



Testing slaughter animals for residues; what is the state of the art?

Leendert A. van Ginkel, RIVM, The Netherlands
Bruno LeBizec, ENVN LABERCA, France

8 & 9 NOVEMBRE 2006 : SAINT-MALO - FRANCE
4^{EME} SYMPOSIUM INTERNATIONAL DE LA FILIÈRE VEAU
"Enjeux et nouvelles perspectives pour la filière veau"



Pf Dr Bruno Le Bizec

Laboratoire d'Etude des Résidus et Contaminants dans les Aliments (LABERCA)

Ecole Nationale Vétérinaire de Nantes (ENVN)

Route de Gachet – BP 50707 - 44307 Nantes Cedex 3

FRANCE

Tél : 33 2 40 68 78 80

Fax : 33 2 40 68 78 78

E-mail : lebizec@vet-nantes.fr



9 November 2006

VEAL SYMPOSIUM 2006 ST. MALO.

Testing slaughter animals for residues; what is the state of the art?

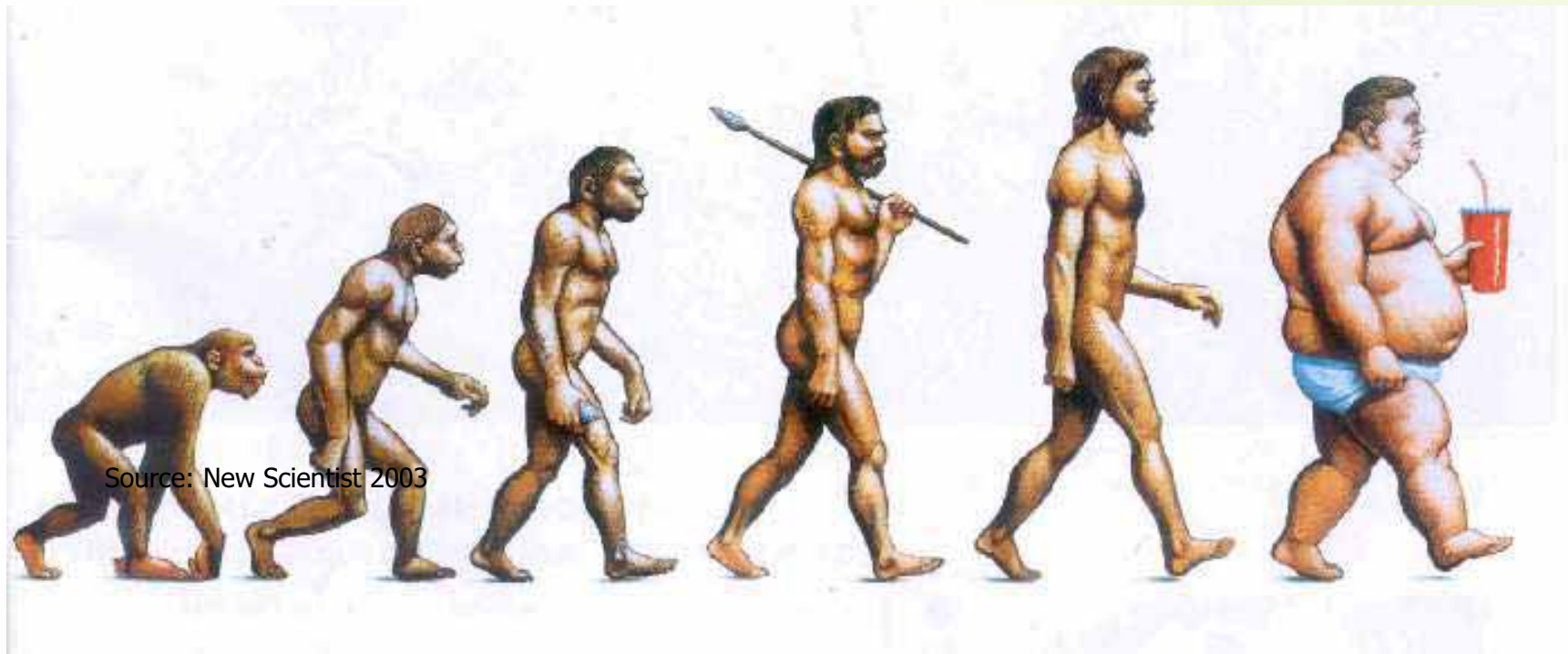


9 November 2006

VEAL SYMPOSIUM 2006 ST. MALO.

Testing slaughter animals for residues; what is the state of the art?

Healthy Food



9 November 2006

VEAL SYMPOSIUM 2006 ST. MALO.

Testing slaughter animals for residues; what is the state of the art?

Healthy food



9 November 2006



VEAL SYMPOSIUM 2006 ST. MALO.

Testing slaughter animals for residues; what is the state of the art?

Safe food



9 November 2006

VEAL SYMPOSIUM 2006 ST. MALO.

Testing slaughter animals for residues; what is the state of the art?

Contaminants and Residues



Natural Toxines



Veterinary Drugs



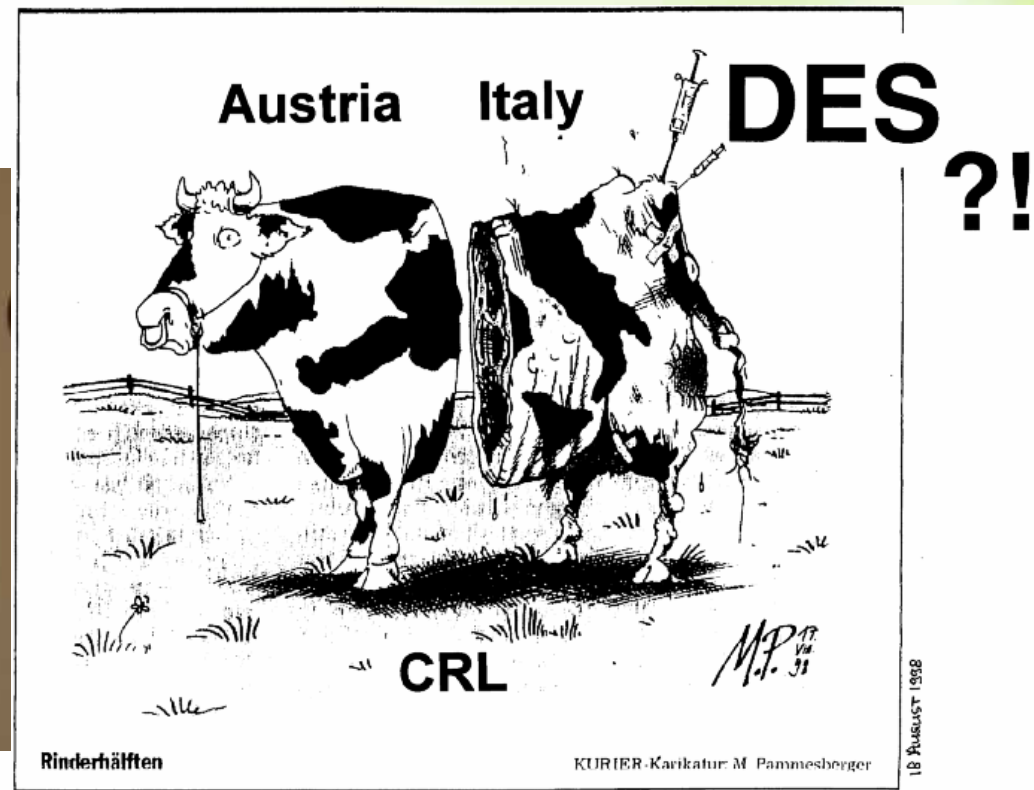
Industrial Contaminants

9 November 2006

VEAL SYMPOSIUM 2006 ST. MALO.

Testing slaughter animals for residues; what is the state of the art?

Hormonal Growth Promoters



9 November 2006

VEAL SYMPOSIUM 2006 ST. MALO.

Testing slaughter animals for residues; what is the state of the art?

Council Directive 96/23



ANNEX I

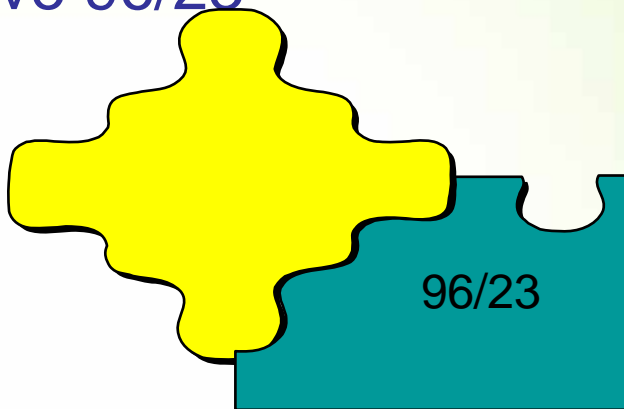
GROUP A

Substances having anabolic effect and unauthorized substances

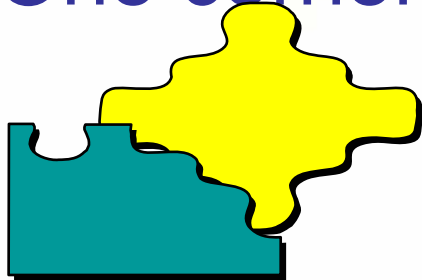
- 1 - Stilbens, stilben derivatives, and their salts and esters
- 2 - Thyrostats
- 3 - Steroids
- 4 - Resorcylic acid lactones including zeranol
- 5 - β -agonists
- 6 - Compounds included in Annex IV of EC n°2377/90

GROUP B

Veterinary drugs and contaminants



One cornerstone: network of laboratories



EU-Member
States

Current and future
(NRLs)



