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Anabolics: the situation in Belgium

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Summary — The illegal use of anabolics in 1989–1990 and its control in Belgium are reviewed. After a short presentation of the Belgian legislation and EEC directives, a recent hormone scandal published by a consumer magazine is related and the composition of 17 hormone cocktails found on the black market is given. The strategy of control involves screening of the main artificial anabolics in urine by radioimmunoassay (RIA) and confirmation by thin-layer chromatography (TLC). Samples of fat and injection sites are also examined by TLC. In the future, RIA will probably be replaced by enzyme immunoassay and TLC by mass spectrometry.

anabolics / anabolizing agents / hormone residues / immunoassay / control strategy

Résumé — **Hormones anabolisantes : la situation en Belgique.** L'utilisation illégale d'hormones anabolisantes en 1989–1990 et son contrôle en Belgique sont passés en revue. Après une brève présentation de la législation belge et des directives CEE, on relate un scandale récent dénoncé par un organisme de défense des consommateurs ainsi que les résultats d'une enquête portant sur l'analyse de 17 fioles d'anabolisants vendues au marché noir. La stratégie du contrôle comporte un criblage des principales hormones anabolisantes artificielles par dosage radio-immunologique (RIA) et une confirmation par chromatographie sur couche mince (CCM). Des échantillons de graisse et des sites d'injection sont aussi soumis à une analyse par CCM. Dans le futur, les RIA seront probablement remplacés par des dosages immuno-enzymatiques et la CCM par la spectrométrie de masse.

hormone anabolisante / résidu d'hormone / dosage immunochimique / stratégie de contrôle

* Correspondence and reprints

INTRODUCTION

The consequences of the application of the Directive (88/146/EEC) prohibiting the use of anabolics and taking into account the limitations of control methods are the following: i), the development of a black market of hormone cocktails including potentially dangerous substances such as synthetic estrogens, androgens, progestagens, corticosteroids and β -adrenergic agonists. ii), As a consequence of this black market, a risk exists for the meat consumer to absorb amounts of residues, at injection sites, of hormonal and toxicological significance. iii), An increased financial effort towards control due to the use of increasingly sophisticated analytical methods. iv), Limitations of therapeutic action in veterinary medicine. v), Disloyal competition between European meat producers and livestock farmers of third-world countries. In fact, control of nude carcasses imported to EEC countries is still impossible in the case of natural hormones and rather difficult for artificial anabolizing agents.

Our presentation will be limited to the illegal use of anabolics in Belgium and its control.

LEGISLATION

Belgian legislation

Belgian legislation is based on a law (July 15, 1985) about "the use of substances having a hormonal or an antihormonal effect in animals". This law planned exceptions to the ban concerning substances that could be used for fattening of meat-producing animals.

The decision of the EEC ministers in December 1985 regarding the complete

ban on anabolics for meat production blocked application of the part of this law concerning tolerance in favor of natural sexual steroid hormones and other substances having a hormonal action that have been recognized as safe for the meat consumer.

EEC ban

Directives (88/146/EEC) replacing (85/649/EEC) (1), (86/469/EEC) (2) and the Commission decision (87/410/EEC, 89//EEC) (3) concern: i), the prohibition of the use in livestock farming of certain substances having a hormonal action and of substances having a thyrostatic action; ii), the control of hormone and thyrostatic residues in living animals and fresh meat; iii), the methods to be used for detecting residues of substances having a hormonal action and of substances having a thyrostatic action.

RECENT EVENT

"Test-Achats" (a consumer magazine)

In January 1990, a consumer magazine, "Test-Achats", published a report about the results obtained after analysis of 81 samples of beef steaks; 16 were considered positive for the presence of hormone residues (medroxyprogesterone acetate, chlormadinone acetate, nortestosterone, or 17β -estradiol). Out of the 16, 11 samples contained only the artificial progestagens. These results contrasted with those of the official controls performed by the Belgian Institut d'Expertise Vétérinaire (IEV) on suspect animals that gave < 10% positive results in 1987 and 1988.

The "Test-Achats" information was largely diffused by the media. Butchers cited in the "Test-Achats" paper criticized the method used and brought an action to court, mainly arguing on the grounds that they were not responsible for the presence of hormone residues in sold meat and that they had no opportunity to perform a contra-expertise. This affair resulted in a dramatic decrease in beef meat consumption and even difficulty in international trade of meat with the Netherlands.

CONTROLS

In Belgium, controls are performed by 3 types of organization: i), IEV for the Department of Public Health; ii), Department of Agriculture; iii), Private organizations.

The IEV

The IEV is responsible for controls in the slaughterhouses. Meat inspectors (veterinarians) collect samples of urine, fat, or injection sites from suspect animals: veal calves, adult cattle, pigs, sheep.

The Department of Agriculture

The Department of Agriculture is responsible for the control of living animals. Its inspectors (veterinarians) collect urine or fecal samples.

Private organizations

The butchers' trade union, supermarkets, "hormone-free" label organizations collect their own samples and organize their own control in collaboration with official or non-official laboratories using official or non-official analytical methods.

