

Residues from veterinary medicinal products, growth promoters and performance enhancers in food-producing animals: a European Union perspective

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Summary

The authors present an overview of the presence of residues from veterinary medicinal products, growth-promoting agents and performance enhancers in food-producing animals, as a result of administering these substances – legally or illegally – on farms. The current situation in the European Union (EU) is represented by an analysis of the 2004 results from the national residue monitoring plans of EU Member States. Aspects of ante-mortem and post-mortem inspection are also considered, as well as the practical challenges facing veterinary inspectors attempting to uncover illegal uses and prevent public health risks. Substances which are considered illegal because their risks have not yet been assessed, such as those employed in minority species or for minor uses, are also discussed.

Keywords

Antibiotics – Beta-agonists – Bovine somatotrophin – European Union – Feed additives – Food safety – Growth promoters – Legislation – Monitoring – Performance enhancers – Prohibited substances – Residues – SPS Agreement – Veterinary legislation – Veterinary medicinal products.

Residues from growth promoters, performance enhancers and veterinary medicinal products: the legal framework

The definition of residues in European Union (EU) legislation includes substances having a pharmacological action, their metabolites and other substances transmitted to animal products that are likely to be harmful to human health.

Safe levels of residues in food of animal origin result from the participation of all parties involved in the food chain – ‘from stable to table’. Toxicological evaluations are developed into agreed limits that determine, with other measures, the level of protection. This occurs through a political decision-making process. Farmers, veterinarians and all those parties involved in the food business have primary responsibility for the quality and safety of food on the market. They need to be sure which substances can safely be used in agricultural production. Regulators must adopt food control measures, taking international trade obligations into account. In cases of non-compliance, inspectors and laboratory analysts must provide evidence and know what can be enforced. Judges decide on penalties for the illegal use of pharmacologically active

substances. Finally, the consumer decides the success of these food products at the shop counter.

Trade in foods of animal origin can be significantly affected by differences in food safety requirements between countries, such as those for veterinary medicine residues. The availability of different analytical methods and differences in performance between laboratories, especially when detecting substances for which no permitted limit has been established, can also lead to technical barriers to trade.

Background to the Agreement on Sanitary and Phytosanitary Measures

The Agreement on the Application of Sanitary and Phytosanitary Measures (the 'SPS Agreement') was signed at the end of the General Agreement of Tariffs and Trade Uruguay Round (1986-1994). This led to the foundation of the World Trade Organization (WTO) to regulate international trade. The SPS Agreement was signed by 132 Member Governments (34) in Marrakesh on 15 April 1994 and entered into force, with the establishment of the WTO, on 1 January 1995. The Agreement deals with the application of food safety and animal and plant health regulations.

The SPS Agreement allows countries to set their own biosecurity standards but encourages governments to 'harmonise' these, i.e. base their national measures on the international standards, guidelines and recommendations developed by:

- the joint Food and Agriculture Organization (FAO)/World Health Organization (WHO) Codex Alimentarius Commission (the Codex) for food safety
- the World Organisation for Animal Health (OIE)
- the FAO International Plant Protection Convention for plant health (23).

The SPS Agreement establishes rules based on scientific measures, which aim to reduce uncertainty in trade. Thus, the Agreement facilitates trade while still enabling each Member State to take the necessary measures to: 'protect human, animal and plant health, subject to the requirement that these measures are not applied in a manner which would constitute a means of arbitrary or unjustifiable discrimination between Members where the same conditions prevail or a disguised restriction on international trade'.

These rules apply to:

- foodstuffs for human consumption
- feed intended for animal consumption

– plants and animals, and any products derived from them.

The basic aim of the SPS Agreement is to ensure that, if a Member State wishes to: 'maintain, introduce or amend a technical regulation or standard, or procedures for conformance, it must be able to justify its actions by verifiable scientific and technical information'.

The Agreement is an 'international obligation', which requires governments to abide by the rules affecting their trade in an: 'open, non-discriminatory and science-based fashion'.

European Union legislation on residue control

As a member of the WTO, the EU must comply with the SPS Agreement (Article 2.3) and attempt to prevent countries from using SPS measures to restrict international trade.

European Union legislation should guarantee to EU consumers that the food available on the EU market is safe, regardless of whether it was produced in the EU or a third country.

In 1996, following a proposal from the European Commission, the European Council published two Directives, Directive 96/22/EC (17) and 96/23/EC (16), which repealed earlier directives and constitute the present legal framework for controlling residues in foods of animal origin. Directive 96/22/EC prohibits the use of beta-agonists and certain substances which have a hormonal or thyrostatic action in livestock farming. Directive 96/23/EC establishes the measures that EU Member States should take to monitor substances and their residues in both live animals and animal products. (See Table I for a list of these substances and residues, as detailed in Annex I of the Directive.)

Together, these Directives describe how to investigate and detect substances in animals, feedingstuffs and animal products.

In addition, Commission Decision 97/747/EC (4) establishes the levels and frequencies of sampling required to monitor such substances and their residues in certain animal products. For instance, this Decision extends residue control from red meat to include poultry, rabbit and game meats, eggs, milk, honey and fish. Decision 97/747/EC also makes substantial changes to the criteria for selecting samples, moving from random to target sampling.

To conform with Annex II of Directive 96/23/EC, all Member States should draw up a plan for the detection of

Table I
Substances and their residues which should be monitored in both live animals and animal products in Member States of the European Union

These substances are listed in Annex I of Directive 96/23/EC, organised by sub-group (A1 – A6) (16, 28)

