

Nandrolone Review

**Report to UK Sport
January 2000**

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NANDROLONE

REPORT TO THE UK SPORTS COUNCIL FROM THE EXPERT COMMITTEE.

INTRODUCTION

1. Recent media reports have suggested that there may be a growing increase in the number of adverse findings concerning the anabolic steroid, nandrolone, following doping controls carried out on sports men and women both in the UK and abroad. This has caused concern to all involved with sport, and particularly to governing bodies and competitors. In an attempt to clarify this situation, UK Sports has brought together a number of experts and has given them the task of inquiring into the problem.

2. The membership of the Committee is shown at Appendix 1. The committee members encompass in their expertise and backgrounds a number of disciplines and includes analytical chemists, biochemists, endocrinologists, pharmacologists, and forensic scientists. In addition the committee was empowered to seek advice where necessary from external organisations and experts, both in the UK and abroad.

3. UK Sport requested the committee to:

Investigate the current situation with regard to the testing of sports men and women for evidence of nandrolone abuse, and to reach conclusions and make recommendations.

The objectives of the committee were to ensure that the scientific methods and standards which are being applied to the task of detecting nandrolone abuse are satisfactory and safe, and that there is sufficient well-based relevant information available to competitors and their advisers, and to governing bodies, to enable them to make fair and informed decisions.

4. The full committee and its sub-committees have met on several occasions. In addition to taking information from the committee members themselves, comment and advice has been sought from a number of other scientists, and from individuals and organisations both in the UK and overseas, the committee is grateful to them for their assistance. The names are listed in Appendix 2.

NANDROLONE

5. Nandrolone (also referred to as 19-nortestosterone) is an anabolic-androgenic steroid and it is regarded as a performance-enhancing drug. The International Olympic Committee (IOC) lists nandrolone as a banned substance and administration by competitors is a doping offence.

6. The IOC Medical Code (January 1999) defines doping as

- 1 *the administration of substances belonging to prohibited classes of pharmacological agents, and/or*
- 2 *the use of various prohibited methods.*

A revised version of the Code will come into effect in January 2000.

7. Nandrolone and related substances are included in the IOC Prohibited Classes of Substances under "Anabolic Agents (Class C)". As at 31st January 1999, two other similar steroids, 19-norandrostenedione and 19-norandrostenediol, were also included in the list of banned steroids. The IOC code represents the most widely accepted international sporting standard, although some international and national sporting organisations may add or change details, and effective dates.

8. After administration, nandrolone is rapidly modified in the body (metabolised) to a number of products (metabolites), which are then excreted in the urine. A major metabolite of nandrolone is 19-norandrosterone. This metabolite can also originate from sources other than administered nandrolone. Possible additional or alternative sources are nandrolone produced naturally within the body, 19-norandrostenedione, 19-norandrostenediol, and norethisterone. Nandrolone, 19-norandrostenedione, and 19-norandrostenediol all produce the same metabolic product, 19-norandrosterone, and the analytical procedure cannot identify the source.

9. In the UK, the urine samples which are provided by competitors for the purpose of drug screening are collected according to a defined IOC protocol, which is designed to ensure that an adequate volume of urine is collected, that there is no possibility of substitution or contamination, and that the sample is kept securely at all times until it reaches the laboratory in which the analysis is to be performed. All these procedures are carefully documented, and are carried out under the direct supervision of an Independent Sampling Officer. On reaching the laboratory, the samples are also kept in such a way as to ensure their integrity and security before and after the analysis. The analytical laboratory has to be approved and accredited by the IOC and has to achieve and maintain specific standards in respect of its working practices and analytical performance.

INCIDENCE OF ADVERSE REPORTS

10. There have been several media articles recently, which have drawn attention to an apparent increase in the number of adverse findings concerning nandrolone. Since we did not have any information to support this contention, we have examined the published results to date from all the tests carried out by UK Sport on competitors from both within and from outside the UK. We did not however have access to the current figures for the IOC laboratories. The data are shown at Appendix 3, and show that there has been an increase in the number of positive findings in relation to nandrolone reported in 1999. However, we did not consider if any of these tests relate to a single competitor who may have been tested on more than one occasion or whether the selection policy applied in each test situation influenced these data. Furthermore the committee considers it should be cautious when comparing figures for 1999 with those available for previous years since without further enquiry we are unable to say if the adoption of "Analytical Criteria for Reporting Low Concentrations of Anabolic Steroids (August 1998)" (**Para. 22**) has contributed to any degree to the increase in the number of adverse reports.

COLLECTION AND HANDLING OF URINE SAMPLES

11. The committee reviewed the procedures currently employed for the documentation, collection, and transport of samples within the United Kingdom and considered that these are of a high standard. It was suggested that UK Sport might wish to review the report form to see if it could be further improved to provide more background information. Such information might be helpful to a subsequent investigation. We consider that the present procedure for visually observing the collection of urine samples is satisfactory.

