

Code of report : 02RAMOS10.u
Client : Mr. Andy Ramos
Date : September 3rd, 2010

Commission :

On August 31th, 2010 Mr. Andy Ramos requested to Dr Douwe de Boer to assist him in a case of an Adverse Analytical Finding of CLENBUTEROL, which was found in a urine sample of the cyclist Mr. Alberto Contador.

This report relates the opinion of Dr de Boer in respect of the origin of the unexpected presence of **CLENBUTEROL** in biological samples in general and in a urine sample of the cyclist in particular.

1.1 Introduction

In principal CLENBUTEROL is classified as a so-called 'beta-2 agonist' and consequently the main therapeutic use of CLENBUTEROL is in the treatment of asthma to relax the smooth muscle in the airways. It is produced for human use generally in tablet form and as such it is accessible worldwide in a limited number of countries. Because alternative 'beta-2 agonists' are available¹ without much less serious secondary effects, CLENBUTEROL is currently being replaced by more save 'beta-2 agonists' in a significant number of countries. In most of the Western European countries it is therefore even an obsolete human medicine.

Non-therapeutic use of CLENBUTEROL in humans includes its anecdotal claimed effects as an anabolic compound. Probably this claim is based on its apparent effective and illegal application in cattle as a growth agent. Unfortunately, this illegal use, if followed by human consumption of CLENBUTEROL containing edible parts of cattle, may also result in the unintentional administration of CLENBUTEROL to humans.

1.2 True *versus* anecdotal use of CLENBUTEROL

While the main true and therapeutic reason to apply CLENBUTEROL in humans is to relax on relatively long term the smooth muscle in the airways i.e. long acting bronchodilator², CLENBUTEROL also has other kinds of effects. Short-term effects of CLENBUTEROL for example are similar to stimulant drugs like amphetamine or ephedrine (i.e. increases heart rate, temperature, perspiration and blood pressure).

Anecdotal³ claimed long-term effects in humans are those of overall anabolic effects i.e. to promote the growth of skeletal muscle ('anabolic effects') and to reduce body fat ('catabolic effects'). It is mainly because of those overall anabolic effects that CLENBUTEROL is banned under the Olympic Movement's *World Anti-Doping Code*⁴ *Prohibited Classes of Substances and Prohibited Methods*⁵.

CLENBUTEROL is also used as a true bronchodilator in veterinary medicine. Additionally, it also has tocolytic⁶ effects. The most common veterinarian preparation is a syrup. However, the main reason why CLENBUTEROL is being applied frequently in cattle, is because its growth promoting effects. In order to achieve growth promoting effects, it must however be applied at higher dosages, namely ten to hundred times the clinically active dose as a bronchodilator⁷.

In principal the use of CLENBUTEROL as a growth promoting agent in cattle is prohibited in most industrial countries. Although its abuse is considered as malpractice, the profits are significant if abused in cattle breeding. When effective counter-measurements are lacking in a country, i.e. testing of cattle, the gate-way to malpractice is basically wide open. Perhaps the main problem of this kind of malpractice is the possibility of creating intoxications caused by human consumption of CLENBUTEROL containing edible parts of cattle.

¹ For example SALBUTAMOL, SALMETEROL and, TERBUTALINE, amongst others.

² For reference, the half-life of the bronchodilator SALBUTAMOL [also known as ALBUTEROL] is 3.5 - 6 hours, while that of CLENBUTEROL is 25 - 39 hours. Source: Clin Toxicol 2001; 39: 339-344.

