Surveillance of **growth promoters**

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Abstract

The use of growth promoters in farm animals has been banned within the European Union since 1988. In order to guarantee to consumers that foodstuffs are free from residues of this type of substance, a European surveillance and control system supports this measure, which has been organised in France since 1988 within the framework of the surveillance and control programmes implemented by the Directorate General for Food. This paper aims to describe the regulatory framework and the terms of implementation regarding compounds of interest, animal species concerned, relevant biological matrices and appropriate analytical strategies. Data obtained from the 2014 plans illustrate the entire system.

Keywords

Growth promoters, Surveillance programme, Mass spectrometry

Résumé

Le dispositif de contrôle des promoteurs de croissance

L'utilisation des promoteurs de croissance est interdite en élevage au sein de l'Union européenne depuis 1988. Afin de garantir au consommateur des denrées exemptes de résidus de ce type de substances, un dispositif européen de surveillance et de contrôle accompagne cette mesure, qui en France est organisé depuis 1988 dans le cadre des plans de surveillance et de contrôle mis en place par la direction générale de l'Alimentation. Le présent article décrit le cadre réglementaire, les modalités de mise en œuvre en termes de composés d'intérêt, d'espèces animales concernées, de matrices biologiques pertinentes et de stratégies analytiques adaptées. Les données issues des plans 2014 illustrent l'ensemble du dispositif.

Mots-clés

Promoteurs de croissances, plan de surveillance, spectrométrie de masse

Growth factors or growth promoters are defined as anabolic substances that increase muscle mass with the aim of improving physical and/or economic performance. Throughout history, humans have attempted to improve their performance by artificial means. The first mentions of doping date back to Antiquity (the Iliad and Odyssey). As early as the 6th century B.C., Greek athletes consumed different meats depending on their sporting discipline: jumpers ate goat's meat, boxers and throwers ate bull meat, while wrestlers preferred fatty pork meat.

The concept of doping in livestock rearing is far more recent and the first scandals related to its use date from the 20th century. Growth stimulants or their synthetic derivatives were used at that time to improve feed conversion and thereby growth in animals. With this type of treatment, animals develop more quickly for the same amount of feed.

Initially, cheap synthetic hormones such as diethylstilbestrol (DES), used at the time in human medicine, were administered to animals. Following a number of scandals and the very strong consumer reactions due to the related health risks, natural hormones were used instead:

- sex hormones (testosterone, oestradiol, progesterone),
- synthetic steroid hormones (trenbolone acetate),
- synthetic antithyroids (thiouracil),
- adrenaline-analogue ß-agonists (β2 adrenergic agonists) (clenbuterol),
- pituitary growth hormone (somatotropin).

Since the concentrations used were very low and did not result in residue levels above those in non-treated animals in the case of natural hormones, the debate then focused on ethical issues. However, residues are still the subject of highly controversial reports, with supporters proving the safety of treatments and detractors arguing that the data are insufficient.

Producers in the United States, Canada and other countries use these stimulants for three main reasons: to improve the quality of meat (treated animals produce leaner meat at the expense of fat), to improve conversion rates (a higher weight is obtained with less feed), and to reduce production costs (the meat price is lower as a higher amount of meat is produced with lower production costs).

In the European Union, the use of growth promoters in livestock rearing is governed by a regulatory framework; its application is monitored through an EU-wide harmonised control system. The system involves the detection and identification of possible residues of these substances or their markers in animal matrices or food of animal origin.

Regulatory references

The use of steroids and thyrostatics has been prohibited in livestock rearing since 1988 (Directive 88/146/EEC). This legislation has been amended over the years and in 1996 led to the implementation of a regulatory system governing the use in livestock rearing of substances with hormonal effects (oestrogens, androgens, progestagens), or with thyrostatic effects, as well as ß-agonist substances (Council Directive 96/22/EC, amended by Directives 2003/74/EC and 2008/97/EC). Prohibited substances are listed in this regulation in Annex II. It is, however, possible for certain Member States to derogate from the ban on these substances for specific therapeutic or zootechnical indications, provided that the substances are used in veterinary medicinal products that have received a marketing authorisation (MA), and that the corresponding analytical tests for residue detection are available.