12. We are not aware of any information, which would suggest that an adverse 19-norandrosterone report has originated because of any defect in the sample collection or transport procedures. Nevertheless every effort should be made to ensure that high standards are maintained during the collection, storage, and transport of the urine sample.

13. IOC and ISO Guide 25 accredited laboratories are required to work with secure intra-laboratory chain of custody procedures. We are satisfied with current procedures.

14. There is no evidence we are aware of to suggest that nandrolone metabolites might be produced by microbiological action in urine samples during storage.

15. The committee considered there would be no advantage in requesting a further urine sample from a competitor who had tested positive for 19-norandrosterone.

ANALYTICAL ISSUES

16. The sampling and analytical procedures are described in more detail in the International Olympic Committee Medical Code

17. The current internationally adopted system of urine collection is that a specimen collected from an athlete is divided between two sample containers, usually designated A and B, and the containers are then sealed in the presence of the athlete. Both containers are sent to the same IOC accredited laboratory, the A-sample seal is broken and the urine analyzed. Should there be an adverse finding for the A-sample, the athlete and/or his representative may choose to witness the breaking of the seal on the B container and the subsequent analysis. Additional tests on the B sample may be requested during that time or at a later date.

18. We have examined this and other procedures and conclude that the current 'two sample' system is satisfactory and raises no specific problem in relation to nandrolone. If a positive result on the A-sample is challenged, the athlete should be encouraged to have an independent expert present at the B-sample analysis.

19. ISO Guide 25 requires that the purity of the reference material used in the analysis of each nandrolone metabolite should be known and fit for the purpose of quantitative work. In the UK, the IOC-accredited laboratory is also accredited by the United Kingdom accreditation service to the ISO Guide 25 accreditation standard. Reference material used for quantification of 19-norandrosterone has a fully specified accompanying certificate of analysis from the supplier. (ISO = International Standards Organisation).

20. We conclude that the current analytical system, which is being operated for nandrolone metabolites, is satisfactory.

21. We conclude that both 19-norandrosterone and 19-noretiocholanolone (which are metabolites of nandrolone) can be effectively identified by the routine methods currently employed within IOC accredited laboratories. If the presence of 19-noretiocholanolone is to be reported then the reporting standard should be the same as that for 19-norandrosterone.

22. To help achieve international consistency in reporting, the IOC produced a document for the guidance of accredited laboratories, entitled 'Analytical Criteria for Reporting Low Concentrations of Anabolic Steroids (August 1998)' (appendix 4). The document makes a number of recommendations and gives criteria for reporting low concentrations of anabolic steroids in urine, using methods such as gas chromatography and mass spectrometry. The section relating to 19-norandrosterone describes a minimum reporting concentration for samples containing 19-norandrosterone. The document recommends that a report should not be issued for male urine if the concentration found in the test sample is less than that in a control urine containing 19-norandrosterone at a nominal concentration of 2 nanograms per millilitre. For the non-pregnant female, the reporting concentration must not be less than two and a half times that of the control, i.e. not less than 5 nanograms per millilitre. Adjustment of the reporting concentration will be made if the specific gravity of the urine sample is greater than 1.020. In addition, for females, it is recommended that a report should not be issued if there is evidence of pregnancy. Notification is also made if the result is considered to be compatible with administration of norethisterone (which is present in some oral contraceptives) (Para. 34).

23. With regard to the minimum reporting concentration, the approach recommended by the IOC to take account of urine concentration by increasing this minimum with regard to the amount by which the specific gravity of the urine samples exceeds 1.020, was deemed appropriate.

24. Although the IOC document entitled 'Analytical Criteria for Reporting Low Concentrations of Anabolic Steroids' is satisfactory in describing analytical criteria for identification, neither this document nor the IOC Anti-Doping Code, as currently formulated, require that information is given on the concentration of 19-norandrosterone present in a positive sample. Currently, all that is required from a laboratory is to demonstrate that the concentration exceeds the minimum reporting concentration (**Para 22**). We consider it would be helpful to governing bodies if the criteria for IOC accredited laboratories stated whether quantification of the samples is required. Full quantification requires several different calibrant concentrations to be prepared and analyzed, and if it is to be implemented, then sports authorities should be made aware of the increased cost of analysis. Due consideration should be given as to whether this is necessary only within a limited concentration range.

25. Existing techniques in IOC accredited laboratories for the identification of nandrolone metabolites use gas chromatography-mass spectrometry, and are described in 'Analytical Criteria for Reporting Low Concentrations of Anabolic Steroids (August 1998)'. We also considered other alternative analytical techniques such as gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS), liquid chromatography-tandem mass spectrometry (LC-MS-MS) and capillary electrophoresis-mass spectrometry (CE-MS), and concluded that, given the current state of technology, these techniques should be kept under review.

