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The illegal practice and resulting risks versus the controlled use of licensed drugs: views on the present situation in Germany

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Summary — The chronicle of misuse of anabolics in veal calf production started with diethylstilbestrol, which was used until 1980. During the following years until 1988, illicit preparations contained various steroidal sex hormones, predominantly estradiol, 19-nortestosterone, testosterone and their esters. Since autumn 1988, the sex hormones have been replaced by anabolic β -agonists such as clenbuterol and salbutamol. The real extent of misuse has not been determined. If sex hormones have been applied, most residue levels in the periphery apart from the injection site will remain $< 10^{-9}$ ng/g. At the injection sites total residues may be in the mg range, and biological effects on the consumer cannot be excluded. The orally active β -agonist clenbuterol is accumulated in most organs and a 0 d withdrawal period is not feasible. Immunoassays are approved methods for screening purposes, whereas confirmation is mostly carried out by gas chromatography coupled to mass spectrometry.

anabolic / illegal practice / cattle / residue

Résumé — **Usage illégal des drogues autorisées et risques qui en résultent : un aperçu de la situation actuelle en Allemagne.** *L'usage illégal des anabolisants dans la production des veaux a débuté avec le diéthylstilbestrol, utilisé jusqu'en 1980. Au cours des années suivantes et jusqu'en 1988, les préparations illicites contenaient diverses hormones sexuelles stéroïdes, principalement l'estradiol, la 19-nortestostérone, la testostérone et leurs esters. Depuis l'automne 1988, les hormones sexuelles sont remplacées par des β -agonistes anabolisants comme le clenbutérol et le salbutamol. On ne connaît pas l'ampleur réelle des abus. Quand il s'agit d'hormones sexuelles, la plupart des résidus se maintient à un taux $< 10^{-9}$ ng/g à la périphérie du site d'injection. Au niveau du site d'injection, la totalité des résidus peut atteindre un taux de l'ordre du mg et on ne peut exclure des effets biologiques pour les consommateurs. Le clenbutérol, β -agoniste actif s'il est administré oralement, s'accumule dans la plupart des organes et un délai d'attente de 0 jour n'est pas acceptable. Les tests immunologiques sont utilisables pour le dépistage alors que la confirmation se fait surtout par chromatographie en phase gazeuse associée à la spectrométrie de masse.*

anabolisant / pratique illégale / résidu / bétail

INTRODUCTION

In Germany the use of anabolic sex hormones for growth promotion in food-producing animals has never been permitted; however, several therapeutic preparations containing estradiol, testosterone or nortestosterone were registered until 1988 and have been used to some extent in growing animals such as veal calves. Many illegal preparations were also applied before the EC ban on anabolic sex hormones.

Anabolic sex hormone preparations exhibit optimal efficiency in veal calves, steers and heifers due to their negligible endogenous sex hormone production, but have little efficiency in bulls that produce endogenous sex hormones. Misuse of anabolics seems to be limited to veal meat production, which contributes only 1.8–1.1% of the total meat consumption (Zentrale Markt- und Preisberichtsselle, 1988). On the other hand, most bovine meat consumed within Germany originates from young bulls and there is no need for misuse of anabolics. Only few controls have been carried out in slaughtered culled cows and heifers that represent a potential target for anabolics. Steer production is negligible in Germany.

MISUSE OF ANABOLICS IN VEAL PRODUCTION

Anabolic hormones have been used to an undetermined extent for at least 10–20 years. Dithylstilbestrol (DES) was the major compound in use until 1980, but its misuse has stopped since the development of efficient, sensitive immunoassays. In the following years we have analysed several illicit preparations (table I). It is obvious that different steroids have replaced DES;

when assays for medroxyprogesterone acetate (MPA) and 19-nortestosterone (NT) were elaborated, a tendency toward the use of physiological hormones was found. Nevertheless, even more complex cocktails with orally active compounds were found in early 1988. The problem became more complicated, because some preparations had incorrect, misleading declarations (vita vet A + D₃ + E), whereas most "cocktails" had no declarations. Since autumn 1988, we have had no further indications on misuse of sex hormones and it seems that they have been replaced by β_2 -agonists such as clenbuterol and salbutamol. These compounds are added to animal feed (eg milk replacer), come in bags with a typical declaration such as the word "Beschleuniger" (= enhancer). These compounds also appeared less frequently on the black market when analytical routine methods became available (Meyer *et al*, 1991). Actually, it is not known whether new compounds are in illegal use; there are rumours regarding bromhexine (*N*-{2-amino-3,5-dibromobenzyl}-*N*-methylcyclohexylamine), another new drug being "analysis proof" or about a superior 6-components food additive.

