

Code of report : 10LANDGH15b.u
Client : Mrs. Ria van Landeghem
Date : October 15th, 2015

Commission :

On September 3rd, 2015 Mrs. Ria van Landeghem requested to Dr. Douwe de Boer to assist her in a case of an Adverse Analytical Finding (AAF) of OXANDROLONE, which was presumed to have been found in a urine sample of Mrs. Ria van Landeghem (indicated hereafter as “athlete”) and which was collected from her as being a athlete during a kind of out-of-competing testing just before the Olympic Games in Seoul 1988.

This report relates the opinion of Dr. de Boer (indicated hereafter as “expert”) in respect of the analytical report of the so-called presence of OXANDROLONE in in the A- and B sample of the athlete in particular. The information was supplied by Mrs. Van Landeghem herself and consisted of a copy of an analytical report and an oral description of what happened during that time.

1.1 Introduction

In principal OXANDROLONE is and always has been classified as a so-called anabolic steroid'. OXANDROLONE is typically indicated as adjunctive therapy to promote weight gain after weight loss following extensive surgery, chronic infections, or severe trauma, and in some patients who without definite pathophysiologic reasons fail to gain or to maintain normal weight, to offset the protein catabolism associated with prolonged administration of corticosteroids, and for the relief of the bone pain frequently accompanying osteoporosis¹.

It has been shown that OXANDROLONE is excreted in urine mainly as unchanged (estimated in the range of 35% of the dosage administered in a case study) and that epi-OXANDROLONE is an important metabolite (estimated in the range

¹ <http://www.drugs.com/pro/oxandrolone.html> (accessed October 14th, 2015).

of 8% of the dosage administered in case study)². Based on that case study, it can be stated that after oral OXANDROLONE administration mainly OXANDROLONE can be found in urine. A ratio between OXANDROLONE and epi-OXANDROLONE, that was found typically in urine samples, is approximately 4:1. Several additional metabolites are currently also known, but in 1988 this was the typical information available at that time.

1.2 What is known in respect with the AAF of OXANDROLONE in this specific case?

The following facts were collected:

- 1) Mrs. Van Landeghem was tested in the Olympic Laboratory of Seoul in 1988 just before the start of the Olympic Games.
- 2) The analytical certificate stated that the A- and B-sample were positive for OXANDROLONE and was signed by Dr. Park.
- 3) The analytical report shows chromatograms of the analysis of the A-sample, a positive sample and the B-sample with ion traces typically for O-trimethylsilyl derivative of OXANDROLONE and epi-OXANDROLONE as well as for the N-heptafluoro-butyryl-O-trimethylsilyl derivative of stanozolol, the internal standard used³. The applied method was published by Dr. Park *et al.* in 1990⁴.
- 4) From the data supplied by the athlete, it is unclear how the 3 samples were analysed, nor it is clear if negative urine was analysed in the same sequence also, nor was the sequence of analysis exactly known. Date and time of analysis of the reported sample analyses are as follows: the A-sample #16-01 [18 SEP 88 4:37 PM], oxandrolon standard [18 SEP 88 5:05 PM] and the B-sample #16-01 [20 SEP 88 4:57 PM].

² Massé R, Bi HG, Ayotte C, Dugal R. Studies on anabolic steroids. II - Gas chromatographic/mass spectrometric characterization of oxandrolone urinary metabolites in man. *Biomed Environ Mass Spectrom* 1989;18:429-38.

³ Analytical report describing the sample collection form [denoted "bijlage 2"] and the chromatograms of "the A-Sample #16-01" [denoted "bijlage 3"], "B-sample #16-01" [denoted "bijlage 3/2"] and "oxandrolone std (standard)" [denoted "bijlage 3/3"].

⁴ Chung BC, Choo HY, Kim TW, Eom KD, Kwon OS, Suh J, Yang J, Park J. Analysis of anabolic steroids using GC/MS with selected ion monitoring. *J Anal Toxicol* 1990;14:91-5.

- 5) From data published by Dr. Park⁵ himself, an example of chromatograms with ion traces typically for O-trimethylsilyl derivative of OXANDROLONE were available for a positive and a negative urine sample [see also Appendix B].
- 6) From the data supplied by the athlete, the quality of the copy of the analytical report was not perfect and not every number could be deciphered. The numbers in Appendix A are based on visual inspections.
- 7) From the data supplied by the athlete, the chromatograms were interpretable at a reasonable level.

1.3 What is known in respect with the state-of-the-art of anabolic steroid screening at the time of this specific case?

The following facts are known to the expert:

- 1) The expert performed at that time himself anabolic steroid screening in the same way as reported by Dr. Park and therefore, the expert is completely familiar with the procedure applied (see also *Curriculum Vitae* of the expert).
- 2) The analytical report was characteristic for that time and to a certain extent that report may be considered as the state-of-the-art of that time.
- 3) At that time and in contrast to the current situation no written procedures were available in respect with identification criteria and identification was based on common scientific sense. The first attempt to establish procedures was undertaken in 1989 by Prof. R. Massé⁶ and Prof. M. Donike⁷ on behalf of the International Olympic Committee (IOC), one year after the Olympic Games in Seoul in 1988.