Groups of substances which have an anabolic effect or are unauthorised	Principal substances in this group
A1– Stilbenes, stilbene derivatives and their salts and esters	Diethylstilboestrol, dienooestrol, hexooestrol
A2 – Antithyroid agents	Thiouracil, methylthiouracil, propylthiouracil, phenylthiouracil
A3 – Steroids	Trenbolone 17-alpha and -beta, 19 nortestosterone 17-alpha and -beta, testosterone, oestradiol and esters, medroxyprogesterone, nandrolone, methyltestosterone, melengestrol, megestrol, ethylestrenol, boldenone, cortisone, dexamethasone/prednisolone, chlormadione, stanozolol, chlortestosterone, 16 OH-stanozolol, norgestrel, methandriol, fluoxymesterone, flumethasone, flugestone, chloroandostedione, caproxyprogesterone, acetoxyprogesterone
A4 – Resorcylic acid lactones, including zeranol	Taleranol, zearalenol, ethinylestradiol, estradiol benzoate
A5 – Beta-agonists	Clenbuterol, salbutamol, cimaterol, mabuterol, ractopamine, terbutaline, brombuterol, isoxsuprine, methyl-clenbuterol, hydroxymethyl-clenbuterol, clenproperol
A6 – Compounds included in Annex IV to Council Regulation (EEC) No. 2377/90 of 26 June 1990 (13)	Chloramphenicol, nitrofurans, chlorpromazine, dimetridazole, ronidazole, metronidazole, phenylbutazone, dapsone, ipronidazole

groups of residues or substances, according to the type of animal. Since 1998, monitoring programmes have been based on this Directive, which takes a different approach from the previous Directive, 86/469/EEC (10). While Directive 86/469/EEC established purely random sampling criteria, Directive 96/23/EC sets targeted sampling criteria (16), effectively meaning that the results obtained from monitoring programmes before 1997 cannot be compared with those obtained after 1998.

Applying targeted criteria means that the selection of samples is oriented towards detecting the maximum number of positive results, based on such factors as:

- previous results
- the current situation in the region
- knowledge of the possible abuse of certain substances in this area, etc.

European Community legislation on the use of growth promoters

Since 1981, Community legislation (Council Directive 81/602/EEC) has banned certain hormones (diethylstilboestrol and other stilbenes and thyrostatics) (9), but Member States were free to ban or authorise the use of hormonal growth promoters. However, melengestrol acetate (MGA) was never authorised by any Member State.

In 1988, the European Community (EC) prohibited the use of six hormones for animal growth promotion:

- 17-beta oestradiol
- testosterone
- progesterone
- zeranol
- trenbolone acetate
- MGA.

This ban applied internally and to imports from third countries, without discrimination, from 1 January 1989 (12). As a result, third countries that want to export bovine meat and meat products to the EC must either have equivalent legislation or operate a hormone-free cattle programme.

Directive 96/22/EC, concerning the prohibition of certain substances with a hormonal or thyrostatic action and beta-agonists, does, however, allow the use of EC-approved veterinary medicines containing hormones (e.g. 17-beta oestradiol, testosterone, progesterone and derivatives) for therapeutic use and reproductive purposes (17). The veterinary medicine must be administered by a veterinarian and treatment of food-producing animals is prohibited.

In the United States of America (USA), according to the Code of Federal Regulations (CFR), Title 21, Parts 522, 556, and 558, such hormones are authorised as growth promoters for food production animals (30). For instance, estradiol, MGA, progesterone, testosterone, trenbolone

acetate and zeranol are all authorised for bovines. The Joint FAO/WHO Expert Committee on Food Additives conducted risk evaluations on these hormones in 1988, 1999 and 2000 (31, 32, 33).

The EU approval procedure for certain substances may lead to the same substance being evaluated through two parallel processes for two different purposes. For example, a hormone may be evaluated for:

a) therapeutic use, based on independent scientific advice from the Committee for Veterinary Medicinal Products (CVMP) (www.emea.eu.int)

b) use as a growth promotant, based on advice from the Scientific Committee on Veterinary Measures Relating to Public Health (SCVPH) and, since 2003, the European Food Safety Authority (EFSA) (www.efsa.eu.int).

Residues of veterinary medicinal products

Article 6 of Directive 2004/28/EC (amending Directive 2001/82/EC) requires the inclusion of a pharmacologically active substance in Annex I, II or III of Regulation 2377/90 (13) as a pre-condition for obtaining marketing authorisation for veterinary medicinal products for food-producing animals in the EU (22).

Administering veterinary medicinal products containing pharmacologically active substances included in Annex IV (such as chloramphenicol and nitrofurans) to food-producing animals is prohibited within the EU. Malachite green is an example of a pharmacologically active substance which has never been evaluated, according to Regulation 2377/90 (13). Therefore, this substance is not authorised for use in food-producing animals in the EU.

Veterinary medicinal products can be authorised and should be used according to the specific marketing authorisation granted. Other substances are authorised for use in some species but cannot be legally used in all food-producing animals.

Since its creation, the European Medicines Evaluation Agency (EMA) (www.emea.eu.int) has managed the technical aspects of this process, notably through the CVMP. No marketing authorisation may be granted for a veterinary medicinal product unless a maximum residue level (MRL) for the active substance has been established by the Commission. This is essentially a food safety measure.

Once an MRL has been set for an active substance, companies may apply for marketing authorisation for veterinary medicines containing this active substance, through either the 'centralised' or 'decentralised' procedure. In the centralised procedure, authorisation is

granted by the Commission on the basis of an opinion from the CVMP. In the decentralised procedure, marketing authorisations are granted by Member State authorities. There is also a process for mutually recognising the decision taken by another Member State. In cases of disagreement, the dossier is referred to the CVMP for an opinion, which is then forwarded to the Commission for adoption.

An MRL is set for each relevant food product (i.e. eggs, milk, meat, liver, etc.) for each relevant species. This is because the MRL is linked to the level of the active substance which remains in the animal tissue at the end of medical treatment, and also to the amount of this particular food which is consumed by the population on a daily basis.

The time period required for the level of the active substance to decrease to the MRL differs, depending on the particular animal product and the formulation of the active substance. This so-called 'withdrawal period' is determined through depletion studies for all substances with MRLs. As defined in Article 1, Point 9, of Directive 2001/82/EC (20), it is the period necessary between the last administration of a veterinary medicinal product to animals, and the production of foodstuffs from such animals, to protect public health. The withdrawal period ensures that these foodstuffs do not contain residues in quantities over the MRLs for active substances laid down under Regulation (EEC) No. 2377/90 (13).

The EU legislation (Directive 96/23/EC) (16) requires that a routine analytical method for the active substance be developed and validated by the pharmaceutical company. This is a sensitive issue, as developing a validated method is time-consuming, costly and benefits the testing laboratories rather than the company.