The use of growth hormone has been banned in Europe since 1990 (Decision 90/218/EEC), this was followed by a moratorium (Decision 94/936/EC), extended since 1999 by Decision 1999/879/EC.

The first controls of the illegal use of these substances were governed by Directive 85/358/EEC. This legislation has changed in parallel with that concerning the use of growth promoters and led to Directive 96/23/EC in 1996, which, in addition to controls on the illegal use of growth promoters, covers and harmonises the surveillance and control of all types of chemical residues in foodstuffs of animal origin that involve a proven or potential hazard for human health (residues of veterinary medicinal products and environmental contaminants).

The legislation emphasises the obligation to designate National Reference Laboratories and their fundamental role in the organisation of laboratory networks carrying out official analyses. The text is supplemented by:

- Decision 97/747/EC fixing the levels and frequencies of sampling for certain sectors,
- Decision 98/179/EC laying down detailed rules on official sampling,
- Decision 2002/657/EC implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results.

European Directives are transposed into national law to become effective in each Member State. In France, Articles 234-1 to R. 234-14 of the Rural and Maritime Fishing Code (CRPM) partially cover the directives regarding growth promoters.

Control plans implemented in 2014 (Table 1)

Directive 96/23/EC, supplemented by Decision 97/747/EC, governs the strategy, level and frequency of sampling for the eight control plans to implement each year in primary production for the detection of growth promoters in the following sectors:

- cattle, swine, and poultry at the farm and slaughterhouse,
- sheep/goats, horses, rabbits, and farmed game at the slaughterhouse,
- farmed fish at the farm or first processing levels.

Samples are targeted and unannounced. The targeting criteria can be related to the production type or any other information that the DDecPPs have. The groups of growth promoters to be tested for annually as part of these control plans are in line with Directive 96/23/EC: stilbenes and stilbene derivatives (Group A1), antithyroid agents (Group A2), steroids (Group A3), resorcylic acid lactones (Group A4), and ß-agonists (Group A5). It is important to note that corticosteroid testing (Group B2f) is traditionally associated with growth promoter testing, because historically in Europe, they were found in the context of investigations related to misuse of ß-agonists and/or steroids.

Outside the scope of regulatory obligations, France has decided to also control for the presence of growth hormones (somatotropins) in cattle and fish.

The choice of matrices to be sampled was defined based on their relevance, either in terms of possible administration routes (feed) or matrices that best concentrate residues of administrated substance.

Sampling and breakdown of samples in 2014

The number of samples to collect by sector and by sampling site (farm or slaughterhouse) for the control plans on growth promoters was calculated (Table 2):

1. to meet the requirements of Council Directive 96/23/EC, i.e. pro rata of:
   - the number of animals slaughtered for meat and large game;
   - the tonnage produced for poultry, small game, and lagomorphs;
   - production volumes for farmed fish.

2. to establish prioritisation based on the number of non-compliant samples detected the previous years.

The regional distribution of these samples was based on pro rata volumes of livestock for production site samples and on pro rata volumes of slaughtered animals for slaughterhouse samples, as illustrated in Figure 1.

Growth promoters to be detected and analytical methods

Directive 96/23/EC requires that Member States develop reliable analytical methods for the control of the fraudulent use of growth factors at production sites, under the coordination of the European Union Reference Laboratories (EURLs) appointed by the European Commission. The RIKILT (Wageningen, Netherlands) is the EURL for growth promoters with hormonal effects, and the BVL (Berlin, Germany) is the EURL for ß-agonist type substances. The missions of these laboratories include contributing to the development and validation of analytical methods, and harmonising performance within the EU.

Box.

Objectives

Verify compliance with the regulatory ban on the use of growth promoters.

Verify the absence of growth promoter residues in animal matrices intended for human consumption.

Detection of fraudulent practices.