RESULTING RESIDUES AND RISKS

After correct application of preparations registered elsewhere and containing physiological steroids only, residue levels remain low in veal calves, the biological range is not exceeded and there is no risk (Meyer *et al*, 1984, 1985). Illegal practice differs in 4 major respects: i), the injection site is often situated in the edible parts of the animal; ii), dosages are up to 100-fold higher; iii), orally active hormones and arbitrary combinations are also used; iv), the pharmacokinetics are almost unknown.

Table I. Illicit anabolic preparations investigated.

<i>Time</i>	<i>Composition</i>	<i>Declaration</i>	<i>Other remarks</i>
May 83	1.5% NT-ester	No	Oily solution in 100 ml bottle
Aug 83	2.5% E ₂ B 1.0% NT-decanoate	No	Oily solution in 100 ml bottle
March 84	1.0% NT-decanoate	No	Oily solution in 500 ml bottle
Aug 85	2.5% E ₂ B 5.0% NT-decanoate 0.5% MPA 1.0% Chloramphenicol	No	Oily solution in 100 ml bottle
Aug 85	5.0% NT-ester	No	Oily solution in 500 ml bottle
Dec 85	2.5% E ₂ B 5.0% NT-decanoate 0.5% MPA 1.0% Chloramphenicol	No	Oily solution in 100 ml bottle
April 86	4.0% Testosteronepropionate 2.5% E ₂ B	No	Yellow oily solution
Jan 88	0.8% Testosteroneester 0.3% E ₂ B	No	Viscous solution polymerising after contact with water
March 88	0.14% 17 α -Ethinylestradiol 0.7% 17 α -Methyltestosterone 3.6% Progesterone 0.5% Testosteroneenantate 1.9% NT-decanoate	VITA-VET A + D ₃ + E	Light yellow emulsion in 100 ml bottle
March 88	1.6% Testosteronepionate 3.0% NT-decanoate	Catosal	Pinkish aqueous suspension in 100 ml bottle
May 88	4.4% Testosteroneester 1.6% E ₂ B	No	Oily solution
Oct 88	125 ppm Clenbuterol	Milk replacer	White powder
Nov 89	20% Salbutamol	"Enhancer"	Yellow powder in plastic bag
Nov 90	16% Salbutamol	No	Brown powder

NT = 19-Nortesterone, E₂B = Estradiol-3-benzoate, MPA = Medroxyprogesterone acetate.

However, most residue levels in the periphery apart from the injection site remain <1 ppb, as documented for DES (Vogt, 1980), NT (Meyer *et al*, 1989) or MPA (Rapp and Meyer, 1989). Whereas DES may be a tumor-inducer (Gass *et al*, 1964), most other steroids seem to have hormonal activity only, and a risk originating from residues \approx 1 ppb is not identifiable. Nevertheless, at the injection sites total hormone residues are in the mg range (table II) as shown by model experiments with a cocktail that has been in illegal use. After a prolonged waiting time (2–3 months) the injection sites are almost depleted and residue levels go down to the μ g range. However, different galenics of the unlicensed cocktails will result in other pharmacokinetics and the necessary waiting times will be quite variable. Residues in the mg range may induce biological effects in the consumer, but after a single consumption of meat that includes an injection site only the gonadotropin secretion may be impaired and the effect will be completely reversible; problems could arise if injection sites containing orally bioactive hormones (ethinylestradiol, DES, 17

Table II. Residues found in injection sites after im application of an oily cocktail containing 2.5% estradiol-3-benzoate (E_2B), 5% 19-nortestosterone (NT)-decanoate plus 0.5% medroxyprogesterone acetate (MPA).

Dose injected (ml)	Waiting time (d)	Residues at injection sites (mg)		
		MPA	E_2B	NT-ester
10	18	0.63	2.9	3.3
4	20	< 0.02	1.9	3.4
4	63	< 0.01	0.3	0.06
4	70	< 0.02	< 0.06	0.12
4	83	< 0.01	0.03	0.03

α -methyltestosterone) are processed and contaminate a number of finished products such as baby food (Karg *et al*, 1989). Consumption on several consecutive days is possible and longer-lasting interference with the child's physiology cannot be completely excluded.