Thus, the pharmaceutical industry is not willing to invest in studies on species for which the market is limited (for instance, horses, rabbits, goats, fish and bees, or in the case of a disease which occurs only rarely). From the industry perspective, the lack of commercial interest means that the costs outweigh the benefits. As a result, many veterinary products previously used in these species have no MRL and their use has become illegal. This has led to 'the lack of availability of medicines for minor uses and minor species'. In the human domain, these types of products are called 'orphan medicines' and receive special support programmes (for instance, government subsidies, tax exemptions, research funding).

Mutual recognition of MRL procedures between Members of the WTO and the exchange of appropriate risk assessments could help to avoid trade problems due to non-harmonised MRLs.

As in marketing authorisations, MRLs do not include any provisions covering the misuse of a substance or product and, of course, the pharmaceutical companies which submit applications to the EMEA do not support or promote the illegal use of their products. Nevertheless, their products may be bought for misuse, a problem which – unless official institutions develop their own validated analytical methods – will remain undetected. It is considered that the appropriate way to avoid misuse is for each Member State to implement its own adequate risk management measures.

Medicines which are authorised for some food-producing animals may not have been granted marketing authorisation for minor uses and minor species (e.g. honey, rabbit meat, game meat, etc.). In addition, some veterinary medicines which were commonly used for food-producing animals may now be banned, due to a lack of MRLs (e.g. sulphonamides), despite the fact that their safety risk assessment is well known. In such cases, an international position should be agreed upon to avoid trade barriers.

Feed additives and performance enhancers

Feed additives are intended to improve feed quality, nutritional aspects, animal health and animal performance. According to Regulation (EC) No. 1831/2003 (21), there is a wide range of substances considered feed additives that may be classified as technological, organoleptic, nutritional and zootechnical (i.e. increasing animal production or performance).

Feed additives cannot be placed on the market in the EU unless they are authorised, based on scientific evaluation of their:

- efficacy
- effect on animal health
- effect on human health
- effect on the environment.

As noted above, zootechnical feed additives (performance enhancers) are substances that have a positive impact on the production of healthy animals, affecting particularly their gastro-intestinal flora, the digestibility of their diet and the environment. (Some current food additives may damage the environment. In this context, an additive which has ‘zero impact’ on the environment is considered positive. Other additives may be designed to, for example, reduce levels of phosphates and other contaminants when the manure containing them is spread as slurry.)

For instance, due to the demonstrated increase of antimicrobial resistance, the use of antibiotics as a feed additive was banned from 1 January 2006, except those authorised as coccidiostats or histomonostats. The fact that

antibiotics have been widely used as feed additives, with possible detrimental effects on animal and public health, is something to be considered when evaluating the risk of substances with pharmacological activity. In the USA (http://www.fda.gov/cvm/animalfeed_info.htm#ingredients), feed containing antibiotics as growth promoters is considered as medicated feed for control purposes, even if it contains sub-therapeutic doses according to authorised use.

The code of good practice for animal feeding in the Codex, published in 2004 (2), does not include the concept of improving animal production in the definition of feed additives. However, the code does not explicitly exclude the use of antibiotics to improve animal growth, provided that there has been a previous risk assessment on their safety for public health.

In the USA, food products are regulated by the provisions of the Federal Food, Drug and Cosmetic Act (FFDCA), and the regulations issued under its authority (29). These regulations are published in the CFR (30). The FFDCA defines food as: ‘articles used for food or drink for man or other animals’. Therefore, any product that is intended to be used as an animal feed ingredient, become part of an ingredient or feed, or be added to the drinking water of an animal is considered ‘food’ and thus subject to regulation.

The Food and Drug Administration (FDA) Center for Food Safety and Applied Nutrition is responsible for regulating human food products. In the USA, any substance intentionally added to an animal feed, including pet food, must be used in accordance with a food additive regulation, unless it is generally recognised as safe for its intended use, among qualified experts (<http://www.fda.gov/cvm/prodregulation.htm>).

Independent of their specific evaluations for efficacy, etc., feed additives can be considered part of the food chain. Therefore, from the point of view of safety evaluation for residues, the same toxicological approach should be taken towards feed additives as towards contaminants and food additives.

In general, the evaluation criteria for performance enhancers or zootechnical products that have no benefit for the animal or the consumer but are used to reduce production costs should respond to new safety standards. As such substances could potentially be used in all types of animal production units, the new standards for evaluating risk should consider all kinds of population groups, including particularly susceptible groups, such as pregnant women, children, the elderly and immuno-compromised, because consumers may be exposed throughout the whole of their lifetime.

Beta-adrenoceptor agonists

Beta-adrenoceptor agonists (β -agonists) bind to β -receptors on cardiac and smooth muscle tissues. They also have important functions in other tissues, especially in:

- bronchial smooth muscle (in relaxation)
- the liver (in stimulating glycogenolysis)
- kidneys (in stimulating the release of renin).

Overall, the effects of the β -agonists are cardiac stimulation (increased heart rate, contractility, conduction velocity, relaxation) and systemic vasodilatation.

Beta-agonists can be used for a number of purposes, including promoting growth. At doses several times higher than therapeutic doses, they induce muscular hypertrophy by decreasing muscular degradation and fat synthesis. As a result, the ratio of muscle to fat is modified (the proportion of muscle in the carcass is increased), with an overall improvement in growth performance.

In the EU, placing β -agonists on the market for use in farm animals intended for human consumption is forbidden under Council Directive 96/22/EC (17). The exception is when they are used for therapeutic treatment, under direct veterinary supervision, in calving cows, foaling horses and companion animals. The Directive prohibits the importation from third countries of farm animals, or their meat, to which β -agonists have been administered (except for the therapeutic uses outlined above) and irrespective of any guarantee that the meat is free from residues. As a category, β -agonists are banned for growth promotion.

Clenbuterol

Clenbuterol is an authorised β -agonist for specific therapeutic uses in the EU (horses and cows) (3), USA (horses) (http://www.fda.gov/cvm/CVM_Updates/clenbut.htm), and Canada. In Australia, some β -agonists, including clenbuterol, are authorised and their MRLs have been established.