Community
Reference
Laboratories



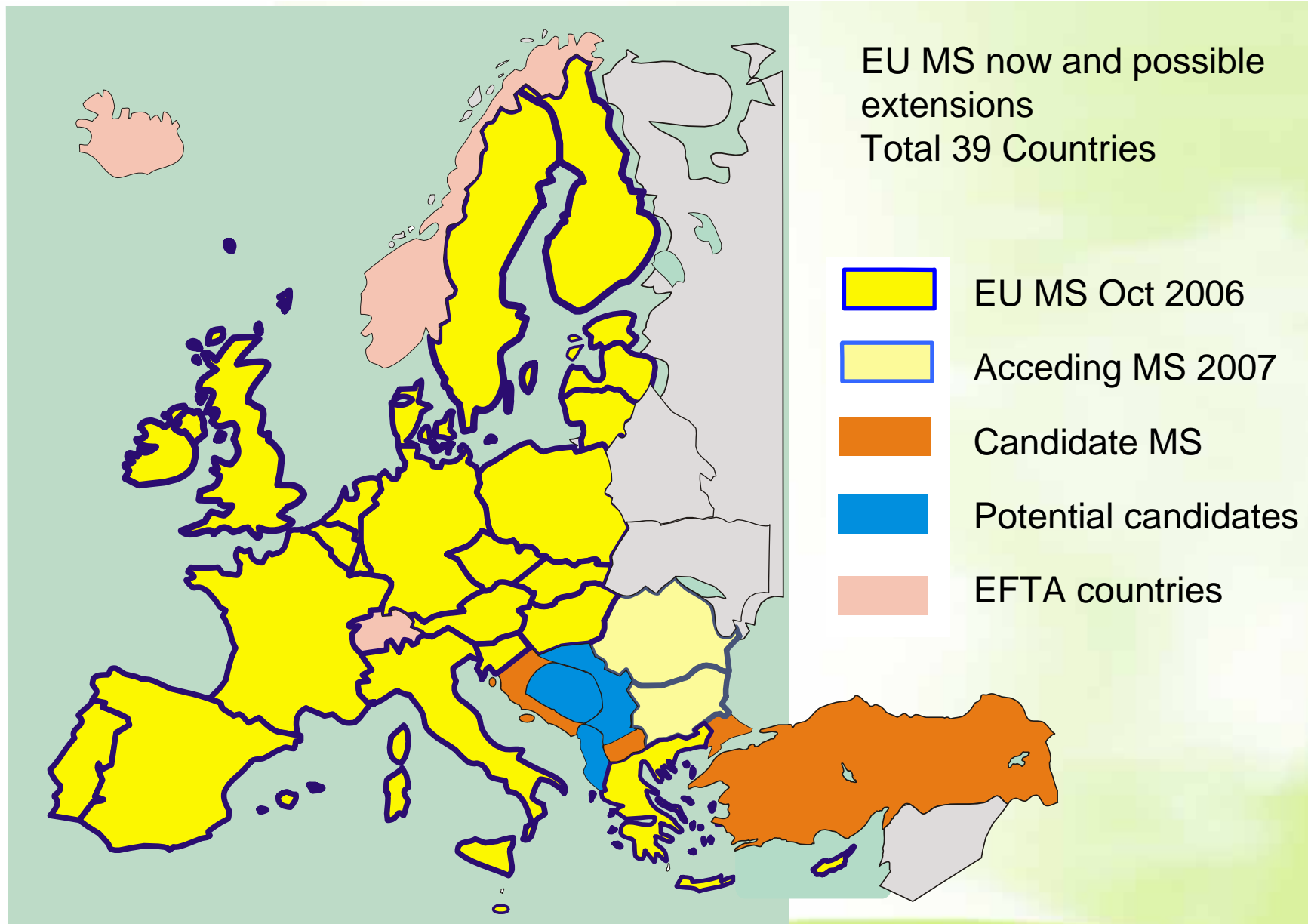
European
Commission



9 November 2006

VEAL SYMPOSIUM 2006 ST. MALO.

Testing slaughter animals for residues; what is the state of the art?

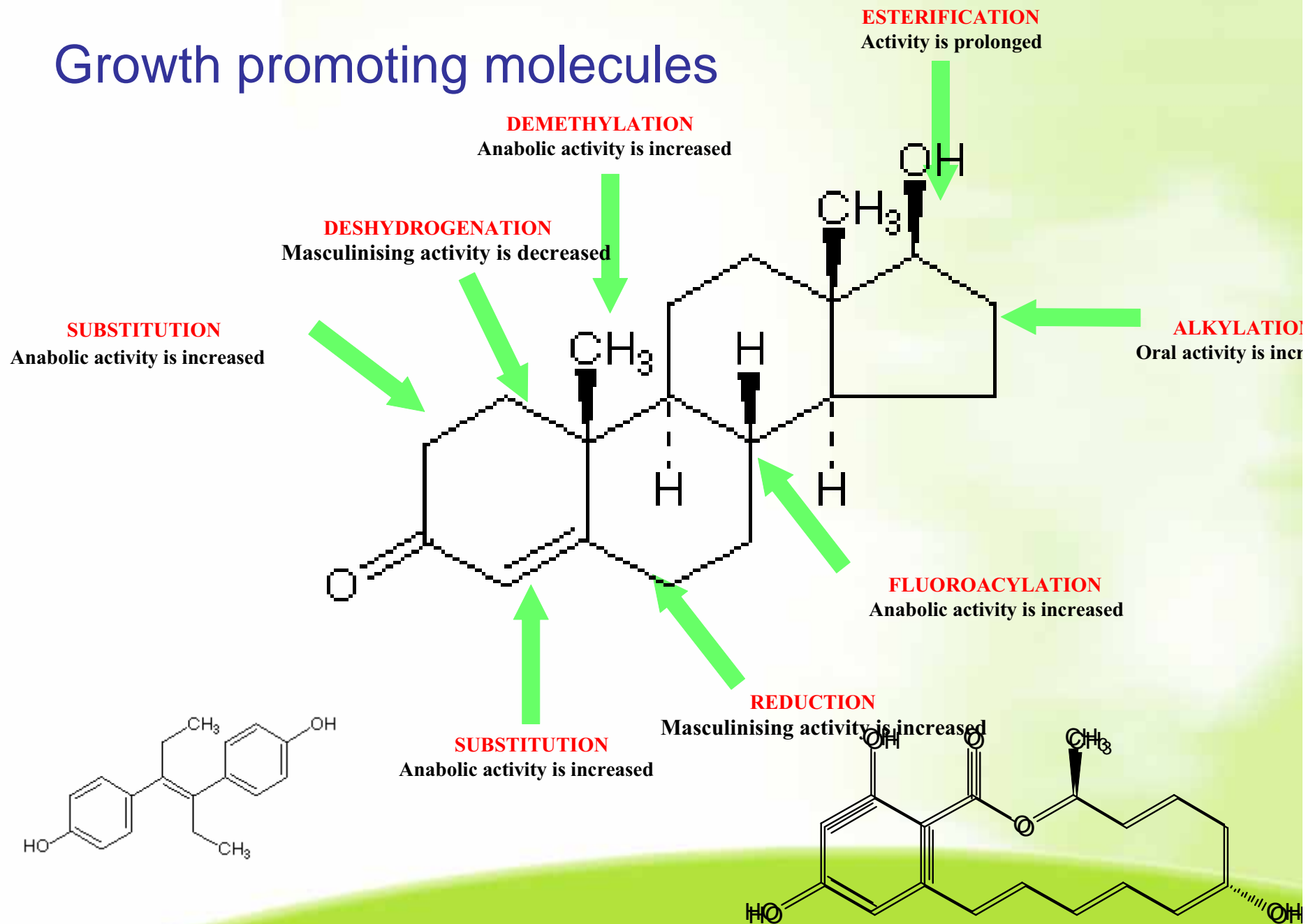


9 November 2006

VEAL SYMPOSIUM 2006 ST. MALO.

Testing slaughter animals for residues; what is the state of the art?

Growth promoting molecules



9 November 2006

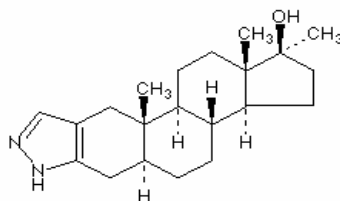
VEAL SYMPOSIUM 2006 ST. MALO.

Testing slaughter animals for residues; what is the state of the art?

Athletes and farmers exchange information on “best practices”



Stanozolol



A problem in sport



A problem in farming

9 November 2006

VEAL SYMPOSIUM 2006 ST. MALO.

Testing slaughter animals for residues; what is the state of the art?

Biotransformation of (pro) hormones

prohormones



excretion

activation and metabolism

9 November 2006

VEAL SYMPOSIUM 2006 ST. MALO.

Testing slaughter animals for residues; what is the state of the art?