³ No human studies are available on whether clenbuterol has true anabolic effects. Body builders may utilise clenbuterol as a 'fat burner' to 'define' muscles (i.e. for its 'catabolic effect'). Clenbuterol has the ability to slightly increase the body's core temperature and metabolism, which users believe assists in the burning of calories. The body will fight this effect however, so clenbuterol may only have an effect over a limited time period. Source: NDARC Fact Sheet Clenbuterol (accessed May 24th, 2010) [http://www.med.unsw.edu.au/NDARCWeb.nsf/resources/NDARCFact_Drugs2/\\$file/CLENBUTEROL+NDARC+FACT+SHEET.pdf](http://www.med.unsw.edu.au/NDARCWeb.nsf/resources/NDARCFact_Drugs2/$file/CLENBUTEROL+NDARC+FACT+SHEET.pdf)

⁴ WADA Anti-Doping Code 2009, available on WADA website www.wada-ama.org

⁵ WADA The 2010 Prohibited List, International Standard, available on WADA website www.wada-ama.org

⁶ Tocolytics compounds are anti-contraction medications or labor repressants

⁷ Source: Toxicology 2003; 187: 91-99.

1.3 CLENBUTEROL intoxications

Various reports in scientific literature and also in common journals from public news sources have indicated the occurrence of CLENBUTEROL intoxications. Merely, a small compilation has been selected random wisely hereafter to illustrate the problem.

In 1990 there were outbreaks of clenbuterol-related poisonings in France and Spain. In France at least 22 persons were affected, while in Spain 135 cases were reported. Veal liver was likewise the source of intoxication⁸.

In 1992 113 cases occurred in association once more mainly with the ingestion of veal liver⁸. Symptoms were nervousness, tachycardia, muscle tremors, myalgia and headache. CLENBUTEROL was detected in 47 urine samples in concentrations ranging from 11 to 486 ng/mL. In one family, the suspected source was meat.

In May 1997 15 persons were found to be intoxicated after the consumption of CLENBUTEROL - containing meat (1140 - 1480 ng/g meat)⁹. Clinical symptoms were distal tremors, palpitations, headache, tachipnoea-dyspnoea as well as moderate hyperglycaemia, hypokalemia and leucocytosis. Observed mean concentrations in urine were initially 18 ng/ mL and after 48 h 13 ng/mL.

In 2001 a report was issued of an intoxication after an accidental intake of CLENBUTEROL – containing powder¹⁰. Observed symptoms were tachycardia, hypokalemia and hypophosphatemia. No information was available in respect to urine.

In the period of April 1998 and April 2002 a total number of 50 persons were associated in Portugal with CLENBUTEROL intoxication (1400 ng/g liver and 1200 ng/g meat)¹¹. Symptoms described were gross tremors as gross tremors of the extremities, tachycardia, nausea, headaches and dizziness. No information was available in respect to urine.

In the period of January and February 2005 a total number of 34 persons were associated in the United States with CLENBUTEROL intoxication of which 13 persons

⁸ Source: Public Health Reports May-June 1996: 110; 338-342.

⁹ Source: Toxicol Lett 2000; 114: 47- 53.

¹⁰ Source: Clin Toxicol 2001: 39; 339-344.

¹¹ Source: Food Addit Contam 2005; 22: 563-566.

were confirmed exposures¹². Symptoms were tachycardia, hypotension, nausea, chest pain, palpitations, dyspnea, tremor as well as hyperglycaemia, hypokalemia and hyperlactaemia. The cause was the intake of CLENBUTEROL- containing heroin. Observed concentrations in urine were 9.4 - 12526 ng/ mL.

In September 2006 over 330 people in Shanghai were reported to have been poisoned by eating pork contaminated by CLENBUTEROL that had been fed to the animals to keep their meat lean^{13,14}. No scientific data were available.

In February 2009, at least 70 people in one Chinese province (Guangdong) suffered food poisoning after eating pig organs believed to contain CLENBUTEROL residue¹⁴. The victims complained of stomach-aches and diarrhea after eating pig organs bought in local markets^{15,16}. No scientific data were available.

1.4 Accidental use of CLENBUTEROL and observed concentrations in urine

Based on the preceding indicated information intoxications with obvious symptoms are associated with CLENBUTEROL if concentrations in urine are greater than 9 ng/mL¹⁷. It is also evident that persons may have concentration of CLENBUTEROL in urine lower than 9 ng/mL and feel no symptoms at all. Therefore, the accidental intake of low amounts of CLENBUTEROL may happen unnoticeable if the concentration of CLENBUTEROL is relatively low.