Programming framework

Directive 88/146/EEC of 7 March 1988 prohibiting the use in livestock farming of certain substances having a hormonal action.

Directive 96/22/EC amended by Directives 2003/74/EC and 2008/97/EC concerning the prohibition on the use in stockfarming of certain substances having a hormonal or thyrostatic action and of beta-agonists.

Directive 96/23/EC of 29 April 1996 on measures to monitor certain substances and residues thereof in live animals and animal products.

Decision 2002/657/EC implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results.

French Public Health Code (CSP) and Rural and Maritime Fishing Code (CRPM).

Protocol

- **Type of compounds of interest:** substances with hormonal effects (oestrogens, androgens, progestagens), stilbenes, resocylic acid lactones, antithyroids, as well as β-agonist substances and corticosteroids.
- **Target production sectors:** cattle, swine, sheep, goat, horse, poultry, aquaculture, lagomorph, and game production sectors.
- **Stage of the food chain:** farms, slaughterhouses.
- **Definition of non-compliance:** a sample is considered non-compliant if the concentration of the analyte of interest measured exceeds the decision limit of the confirmation method (Article 6, Decision 2002/657/EC).
- **Number of samples and sampling method:**
- The number of samples to collect by sector and sampling site (farm or slaughterhouse) was calculated to meet the requirements of Directive 96/23/EC. The number of samples to collect depends on:
  - > the number of animals slaughtered for meat and large game,
  - > the tonnage produced for poultry, small game, and lagomorphs,
  - > production volumes for farmed fish.
- **Sampling strategy:** targeted (conformation of the animals, for example).
- **Analytical methods:** multi-dimensional mass spectrometry (MS/MS) for screening and confirmation analyses. Specific techniques such as high-resolution mass spectrometry (HRMS) and isotope-ratio mass spectrometry (IRMS) are also used in the context of confirmation analyses.
- **Types of samples:** biological matrices such as urine, appendages, tissues, retinas, faeces, and blood.

Table 1. Control plans for growth promoters in animal matrices for 2014

Sector	Promoter group	Animal feed	Blood	Urine	Hairs	Lungs	Eyes	Thyroid	Muscle or liver
Cattle	Stilbenes	X		X	X				X
	Antithyroids	X		X				X	
	Steroids	X		X	X				X
	Steroid esters				X				
	Resorcylic acid	X		X	X				X
	ß-agonists	X		X	X	X	X		
	Glucocorticosteroids				X				X
	Recombinant bovine somatotropin		X						
Pigs	Stilbenes	X		X					X
	Antithyroids	X		X					
	Steroids	X		X					X
	Steroid esters				X				
	Resorcylic acid	X		X					X
	ß-agonists	X				X	X		
	Glucocorticosteroids				X				X
Sheep, goats, horses	Stilbenes			X					
	Antithyroids			X					
	Steroids			X					
	Resorcylic acid			X					
	ß-agonists					X			
	Glucocorticosteroids				X				X
Poultry	Stilbenes	X							X
	Steroids	X							X
	Resorcylic acid	X							X
	ß-agonists	X				X			
Rabbits, Game	Stilbenes								X
	Steroids								X
	Resorcylic acid								X
	ß-agonists					X			
Fish	Stilbenes								X
	Steroids								X
	Resorcylic acid								X
	Somatotropin		X						

In addition to the classes listed in Annex I of Directive 96/23/EC, other non-regulated substances may also be monitored on the basis of information emanating from the National Veterinary and Plant Protection Squad (BNEVP) or the National Reference Laboratory. An example is selective androgen receptor modulators (SARMs). These substances are currently tested for as part of an exploratory plan.

The official methods used today can detect and identify about 70 different growth promoters. The first-line (screening) analysis of a sample must be rapid, easy to implement, cheap, sensitive and robust. These methods have a high processing capacity and are applied by the eleven laboratories in the growth promoters network, covering the whole country, to screen multiple samples in order to rapidly distinguish between "compliant" and "suspect" samples. A sample is considered suspect when the identity of the compound is confirmed following screening and, if relevant, when the compound has a maximum residue level, its concentration exceeds this threshold.