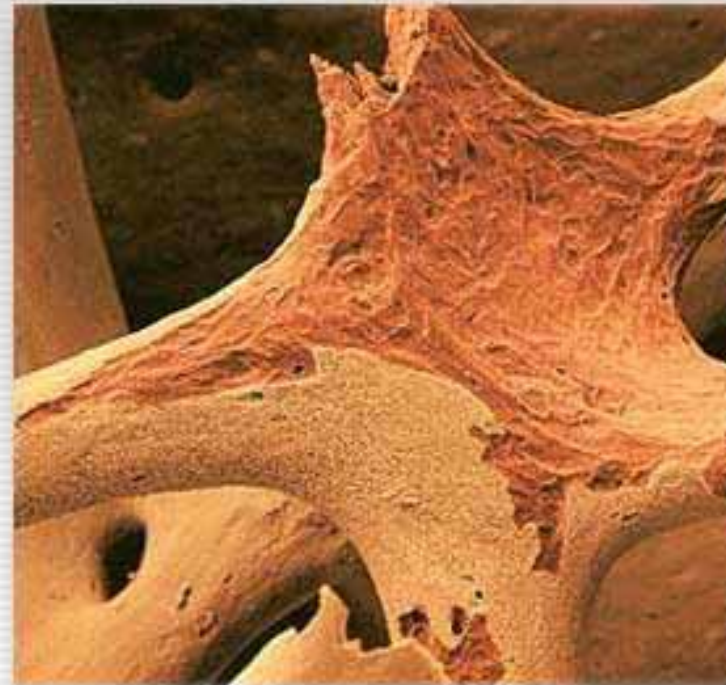
Different approaches for residue testing

- Using the activity: functional test
- Using the chemistry: (fysical) chemical test

Functional tests

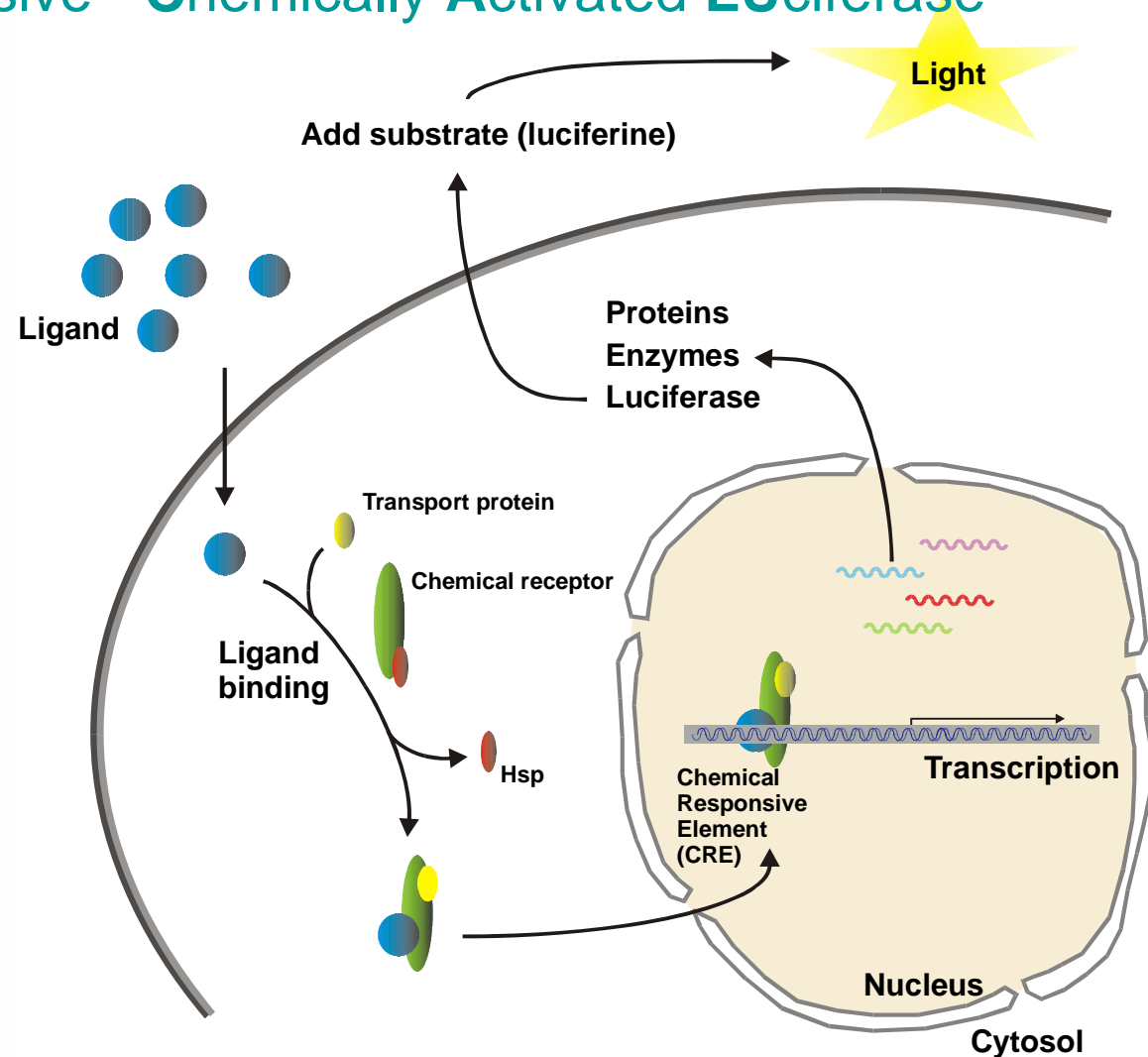
Effect assays : e.g. hormonal effects

- Mouse uterus weight test for oestrogens
- Rat seminal vesicle test for androgens



The Estrogen Responsive - Chemically Activated **LU**ciferase eXpression (ER CALUX®) assay

ER-CALUX uses a genetically modified T47D human breast adenocarcinoma cell expressing an endogenous estrogen receptor.



Hormonal Effect Assay

Advantages:

- * total estrogenic effect
- * sensitive and rapid
- * high sample throughput
- * also unknown compounds



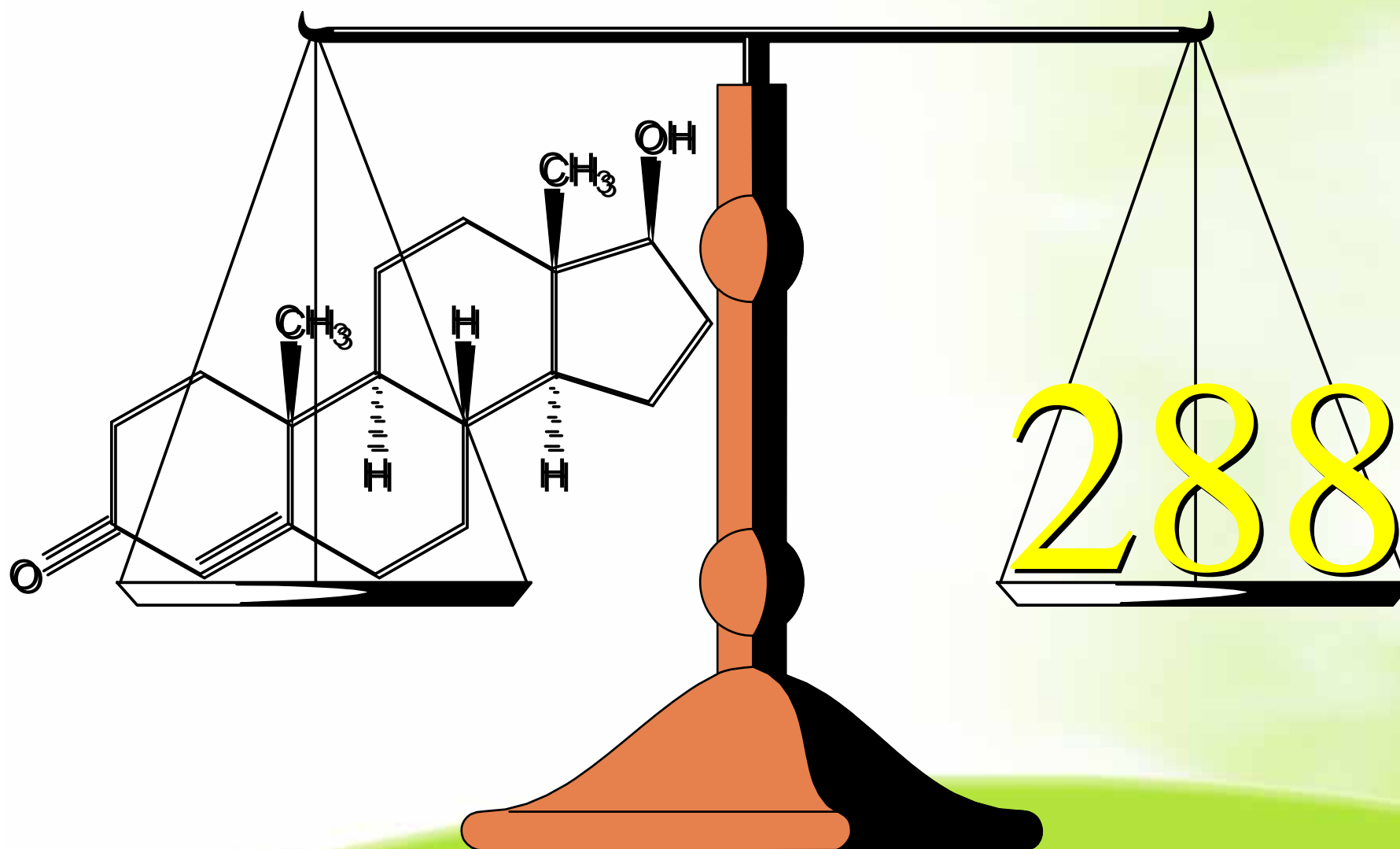
Disadvantages:

- * no absolute amount per substance
- * endocrine disruptors also measured
- * biological effect, no identification individual compounds



Chemical test

- Use physical chemical properties of molecules to determine the identity and concentration
 - Several possibilities, but in residue analyses Mass measurements are the most important