⁵ Park J, Kwon O, Choo Hy, Suh J. Quantitation of oxandrolone (a synthetic anabolic steroid) in human urine by GC/MS. *Korean J Toxicol* 1988;4:117-29.

⁶ Prof. Massé was also the person who studied the analysis of oxandrolone [see footnote 2]

⁷ Prof. Donike was who studied and developed the analysis of stanozolol [anabolic steroid for which Ben Johnson was found positive], who at that time was the leading scientific expert within the Medical Commission of the IOC and who advised and helped the anti-doping laboratory of Seoul of Dr. Park.

- 4) The current procedure in respect with identification criteria is available at the website of the (World Anti-Doping Agency) WADA⁸, but already since the first version in 2003 (TD2003IDCR) it is evolving continuously. In that respect it must be stated for the identification of anabolic steroids, that the criteria has been constant for at least one decade and can be extrapolated retrospectively to the situation of 1988.

Conclusions

- 1) **The found concentration of what was assigned by Dr. Park in the A-sample as OXANDROLONE in the urine sample of Mrs. Van Landeghem was relatively low [see copy Report Seoul 1988 with original data; footnote 3]. No indications in the A-sample were found for and of the presence of the metabolite epi-OXANDROLONE.**
- 2) **The chromatogram of the ion traces as reported by Dr. Park in the A-sample was comparable to the positive urine sample as reported by Dr. Park in the same analytical report [see copy Report Seoul 1988 with original data; footnote 3].**
- 3) **According to the retrospective extrapolation of the current identification criteria to the situation of 1988 it is not unreasonable to assume that the ion traces as reported by Dr. Park in the A-sample were similar to those of the positive sample (based on relative retention time and ratios of ion trace abundances [see Appendix A]). Therefore, it is rationally to conclude that sufficient prove was collected to state that OXANDROLONE was present in the A-sample.**

⁸ WADA Technical Document – TD2015IDCR Minimum criteria for chromatographic-mass spectrometric confirmation of the identity of analytes for doping control purposes. https://wada-main-prod.s3.amazonaws.com/resources/files/wada_td2015idcr_minimum_criteria_chromato-mass_spectro_conf_en.pdf

- 4) **While the found concentration of what was assigned by Dr. Park in the A-sample as OXANDROLONE in the urine sample of Mrs. Van Landeghem was relatively low, in the B-sample it was even lower [see copy Report Seoul 1988 with original data; footnote 3]. Also no indications in the B-sample were found of the presence for and of the metabolite epi-OXANDROLONE.**

- 5) **According to the retrospective extrapolation of the current identification criteria to the situation of 1988 it is not unreasonable to assume that the ion traces as reported by Dr. Park in the B-sample were NOT very similar to those of the positive sample (based on relative retention time and missing ratios of ion trace abundances [see Appendix A]). Dr. Park himself published an example of chromatograms with ion traces typically for O-trimethylsilyl derivative of OXANDROLONE in a positive and a negative urine sample [see Appendix B]. Consequently, Dr. Park proved himself that the analysis of OXANDROLONE could be interfered by signals in a negative urine sample at very low levels of OXANDROLONE. Therefore, it is rationally to conclude that insufficient prove was collected to state that OXANDROLONE was present in the B-sample.**

- 6) **Finally and once more, it must be stated that the analytical report was characteristic for that time. However, despite a lack of well-defined identification criteria, this does not automatically justify the fact of insufficient prove. A more adequate identification could and should have been applied.**

Therefore and with up-to-date awareness by looking back in time, it must be regrettably acknowledged and admitted, that sometimes certain serious mistakes of insufficient prove were made in those days. Such serious mistakes justify retrospectively why the anti-doping regulations currently have implemented requirements with adequately defined identification criteria.

The fact of having defined identification criteria nowadays does not compensate for the impact that ancient serious mistakes of insufficient prove had on the career of athletes. After all, it implies that some athletes wrongly have been accused for applying doping and have suffered potentially severe consequences.

An appropriate action that at least should be taken towards wrongly accused athletes is to acknowledge and admit that the occurrence of serious mistakes of insufficient prove in the past cannot be excluded. This case is an example of one of those mistakes. As a scientific expert of that decade himself, the expert Dr. de Boer himself recognizes and acknowledges that situation.

While Dr. de Boer cannot ignore that the state-of-the-art of identification in sport doping control in the eighties was not perfect, Dr. de Boer even considers that nowadays it is still not perfect. Therefore, also today athletes should never accept automatically a positive finding in sport doping control and whenever possible and reasonable should go into appeal.