This first step is used to identify suspect samples that will then need to be assessed as compliant or not using a confirmation method. The sample is then re-extracted to ensure that the results are not false (contamination, sample switch, etc.). Non-compliance is reported when the concentration of the identified compound is higher than the decision limit or CCa. The performance of the methods developed must have a false-compliant (false-negative) rate below 5% for the screening step, and a false-non–compliant (false-positive) rate below 1% for the confirmation step. The requirements concerning method performance and interpretation of the results are described in Decision 2002/657/EC.

While screening methods can use various analytical techniques (immunoassays, mass spectrometry), confirmation methods require targeted analysis of the administered compound and/or its direct metabolites by chromatography coupled with detection by mass spectrometry for non-ambiguous identification and quantification of the analyte of interest.

Screening methods

Official screening analyses are performed by the network of first-line laboratories approved by the General Directorate for Food. These establishments have official multi-residue methods developed and validated by the NRL, in accordance with Decision 2002/657/EC. These methods are used to test for growth promoters in complex biological matrices such as urine, appendages (e.g. hairs), or other matrices retained for their relevance. For example, the retina is a useful biological matrix because it durably fixes residues of ß2-adrenergic agonists and can be used to demonstrate fraud a long time after administration of the substance. This matrix is preferred at the slaughterhouse. Hairs are also able to fix residues of steroids or ß-agonists, thus extending the detection window. This matrix is used both on the farm and at the slaughterhouse. There are in fact several matrix/compound pairs that increase the effectiveness of the control (e.g. ß-agonists/lung or retina, steroids/faeces, progestagens/fat tissue, thyrostatics/thyroid, etc.).

The nature of the biological samples, which are often complex, means that they generally require several extraction and purification steps before characterisation of their contents. The measurement

Table 2. Number of samples to collect by sector and sampling site

	2014 target population	Size of the minimum annual national sample required by regulations for the detection of growth promoters and other prohibited substances (Group A)		Minimum size of the national sample by sub-group				Remainder to distribute depending on prioritisation of the MS (reference year 2014)	DGAL scheduling 2014		
									Farm	Slaughter-house	Total
Cattle	4,775,000 (total number of cattle slaughtered over 12 months)	0.25% of production, including half on the farm	11,937 samples, including 6,000 on the farm	A1	Stilbenes	5%	597	8,356	2,100	2,100	**4,200**
				A3	Steroids (+esters)	5%	597				
				A4	Resorcylic acid	5%	597				
				A2	Antithyroids	5%	597		400	300	**700**
				A5	ß-agonists	5%	597		1,800	1,900	**3,700**
				A6	Substances included in 37/2010 - Table 2	5%	597		1,700	1,700	**3,400**
		Abs*	Abs*	B2f	Glucocorticosteroids	Abs	Abs			600	**600**
					Somatotropin	Abs	Abs		200		**200**
Total promoters, cattle											**9,400**
Pigs	23,933,000 (total number of pigs slaughtered over 12 months)	0.02% of the production with a minimum of 0.001% on the farm	4,787 samples (different animals), including 239 on the farm	A1	Stilbenes	5%	239	3,351	130	190	**320**
				A3	Steroids (+esters)	5%	239				
				A4	Resorcylic acid	5%	239				
				A2	Antithyroids	5%	239		40	200	**240**
				A5	ß-agonists	5%	239		40	200	**240**
				A6	Substances included in 37/2010 - Table 2	5%	239		90	3,910	**4,000**
		Abs*	Abs*	B2f	Glucocorticosteroids	Abs	Abs			200	**200**
Total promoters, pigs											**1,000**
Small ruminants	4,472,000 (total number of sheep-goats slaughtered over 12 months)	0.01% of production	447 samples	A1	Stilbenes	5%	22	313		100	**100**
				A3	Steroids	5%	22				
				A4	Resorcylic acid	5%	22				
				A2	Antithyroids	5%	22			30	**30**
				A5	ß-agonists	5%	22			100	**100**
				A6	Substances included in 37/2010 - Table 2	5%	22			220	**220**
		Abs*	Abs*	B2f	Glucocorticosteroids	Abs	Abs			140	**140**
Total promoters, small ruminants											**370**
Horses	19,000 (total number of horses slaughtered over 12 months)	No minimum requirement but obligation to test for substances in Group A		A1	Stilbenes	Abs	Abs		4	4	**4**
				A3	Steroids	Abs	Abs				
				A4	Resorcylic acid	Abs	Abs				
				A2	Antithyroids	Abs	Abs		4	4	**4**
				A5	ß-agonists	Abs	Abs		4	4	**4**
				A6	Substances included in 37/2010 - Table 2	Abs	Abs		4	4	**4**
				B2f	Glucocorticosteroids	Abs	Abs		4	4	**4**
Total promoters, horses											**16**
Poultry	1,703,000 tonnes produced over 12 months	0.25% of the tonnage produced with a minimum of 0.05% on farms	4,269 samples (different batches)	A1	Stilbenes	5%	213	3,204	68	247	**315**
				A3	Steroids	5%	213				
				A4	Resorcylic acid	5%	213				
				A5	ß-agonists	5%	213		187	695	**882**
				A6	Substances included in 37/2010 - Table 2	5%	213		616	2,444	**3,060**
Total promoters, poultry											**1,197**
Rabbits	46,000 tonnes produced over 12 months	30 samples + 0.1% of "tonnage produced -3000 t"	73 samples (different batches)	A1	Stilbenes	30%	22			5	**5**
				A3	Steroids						
				A4	Resorcylic acid						
				A5	ß-agonists					10	**10**
				A6	Substances included in 37/2010 - Table 2	70%	51			60	**60**
Total promoters, rabbits											**15**