The Belgian system of control is more stringent than in other EEC states: i), it is applied to all suspect meat-producing animals; ii), urine, fat, injection sites and fecal samples are taken instead of urine in other EEC countries; iii), in 1988 and 1989 the number of analyses was about twice that imposed by the EEC.

OFFICIAL ANALYTICAL METHODS

Radioimmunoassay (RIA) is applied as a screening method to the urine and fecal samples. Samples identified as positive by RIA are confirmed by high performance thin layer chromatography (HPTLC) (Verbeke, 1979). Fat, injection sites, and fecal samples are also directly examined using HPTLC.

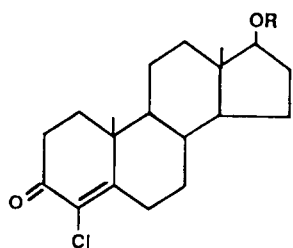
Methods under development

We have developed enzyme immunoassays (Degand *et al*, 1989) as a screening method in urine samples for the presence of residues of the main artificial anabolics and β -agonists: diethylstilbestrol and related substances, ethinylestradiol, nortestosterone, methyltestosterone, trenbolone and clenbuterol. Some of these assays will soon be commercialized by Techland SA, a Belgian company. Urine and meat samples can be analyzed by gas chromatography coupled to mass spectrometry (GC-MS). Rapid and sensitive GC-MS methods are in progress at our 2 laboratories to better satisfy the need for practical control.

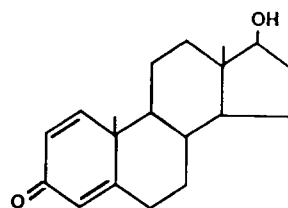
RESULTS OF CONTROL

Black market vials

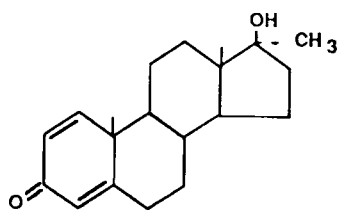
In 1989–1990, an analysis of 17 cocktails was performed. They contained:



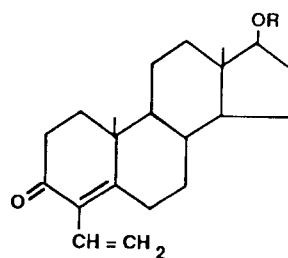
**4-Chlorotestosterone acetate
clostebol acetate**



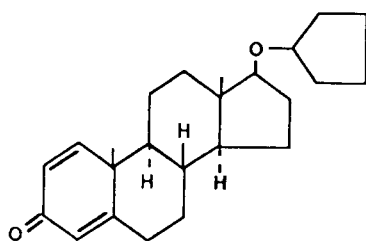
BOLDENONE



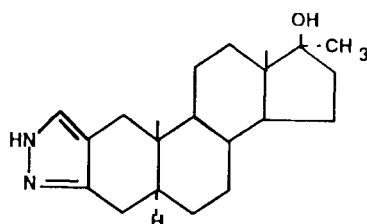
**METHANDIENONE
METHANDROSTENOLONE**



4-vinyl-testosterone esters



QUINBOLONE



STANOZOLOL

Fig 1. Formulae for some anabolics found on the black market.

- ethinyl-estradiol + dexamethazone isonicotinate
- dexamethazone isonicotinate
- ethinyl-estradiol + dexamethazone isonicotinate + chlormadinone acetate
- dexamethazone
- estradiol benzoate + cortisone acetate
- 4-vinyl-testosterone esters
- estradiol benzoate
- estradiol benzoate + testosterone
- nortestosterone decanoate + testosterone propionate + ethinyl-estradiol
- ethinyl-estradiol + nortestosterone decanoate
- chlormadinone acetate + ethinyl-estradiol
- estradiol-benzoate + nortestosterone decanoate + testosterone cypionate
- estradiol benzoate + testosterone cypionate
- chlormadinone acetate + testosterone cypionate
- estradiol benzoate
- estradiol benzoate + testosterone
- estradiol + methandienone + methyltestosterone + progesterone + (isomer) + pregnenedione + chlor-testosterone acetate (clostebol) + stanozolol (composition established in collaboration with H De Brabander, Faculty of Veterinary Medicine, University of Ghent, Belgium).

Boldenone, quinbolone were also sometimes found in black market cocktails (formulae shown in fig 1). Most of these substances were also identified at injection sites collected in slaughterhouses.

Less than 10% of urine samples were found positive for the presence of anabolic

residues in 1987–1988. Lower positive percentages were recorded in 1989–1990.

CONCLUSION

With a complete ban on anabolic hormones, control is very difficult and expensive: using immunoassay, it takes at least 6 months to set up an assay and another 6 months to perform an inter-lab validation; HPTLC is not sensitive enough and sometimes gives false positive results; high performance mass spectrometry (multiple MS) is required in addition to GC-MS for a rapid response to the illegal use of new molecules.

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- Verbeke R (1979) Sensitive multi-residue method for detection of anabolics in urine and in tissues of slaughtered animals. *J Chromatogr* 177, 69-84