Ractopamine

On 22 December 1999, the US FDA authorised (29) the use of ractopamine as a growth promoter for pigs during the finishing period, with zero days of withdrawal time. The drug is sold in the form of a medicated feed preparation to be added to pig feed without any veterinary supervision. The high potential exposure of all kinds of at-risk population groups to ractopamine residues must be clearly analysed when evaluating its risk. Ractopamine has not yet been authorised for use in the EU.

Bovine somatotrophin

Bovine somatotrophin (BST) is a bovine growth hormone produced by the pituitary gland of the cow. This hormone is a protein, like insulin, not a steroid hormone, such as sex hormones or cortisone. During lactation, BST mobilises body fat for use as energy and diverts feed energy towards milk production rather than tissue synthesis. In fact, BST increases efficiency in milk production by 10% to 15%.

Council Decision 90/218/EEC (14) banned the placing of BST on the market and its administration to dairy cows by any means whatsoever. Council Decision 94/936/EC (15) extended the moratorium on marketing and using BST in the EU until 31 December 1999.

The Treaty of Amsterdam (1), in force since 1 May 1999, defines new ground rules on animal welfare in the EU in a special 'Protocol on the Protection and Welfare of Animals'. This protocol recognises that animals are sentient beings and obliges European institutions to pay full regard to the welfare requirements of animals when formulating and implementing Community legislation.

Council Directive 98/58/EC on the protection of animals kept for farming purposes is motivated by the spirit expressed in the European Convention (8, 18). In point 18 of the Annex, the Directive states that: '... no other substance with the exception of those given for therapeutic or prophylactic purposes, shall be administered to an animal unless it has been demonstrated by scientific studies of animal welfare or established experience that the effect of the substance is not detrimental to the health or welfare of the animal'.

Bovine somatotrophin is not used for therapeutic purposes but only to enhance milk production. The Scientific Committee on Animal Health and Animal Welfare (SCAHAW) adopted a report on animal welfare aspects of the use of BST on 10 March 1999 (26). The report stated that BST:

- increases the risk of clinical mastitis, as well as the duration of mastitis treatment
- increases the incidence of foot and leg disorders
- can adversely affect reproduction
- can induce severe reactions at the injection site.

The SCAHAW opinion concluded that BST should not be used in dairy cows because it is not a treatment for disease (on the contrary, it can cause disease) and hence is likely to increase the use of veterinary medicines. Council Decision 1999/879/EC (19) permanently banned the marketing and use of BST in the Community from 1 January 2000, in accordance with the provisions laid down in Council

Decision 98/58/EC on the protection of animals kept for farming purposes (18).

The Codex has not yet established maximum residue limits or an acceptable daily intake for BST. A draft standard has been retained in the last step of the Codex procedure for several years but, though proposed, has not yet been approved. In addition, BST-treated cows have been found to have much higher levels of insulin-like growth factor 1 (IGF 1) in their milk than normal cows. According to the conclusions on BST accepted by the EU SCVPH (27), further studies are needed to establish whether there is a correlation between IGF 1 and breast and prostate cancer in humans.

When a growth promoter is clearly toxic or has unambiguously negative animal welfare effects, the risk analysis may be relatively obvious but as soon as the risks are uncertain, the situation becomes difficult to assess. Bovine somatotrophin is an example of a substance which may be used in a continuous and systematic way to enhance yield. In risk terms, this is qualitatively quite different from occasional therapeutic use. Evaluating the toxicological risks associated with long-term exposure is a challenging exercise.

Controlling residues of veterinary medicines and illegal substances in the European Union: current situation

National residue monitoring plans

In the EU, Council Directive 96/23/EC (16) requires Member States to adopt and implement a national residue monitoring plan for specific groups of residues. Member States must assign the task of implementing these controls to a central public department or body. This department is responsible for:

- drawing up the national plan
- co-ordinating the activities of the central and regional departments responsible for monitoring the various residues
- collecting data
- sending the results of the surveys undertaken to the European Commission each year.

National monitoring plans should be targeted. That is, samples should be taken with the aim of detecting illegal treatment or controlling compliance with:

- the MRLs for veterinary medicinal products set out in Annexes I and III of Council Regulation (EC) 2377/90 (13)
- the maximum levels for pesticides set out in Annex II of Council Directive 86/363/EEC (11)
- the maximum levels laid down in the relevant legislation on contaminants.

This means that, in the national plan, Member States target the groups of animals and the sex/age combinations where the probability of finding residues is highest. This approach is different from random sampling, where the objective is to gather statistically significant data, for instance, to evaluate consumer exposure to a specific substance.

In addition, suspect samples are those samples in the national monitoring plans taken as a consequence of:

- a) non-compliant results on samples taken in accordance with the monitoring plan
- b) the possession or presence of prohibited substances at any point during manufacture, storage, distribution or sale throughout the food and feed production chain
- c) suspicion or evidence of illegal treatment or non-compliance with the withdrawal period for an authorised veterinary medicinal product.

Results of residue monitoring in food of animal origin in European Union Member States in 2004

Approximately 807,000 targeted samples and 64,000 suspect samples were taken for the purpose of residue control by Member States in 2004 (6). These samples were taken from all food commodities, including bovines, pigs, horses, sheep and goats, poultry, milk, eggs, rabbit meat, game and honey. A total of 806,525 samples were taken in 2003.

For hormones (including stilbenes, steroids and zearanol derivatives), in terms of absolute results, a total of 61,623 targeted samples were taken in 2004. Seventy-five non-compliant results were found for steroids and zearanol derivatives in bovines, which means 0.12% of samples were non-compliant for hormones in the EU, the same percentage as in 2003. A total of 84 targeted samples (75 in 2003) out of 27,709 (17,474 in 2003) were non-compliant for steroids and zearanol derivatives in pigs, which means that 0.3% of the results were non-compliant for hormones in pigs (mainly due to the presence of nandrolone and contamination with the metabolite zearalene), compared to 0.43% in 2003. There were no non-compliant results for stilbenes and derivatives or for thyrostatic agents in 2004.