26. There are as yet insufficient data to warrant the consideration of alternative analytical matrices to urine, such as hair, blood, or saliva.

INTERPRETATION OF THE REPORT

27. The testing laboratory will perform a screen for the presence of banned substances. In the case of 19-norandrosterone, it will only issue an adverse report for possible sanction if the concentration of this metabolite is in excess of two nanograms per millilitre of urine in the case of a male competitor, and five nanograms per millilitre in the case of a female competitor. As explained above (Para. 22), these concentrations have been laid down in the document of analytical criteria, which has been made available to IOC accredited laboratories to enable them to achieve international harmonisation. The laboratory does not report a specific concentration, only that a metabolite of nandrolone has been detected.

28. A possible area of controversy lies in the interpretation of the results of the urine analysis. Until recently, it has been assumed that the only source of routinely detectable urinary 19-norandrosterone was administered steroids, such as nandrolone. However, there is now evidence that 19-norandrosterone is present naturally in low but detectable concentration in urine from some men and women. Hence, the need arises to establish the maximum concentration of 19-norandrosterone, which may be found in a urine sample collected from individuals who are not using nandrolone, or similar substances, which can produce the common metabolite, 19-norandrosterone. The IOC Medical Code makes no statement on this concentration and offers no guidance. The committee has therefore carried out a survey of the scientific literature. The three published studies of the excretion of 19-norandrosterone by normal subjects (1,2,3) agree in finding no urine concentration greater than 0.6 nanograms per millilitre. However, the significance of these findings is limited, because the test population was small and comprised only 47 individuals in total, was not a representative racial mix and was composed of non-athletes.

29. Many more data were obtained from the 1996 Winter Olympics in Nagano (4). Here of the 370 male competitors tested, only 5 gave urinary concentrations of 19-norandrosterone exceeding 0.1 nanograms per millilitre, and no concentration exceeded 0.4 nanograms per millilitre. It is noteworthy that this sample population comprised healthy athletes mainly under intense physical exertion. It cannot be excluded that some of these competitors might have been taking nandrolone, but even if this had been the case, it would have had the effect of increasing the urinary values and so the true maximum concentration could have been smaller. Of the 251 female competitors tested none exceeded the 5 nanograms per millilitre threshold. We would have liked to have had sight of the complete data from the Nagano study. We understand that there is additional information from a FIFA study in Switzerland but these data were not available to us. It would be beneficial if all the information available from these and similar studies could be brought into the public domain. We recommend that steps should be taken to broaden the data base of available information on the urinary excretion of 19-norandrosterone in healthy male and female subjects, so as to add further weight to the conclusion that only very small concentrations of 19-norandrosterone occur in normal urine and to establish a definitive upper limit of normal.

30. During intense exercise, some degree of dehydration may occur, causing the urine to become concentrated. This will, to a degree, increase the measured concentration of excreted substances. This is allowed for in the laboratory analysis by correcting for the specific gravity of the urine sample if this exceeds a specified concentration. We consider that this is the most appropriate method of correction when necessary.

31. It has been suggested that additional and possibly more definitive information might be obtained from the urine analysis if other steroid metabolites, in particular 19-noretiocholanolone, were to be measured in the test sample. We have found no convincing evidence to support this suggestion and consider that the measurement of 19-norandrosterone alone, as carried out at present, is adequate. The exceptions to this conclusion are that measurement of 19-noretiocholanolone would assist in the detection of the possible abuse of 5-alpha reductase inhibitors (vide Para. 33), and the identification of tetrahydronorethisterone is helpful in some cases (Para. 34).

32. We have concluded that in the light of information currently available, the reporting policy under which the IOC laboratories are required to work in regard to 19-norandrosterone is satisfactory. However, it would be helpful if the IOC publicised and made available the analytical criteria (Appendix 4) issued to laboratories, for the benefit of competitors and their advisers. In particular, the rationale for guidance to laboratories on issuing adverse reports should be explained. We also recommend that the IOC should be asked correlate and publish the available data on the normal excretion of 19-norandrosterone, and should clearly define the maximum urinary concentration of this steroid above which it is considered that a doping offence may have been committed.

REDUCTASE INHIBITORS

33. Drugs, which are described chemically as 5-alpha-reductase inhibitors (such as finasteride) will, if taken by a competitor, reduce the excretion of 19-norandrosterone formed from administered nandrolone. In parallel with this effect, there is an increase in the excretion of 19-noretiocholanolone. Such a metabolic pattern would alert the laboratory to possible administration of the drug. We recommend that the IOC Medical Committee should consider adding this and similar inhibitors to the list of banned masking agents. If a competitor is using them for medical purposes, this could easily be established by documentation because these drugs are Prescription Only Medicines in the UK and are only available legitimately on a medical prescription issued for treatment of a medical condition.