Samenvatting en vrije vertaling “Conclusions”

- 1) De waargenomen concentraties van OXANDROLON, zoals Dr. Park suggereert dat voor zover deze aanwezig zouden zijn in het A- en B-staal, waren laag tot zeer laag.
- 2) Terwijl voor OXANDROLON in het A-staal wel redelijkerwijs bewijs wordt aangevoerd door Dr. Park, wordt dat voor het B-staal niet redelijkerwijs aangevoerd.
- 3) De bewijsvoering is verder onvolledig qua informatie met betrekking tot met name negatieve kwaliteitsstalen en qua informatie met betrekking tot de volgorde van alle eventueel geanalyseerde stalen.
- 4) De manier van rapporteren is karakteristiek voor de tijdsgeest van de analyses, wat echter geen excuus is de onvolledige bewijsvoering. Dr. Park zelf heeft in wetenschappelijke literatuur laten zien aan de hand van negatieve kwaliteitsstalen dat de analyse geïnterfereerd kan worden door een onbekende achtergrondsfactor, wat indirect aangeeft dat een uitgebreidere bewijsvoering op z'n plaats zou zijn geweest.
- 5) Ongelukkigerwijs en achteraf betreurigswaardig is het niet uit te sluiten dat in het verleden een dergelijk manier van werken soms vaker plaats heeft gevonden. Dit is dan ook één van de redenen waarom de WADA bijv. het technisch document aangaande identificatiecriteria heeft ontwikkeld. Zelfs heden ten dage moet dit aspect kritisch vervolgd en geverifieerd worden, want het is nooit altijd perfect.

Appendix A

Identification evaluation (the numbers of RT and abundance are based on visual inspections)

ION	ISTD ⁹	OXANDROLONE		STANDARD		
		RT	RRT ⁹	ABUNDANCE	RELATIVE ABUNDANCE	
M/Z	RT ⁹	RT			maximum tolerance ¹⁰	
	(MIN)	(MIN)				
581	8.14	-	-	not relevant	not relevant	not relevant
378	-	-	-	11341	2.4	< 7.4
363	-	-	-	48551	10.3	5.3-15.3
321	-	-	-	37690	8.0	3.0-13.0
308	-	-	-	87349	18.5	13.5-23.5
143	-	7.37	0.905	473172	100.0	-

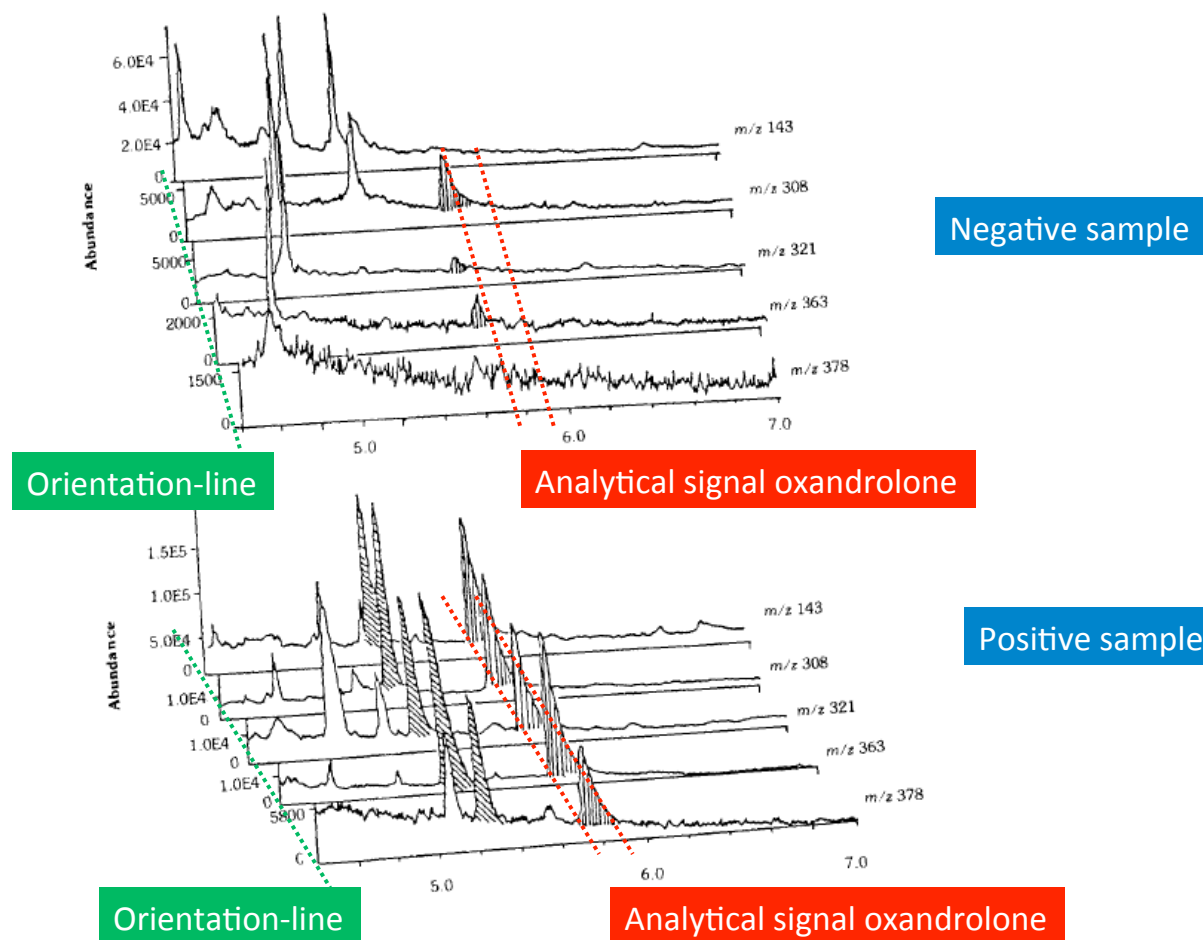
ION	ISTD	OXANDROLONE		A-SAMPLE		
		RT	RRT	ABUNDANCE	RELATIVE ABUNDANCE	
M/Z	RT	RT			maximum tolerance	
	(MIN)	(MIN)				
581	8.17	-	-	not relevant	not relevant	not relevant
378	-	-	-	128	2.3	< 7.4
363	-	-	-	561	9.9	5.3-15.3
321	-	-	-	464	8.2	3.0-13.0
308	-	-	-	1017	17.9	13.5-23.5
143	-	7.37	0.902	5677	100.0	-