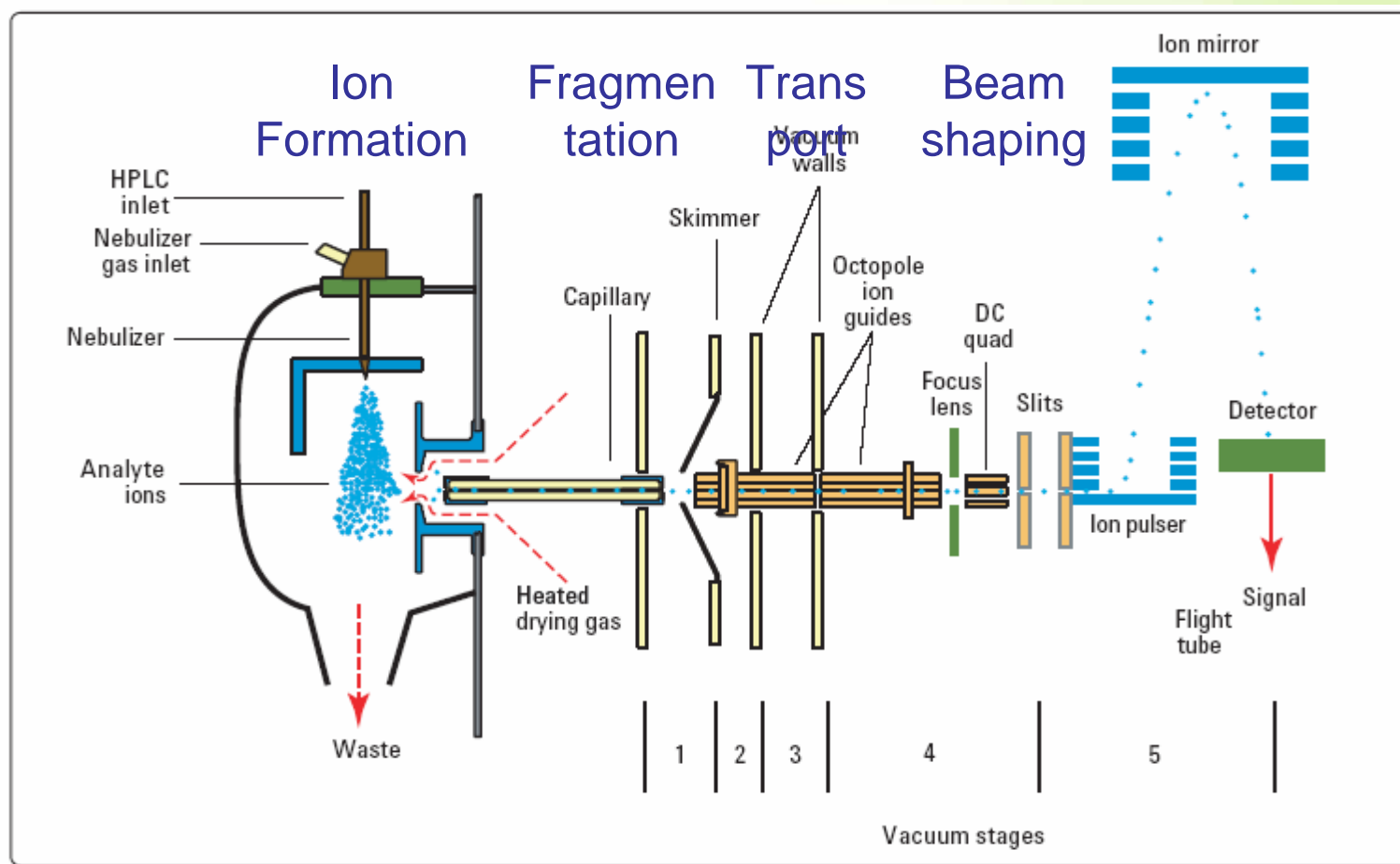
9 November 2006

VEAL SYMPOSIUM 2006 ST. MALO.

Testing slaughter animals for residues; what is the state of the art?



Technique LC-TOF-MS

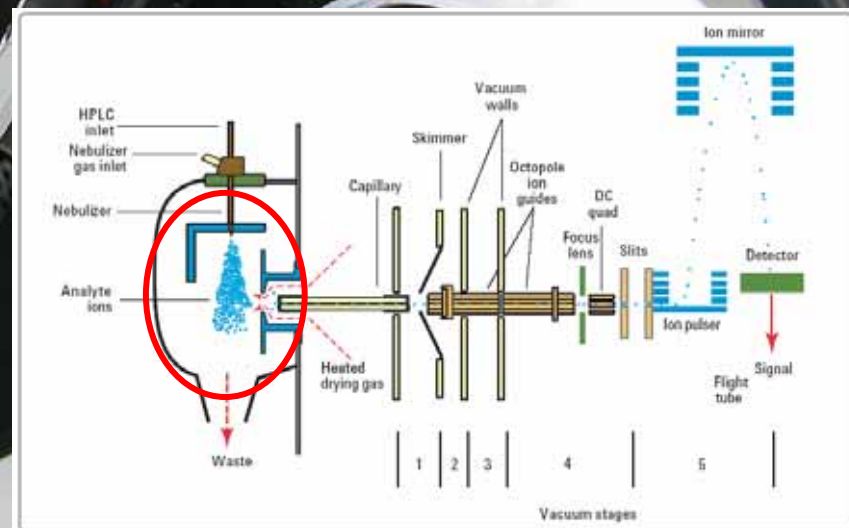


9 November 2006

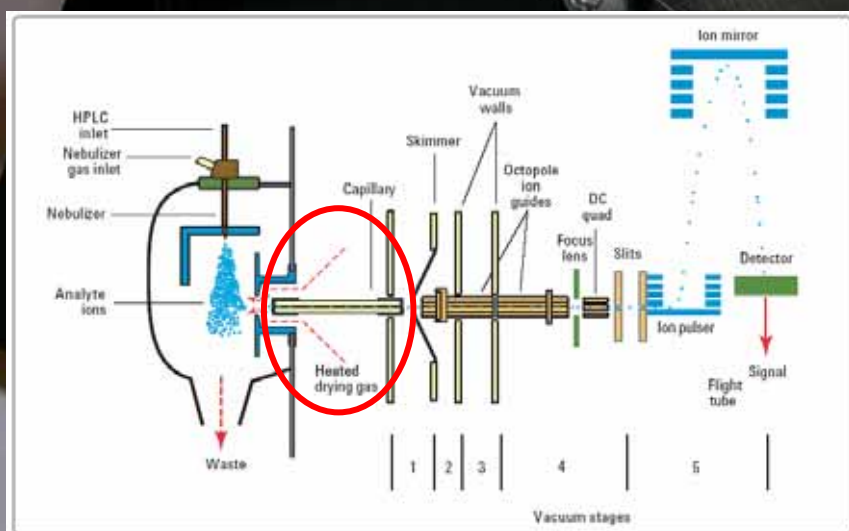
VEAL SYMPOSIUM 2006 ST. MALO.

Testing slaughter animals for residues; what is the state of the art?

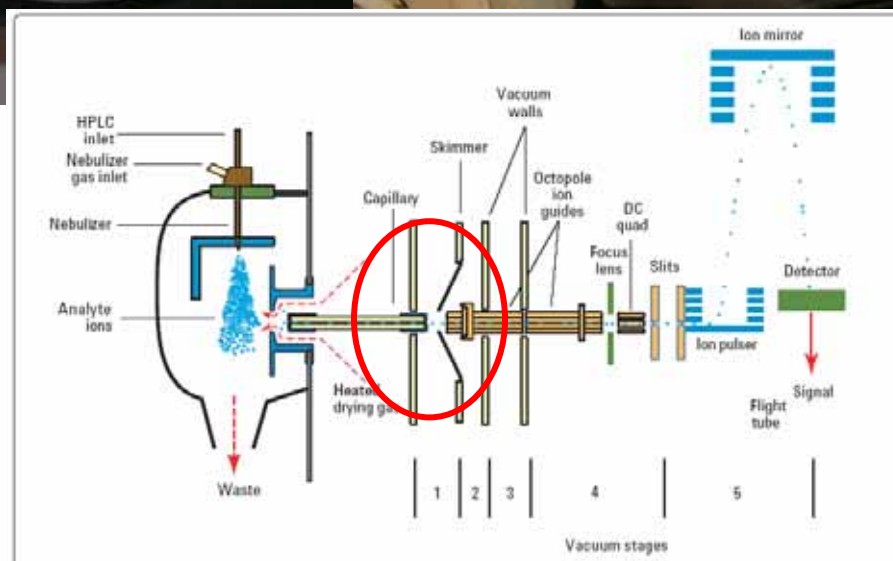
From the ion source into the vacuum chamber



Through the heated capillary to the first stage vacuum chamber



Fragmentation

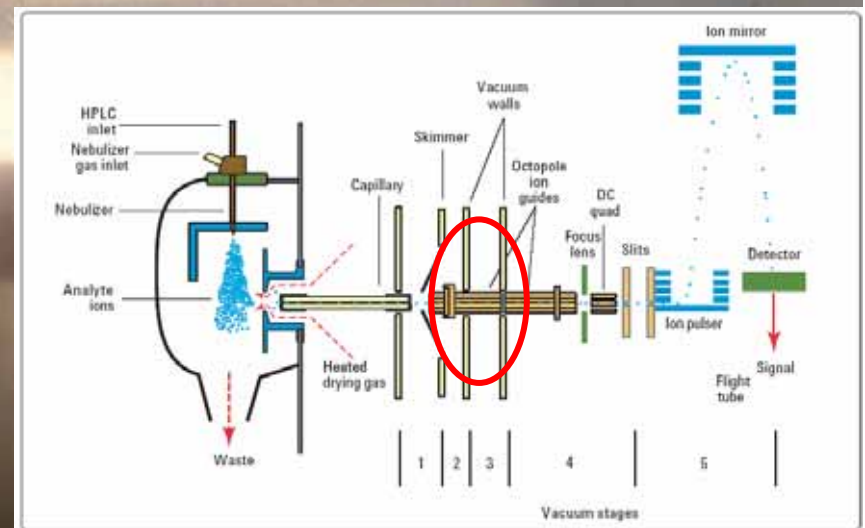


9 November 2006

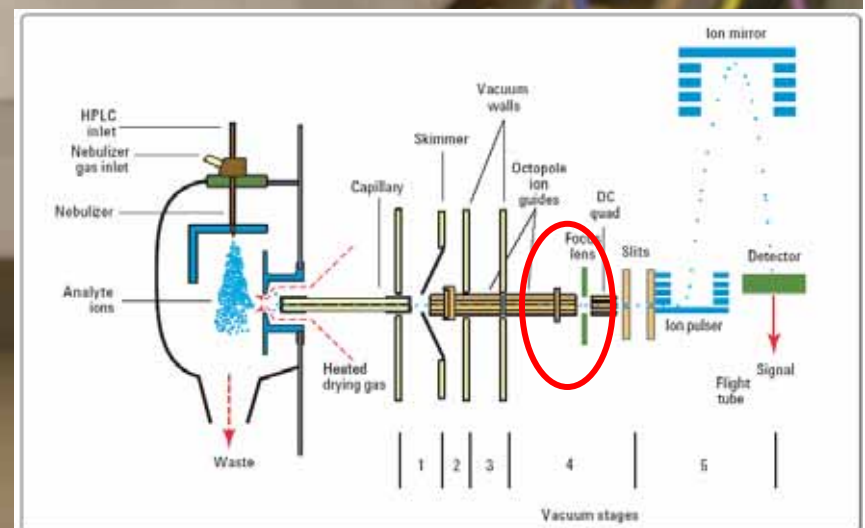
VEAL SYMPOSIUM 2006 ST. MALO.