NORETHISTERONE

34. Norethisterone is an orally active steroid, which is used by women for the purpose of contraception and for the treatment of menstrual dysfunction. The amount of 19-norandrosterone derived from contraceptive doses of norethisterone is small and should not cause a problem with interpretation if the report of the accredited laboratory also notes the presence of another metabolite of norethisterone, i.e. tetrahydronorethisterone. Larger doses being used therapeutically might lead to a urinary concentration of 19-norandrosterone resulting in a notifiable concentration, but such cases could be investigated individually and the use of the drug would be supported by medical evidence of a prescription issued for treatment of a medical condition.

OTHER BANNED STEROIDS

35. In addition to nandrolone, two other currently banned steroids, 19-norandrostenedione and 19-norandrostenediol are metabolised to 19-norandrosterone, which will appear in the urine after they have been administered. It is likely that these two compounds are converted in the body transiently to nandrolone before they are metabolised to 19-norandrosterone. These steroids are offered for sale very widely and advertised as being converted to nandrolone in the body after ingestion (although we are not aware of any evidence for this). Some advertisers, but not all, warn the purchaser that these steroids are banned by the IOC.

36. We consider that because of the labelling used in some cases, there is a risk that it may not be apparent to purchasers of these materials that they are banned steroids, and we advise that users should be reminded to ensure that they understand what is being offered in these preparations.

37. At present, 19-norandrostenedione and 19-norandrostenediol are not considered in the UK to be medicinal products and so their supply is not controlled. Equally, they are not included in the Misuse of Drugs Act 1971. Thus, it is not an offence to supply them. However, the committee has been advised that the Medicines Control Agency is about to classify these compounds as medicinal products, and when that has been done, to manufacture or supply them in the UK will require an appropriate licence.

38. We recommend that the sports community should be made aware that if these products (19-norandrostenedione and 19-norandrostenediol) were classified as medicinal products, they would be subject to statutory controls in the UK.

39. The rules of sport in relation to the prohibition of nandrolone and similar steroidal substances were considered by the committee at some length. The recent addition of 19-norandrostenedione and 19-norandrostenediol to the IOC list of banned substance has clarified the position over these steroids. Previously, it had been a matter of interpreting the rules and deciding whether these compounds were banned substances by virtue of inclusion in the phrase "and related substances". Any possible ambiguity in this respect has now been removed by direct reference to these steroids in the IOC Medical Code. The committee felt that

it would be helpful if the IOC and other regulatory bodies were more proactive in assessing the availability of new substances and ensuring that the list of banned drugs was kept as up-to-date as possible. This would require active monitoring of the market and taking scientific advice as early as possible.

NUTRITIONAL SUPPLEMENTS AND HERBAL PREPARATIONS

40. We are unaware of any evidence that a dietary substance might influence the production of endogenous (i.e. produced naturally within the body) nandrolone. This had been suggested to us, since preliminary studies with experiments carried out on rats (5) appear to have shown that the administration of acetyl carnitine might protect these animals against the diminution of testosterone production caused by severe exercise. These reports were not considered to be in any way relevant to the situation in human subjects. We are unaware of any relevant human studies.

41. Whilst some substances marketed as nutritional supplements contain a notification on the label that they contain steroids, it may not always be obvious from the labelling that they contain banned substances. It has also been reported to us (6,7) that cases have arisen in which the steroid content differed from that stated on the label, or there was no indication at all on the label that the supplement or herbal preparation contained steroids, whereas they were in fact present. If this is so, a sportsperson who uses these products may unknowingly ingest steroid preparations and so put themselves at risk.

42. It is apparent that the market for nutritional supplements in sport is considerable and represents a substantial commercial business. Even a cursory review of sports magazines reveals the huge advertising exposure that exists. Competitors should be aware and appreciate the possible risks involved in using some of these supplements.

43. We make the following recommendations:

a) Sportspersons should not take any dietary or herbal preparation unless they are entirely satisfied that they are aware of and understand the nature of the contents. Competitors should discuss the preparation fully with their team doctor **before use**.

b) UK Sport should investigate the possibility that an appropriate national or international organisation should scan the press and Internet advertisements for new preparations, so that, after taking professional advice, these advertisements should be brought to the notice of team doctors with an appropriate warning. Any new steroid preparation encountered in the process should be brought to the notice of the IOC Medical Commission.

FOOD SOURCES

44. Nandrolone is produced naturally in some animals (e.g. boar, horse) and will accumulate naturally in organs such as liver and testes. A relatively small-scale study of 25 animals demonstrated nandrolone to be present in boar liver from non-castrated animals and lower concentrations were present in muscle (8). However, larger scale studies are required to determine whether the reported levels are typical.

45. Nandrolone residues in animal products can be evaluated through the results of national surveillance programmes and there is good evidence that food available in the UK is not a significant dietary source of this steroid.