Although CLENBUTEROL is a so-called non-threshold compound, i.e. substance for which no quantitative threshold is specifically identified in the prohibited list, the WADA has defined a specific Minimum Required Performance Level (MRPL) for CLENBUTEROL at which all laboratories shall operate. The WADA in that respect

¹² Source: Toxicology 2008; 52: 548-552.

¹³ "Pigs fed on bodybuilder steroids cause food poisoning in Shanghai" (in English). *AFP*. September 19th, 2006. <http://www.breitbart.com/news/2006/09/19/060919065258.qtzm4eom.html>. Accessed on May 24th, 2010.

¹⁴ MoniQA Fact Sheet No 3 Clenbuterol, March 2009. MoniQA is an EU-funded project connecting global players in the field of food safety and quality, addresses the melamine crisis and other emerging issues in food safety

¹⁵ "China: 70 ill from tainted pig organs". *CNN.com/asia*. February 23rd, 2009. <http://www.cnn.com/2009/WORLD/asiapcf/02/22/china.poisonings/index.html>. Accessed on May 29th, 2010.

¹⁶ <http://bbs.chinadaily.com.cn/viewthread.php?tid=628213> Accessed on May 29th, 2010.

states¹⁸: *‘In order to ensure that all WADA-accredited Laboratories can report the presence of prohibited substances, their metabolite(s) or their marker(s) in a uniform way, a minimum routine detection capability for testing methods has been established. It is recognized that some laboratories will be able to identify a wider range or lower concentrations of prohibited substances than other laboratories. While such individual capabilities are encouraged in order to improve the overall system, it is also recognized that there are Minimum Required Performance Levels (MRPL) at which all laboratories shall operate. The MRPL is an analytical parameter of technical performance with which the laboratories shall comply when testing for the presence of a particular prohibited substance, its metabolite(s) or marker(s). The MRPL is not a threshold, nor is it a limit of detection (LOD) or a limit of quantification (LOQ)’. This specific MRPL for CLENBUTEROL is 2 ng/mL¹⁹.*

Therefore, Adverse Analytical Findings for CLENBUTEROL may result from concentrations below the MRPL even if such concentrations are far below the MPRL. The reason of course is that CLENBUTEROL may be abused especially during training periods and if testing is performed after such training periods, that CLENBUTEROL abuse should still be achievable. It is also obvious that accidental intake of low amounts of CLENBUTEROL therefore, may result in an Adverse Analytical Finding.

1.5 Origin of the unexpected presence of CLENBUTEROL

In principal the origin of CLENBUTEROL cannot be determined just from an observed CLENBUTEROL concentration in urine. Also no other scientific data are available, which could facilitate to determine this. Therefore, any unexpected presence of CLENBUTEROL offers the doping authorities legally to accuse an athlete in any case, while the athlete has restricted possibilities to defend himself legally. While it may be very easy said than done, only if the athlete proves beyond all doubt otherwise, a defence may be succesful.

¹⁷ The value of 9 ng/mL is not based on an epidemiological evaluation, but is merely a rough distinction between having obvious symptoms *versus* no obvious symptoms in the small compilation of literature, that has been selected random wisely.

¹⁸ WADA Anti-Doping Code 2009, available on WADA website www.wada-ama.org

¹⁹ TD2009MRPL, WADA’s Technical Document for minimum required performance levels for detection of prohibited substances, version 2.0, available on WADA website www.wada-ama.org

1.6 Should any concentration be an Adverse Analytical Finding?

Under normal conditions it is assumed that where relevant that an Adverse Analytical Finding is coupled to a `fair and reasonable` concentration. Because of that, for the non-threshold compounds stimulants, narcotics and β -blockers prohibited in-competition only, it is not recommended that WADA-accredited laboratories report below 10% (1/10th) of the MRPL²⁰. Also because of that a concentration of the threshold compound 19-norandrosterone lower than 2 ng/mL is not sanctioned²¹ nor are laboratories to report concentrations of glucocorticosteroids below the MRPL²². In this context a `fair and reasonable` concentration signifies a concentration at which a sanction is justified.