Testing slaughter animals for residues; what is the state of the art?

Focussing the ions and transport to the third stage

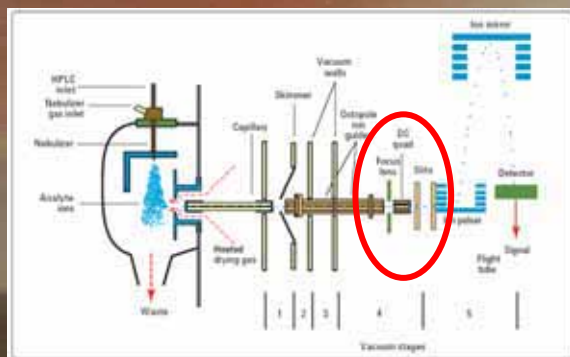


Acceleration into the fourth stage



Quad + Slits (Beam shaping)

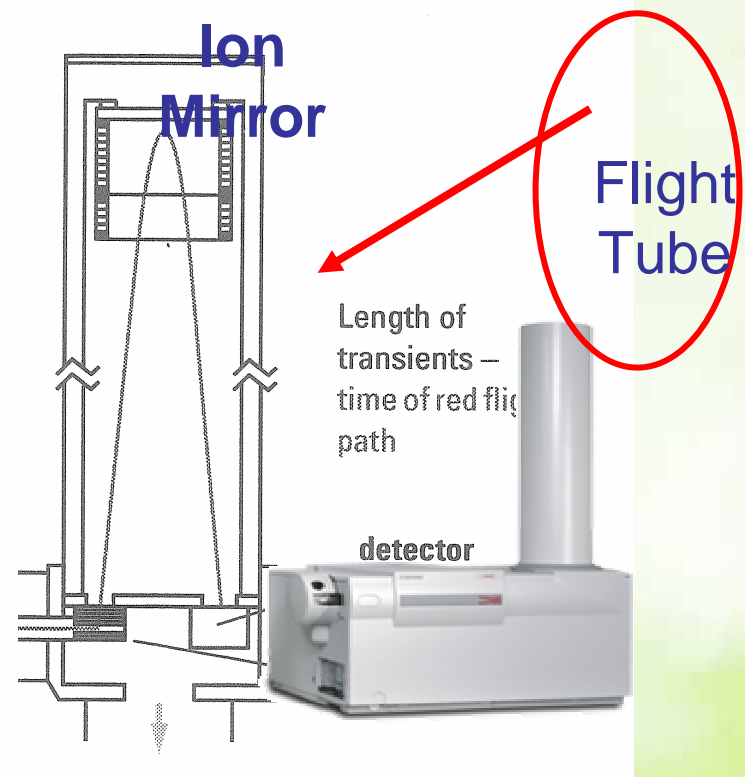
**Slits (Beam
shaping)**



Into the Flight Tube (Separation/Detection)

ions travel through the flight tube, which is about 1 meter of length. At the opposite end of the flight tube is an ion mirror, which reflects the ions that arrive to the detector

m/z	Flight time (μsec)	Transients/sec
800	50	20,000
3200	100	10,000
6400	141	7,070

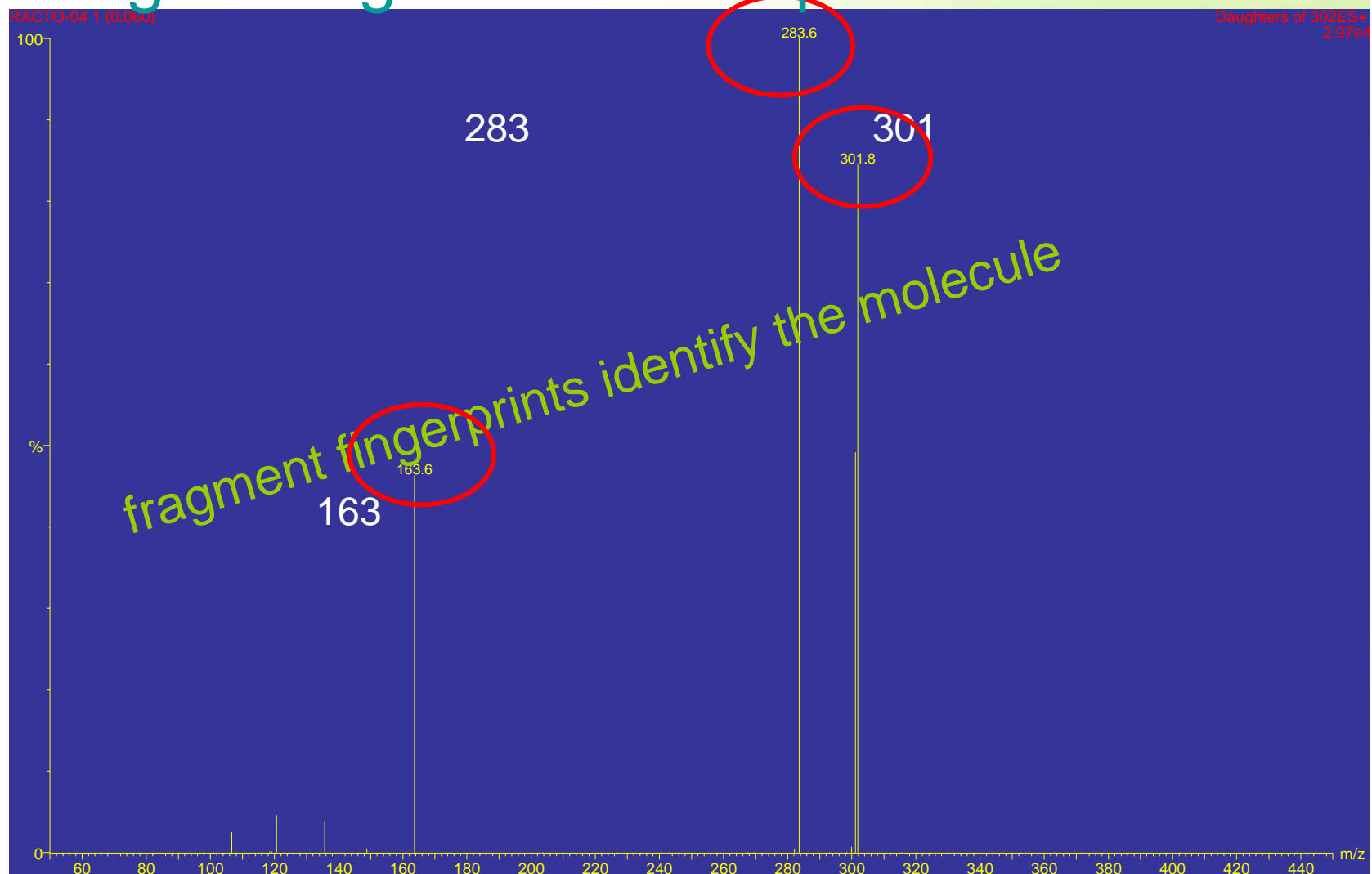


9 November 2006

VEAL SYMPOSIUM 2006 ST. MALO.

Testing slaughter animals for residues; what is the state of the art?

Mass fragmentogram : mass spectrum

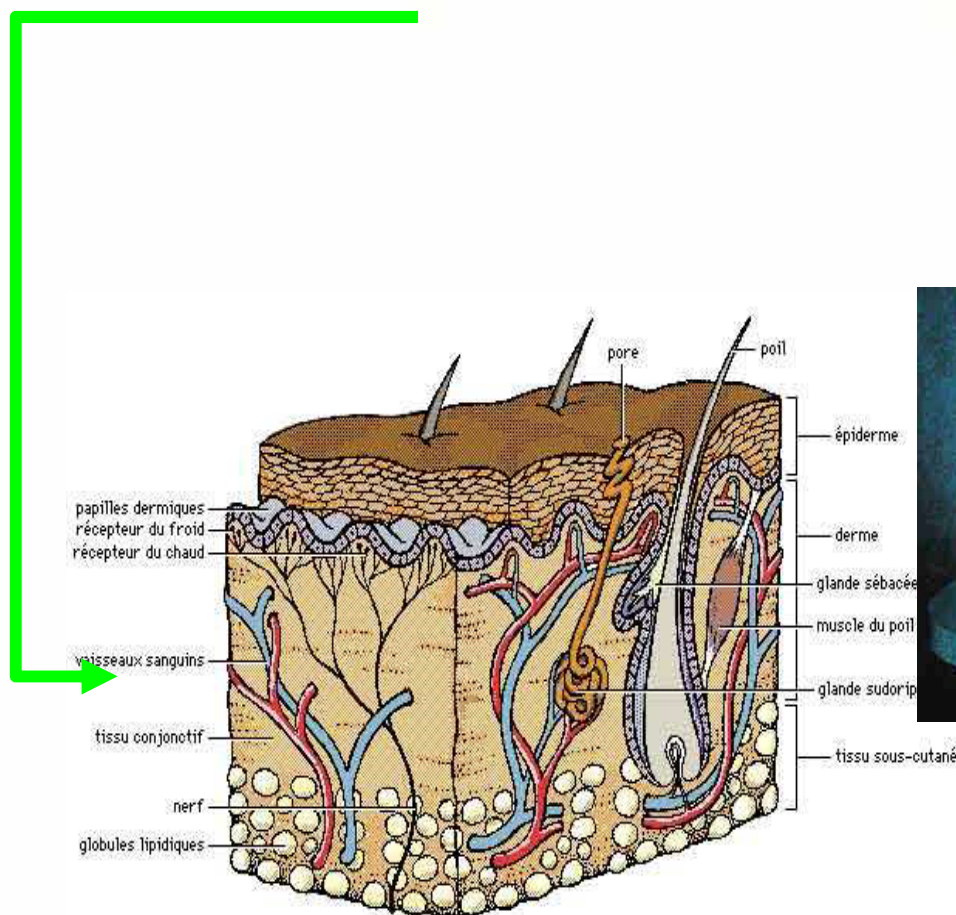


9 November 2006

VEAL SYMPOSIUM 2006 ST. MALO.