46. We have been advised by the Ministry of Agriculture, Fisheries and Food Veterinary Medicines Directorate that in the last full year of testing of home grown and imported foods in

the UK (1998), no measurable nandrolone was found in edible tissues. In addition, recently reported data show no positive results from nandrolone analyses performed between January and September 1999 (9, 10). Consequently, animal products consumed in the UK up to this date seem unlikely to give rise to positive urine tests in athletes.

47. The development of national plans for analysis of residues across the EU has only become mandatory in recent years and consequently statistics for the EU as a whole are not yet available. In addition, the high standards established by the EU may not apply else where in the world. Consequently, it is not possible at present to assess reliably the levels of nandrolone in the global diet and to determine if there have been any significant changes in recent years.

48. We have concluded that it is unlikely that eating good quality unprocessed muscle meat could cause a positive urine test. In the present state of knowledge it seems prudent to avoid offal from boar and horse.

49. A review of the literature has failed to support the suggestion that nandrolone may be present in vegetables.

The Committee wishes it to be noted that this report provides an overview of the general position and not of any specific case(s,) and that it expresses the opinion of the Committee at the date the report is made.

The Committee acknowledges with thanks the efficient support of Ms Sheridan Jones, Special Projects Officer, Ethics and Anti-Doping Directorate in the co-ordination of the review and preparation of the report.

CONCLUSIONS AND RECOMMENDATIONS

1. The data suggests an increase in 1999 in the number of adverse reports concerning the anabolic steroid nandrolone.
2. We consider that the IOC recommended sample collection procedures are satisfactory. We have examined in particular the arrangements in the UK and are satisfied that they are of a high standard. Storage and transport arrangements, together with associated chain of custody documentation should be strictly adhered to.
3. The committee has examined the analytical procedures that are employed for the detection of nandrolone metabolites in urine and is of the opinion that they are satisfactory. The laboratories are accredited and approved, and employ analytical techniques and laboratory practices which are of a high standard.
4. We suggest that an indication of the concentration of nandrolone metabolites in a sample that is declared positive should be included in the adverse report that the laboratory issues. We recognise that this has to be done with due regard for the cost involved and that full quantification may not be necessary.
5. We consider that it would be helpful if the IOC would define the urine concentration of 19-norandrosterone above which it considers that a doping offence may have been committed. Further studies should be carried out to investigate generally the factors influencing the endogenous production of nandrolone in human subjects.
6. We **recommend** that the IOC should publicise and make available the analytical criteria issued to laboratories (Analytical criteria for reporting low concentrations of anabolic steroids) (Appendix 4).
7. Some dietary supplements contain compounds similar to nandrolone or its metabolic precursors, which produce the same metabolites as does nandrolone. It may not be obvious from the label that such substances are present and are banned substances. Users of inadequately or incorrectly labelled products are at risk of unknowingly ingesting a banned substance. **We therefore recommend** that the sports community should be reminded they must maintain a high level of awareness of the possible hazards of using some nutritional supplements and herbal preparations.
8. We have not seen any evidence that suggests that a dietary substance can influence the production of nandrolone within the body.
9. We **recommend** that the availability of new nutritional supplements or substances purporting to be performance-enhancing drugs should be monitored and the rules that include the list of banned substances should be up-dated regularly in response to this information.
10. We are unable to assess fully the possible risk that consumption of meat may cause a notifiable urine concentration of 19-norandrosterone, but we believe that the possibility is remote from eating good quality unprocessed muscle meat from commoner animal species. It may be prudent to avoid offal from boar and horse.

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Appendix 1**Committee Membership***Chairman:*

Prof. V H T James, Emeritus Professor of Chemical Pathology, University of London.

Scientific Secretary:

Dr A Kicman, Head of Research & Development, Drugs Control Centre, Kings College London.

Members:

Prof. R V Brooks, Emeritus Professor of Chemical Endocrinology, University of London.

Prof. D A Cowan, Head of Drug Control Centre, Kings College London.

Prof. V Garner, Scientific Director of Hall Analytical Laboratories, Manchester.

Prof. S Gaskell, Head of Department of Chemistry, UMIST, Manchester.

Dr. J Honour, Clinical Scientist, Department of Chemical Pathology, University College London.

Dr. E Houghton, Senior Assistant Director & Head of Research & Development Department, Horse Racing Forensic Laboratory, East Midlands.

Prof. H Jacobs, Emeritus Professor of Reproductive Endocrinology at University College London Medical School.

Prof H L J Makin, Professor of Analytical Biochemistry, University of London at St. Bartholomew's and Royal London School of Medicine.

Prof. T Moffat, Chief Scientist of The Royal Pharmaceutical Society of Great Britain, Professor at The School of Pharmacy, University of London.

Dr. P Robb, Senior Analytical Scientist, Ministry of Agriculture, Fisheries and Food. The Central Science Laboratory, York.

Dr. B Sheard, Chief Executive, Horse Racing Forensic Laboratory, East Midlands.