Abs: No minimum sample imposed by regulation

Table 2. Number of samples to collect by sector and sampling site (cont'd)

	2014 target population	Size of the minimum annual national sample required by regulations for the detection of growth promoters and other prohibited substances (Group A)		Minimum size of the national sample by sub-group				Remainder to distribute depending on prioritisation of the MS (reference year 2014)	DGAL scheduling 2014		
									Farm	Slaughter-house	Total
Farmed game	3,000 heads of large game 9,000 tonnes of small game produced over 12 months	20 samples	20 samples (different batches)	A1	Stilbenes	Abs	Abs			4	**4**
				A3	Steroids	Abs	Abs				
				A4	Resorcylic acid	Abs	Abs				
				A5	ß-agonists	Abs	Abs			4	**4**
				A6	Substances included in 37/2010 - Table 2	Abs	Abs			28	**28**
Total promoters, game											**8**
Farmed fish	50,000 tonnes produced over 12 months	0.333%	165 samples (different batches)	A1	Stilbenes	Abs	Abs			50	**50**
				A3	Steroids (+esters)	Abs	Abs				
				A4	Resorcylic acid	Abs	Abs				
				A6	Substances incluses dans 37/2010 - Tableau 2	Abs	Abs			120	**120**
				B2f	Somatotropin	Abs	Abs			50	**50**
Total promoters, fish											**100**
Total promoters, all sectors											**12,106**

Abs: no minimum imposed specifically

methods must combine selectivity and sensitivity because the residues of these substances are mostly found at ultra-trace amounts ($ng.kg^{-1}$ to $pg.kg^{-1}$). One of the most commonly used methods today is chromatography coupled with mass spectrometry. The technique may use gas chromatography for small thermostable and volatile compounds (steroids, stilbenes, resorcylic acid lactones), or liquid chromatography for the others (ß-agonists, thyrostatics, somatotropin, corticosteroids). To increase the specificity of detection, mass spectrometry is systematically of the multi-dimensional type (MS/MS); high-resolution mass spectrometry (HRMS) may sometimes be used.