Testing slaughter animals for residues; what is the state of the art?

A new possibility: detection of steroid-(esters) in hair



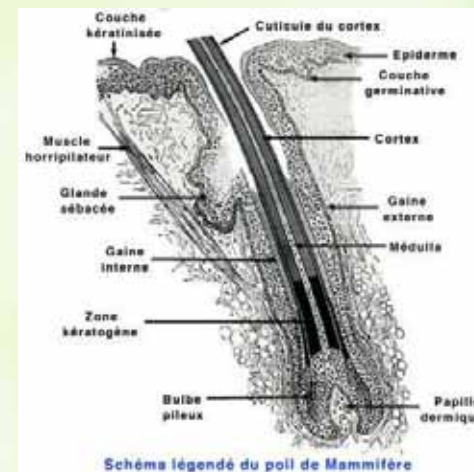
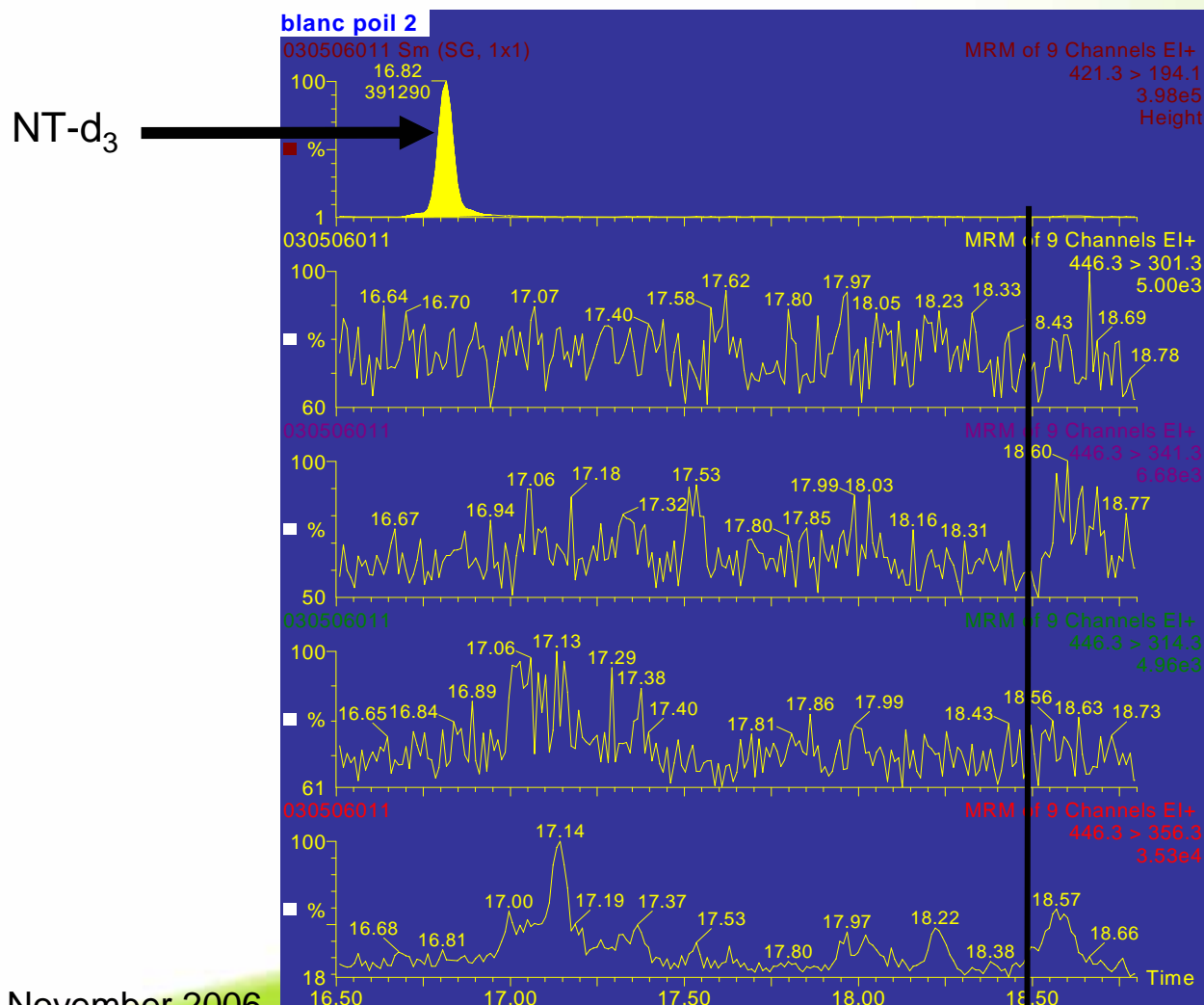
9 November 2006

VEAL SYMPOSIUM 2006 ST. MALO.

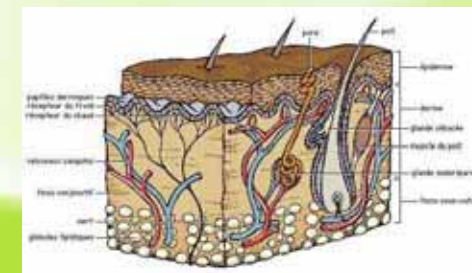
Testing slaughter animals for residues; what is the state of the art?

METHYLTESTOSTERONE. Long term detection in hair.

- GC-MS/MS, EI, SRM, compliant hair sample



MT



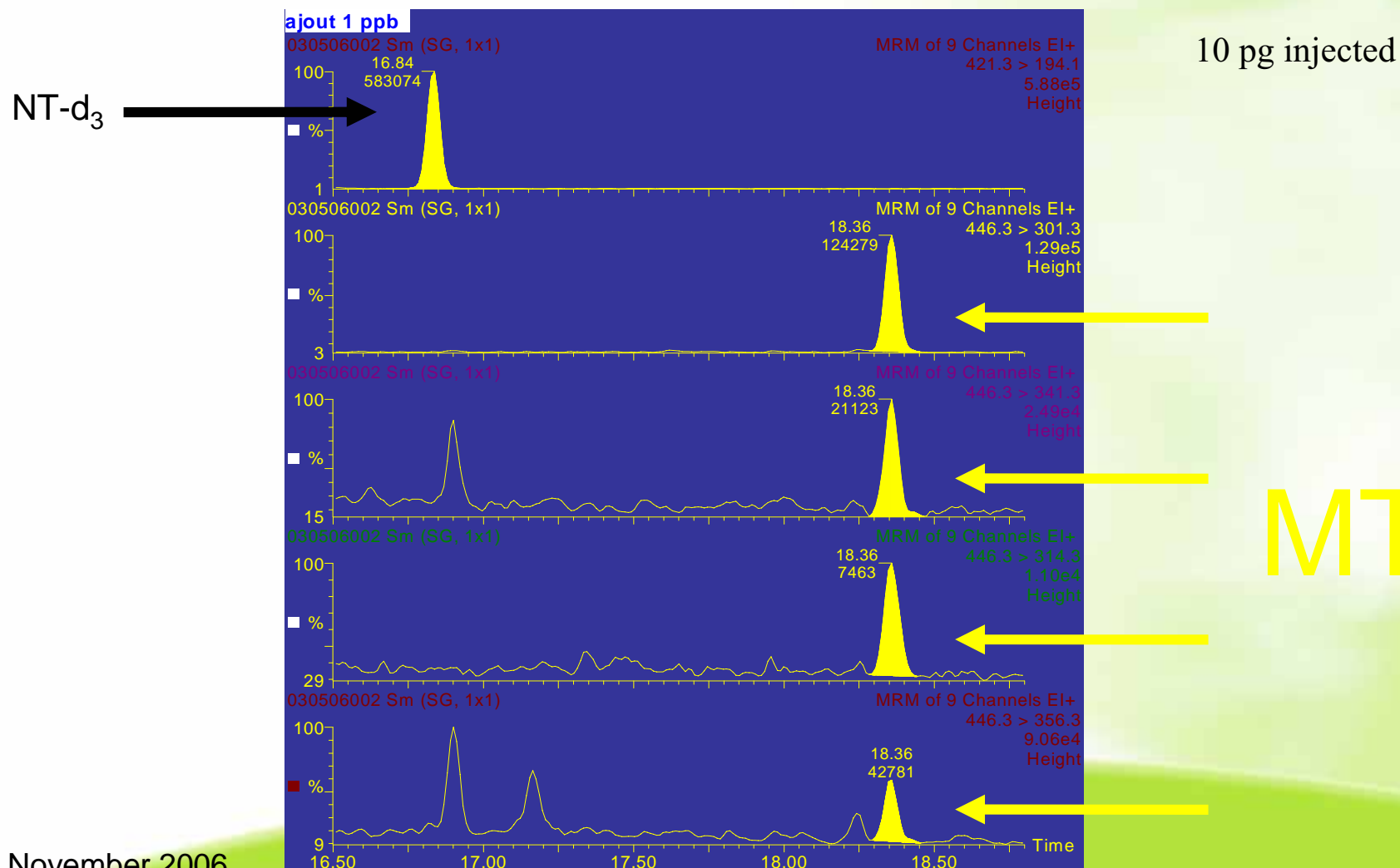
9 November 2006

VEAL SYMPOSIUM 2006 ST. MALO.