Dr. M Wallace, Principal Scientist, Endocrine Section, Department of Clinical Biochemistry, Royal Infirmary, Glasgow.

Dr. M Wheeler, Consultant Clinical Scientist at Guy's and St Thomas' Hospitals and Honorary Senior Lecturer, Kings College, London.

Adviser on Anti-Doping Procedures

Michele Verroken, Director of Ethics & Anti Doping, UK Sport, London

Committee Secretariat:

Sheridan Jones, Special Projects, Ethics & Anti Doping, UK Sport, London

Appendix 2***Assistance received from External Organisations and Experts***

Mr. D Mackintosh, Principal Lecturer in School Life Sciences, Kingston University

Mr. D Carter, Manager Borderline Department, Medicines Control Agency, London

Mr. T Morton-Hooper, Solicitor, Mishcon de Reya, London.

Prof. C Ayotte, Professor et Directrice de laboratoire de controle du dopage, INRS Institute, Quebec

Dr. L Dehennin, Laboratoire de la Federation des Courses Francaises, France

Prof. L Bowers, Director of Drug Analysis Laboratory for Athletic Drug Testing & Toxicology, Indiana University Medical Centre, United States.

Prof. A Ljungqvist, Chairman & Vice President of IAAF Medical Committee, Sweden

Dr. W Schaenzer, Director Deutsche Sporthochschule, Institute of Biochemistry, Germany.

Dr. R Stephany, National Institute of Public Health and the Environment (RIVM), The Netherlands.

Dr. P Schamasch, Medical Director, International Olympic Committee, Switzerland.

Dr. J Segura, Institute Municipal d'Investigacio Medica IMIM, Spain.

Prof. R Maughan, University Medical School, Aberdeen, United Kingdom.

Dr Alan Hayes, Pure and Applied Chemistry Journal, United Kingdom.

International Amateur Athletics Federation

International Badminton Federation

New Zealand Sports Drug Agency

IOC Laboratory, Portuguese

Home Office

International Weightlifting Federation

Australian Sports Drug Agency

IOC Laboratory, Prague

IOC Laboratory, France

Appendix 3**Table 1. Data from UK Sport's Drug Testing Programme on UK and Non UK Competitors**

Competitors tested within UK Sport's Testing Programme include UK competitors and non-UK competitors. Number of Findings does not indicate number of different athletes providing a positive urine sample and/or committing a doping offence. The data may also include competitors tested on more than one occasion.

Year	Total number of A Samples Analysed	Number Positive Nandrolone Findings	% Positive Findings versus Total A Samples analysed
1999	5771	17	0.29
1998	4669	4	0.09
1997	4573	4	0.09
1996	4395	3	0.07
1995	4228	5	0.12
1994	4435	1	0.02
1993	3829	5	0.13
1992	4046	1	0.02
1991	3421	6	0.18
1990	3708	2	0.05
1989	3172	7	0.22
1988	2798	2	0.07

Table 2. Data from IOC Accredited Laboratories Worldwide

Year	Total number of A Samples Analysed	Number Positive Nandrolone Findings	% Positive Findings versus Total A Samples Analysed
1999	N/A	N/A	N/A
1998	105250	259	0.25
1997	106561	262	0.25
1996	96454	232	0.24
1995	93938	212	0.23
1994	93680	207	0.22
1993	89166	227	0.25
1992	87808	152	0.17
1991	84088	165	0.20
1990	71341	192	0.27
1989	52371	224	0.43
1988	47069	304	0.65
1987	37882	N/A	N/A
1986	32982	N/A	N/A

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Appendix 4

INTERNATIONAL OLYMPIC COMMITTEE

ANALYTICAL CRITERIA FOR REPORTING LOW CONCENTRATIONS OF ANABOLIC STEROIDS

INTRODUCTION

A working group of directors of IOC Accredited Laboratories met:

To harmonise, in a defensible way, the analytical part of reporting for low concentrations of anabolic steroids, when using the recently implemented techniques of HRMS and MS/MS.

The group took into account the different reliable analytical strategies, the different techniques available (including LRMS) and the interpretation of the data. The present document provides the draft of the minimum criteria determined by the group as necessary and focuses on the identification of compounds by MS techniques. The minimum criteria are listed under the heading "criteria" and the main conclusions of the discussion are included in the sections labelled "recommendations". A glossary of the terms used is provided on the final page of this document.

ANALYTICAL STEPS REQUIRED

At a minimum an analysis that results in an adverse report shall consist of two steps:

screening the A-sample and
performing an A-sample confirmation

Either step may be on a single A-sample or in a batch of other samples.