Because CLENBUTEROL is no stimulant, narcotic or β -blocker, can never be of endogenous origin and is forbidden independent of its way of administration, the preceding argumentation officially does not apply. However, if normal conditions justifies to a `fair and reasonable` concentration under certain circumstances for specific compounds, it also implies that abnormal conditions may exist that does not justify a sanction for some other compounds. If a WADA-accredited laboratory applies an extreme lower LOD (LLOD) for CLENBUTEROL, the probability that an accidental intake of low amounts of CLENBUTEROL results in an Adverse Analytical Finding increases dramatically. Therefore, it can be questioned if any concentration should be considered an Adverse Analytical Finding and if abnormal conditions are not met in this specific case of Adverse Analytical Finding of CLENBUTEROL. After considering all circumstances it would not be unlogical also to consider a `fair and reasonable` concentration for CLENBUTEROL and perhaps WADA-accredited laboratories should not report CLENBUTEROL below 10% (1/10th) of its MRPL of 2 ng/mL, i.e. 0.2 ng/mL.

²⁰ TD2009MRPL, WADA's Technical Document for minimum required performance levels for detection of prohibited substances, version 2.0, available on WADA website www.wada-ama.org

²¹ The reason is that it cannot be excluded reasonably that that 19-norandrosterone concentrations lower than 2 ng/mL in urine are of endogenous origin, while it can be excluded reasonably that those concentrations are caused by the consumption of contaminated meat.

²² The reason is that the origin of glucocorticosteroids cannot be established just based on a concentration in urine and some ways of administrations are not prohibited.

1.7 Is the CLENBUTEROL concentration in Mr. Contador's urine sample an Adverse Analytical Finding?

The following facts were collected:

- 1) Mr. Contador took part in the Competition "Tour de France 2010", which took place in the period of July 3rd to July 25th.
- 2) Mr. Contador underwent sport drug testing at several days, amongst others at July 19th, 20th, 21st, 22nd, 23rd and 24th, all at which a urine sample was taken at the end of the afternoon or the beginning of the evening²³.
- 3) In the period of July 19th to 20th no CLENBUTEROL was found above the Limit of Identification (LOI) nor above the LLOD of the respective WADA –accredited laboratory²³.
- 4) The concentration of CLENBUTEROL found in the urine sample taken at July 21st was very low; the applied method was not quantitative, but it may be estimated that the concentration was in the range of 50 pg/mL, which is equivalent 0.05 ng/mL^{24,25}.
- 5) The concentration of CLENBUTEROL found in the urine sample taken at July 22nd was even lower; the applied method was not quantitative, but it may be estimated that the concentration was in the range of 20 pg/mL, which is equivalent 0.02 ng/mL^{23,25}.
- 6) In the period of July 23rd to 24th no CLENBUTEROL was found above the Limit of Identification (LOI) of the respective WADA –accredited laboratory, although traces were observed above the LLOD²³.
- 7) The half-life of CLENBUTEROL is 25 - 39 hours (mean half-life 32 hours).
Source: Clin Toxicol 2001; 39: 339-344.

Based on the indicated facts it must be concluded that the CLENBUTEROL has been administered somewhere in period after the urine testing on July 20th. If it is

²³ Information orally received by Dr Douwe de Boer from Mr. Andy Ramos

²⁴ Laboratory Document Package of Sample A 2512045; apparently, the WADA-accredited laboratory of Cologne applies an extreme LLOD for CLENBUTEROL.