Testing slaughter animals for residues; what is the state of the art?

Case study 3 = METHYLTESTOSTERONE. Long term detection in hair.

- GC-MS/MS, EI, SRM, 1 ng.g⁻¹ in hair



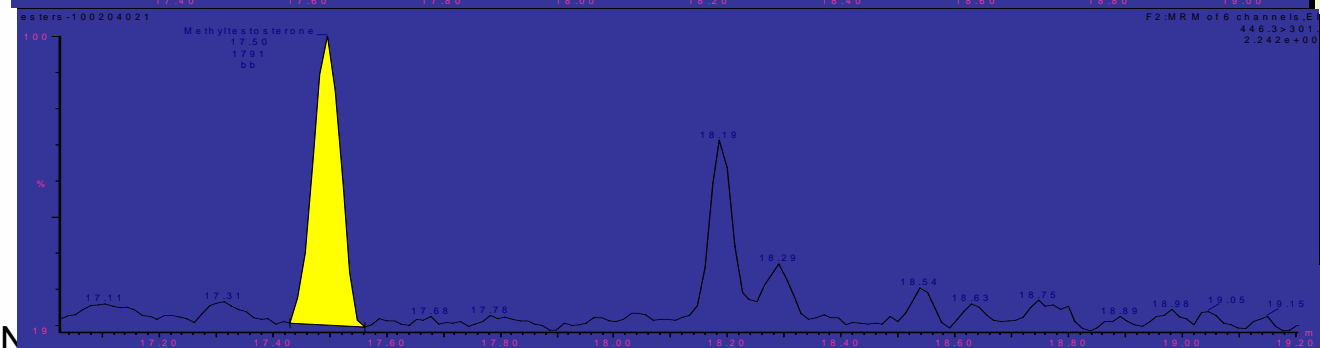
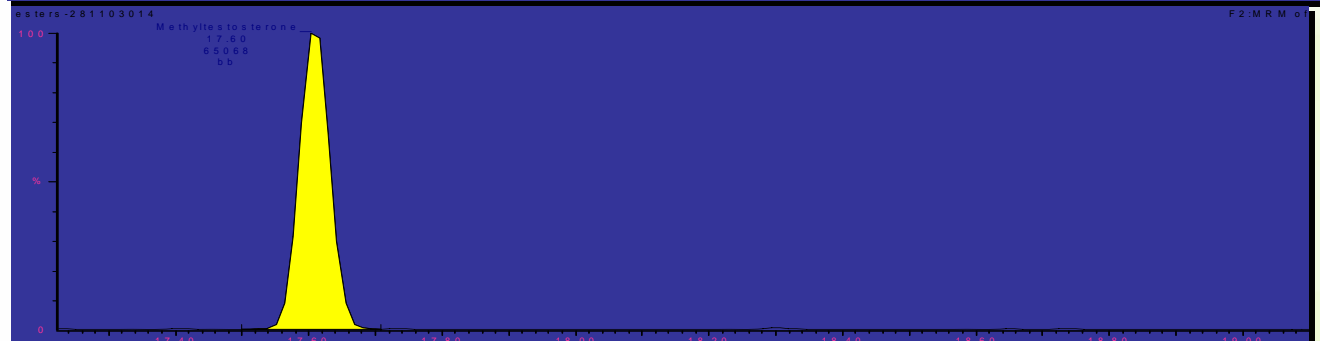
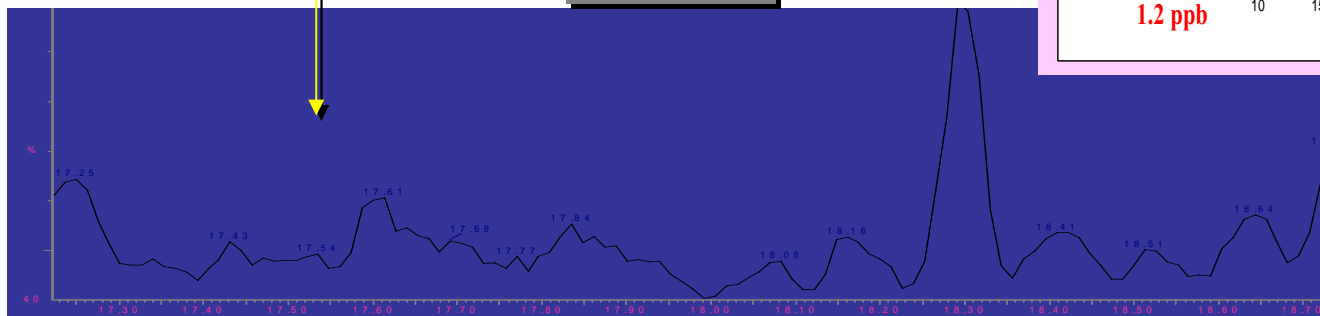
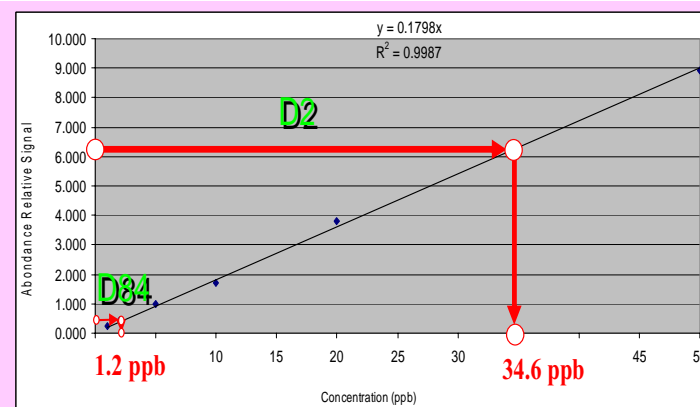
9 November 2006

VEAL SYMPOSIUM 2006 ST. MALO.

Testing slaughter animals for residues; what is the state of the art?

■ GC-MS/MS, EI, SRM, kinetic of fixation

446>301



DAY + 2
34.6 ppb

DAY + 84
1.2 ppb

9 N

VEAL SYMPOSIUM 2006 ST. MALO.

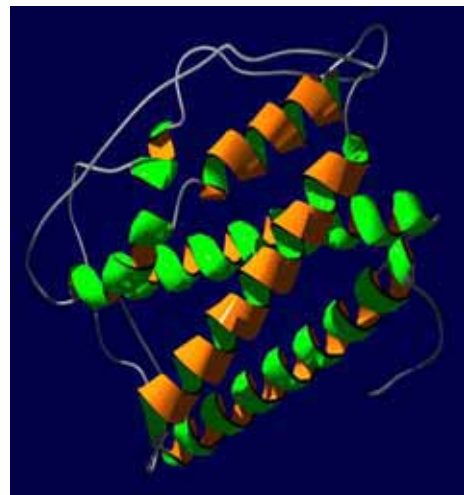
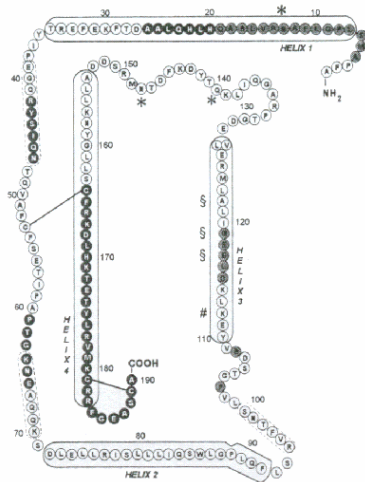
Testing slaughter animals for residues; what is the state of the art?