Confirmation methods

A confirmation analysis may be performed when the network laboratory suspects the presence of one of the target compounds after the screening analysis. The confirmation strategy and analytical technique used are defined specifically based on the type of suspect analyte and its concentration. In this context, there are two sub-groups of substances among the growth promoters: xenobiotic substances for which simple detection clearly demonstrates fraudulent use of chemical substances in animals, and endogenous substances, such as oestradiol or testosterone, for which detection does not necessarily imply non-compliance of the sample. This is because androgenic steroids (testosterone, nandrolone, boldenone) and oestrogenic steroids (oestradiol) can be detected at highly variable concentrations depending on the animal's age, sex and physiological state. In the case of testosterone and oestradiol, measurement of the carbon $^{13}C/^{12}C$ isotope composition by isotope-ratio mass spectrometry (GC-C-IRMS) is used to determine the endogenous or exogenous nature of the residues, in particular in the animal's urine. Hairs can also be used for this type of compound since residues of ester forms of steroids administered can bind to this matrix, demonstrating without a doubt that the substance of interest was used because the animal's body does not produce this type of derivative (e.g. boldenone undecylenate, nandrolone cypionate, etc.). The presence of some substances may also be attributed to the animal's diet. This is the case specifically for zeranol (Group A4) or thiouracil (Group A2), which can be related to feed contaminated with a mycotoxin (zearalenone) or feed fortified with *Brassicaceae*, respectively (Pinel *et al.*, 2006). For these sensitive situations, the NRL handles confirmation and interpretation of results.

Results - 2014 review

Following the screening step performed on all samples, the analyses performed for confirmation purposes mainly involved compounds considered potentially endogenous, such as boldenone, nandrolone, oestradiol, testosterone, zeranol and taleranol, classed as natural hormones, but also strictly xenobiotic compounds such as β-agonists and steroid esters (A1 and A3). The breakdown of confirmation analyses by substance group of interest for 2014 is shown in Figure 2.

The observed non-compliant cases included values exceeding the MRL for dexamethasone in the liver matrix for the B2f substance group, and the presence of thiouracil identified in urine at variable concentrations higher than 10 $\mu g.L^{-1}$ in two samples. These thiouracil concentrations are, however, not incompatible with feed enriched with *Brassicaceae* (Pinel *et al.*, 2006).

At the European level, we should point out that most of the Member States carry out the minimum number of samples required by Directive 96/23/EC and Decision 97/747/EC. The sampled matrices are essentially the same among the Member States. Samples of urine, tissue and appendages, and feed are the most common for the detection of growth promoters.

The trend since 2013 appears to indicate an increase in cases of non-compliance reported by the Member States. However, the summary report issued by the European Food Safety Authority (EFSA, 2016) points out that the detected substances are not systematically attributed to illegal use, but are rather the result of notifications for natural hormones, particularly the A3 substance group, i.e. steroids, and for which non-compliance represents 0.08% of the measurements associated with this group of compounds. This is because some of the detected compounds can be found in the relevant species endogenously, without any illegal treatment. This is the case for instance for boldenone (a and b forms), 17a-nandrolone and 17a-testosterone. These results can be explained by the fact that the Member States still do not have specific, adapted confirmation methods and/or techniques for the particular case of natural hormones. Concerning thyroid agents (Group A2), 0.59% of the analysed samples were reported to be non-compliant and concern exclusively thiouracil. The B2f group is also represented at the European level by 28 reported non-compliant samples.