The number of non-compliant results for corticosteroids in bovines decreased from 73 targeted and 57 suspect samples in 2003 to 42 targeted and 22 suspect samples in 2004. Dexamethasone was the most frequently found substance for corticosteroids.

The incidence of non-compliant samples increased from 0.02% of the bovines analysed in 2003 to 0.06% in 2004. Six Member States reported findings of β -agonists (only one was a new Member State) and only two Member States had more than one case each.

In terms of absolute results, five targeted and seven suspected non-compliant samples were found in 2003 and 17 targeted and 28 suspected samples in bovines in 2004. In pigs, ten targeted and three suspected non-compliant results were found in 2003 and 11 targeted non-compliant samples in 2004. In addition, one targeted non-compliant result was found for poultry (salbutamol) and four for sheep. Apart from one sample which was non-compliant for salbutamol and one for isoxsuprine (from targeted samples in bovines), plus one non-compliant result for isoxsuprine (from suspect samples in bovines), all the remaining samples from bovines were non-compliant for clenbuterol.

For prohibited substances, the percentage of non-compliant results in bovines increased from 0.05% in 2003 to 0.11% in 2004. In pigs, the percentage of non-compliant results for the A6 group of substances (see Table 1) was 0.9% (the same as in 2003). Some non-compliant results were found for chloramphenicol in different food commodities, such as in:

- bovines: 14 targeted samples and two suspected samples
- pigs: seven targeted samples and one suspected sample
- poultry: 18 targeted samples and six suspected samples
- sheep: two targeted samples
- aquaculture: two targeted samples and 40 suspected samples
- milk: five targeted samples.

In the case of nitrofurans, the following samples were non-compliant:

- in bovines: three suspected samples
- in pigs: one targeted sample and 64 suspected samples
- in sheep: seven targeted samples
- in poultry: seven targeted samples and 58 suspected samples
- in rabbits: one targeted sample.

For nitromidazoles: two targeted samples from poultry were found to be non-compliant; five targeted samples from eggs were non-compliant; and one targeted sample from rabbits did not comply.

For veterinary medicinal products, most of the non-compliant results in bovines were for anti-inflammatory drugs, such as dexamethasone, which has an MRL for meat, liver and milk but can also be used illegally as a growth-promoting agent.

Additional investigations should be conducted when detecting residues to rule out the possibility that the substance is present because of its illegal use as an anabolic substance. There were also some non-compliant results for non-steroid anti-inflammatory drugs (NSAIDs) (carprofen and phenylbutazone), and for sedatives in pigs (acepromazin, carazolol, xylazine, azaperone).

Non-compliant results for anticoccidials were reported in bovines, pigs, poultry, eggs and rabbit meat. The most commonly found substances were lasalocid, nicarbazin and salinomycin.

Anthelmintic residues were found in cattle, sheep and goats, and aquaculture. The most commonly found substance was ivermectin.

Residues of malachite green were found in aquaculture products in 14 Member States. The number of non-compliant results increased from 41 targeted and 40 suspected samples in 2003 to 58 targeted and 190 suspected samples in 2004.

In milk, most of the non-compliant results, apart from antibiotics, were for aflatoxin M1. In eggs, they were for anticoccidials, which are not authorised as feed additives for laying hens older than 16 weeks. However, residues are often found in eggs, possibly due to cross-contamination of the feed in the feed mill.

The use of antibacterials in bees is not authorised. Several non-compliant results for antibacterials were reported in honey, as well as pesticides and heavy metals.

Figure 1, shows the overall distribution of non-compliant results for bovines, sheep, goats, pigs, poultry and horses in the EU in 2004. With regard to targeted samples:

- 51% did not comply for antibacterials
- 25% did not comply for environmental contaminants
- 10% did not comply for veterinary medicinal products
- 9% did not comply for hormones

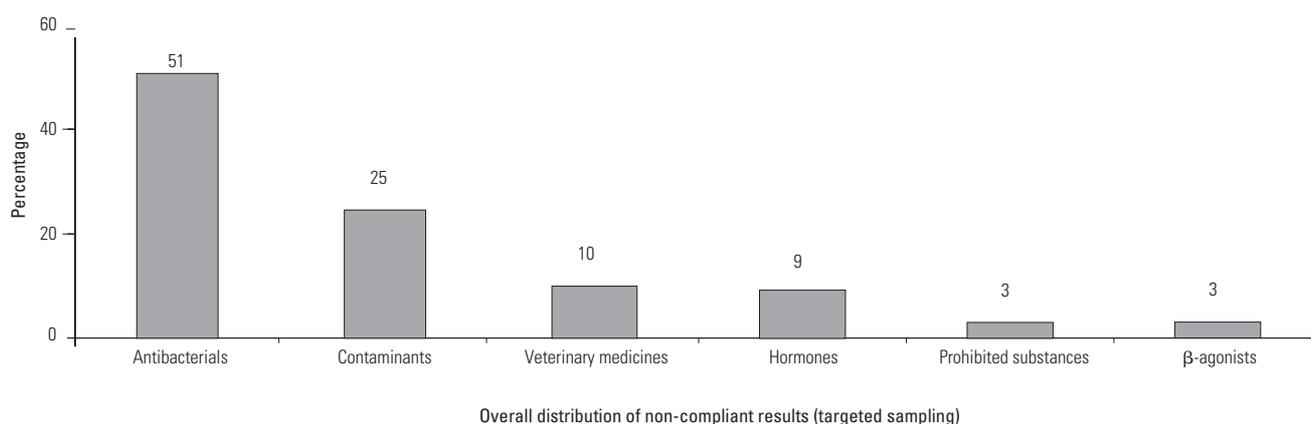


Fig. 1
Overall distribution of non-compliant results (by targeted sampling) for bovines, sheep, goats, pigs, poultry and horses in the EU in 2004

- 3% did not comply for β-agonists
- 3% did not comply for prohibited substances.