BATCHING OF SAMPLES FOR CONFIRMATORY ANALYSIS

At a minimum the batch shall consist of the sample of interest, a certified negative control urine and a certified positive control urine all prepared for analysis identically at the same time. The practice of using a positive control from a different batch, and stored in the desiccator is not recommended. The term certified negative urine refers to a human urine sample that has been determined in the laboratory not to contain the substance(s) of interest. This will be performed by the screening or confirmation method(s) of interest, including sample preparation, derivatisation and MS analysis. The certified positive urine may be a certified negative urine to which the appropriate reference material has been added, or a urine collected from a person following documented administration of the authenticated substance, that has been determined in the laboratory and by the method of interest, to contain the substances of interest.

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ORDER OF INJECTION INTO THE ANALYTICAL INSTRUMENT

The recommended order of injection into the analytical instrument for confirmatory analysis is as follows:

Reagent blank, if pertinent
Negative control urine
Sample being confirmed
Negative control urine
Positive control urine

CHROMATOGRAPHY

Criteria: The retention time (RT) or relative retention time (RRT) of the analyte shall not differ by more than one (1) per cent from that of the same substance in the positive control urine analysed in the same batch. In those cases where shifts in retention can be explained, for example column overload, the retention time criteria can be relaxed.

Recommendation: It is preferable, but not mandatory, to use an internal standard. It is recognised that peak shape is important and should be similar to that of the standard. The concentration of the analyte in the positive control urine should not differ by more than a factor of five from the expected concentration of the analyte in the sample to minimise possible sources of variation in results. For example, if the anticipated concentration of analyte is 5 ng/ml, the concentration of the positive control urine should be in the range of 1-25 ng/ml.

MASS SPECTROMETRY

1. Scan Mode (low resolution)

Criteria: Evaluation of a scan must include consideration of a minimum of three diagnostic ions. If three diagnostic ions are not available a second derivative shall be prepared, or a second ionisation or fragmentation technique shall be used. In any case, a minimum of two diagnostic ions is mandatory in each mass spectrum. The signal to noise ratio of the diagnostic ions must be greater than three to one. The relative abundance of any of the ions shall not differ by more than 5 per cent (absolute) or twenty per cent (relative), whichever is greater, from that of the positive control urine; values of zero and less are not valid, i.e. an expected ion must be present.

Recommendations: The scan may begin at a m/z value greater than any abundant ion due to the derivatising agent or chemical ionisation reagent. Preferably the relative abundance of a diagnostic ion shall be determined from the integrated extracted ion chromatograms.

2. Selected ion monitoring (HRMS & LRMS)

Criteria: A minimum of three diagnostic ions must be monitored in a single mass spectrum. If three diagnostic ions are not available a second derivative shall be prepared, or a second ionisation or fragmentation technique shall be used. In any case, a minimum of two

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diagnostic ions is mandatory in each mass spectrum. The signal to noise ratio of the diagnostic ions must be greater than three to one. The relative abundance of any of the ions shall not differ by more than five per cent (absolute) or twenty per cent (relative), whichever is greater, from that of the positive control urine; values of zero and less are not valid, i.e. an expected ion must be present.

3. MS/MS and related techniques

Criteria: There must be a minimum of three diagnostic ions which may include the precursor ion. The precursor ion must have an abundance equal to or greater than five per cent of that of the most intense diagnostic ion of the MS/MS spectrum.

If three diagnostic ions are not available, a second derivative shall be prepared, or a second ionisation or fragmentation technique shall be used. In any case, a minimum of two diagnostic ions is mandatory. The signal to noise ratio of the diagnostic ions must be greater than three to one. The relative abundance of any of the ions shall not differ by more than ten per cent (absolute) or twenty-five per cent (relative), whichever is greater, from that of the positive control urine; values of zero and less are not valid, i.e. an expected ion must be present.

ADDITIONAL CONSIDERATIONS FOR REPORTING SPECIFIC COMPOUNDS

These considerations have been developed based on current knowledge and experience gained in analysing the following substances and, at present, are limited to the following five analytes:

- clenbuterol
- 19-norandrosterone (metabolite of nandrolone)
- 17 α -methyl-5 β -androstane-3 α ,17 β -diol (metabolite of methyltestosterone)
- 17 β -methyl-5 β -androst-1-ene-3 α ,17 α -diol (metabolite of methandienone)
- 3'-hydroxystanozolol (metabolite of stanozolol)

The intention of these considerations is to provide a harmonised approach between IOC accredited laboratories.

CRITERIA TO BE FOLLOWED EXCEPT FOR 19-NORANDROSTERONE.

The relative signals (i.e. to internal standard) obtained for the analyte under consideration in the screening analysis of the sample should be compared with those obtained from a positive control urine (see below). If the relative signals obtained from the sample are less than those obtained from the positive control urine the sample should not be reported for sanction unless the urine specific gravity is <1.005 . The positive control urine should be prepared by adding sufficient substance to a negative control urine to produce a positive control urine with a nominal concentration of 2 ng/ml.