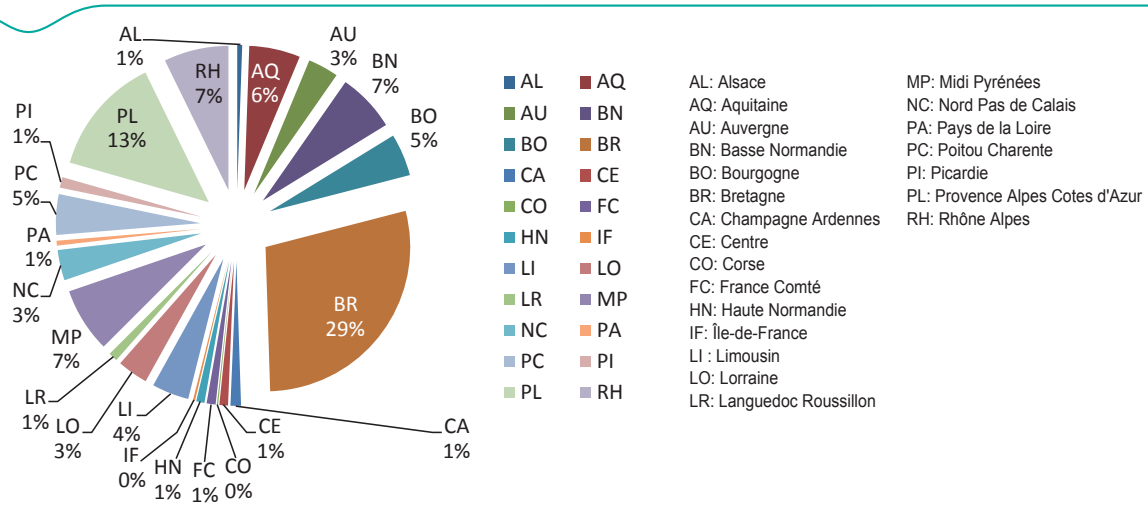

Figure 1. Regional distribution of samples taken for substance groups A1 to A5 and B2f (2014)

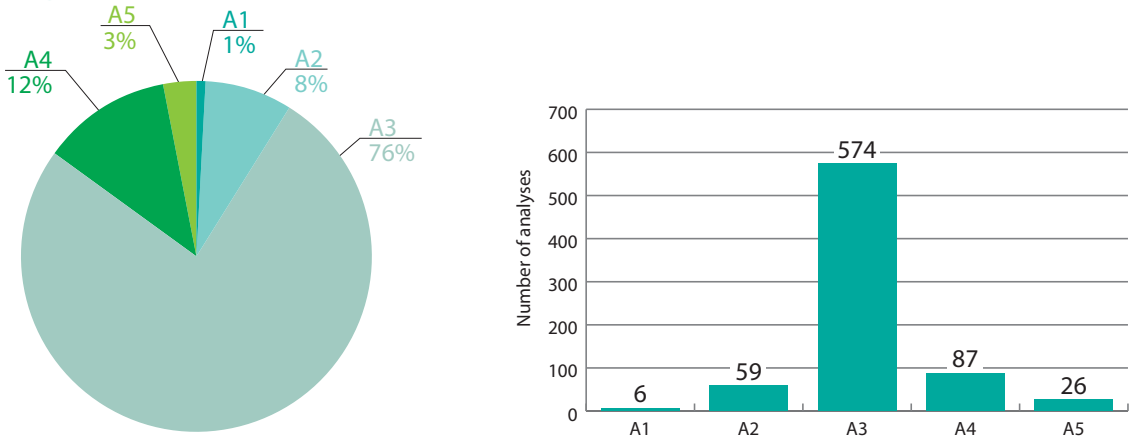

Figure 2. Distribution of the number of confirmation analyses performed by substance (2014)

Conclusion - Outlook

Control of the use of growth promoters is currently based on a range of competencies bringing together:

- the DDecPPs, which help to target the sampling;
- the network of official laboratories, which implement screening methods to test for about 70 different compounds belonging to various groups of growth promoters;
- the NRL, which develops and implements effective and specific confirmation methods.

There are three main types of obstacles that currently hinder even more effective control of the use of growth promoters:

- difficulties related to sampling certain matrices in target animals;
- detection of natural substances that have anabolic properties;
- identification of unknown compounds.

Concerning the first obstacle, the problem is considerable because if these sampling issues are not taken into account, it is highly unlikely that targeting is appropriate and thus that testing in fact identifies fraudulent practices, irrespective of the method used and its performance. An experimental plan involving the sampling of faeces has been implemented to evaluate the scientific usefulness of this matrix in testing for steroid substances, and to consider collecting faeces rather than urine, which is difficult to sample in target animals.