Follow-up of non-compliant results

When non-compliant results are found, follow-up measures are of the utmost importance in residue control. Article 16 and Articles 22-28 of Directive 96/23/EC prescribe a series of actions to be taken in the case of non-compliant results or infringements (16). Some measures are aimed at investigating the origin of the infringement, such as verifying the records on the non-compliant farm. Others are to avoid products containing residues from entering the food chain. Depending on the nature of the identified substance, the animals or animal products must be held on the farm until additional tests prove that the other animals are free of residues. If illegal treatment is confirmed, animals should be slaughtered and sent to a high-risk processing plant. When non-compliant results are found at the slaughterhouse, carcasses can be impounded and the products declared unfit for human consumption.

Finally, when the person (or persons) responsible for the presence of the residue in the food has been identified, and the breach of EU legislation proved, penalties can be imposed, including:

- fines
- loss of the ability to apply for Community aid for a period of 12 months
- cancellation of the farming licence
- sanctions against the veterinarian
- criminal sanctions against the person responsible, including jail.

Aspects of ante-mortem and post-mortem inspection

Slaughterhouse findings on bovine animals suspected of having potentially harmful residues

All animals arriving at a slaughterhouse are submitted to an ante- and post-mortem inspection by a competent and qualified authority (i.e. veterinarian) to identify any risk to public health.

In terms of identifying the use of illegal substances or the misuse of legal ones, it is possible to distinguish between:

- animals with clinical signs of disease
- animals showing signs of the possible use of substances (legal or illegal) for growth promotion.

Animals showing clinical signs or lesions

Suspect animals showing clinical signs or lesions may have been treated with different pharmacological products, such as antibiotics or anti-inflammatory substances, without following the recommended withdrawal times. In any case, those animals will be submitted to a very careful post-mortem inspection (Table II) to try to establish a correlation between clinical and post-mortem findings.

Animals showing signs of the possible use of legal or illegal growth promoters

Suspect animals showing signs of the possible use of legal or illegal substances for growth promotion (i.e. hormones, β-agonists, thiouracils, etc.) may have appropriate samples taken and analysed for confirmation. However, these animals are usually in good health, so identifying such

Table II
Typical signs indicating probable residues of veterinary medicinal products in animals identified during ante-mortem and post-mortem inspection at the slaughterhouse

Necessary withdrawal times are established by Regulation EEC No. 2377/90 (13)

Type of inspection	Clinical signs or lesions
Ante-mortem	Signs of disease, including coughing, mucus, breathing difficulties, lameness, dehydration and/or trauma Weak animals, animals which are smaller than usual for their age and breed, apathetic animals, very excited animals Animals showing skin disorders (raised hair, dry skin, alopecia, etc.)
Post-mortem	Lesions in vital organs (e.g. lungs, liver, kidney) or joints Signs of affected lymph nodes Other general signs: possible oedemas, abnormal colours in meat (too bright), unusually dry meat Visible injection sites

Table III
Typical signs indicating probable residues of growth-promoting substances identified during ante-mortem and post-mortem inspections of animals at the slaughterhouse

Type of inspection	Signs or lesions shown by animals treated with growth promoters		
	Corticosteroids	Hormones	Beta-agonists
Ante-mortem	Animals usually show a large abdominal volume (pendulous abdomen) Abnormal skin appearance, alopecia Oedemas detected in the extremities An increase in water retention Abnormal amounts of urine in the lairage pens Animals are usually well fattened for their breed and sex	Heifers showing over-developed mammary glands, with leaking milk or milk in the lairage pens Males with abnormally small testicles for their breed and age	Animals showing above-average muscular development for their breed, sex and age
Post-mortem	Bladders full of urine in several animals from the same production unit, sometimes also containing sand and calculus Kidney lesions Abnormal vascular permeability which results in formation of oedemas	Highly developed mammary glands (galactophorous conducts with or without serum and/or milk) Increased perirenal and pelvic fat in both males and females Males with unusually small testicles for their age and breed Injection sites or implants, liquids or pellets	Unusually improved carcass conformation for their breed, sex and age Carcasses with an abnormally low fat level, especially in the intercostal and diaphragmatic muscles, and in perirenal and dorsal subcutaneous fat

signs (Table III) and consequent intervention by the inspection services is very difficult.

Owing to the difficulties in detecting specific signs (Table III), additional information may be useful, such as recent findings from the same herd or information provided by other inspectors in other slaughterhouses receiving animals from the same herd/farm. Data collected at the ante-mortem inspection must be correlated with the post-mortem findings, using appropriate protocols.

Two examples at slaughterhouse

Figure 2 shows a Holstein Friesian calf of 11 months, from a production unit of eight calves, from which seven showed testicular atrophy. Clinical findings: there was suspicion during the ante-mortem inspection of possible testicular atrophy (confirmed in the post-mortem inspection), and an increase in the perirenal and pelvic fat. Injection sites or implants were not detected.

Figure 3 shows the testicles of another Friesian calf of the same age, from a different herd, for comparison purposes with Figure 2. The testicles in Figure 2 show an evident testicular atrophy.

Inspectors should have technical information about the normal sizes of various organs (e.g. testicles) in regional breeds, according to age and sex, and the tools to measure them (25).

It is important to emphasise that, for some of the signs listed in Tables I and II, there could be other causes, not related to any treatment, which have the same consequences. For instance, the mammary gland in heifers may be abnormally developed if the female has been suckled by other animals on the farm. Other examples are:

- ovarian tumours (to be confirmed at the post-mortem inspection)
- animals being fed with forage which has a high content of phyto-oestrogens (clover)
- precise hormonal treatments to induce lactation, combining oestrogens and progestagens (24).



Fig. 2
A Holstein Friesian calf, aged eleven months, from a production unit of eight calves, of which seven showed testicular atrophy



Fig. 3
Comparison between the testicles of one calf from the herd shown in Figure 2 and the testicles of another Holstein Friesian calf of the same age, from a different herd

The testicles on the left-hand picture show evident testicular atrophy

However, these are very special cases and do not involve a large number of animals.

Figures 4 and 5 show the mammary glands of two fleckvieh heifers, each 11 months old, for comparison. Nine out of 16 heifers from the same production unit showed abnormal mammary gland development. Six heifers displayed the presence of serum and three of milk. Clinical findings for Figures 4 and 5: the mammary gland (hyperplasic tissue) contained a large amount of milk (some has been collected in a glass). There was also an increase in perirenal and pelvic fat. Injection sites or implants were not detected.