The illegal application of anabolic dosages of clenbuterol, which are 5–10-fold higher than the therapeutic dose for calves will cause accumulation from blood into most tissues (table III). Without withdrawal 200 g of lung or liver contains \approx 10 μ g clenbuterol (Rinke and Meyer, 1990), which represents the therapeutic dose for man (Goebel, 1975). Maximal accumulation was found in the eye, a finding which may become of diagnostic importance. Whether other β -agonists have similar behaviour has not yet been investigated.

Table III. Clenbuterol residues in veal calf tissues (ng/g) after treatment with 10 μ g/kg body-weight daily for 3 wk.

Tissue	Withdrawal (d)		
	0	3.5	14
Lungs	76.0 ^a	2.40 ^b	< 0.08 ^b
Liver	39.0	1.60	0.60
Kidney	29.0	1.20	< 0.08
Spleen	24.0	1.20	< 0.08
Brain	12.0	0.40	< 0.08
Heart	11.0	0.30	< 0.08
Masseter muscle	8.6	0.30	< 0.08
Trapezius muscle	4.8	0.20	< 0.08
Fat	4.4	0.30	0.20
Eye	118.0	58.00	15.10
Ovary	13.0	0.40	< 0.08
Uterus	8.2	0.40	< 0.08
Blood	1.1	0.05	< 0.04
Urine	47.0	0.50	< 0.05

^a Mean of 3 calves. ^b Mean of 2 calves.

MONITORING

In most institutes the monitoring programmes now consist of components: i), screening that should be sensitive enough for the detection of critical injection sites by analysis of materials apart from the injection site; false negative results must be avoided; ii), confirmation with a similar sensitivity; false positive results must be avoided.

Screening should be as efficient as possible and incorporate the following criteria: economy, practicability, large sample throughput, reduced specificity concerning different xenobiotics, but good discrimination of physiologic compounds. For this purpose immunoassays (IA) with suitable antibodies and tracers are mostly superior. A number of radioimmunoassays (RIA) have been developed earlier (Karg and Rapp, 1988), but enzyme immunoassays (EIA) are now available which provide better sensitivity, are applicable in any laboratory, and also for compounds not available in radiolabelled form. Confirmation is mostly carried out with gas chromatography coupled to mass spectrometry (GC-MS) when excreta containing >1 ppb of the residue are investigated. However, in tissues lower detection limits are necessary (10–50 ppt) and high performance liquid chromatography followed by IA has been applied for the confirmation of NT or MPA in adipose tissue (Meyer *et al*, 1989; Rapp and Meyer, 1989).

Nowadays, illicit preparations are becoming more complex, are changing more rapidly and there is an urgent need for multi-residue methods. However, many limitations exist regarding the latter: not all compounds are found in urine (for example, MPA) and other materials have to be investigated; different groups will be separated during clean-up (eg phenolic from neutral steroids, resorcinic from non phenolic

β -agonists); good antibodies always have a limited aspecificity, and prior to GC-MS different derivatisation procedures are necessary. A recently-developed EIA for 3 β -agonists within 1 test (Meyer *et al*, 1991) and a GC-MS technique for several hormones such as stilbenes, steroidal estrogens, zeranol, NT and trenbolone (Santarius and Roesel, 1989) have provided some progress for simultaneous estimation of ppb residue levels in urine.

CONCLUSION

Problematical residues only occur after misuse of illicit anabolic preparations, especially if compounds with good oral bioavailability are included. The real risk for the consumer can hardly be estimated, due to the unknown types and amounts of residues that may be retained at injection sites or which could accumulate in certain tissues. The rapid design of new multicomponent "cocktails" or "enhancers" containing undetected bioactive substances and impurities will create more and more imponderables; the recommendation regarding withdrawal times for illegally-treated animals will be a compromise between the legal situation, practicability and expected safety; the decisions will always be based on fragmentary knowledge. In this respect it becomes obvious that the EC ban has provoked new safety problems rather than solved existing problems. Possibilities for expanding the analytical methods always take time, and it will not prove possible to screen the samples for a multitude of compounds. This situation shows the need either to change the EC legislation, or adopt more general approaches to screening; sex hormones and β -agonists affect endocrinology and metabolism in the animal, and methods for "endocrine and metabolic profiling" may possibly present a temporary way out.

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