliant samples	n samples EU28
A1 Stilbenes	0	0	B: 13148 P: 6182
A2 Antithyroids	63	62	B: 4403 P: 3114
A3 Steroids	38	33	B: 27882 P: 9729
B2f Corticosteroids	20	20	B: 10838 P: 6218
A4 RALs	92	71	B: 11079 P: 5160
Total	**213**	**186**	

Incidence EURL data (3)
A3 Steroids:2014 (t + s + i)

Substance	Species (sampling)	Member State (NC)	NC results
	Subtotal steroids	**7**	**50**
Boldenone	Bovines (target) Bovines (others) Pigs (target)	AT(1), HR(1) IT(2) PL(5)	2 2 5
17α Boldenone	Bovines (target) Bovines (others)	AT(1), HR(1), NL(1) IT(2)	3 2
Epi-nandrolone	Sheep/goats (target)	NL(1)	1
Nandrolone	Bovines (target) Bovines (suspect) Pigs (target) Pigs (suspect)	HR(8) HR(1) CZ(1), NL(6), PL(7) CZ(1)	8 1 14 1
Methyl-testosterone	Bovines (target) Bovines (suspect)	BE(1) BE(6)	1 6
17β-Trenbolone	Bovines (target) Bovines (suspect)	HR(3) HR(1)	3 1

Incidence EURL data (4)
A3 Steroids:androgens 2015 (target)

Substances	Species	Member states NC	NCf	NCs
A3 total	**Bovines** **Pigs**		**23** **15**	**18** **15**
Boldenone	Bovines	HR (1)	1	
	Pigs	PL (2)	2	
17 α-Boldenone	Bovines	NL (5)	5	
Epinandrolone	Bovines	HR (6), CZ (1)	7	
Nandrolone	Bovines	HR (4), PL (1)	5	
Testosterone-17-beta	Bovines	AT (1)	1	
17-β-trenbolone	Bovines	HR (2)	2	
	Pigs	CZ (2), PL (2)	4	
Nortestosterone acetate	Bovines	FR (1)	1	
	Pigs	NL (9)	9	

Overview

- Introduction
- Compounds concerned
- Incidence
- Conclusion

Conclusion

- Use of hormones and growthpromoters banned in EU by law
- Residue Control in EU for steroids, glucocorticoids and beta-agonists in meat (edible tissues) only a small % of the production.
- Non-compliant results over past 5 years very low and stable
- Exogenous steroids only very small number non-compliants. Beta-agonists somewhat higher
- Outside EU some countries use of steroids and growthpromoters allowed.
- Residue control in these countries on small numbers of samples

Conclusion continued

- Illegal use relatively easy due to availability of preparations on internet
- Difficult to combat in certain countries (eg. Mexico)
- Chance of adverse analytical finding in anti doping analysis small in EU higher in other hot spot parts of the world South America, Asia)

Thank you for your attention!