It is well known that: 'Oestrogenic substances induce the galactophorous conduct of the heifer from the beginning of puberty in bovines (average nine to ten months – deviation +/- 6-18 months), while the alveolar synthesis is correlated with the synergic action of oestrogens and progestagen substances. Lactation may be induced after applying seven days of 0.1 mg/kg of 17- β estradiol + 0.25 mg/kg of progesterone; the administration of 0.03 mg/kg of dexamethasone during days 17, 18 and 19 also helps milk induction so that production may begin on day 21 of treatment' (24).

Factors to consider at inspection: suspicion and sampling

Control measures to avoid exceeding the MRLs of authorised substances, or the non-authorised use of legal substances, should be focused on the distribution of veterinary medicinal products, both at wholesale and retail level.



Fig. 4
Mammary gland and milk of a fleckvieh heifer, eleven months old

In feed-mills that manufacture medicated feedingstuffs, controls should be established to avoid 'top dressing' or inadequate mixing of animal feedstuffs. Other measures should be introduced to:

- control the sale and distribution of veterinary medicines
- control the administration of veterinary medicines
- ensure that withdrawal times are followed, etc.

The most efficient measures to decrease illegal residues in food are those aimed at avoiding their use in the first place (at the farm level).

Table IV details the main factors to consider when trying to improve detection of unauthorised substances or the unauthorised use of legal substances.

Possible negative effects of undue suspicion

Inside the slaughterhouse

Investigating suspicions of infringement can have negative effects, as follows.

- a) Interruptions in the production line may affect the production process. The corresponding increase in slaughter times will probably be unpopular among slaughterhouse operators, the owners of the animals and workers, due to its financial consequences;
- b) Considerable time is needed to conduct tests and obtain analytical results. If those results subsequently prove negative, the consequences could be:
 - depreciation in the quality of the meat and its price
 - loss of confidence in the competence of the inspectors;



Fig. 5
Mammary gland of a fleckvieh heifer, eleven months old

- c) If no prosecutions are taken, the system loses credibility. Thus, an over-protective attitude simply decreases confidence in the industry and among the different stakeholders.

For these reasons, inspectors may avoid instigating any stoppages at the slaughterhouse unless the evidence of illegal treatment is very clear.

Outside the slaughterhouse

Negative effects may also occur outside the slaughterhouse, as follows.

- a) National policies may be established which divide farm and slaughterhouse inspection responsibilities among several different departments or agencies, discouraging integrated approaches (i.e. the 'from farm to fork' approach). Some EU countries already divide these responsibilities between the departments of Health and Agriculture. In the USA, there are three agencies: the US Department of Agriculture, the FDA and the Environmental Protection Agency;

- b) Governments may impose budget limitations on control measures and restrict general policies and strategies on residue controls.

It must be stressed that success in detecting residues of misused or illegal substances in animal carcasses is not simply the result of individual veterinarians, competent authorities or inspectors, but of team work. The process starts with government action on the food chain and effective co-ordination between the departments of agriculture (at the farm level) and public health (at the 'fork' level). Evaluating the risks of legal or illegal substances in animals should also take into consideration

Table IV
Principal factors which should be considered when trying to improve the monitoring and policing of unauthorised substances in animals or products of animal origin

Area of improvement	Factors for consideration
Illegal trade ('black market') in illegal substances	<ul style="list-style-type: none"> – Food inspectors, agricultural departments, public prosecutor offices and police services should work together to improve the exchange of information and experience at European Union level – Black market networks operate in several countries. Illegal substances may be manufactured in one country, mixed with feed in another and finally illegally distributed to other Member States and third countries
Design of slaughterhouse facilities (to make ante- and post-mortem inspections easier)	<p>Factors to be considered for identification and follow-up of any suspected carcass and its offal:</p> <ul style="list-style-type: none"> – lighting – position and structure of lairage pens in relation to the position of the inspector – correct correlation of carcass with its offal – slaughterhouse facilities readily accessible to workstations for post-mortem inspections
Human resources	<ul style="list-style-type: none"> – Veterinary assistants are needed, to help inspectors with sampling, and identification and correlation of carcasses and offals. For instance, they can continue with the post-mortem inspection while the inspector investigates the presence of residues. – Scheduled slaughter times should be co-ordinated with inspection resources, especially during weekends and at nights – Palpation of both ears and other parts of the carcass should be conducted on every suspect animal (i.e. 100% of suspicious carcasses should be examined in this way)
Further training needed for inspectors and farmers	<ul style="list-style-type: none"> – Periodical update of the trends for new substances and their effects on animals and carcasses – Some farmers may become confused about the legal use of some products from well-known companies (e.g. melengestrol acetate in the United States of America) since limitations on such use may depend on the species, sex and age of the animal
Laboratory infrastructure necessary for a reliable residue control system	<ul style="list-style-type: none"> – Basic requirements are: accredited laboratories, appropriate analytical techniques for the various substances, recommended detection limits and validated methods – The list of available methods should be well disseminated and publicised by the competent authority – The results of the analytical methods should be consistent with the current authorised limits (e.g. a result indicating that a residue has been found at fewer than 15 parts per million (ppm) is not relevant if the maximum authorised limit is 2 ppm) – Better analytical techniques should be developed to improve the existing capacity to validate some illegal substances. Sometimes this is slower than producing and testing new substances for non-authorised use. Endogenous-like substances (such as 17-beta oestradiol) are more difficult to identify; thus, so are abnormal levels of these substances
Sampling at farm level (at production units suspected of infringement)	<p>This is the most efficient way to follow up suspicious animals and conduct tests. Council Directive 96/23 provides that, for bovines, half the samples should be taken at farm level (16)</p>
Legal advice should be sought at an early stage	<p>To ensure the correct administrative process and appropriate prosecution measures are followed, there should be effective feedback among laboratories, the police, health officials and prosecutors (e.g. sometimes a prosecution cannot be conducted due to an error in the administrative procedure)</p>
Technical facilities and communication systems should be available at the inspection point	<p>To ensure effective communication:</p> <ul style="list-style-type: none"> – telephone, e-mail, fax, digital cameras and intranet systems connecting all inspectors in all relevant regions should be available – all information should be shared with the appropriate research centres, government institutions and among slaughterhouse veterinarians

the risks arising from the misuse of these substances and the difficulties of controlling such abuse.