ION	ISTD	OXANDROLONE		B-SAMPLE		
		RT	RRT	ABUNDANCE	RELATIVE ABUNDANCE	
M/Z	RT	RT			maximum tolerance	
	(MIN)	(MIN)				
581	8.11	-	-	not relevant	not relevant	not relevant
378	-	-	-	?	?	< 7.4
363	-	-	-	?	?	5.3-15.3
321	-	-	-	?	?	3.0-13.0
308	-	-	-	?	?	13.5-23.5
143	-	7.42	0.915	?	100.0	-

⁹ ISTD = Internal Standard, which is stanozolol; RT = Retention Time; RRT = Relative Retention Time

¹⁰ based on TD2015IDCR of the WADA (see footnote 8)

Appendix B



Orientation-line

Analytical signal oxandrolone

Negative sample

Positive sample

Orientation-line

Analytical signal oxandrolone

Curriculum Vitae of Dr Douwe de Boer

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Academic degrees and fields of study

1992 Ph. D, Cum laude, Pharmacy, University of Utrecht, The Netherlands
1986 Graduation in Biochemistry, University of Groningen, The Netherlands

Present position and Institution

2012-today Head of Cluster "Protein Chemistry", Central Diagnostic Laboratory, Maastricht University Medical Centre, The Netherlands

Previous positions and Institutions

2009-2012 Biochemist, Laboratory of Clinical Chemistry, Maastricht University Medical Centre, The Netherlands
2004-2012 Senior Investigator, Laboratory of Clinical Chemistry, Maastricht University Medical Centre, The Netherlands
2003-2004 Scientific and Technical Director of the Doping department, Laboratório de Análises e Dopagem, Instituto do Desporto Portugal, Lisbon, Portugal
1998-2003 Technical Director of the Doping department, Laboratório de Análises e Dopagem, Instituto do Desporto Portugal, Lisbon, Portugal
1992-1998 Assistant Professor and Senior Investigator at the Department of Human Toxicology, Faculty of Pharmacy, University of Utrecht, The Netherlands
1991-1998 Technical Director of the Netherlands Institute for Drugs and Doping Research, Faculty of Pharmacy, University of Utrecht, The Netherlands
1987-1992 Junior Investigator at the Netherlands Institute for Drugs and Doping Research, University of Utrecht, Faculty of Pharmacy, The Netherlands
1986-1987 Junior Investigator at the Netherlands Doping Research Center, Catholic Radboud University, Nijmegen, The Netherlands

Prizes

1997 The Manfred Donike Award for Scientific Excellence in Doping Control

Professional memberships

Koninklijke Nederlandse Chemische Vereniging (KNCV)
Nederlandse Vereniging voor Massaspectrometrie (NVMS)
Nederlandse Vereniging voor Klinische Chemie en Laboratoriumgeneeskunde (NVKC)
The International Association of Forensic Toxicologists (TIAFT)
European Academy of Allergy and Clinical Immunology (EAACI)