²⁵ Assuming that a `fair and reasonable` concentration for CLENBUTEROL for which no adverse finding should be reported by WADA laboratories could be fixed at 0.2 ng/mL, the found concentration is far below the limit of 0.2 ng/mL.

assumed that CLENBUTEROL has been administered directly after the testing on July 20th, it can be calculated what the maximum concentration of CLENBUTEROL might have been in the urine directly after administration. With a very simple pharmacokinetic model²⁶ some rough calculations can be performed. Assuming a half-life of 24 hours that maximum concentration might have been 0.1 ng/mL (= 100 pg/mL) [Appendix 1]. Assuming a half-life of 32 hours it might have been approximately 0.09 ng/mL (=90 pg/mL) [Appendix 2]. Assuming a half-life of 40 hours it might have been approximately 0.08 ng/mL (=80 pg/mL) [Appendix 3]. These maximum concentrations are far below the CLENBUTEROL concentration of 9 ng/mL, which may be the limit at which symptoms might be felt. Consequently, if an accidental intake of low amounts of CLENBUTEROL occurred directly after the testing on July 20th, it must have been happened unnoticeable for Mr. Contador. Moreover, based on what is known in literature it is extremely unlikely that such maximum concentrations resulted in any pharmacological effects. It should also be noted that the amounts of CLENBUTEROL found at the days after adequately fit in the applied pharmacokinetic model and as such it validates the application of that model.

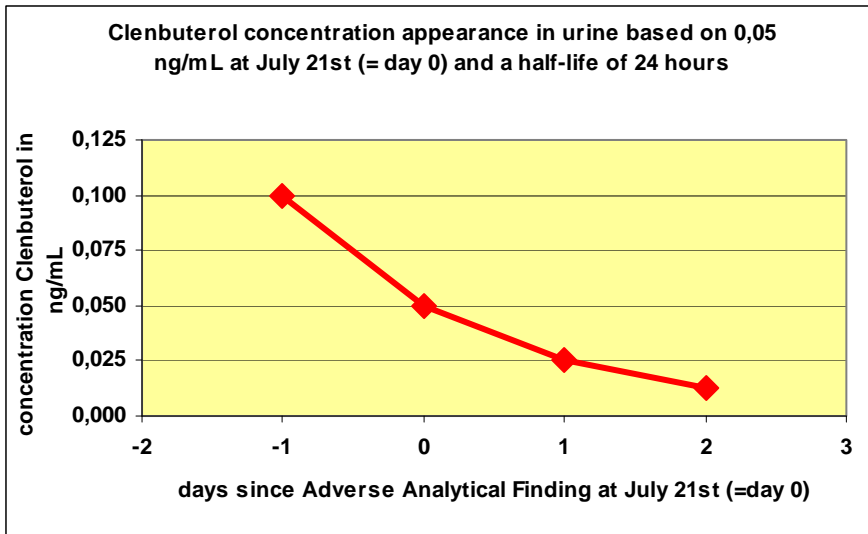
Being concluded this and after consulting Mr. Ramos, it became clear that Mr. Contador consumed some meat from Spain directly after the testing on July 20th. Knowing the history of CLENBUTEROL intoxications in Spain and knowing the fact nowadays CLENBUTEROL can be detected in extremely low amounts, it is obvious that in this particular case the scenario of an accidental intake of CLENBUTEROL by consumption of meat is extremely likely. This case also demonstrates that WADA-accredited laboratories should not report CLENBUTEROL below 10% (1/10th) of its MRPL of 2 ng/mL, i.e. 0.2 ng/mL. as being a 'fair and reasonable' concentration for an Adverse Analytical Finding of CLENBUTEROL

²⁶ The simplest model is that after each period of the half-life the concentration decreases with a factor of 2. In this model it is assumed that the concentration is not influenced by fluctuating intake of fluid or fluctuating clearance in the urine. In principle more sophisticated models are not justified because of the limited amount of information and analytical uncertainties