In the second case, and in line with an agreement between the DGAL and Laberca, specific analytical methods were developed recently. These methods rely on isotope-ratio mass spectrometry enabling high-precision measurement of the carbon 12/carbon 13 ratio in the compound, a proportion that differs depending on whether the compound is endogenous or synthetic (Buisson *et al.*, 2005; Janssens *et al.*, 2015). This strategy is, however, only present in a limited number of Member States (three laboratories). Alternative, more affordable strategies for all Member States are also under investigation and rely specifically on the combination of relevant matrix/residue pairs, for example blood/steroid esters, or hairs/steroid esters (Kaabia *et al.*, 2013).

Thirdly, concerning the detection of unknown compounds or more generally fraudulent physiological manipulation, overall exploratory approaches to the functioning of the species' bodies, implemented over the last decade, have already proven their worth. These strategies do not aim to detect the actual presence of suspect compounds or their direct metabolites, but rather to highlight a specific metabolic or physiological signal that could be associated with anabolic practices. These "indirect" or "non-targeted" approaches (Nebbia *et al.*, 2011; Pinel *et al.*, 2010) are based on methods such as transcriptomics (Riedmaier, 2015; Riedmaier *et al.*, 2009a; Riedmaier *et al.*, 2012; Riedmaier and Pfaffl, 2013; Riedmaier *et al.*, 2009b, c), proteomics (Cacciatore *et al.*, 2009; Cunningham *et al.*, 2009; Kinkead *et al.*,

2015), or metabolomics (Dervilly-Pinel *et al.*, 2015a; Dervilly-Pinel *et al.*, 2012; Gallart Ayala *et al.*, 2015; Jacob *et al.*, 2014; Kouassi Nzoughet *et al.*, 2015b), including derivative areas such as lipidomics (Kouassi Nzoughet *et al.*, 2015a) and steroidomics (Dervilly-Pinel *et al.*, 2011; Kaabia *et al.*, 2014). These new approaches are used to discover molecular markers of effects, which can then be monitored in a targeted way in a context of screening for anabolic practices. The first example of a monitoring method for biomarkers identified using a metabolomics approach (Dervilly- Pinel *et al.*, 2015a), and focusing on the screening of ß-agonist compound use in calves, has been implemented in France since 2013 for official controls (Dervilly-Pinel *et al.*, 2015b). The method is a world-first in this area.

These recent changes could prove to be effective in increasing the control pressure, and ultimately enable detection of a broader, realistic panel of anabolic practices.

In addition, concerning changes to the regulatory context, it is expected that the European regulations on the control of growth factor use will integrate new parameters that could be used to organise control plans even more effectively. This involves specifically the integration of technical progress regarding detection and new uses or substances with hormonal activity.

Against this backdrop, a review of Decision 2002/657/EC concerning the performance of analytical methods and the interpretation of results is currently under discussion at the European level to take into account new innovations and knowledge generated since its release.

It is also expected that changes will take into consideration possible harmonisation of procedures implemented in the various Member States in order to guarantee consistency of practices and decisions for greater quality of control. For example, Decision 2002/657/EC defined the concept of minimum required performance limits (MRPLs) which correspond to a fixed concentration that any control laboratory must be able to reach in a context of screening and confirmation, but only a few values have been published to date (e.g. MRPL for medroxyprogesterone acetate).

Regulation (EC) No 470/2009 indicates the possibility of determining reference points for action (RPAs) for non-authorised, pharmacologically active substances, when necessary, to ensure the control of imported or marketed foodstuffs of animal origin. RPAs are defined as action limits combining analytical possibilities that are both reasonable (i.e. that official laboratories can maintain) and compatible with residue levels which do not involve a risk for the consumer's health. Foodstuffs that contain residues of

substances at a concentration greater than or equal to the RPA are thus considered unfit for consumption. If the concentration is below this limit, the non-compliance is recorded but does not warrant management measures concerning the food. The outlook in this area therefore involves considering analytical and toxicological aspects to determine these values, but without replacing the full process of associated risk assessments (EFSA 2013).

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