Somatotropine (growth hormone) analysis

PST

190 amino acids / Two
disulphide bonds 53-164, 181-
189

Mw 21731



Estradiol

Mw 272,4



9 November 2006

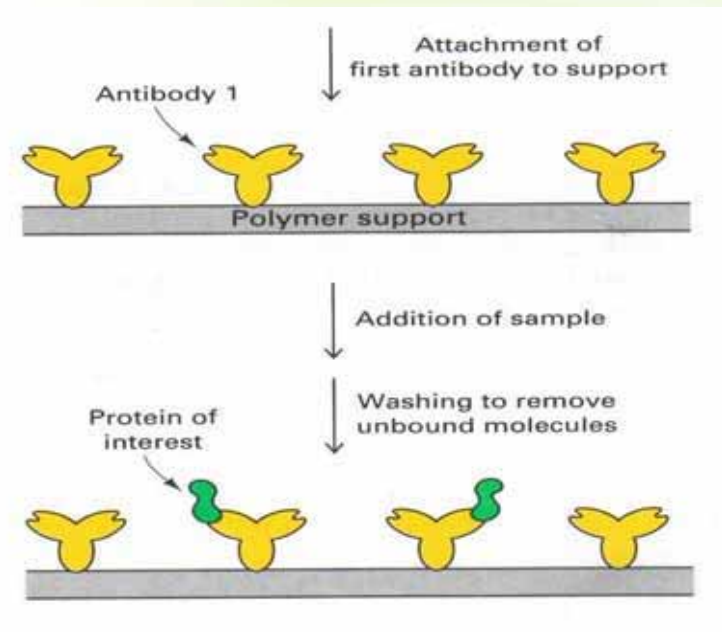
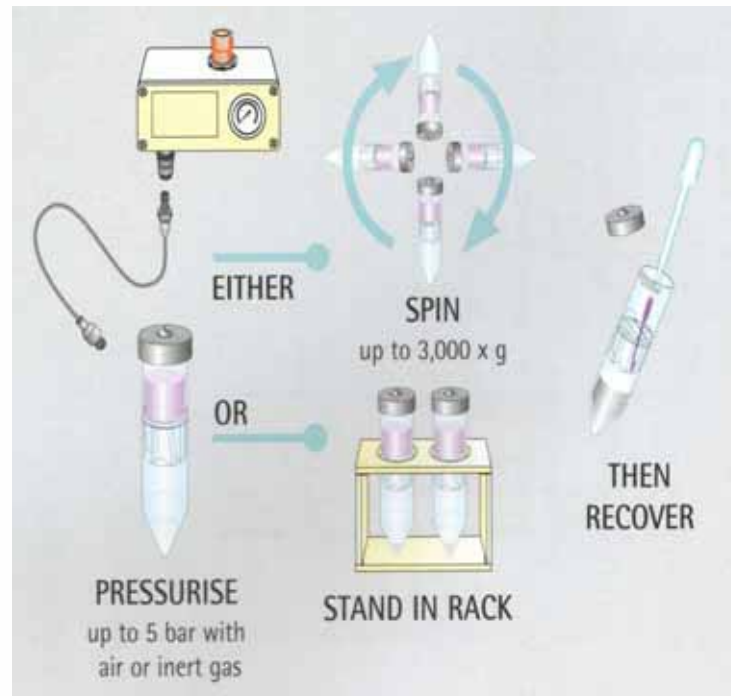
VEAL SYMPOSIUM 2006 ST. MALO.

Testing slaughter animals for residues; what is the state of the art?

Somatotropine (growth hormone) analysis

Dia-filtration: vivaspin devices

Immuno affinity Chromatography



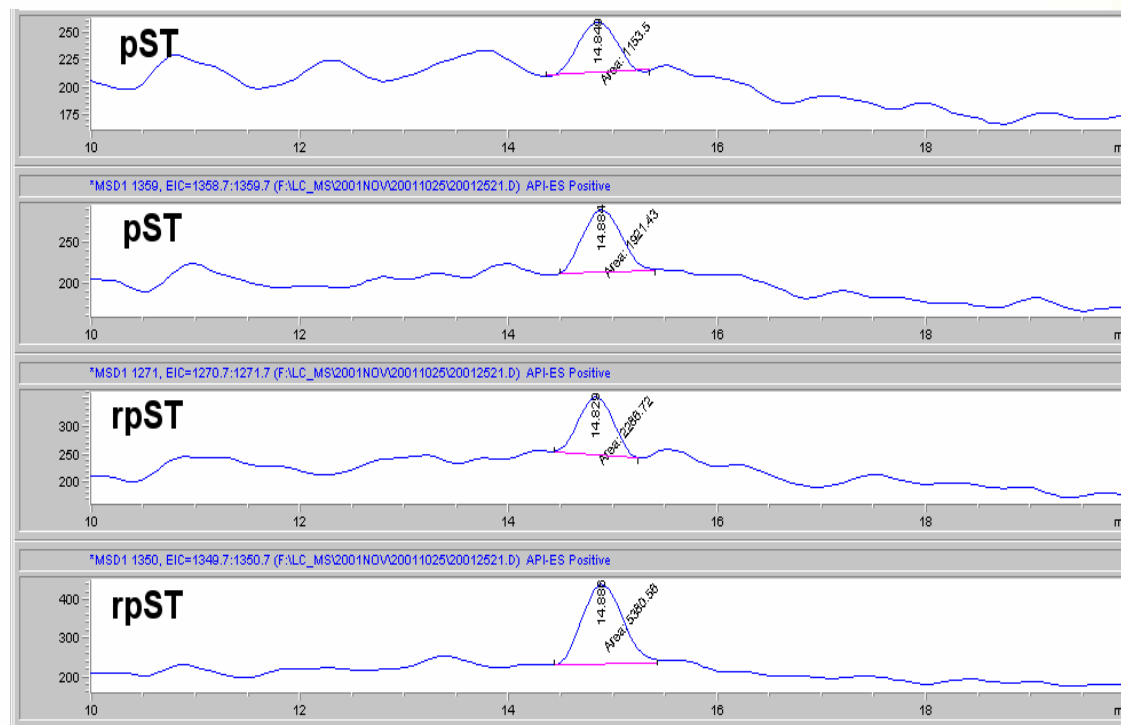
9 November 2006

VEAL SYMPOSIUM 2006 ST. MALO.

Testing slaughter animals for residues; what is the state of the art?

Somatotropine (growth hormone) analysis

Serum 10 microgr/ml



LC-MS/MS separation
on mass difference
between rPST and PST

9 November 2006

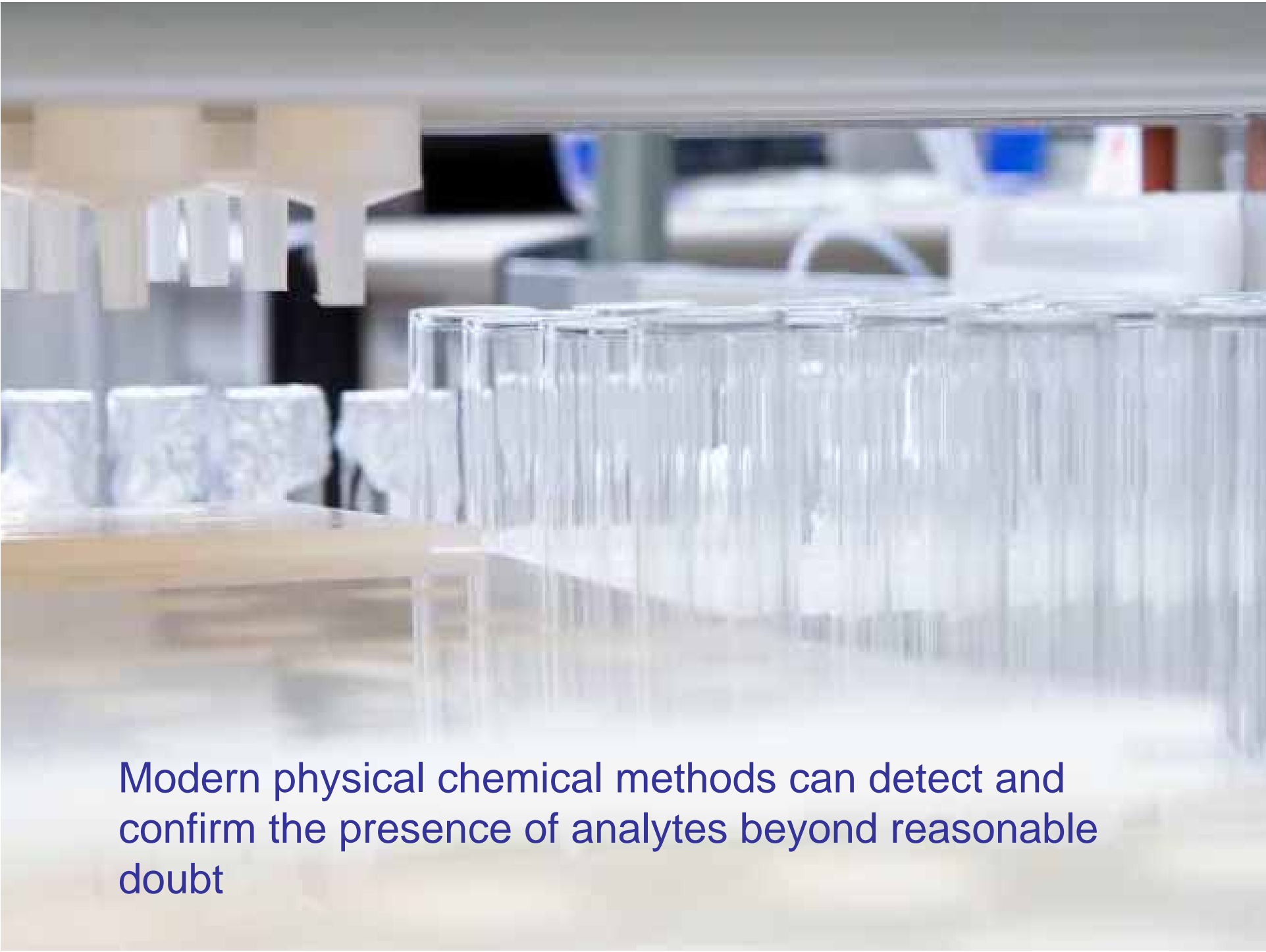
VEAL SYMPOSIUM 2006 ST. MALO.

Testing slaughter animals for residues; what is the state of the art?

Other new possibilities

- Discriminating “natural” from “synthetic” steroids (GC-C-MS)
- Measuring conjugated steroids with LC-MSMS

- 
- Testing samples for their biological (hormonal) effect can also detect unknown compounds.

A photograph of a laboratory environment. In the foreground, several rows of clear plastic test tube racks are visible, each containing multiple test tubes. The racks are arranged on a light-colored wooden surface. The background is slightly blurred, showing more laboratory equipment, including what appears to be a pipette and other glassware, suggesting a professional scientific setting.

Modern physical chemical methods can detect and confirm the presence of analytes beyond reasonable doubt

The misuse of natural hormones can be detected using GC-MS





Analysing samples
of hair greatly
improves the
possibilities of
detecting abuse of
hormones

Community Reference Lab
RIVM-ARO/CRL
94M3433 batch 1
Reference BLANK BOVINE
code bov19 : 5 ml, lyophilized



9 November 2006

VEAL SYMPOSIUM 2006 ST. MALO.

Testing slaughter animals for residues; what is the state of the art?