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NOTE: Results with relative signals less than those obtained from the positive control urine may be reported informally for information, and for possible additional (target) testing of the individual who provided the sample, but should not be used (alone) for possible sanction. More than one metabolite may be detected, but the identification of only one metabolite is sufficient to report for possible sanction.

19-NORANDROSTERONE IN FEMALE URINE

In the case of the detection of 19-norandrosterone in the urine of a female the following four aspects should be assessed:

1. Whether the 19-norandrosterone could have arisen because the female is pregnant, check for example the presence of large signals from the pregnanes, the presence of large amounts of estriol, an abnormally large hCG concentration, or information on pregnancy from the governing body or competitor's declaration form.
2. Whether it is reasonable that the 19-norandrosterone could have arisen as a metabolite of a progestogen (progestin), e.g. norethisterone. Check whether tetrahydronorethisterone is present.
3. Whether the relative signal(s) in the mass spectrometry screening analysis for 19-norandrosterone is less than two and a half (2.5) times that obtained from the positive control urine (nominal concentration 2 ng/ml) described above.
4. If the urine specific gravity is greater than 1.020, multiply the relative signal from the positive control urine by $2.5 \times (\text{sg} - 1)/0.02$ and determine whether the relative signal obtained from the sample is less.

If any of conditions 1, 3 or 4 is met, do not produce an adverse report for possible sanction. If only aspect 2 is met, notify the report as "could be compatible with contraceptive medication declared by the athlete".

Before issuing an adverse report, repeat the measurements on a further two aliquots of the sample. More than one metabolite may be detected, but the identification of only 19-norandrosterone is sufficient to report for possible sanction.

19-NORANDROSTERONE IN MALE URINE

In the case of the detection of 19-norandrosterone in the urine of a male the following two aspects should be assessed:

1. Whether the relative signal(s) in the mass spectrometry screening analysis for 19-norandrosterone is less than that obtained from the positive control urine (nominal concentration 2 ng/ml) described above.
2. If the urine specific gravity is greater than 1.020, multiply the relative signal from the positive control urine by $(sg - 1)/0.02$ and determine whether the relative signal obtained from the sample is less.

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If either condition is met, do not produce an adverse report for possible sanction. Before issuing an adverse report, repeat the measurements on a further two aliquots of the sample. More than one metabolite may be detected, but the identification of only 19-norandrosterone is sufficient to report for possible sanction.

Note: If density (grams/ml) is used instead of specific gravity, then adjust the equation accordingly for the temperature used, e.g. $(\text{density}-0.998)/0.02$ at 20 °C. No temperature adjustment should be necessary when specific gravity is used.

GLOSSARY OF TERMS USED IN THIS DOCUMENT

DIAGNOSTIC IONS. Molecular ion or fragment ions whose presence and abundance are characteristic of the substance and thereby may assist in its identification. A second ion belonging to the same isotopic cluster may also be used as diagnostic only when the peculiarity of the atomic composition of the fragment so justifies (e.g. presence of Cl, Br, or other elements with abundant isotopic ions).

HRMS. High resolution mass spectrometry. HRMS is defined as mass spectrometry at a resolving power (10 % valley definition) in excess of 3,000.

LRMS. Low resolution mass spectrometry. LRMS is defined as mass spectrometry at a resolving power (10 % valley definition) lower than 3,000.

RELATIVE ABUNDANCE. The abundance of a particular ion relative to the most abundant ion monitored expressed as a percentage.

MAXIMUM PERMITTED DIFFERENCE IN RELATIVE ABUNDANCE. The maximum permitted difference between the relative abundance of a particular ion obtained from the sample and that obtained from the positive control urine. This may be expressed in ABSOLUTE or RELATIVE terms.

ABSOLUTE. Calculated by subtracting the stated percentage from the relative abundance obtained for the studied ion from either the sample or from the positive control urine, whichever is the greater. For example, if the relative abundance of an ion is larger in the positive control urine and appears as 50 % and the stated maximum permitted difference is 5 % (absolute), then a relative abundance of down to 45 % (50 - 5 %) for the sample would be acceptable.

RELATIVE. Calculated by multiplying the stated percentage by the relative abundance obtained for the studied ion from either the sample or from the positive control urine, whichever is the greater. For example, if the relative abundance of an ion is larger in the positive control urine and appears as 50 % and the stated maximum permitted difference is 20 % (relative), then a relative abundance of down to 40 % (50 - 50 x 20 %) for the sample would be acceptable.

SCAN. Acquisition of ions of a continuous range of m/z values.

SIGNAL TO NOISE. Magnitude of the instrument response to the analyte (signal) relative to the magnitude of the background (noise).

SIM. Selected ion monitoring. Acquisition of ions of one or more pre-determined discrete m/z values for specified dwell times.

MS/MS. Tandem mass spectrometry either in space (e.g. triple quadrupole MS) or in time (e.g. ion trap MS).

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