Analytical methods

Analytical methods are crucial in detecting illegal residues. To ensure reliable results, all analyses should be conducted by accredited laboratories (ISO 17025) using validated

analytical methods. Commission Decision 2002/657/EC (5), concerning the performance of analytical methods and the interpretation of results, provides guidelines for the appropriate analytical methods to be used when testing official samples and specifies common criteria for interpreting the results. Variations when performing such tests mean that, in practice, results could vary when the same samples are analysed in different laboratories.

Table V
Analyte testing profile for some banned substances (with no maximum residue level or established tolerance limit) in the European Union

Analytes	Analytical methods for screening	Target tissue	Detection limit: µg per kg (current recommended tested values)
Diethylstilboestrol and other steroids, hexoestrol/dienoestrol	GC/MS	Muscle	0.5
		Urine/liver + IS	1.0
2-Thiouracil and other thyrostatics	HPLC/MS	Urine + IS	100
Melengestrol acetate	GC	Kidney/fat	1
		Muscle	0.5
Trenbolone acetate and epimers/nortestosterone	GC/MS	Urine or liver + IS	2
		Muscle	0.5
Zeranol/talaranol	GC/MS	Urine or liver + IS	2
Clenbuterol (accepted for: parturient cows: 0.5 µg per kg in liver; equines: tocolysis and respiratory treatments: 0.5 µg per kg in liver), cimaterol, salbutamol and other beta-agonists	HPLC*/MS	Retinal tissue	2
		Liver	0.2

GC: gas chromatography

MS: mass spectrometry

IS: injection site on suspected animals

HPLC: high pressure liquid chromatography

HPLC*: HPLC alone or in combination with MS

For control purposes and to ensure harmonisation and transparency, Commission Decision 2004/34/EC (7) established minimum required performance limits for chloramphenicol, nitrofurans, and malachite and leuco-malachite green residues in aquaculture products. In the absence of harmonised limits for banned substances, Member States must apply available analytical methods for detecting minimum levels on the basis of other legitimate factors. Therefore, it is possible that, when trade occurs between countries with different detection limits, the exporting country may consider samples negative (according to EU rules), while the same products are classified as positive in the destination country. Table V lists the different analytical methods available for some banned substances (those which have no MRL and no

established tolerance limit) and the target tissues for analysis, as well as the recommended detection limits. The current use of 'cocktails' containing mixtures of different illegal substances in lower doses may challenge the ability of the existing analytical methods to detect some residues. This may also decrease the clinical signs listed in Tables I and II above, making suspicion and identification of illegal use much more difficult for the veterinary inspector at the ante-mortem inspection.

For substances of endogenous origin, such as 17-β oestradiol, it is analytically difficult to show whether the substance is endogenously produced or has been injected for growth promotion, if the correct withdrawal time has been applied.



Résidus de médicaments vétérinaires, de promoteurs de croissance et d'additifs zootechniques chez les animaux d'élevage destinés à la consommation : le point de vue de l'Union européenne

J. Serratosa, A. Blass, B. Rigau, B. Mongrell, T. Rigau, M. Tortadès, E. Tolosa, C. Aguilar, O. Ribó & J. Balagué

Résumé

Les auteurs brossent un tableau de la présence de résidus de médicaments vétérinaires, de promoteurs de croissance et d'additifs zootechniques chez les animaux d'élevage destinés à la consommation, résultant de l'administration (légale ou illégale) de ces substances dans les fermes. La situation actuelle dans l'Union européenne (UE) est décrite en analysant les résultats, pour l'année 2004, des plans de contrôle nationaux des résidus conduits par les États membres de l'UE. L'analyse aborde également les aspects relatifs à l'inspection *ante mortem* et *post mortem* ainsi que les défis concrets qui se posent aux vétérinaires soucieux de mettre en lumière les cas d'utilisation illégale et de prévenir les risques pour la santé publique. Les auteurs examinent enfin certaines substances qui, pour être peu utilisées, ou seulement chez une minorité d'espèces, sont considérées comme illégales tant qu'elles n'ont pas fait l'objet d'une évaluation des risques.

Mots-clés

Accord sur l'application des mesures sanitaires et phytosanitaires – Additif alimentaire – Additif zootechnique – Antibiotique – Béta-agoniste – Contrôle – Législation – Législation applicable à la médecine vétérinaire – Médicament vétérinaire – Promoteur de croissance – Résidu – Sécurité sanitaire des aliments – Somatotropine bovine – Substance prohibée – Union européenne.



Presencia de residuos de medicamentos veterinarios, promotores de crecimiento y potenciadores de rendimiento en animales destinados a la producción de alimentos en la Unión Europea

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Resumen

Los autores presentan un panorama general de la presencia de residuos de medicamentos veterinarios, promotores de crecimiento y potenciadores de rendimiento en animales destinados a la producción de alimentos debida a la administración, legal o ilegal, de esas sustancias en las explotaciones. Ilustran la situación actual en la Unión Europea (UE) mediante el análisis de los

resultados de los planes nacionales de vigilancia de residuos de sus Estados Miembros, realizados en 2004. También examinan distintos aspectos de la inspección *ante mortem* y *post mortem*, así como los problemas prácticos que han de enfrentar los inspectores veterinarios a la hora de descubrir la administración de sustancias ilegales y prevenir riesgos para la salud pública. Asimismo, se analizan las sustancias cuya administración se ha prohibido hasta que se hayan evaluado los riesgos que pudieran presentar, tales como las que se administran a especies minoritarias o para indicaciones poco frecuentes.

Palabras clave

Acuerdo MSF – Aditivo para alimentación animal – Antibiótico – Beta-agonistas – Inocuidad de los alimentos – Legislación – Potenciador de rendimiento – Producto medicinal veterinario – Promotor de crecimiento – Reglamentación veterinaria – Residuo – Somatotrofina bovina – Sustancia prohibida – Unión Europea – Vigilancia.

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