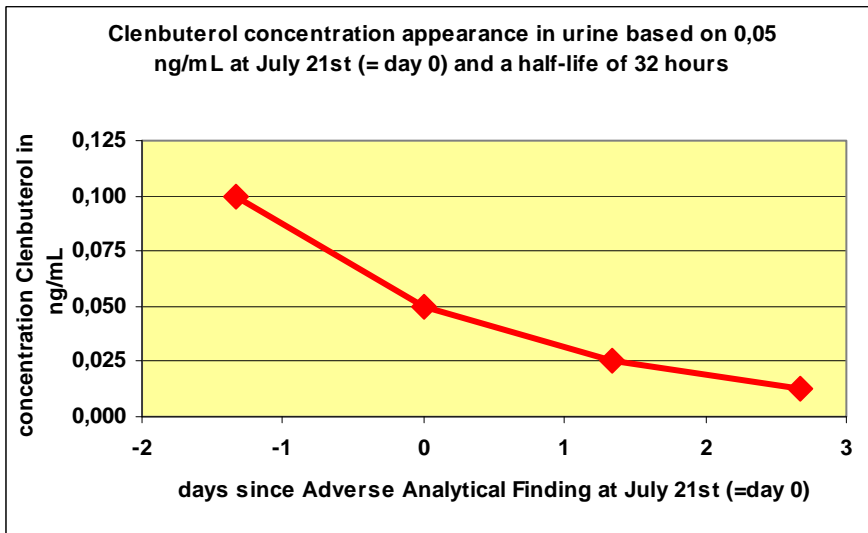
Conclusions

- 1) The found concentration of CLENBUTEROL in the urine sample of Mr. Contador is extremely low.
- 2) It is extremely likely and would be only fair to regard the scenario of accidental intake low amounts of CLENBUTEROL by meat consumption.
- 3) WADA-accredited laboratories should not report CLENBUTEROL below 10% (1/10th) of its MRPL of 2 ng/mL, i.e. 0.2 ng/mL (= 200 pg/mL) as being a `fair and reasonable` concentration for an Adverse Analytical Finding of CLENBUTEROL.

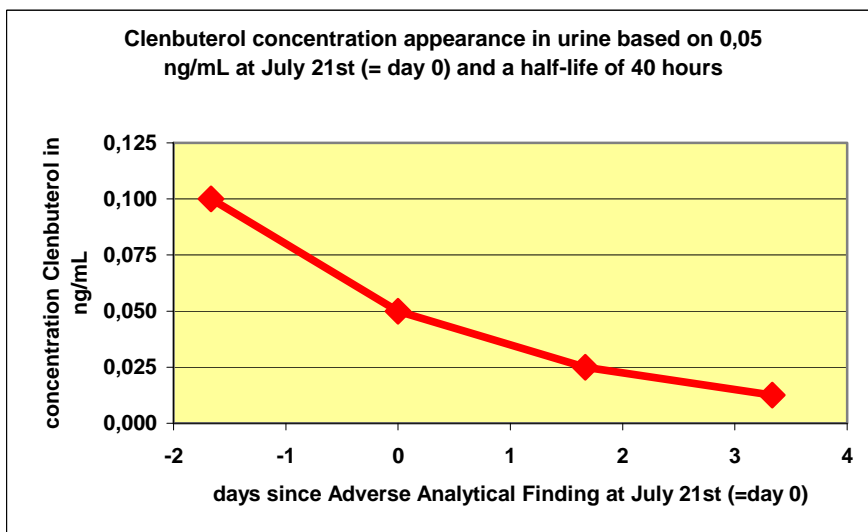
Appendix 1



Appendix 2



Appendix 3



Curriculum Vitae of Dr Douwe de Boer

Name Douwe de Boer
Place of birth Groningen, The Netherlands
Date of birth 01/01/1961
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Institutional address

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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

2009-today Biochemist, Laboratory of Clinical Chemistry, Academic Hospital
Maastricht, The Netherlands
2004-today Senior Investigator, Laboratory of Clinical Chemistry, Academic Hospital
Maastricht, The Netherlands

Previous positions and Institutions

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)