IN THE COURT OF ARBITRATION FOR SPORT

FLOYD LANDIS	)	
	)	
Appellant,	)	
	)	
V.	)	**CAS 2007/A/1394**
	)	
UNITED STATES ANTI-DOPING AGENCY	)	
	)	
Respondent.	)	
	)	

WITNESS STATEMENT OF J. THOMAS BRENNA, Ph. D.

My name is J. Thomas Brenna. My address is 32 Deerhaven Drive, Ithaca, New York, USA.

Background and Qualifications

My curriculum vitae has already been submitted in the prior case. To summarize my background briefly, I am professor in the Division of Nutritional Science at Cornell University in Ithaca New York, and have joint appointments in the Cornell Graduate Fields of Chemistry and Chemical Biology, of Geological Sciences, and of Food Science and Technology. Graduate field membership qualifies me to sponsor graduate students for the PhD in these fields. I hold the PhD in Chemistry from Cornell, specializing in mass spectrometry, and have been an active researcher in a variety of different areas of mass spectrometry since 1980. Since joining the Cornell faculty in 1989, I have specialized in high precision isotope ratio mass spectrometry, especially instrumentation and methods development, and applications to biomedical sciences. I

also have an active program in molecular mass spectrometry directed primarily but not exclusively toward lipids. My research funding over the years has come principally from competitive grant programs at the National Institutes of Health, and from private industry. I currently hold a major grant from USADA. Our research is on metabolism of fats and, for USADA, on development of methods for extending the use of isotope ratio mass spectrometry in antidoping science by making methods more rapid.

Materials Reviewed and Summary of Conclusions

I attended the prior hearing in Malibu, California in May 2007, and gave testimony before that Panel. (My testimony is in the transcript of the prior hearing from pages 230-388 and 1920-1979.) In addition, I have reviewed the documentation packages and relevant sections of exhibits in my field of expertise, and reviewed the briefs filed by the parties for the prior hearing as well as the upcoming hearing. I also attended the reprocessing of the electronic data files in this case from May 4-5 2007.

Based on my knowledge of the field and my expertise, after review of the relevant data, I conclude that the difference in carbon isotope ratio between the 5adiol and the pdiol endogenous reference compound supports the AAF. In particular, I find that:

(1) the peaks are properly and unambiguously identified;

(2) the IRMS instrument was in good working order; and

(3) the LNDD technicians followed the relevant standard operating procedures (SOPs) when analyzing Appellant's samples.

My conclusions are set forth below and, where relevant, I also discuss instances where I disagree with arguments made by Appellant and his experts. In order to provide context for my

conclusions, I will first discuss in general how the science underlying these conclusions works, and how data obtained in these analyses is properly interpreted.

Use of IRMS to detect testosterone

There are two stable forms of carbon in nature. On earth, ^{12}C constitutes 98.9% of carbon, with the balance of 1.1% being ^{13}C. Many decades ago, mass spectrometers were developed that could measure the isotope ratio of carbon with great precision, so that subtle natural variability could be detected. The earliest measurements of natural isotope variability were done in carbon and showed that carbonates had a high isotope ratio relative to living creatures. As the decades passed, many physico-chemical mechanisms that induce variation in natural carbon isotope ratio were discovered. The one most relevant to testosterone testing involves the two different photosynthetic biochemical pathways used by plants. Soy and most human food plants use the "C3" pathway, which give the plant a slightly lower ^{13}C content than the "C4" pathway, used by corn and sugar cane. For various reasons of convenience, high precision carbon isotope ratios are expressed on the delta (permil) scale. C4 plants have an isotope ratio around -15 permil, while C3 plants have an isotope ratio around -32 permil. The mix of foods eaten by a person determines his body's isotope ratio, and it will usually be between these extremes. Most of the chemicals in his body will also have values between these extremes, including the steroids. Since all the steroids in the body derive from one steroid, cholesterol, all the steroids will have similar isotope ratios, often with small, but constant, differences.

Synthetic testosterone is usually derived from C3 plant sterols after some chemical alteration. Because the starting material is from a C3 plant, it has a low delta permil value.

Synthetic testosterone enters the body by doping and dilutes the endogenous testosterone pool, giving all body testosterone a lower isotope ratio than it would have relative to the other steroids had doping not taken place. Measurement of the testosterone isotope ratio, either directly, or more commonly via a metabolite, and comparison with an endogenous reference compound, will reveal a larger than normal difference in carbon isotope ratio, and indicate doping.

Science of IRMS, and GCC-IRMS

The science of high precision carbon isotope ratio measurements by IRMS dates to 1939 when the first carbon isotope ratio measurements showed natural variability. The configuration of modern instruments is usually dated to 1950 for the mass spectrometer, and 1978 of the coupling of GC to IRMS. IRMS instruments are highly specialized for precise measurement of isotope ratio of a very small number of analysis gases. For carbon, only CO2 can be admitted to the IRMS for analysis. To measure steroid isotope ratios, compounds must first be chemically converted to CO2 by combustion. To obtain isotope ratios of individual compounds that originate in mixtures, it is common to perform a GC (gas chromatography) separation, then combust and dry the steroids prior to admission to the IRMS.

The GC is a device that accepts injection of a mixture of steroids, where they emerge one by one for detection by many different means. In steroid testing, either a MS (mass spectrometer) or a combustion interface with an IRMS is used, each with a different analytical purpose. The MS used in GC/MS accepts the steroids as intact molecules, breaks them into pieces, and analyzes the mass of the pieces. The masses of the pieces are highly characteristic of molecular structure, and thus the molecular mass spectrum can be used to identify the chemical structure. An analyst is then able to examine the mass spectra, either by manual inspection or

automated analysis, and confirm the chemical identity of a peak. In the present case, the relevant peaks are the target steroids.

The IRMS instrument is a physically distinct machine from the GC/MS, and is designed to measure a different property of the steroids. The IRMS, in accepting only CO2 for isotopic analysis, measures the carbon isotope ratio of the steroid that would have been identified in GC/MS. In this case, the IRMS is used to measure the carbon isotope ratio at high precision, sufficient to detect variability due to processes that occur in nature. As discussed above, chemical compounds with a common source have similar carbon isotope ratios. Steroids within the human body are all synthesized from cholesterol within the body, and therefore have very similar carbon isotope ratios. If testosterone is ingested from a synthetic source, that testosterone has a lower carbon isotope ratio than testosterone produced metabolically. The basis of the carbon isotope ratio test for testosterone is to measure the carbon isotope ratio of an endogenous reference compound to establish the natural isotope ratio for the athlete, and then compare the carbon isotope ratio of a testosterone metabolite. If the two carbon isotope ratios do not match within acceptable limits, an AAF is made. In this case, the endogenous reference compound is pdiol, and the testosterone metabolite is 5adiol. In Appellant's sample, the carbon isotope difference between these two was much greater than normal, leading the Laboratory to declare an AAF.

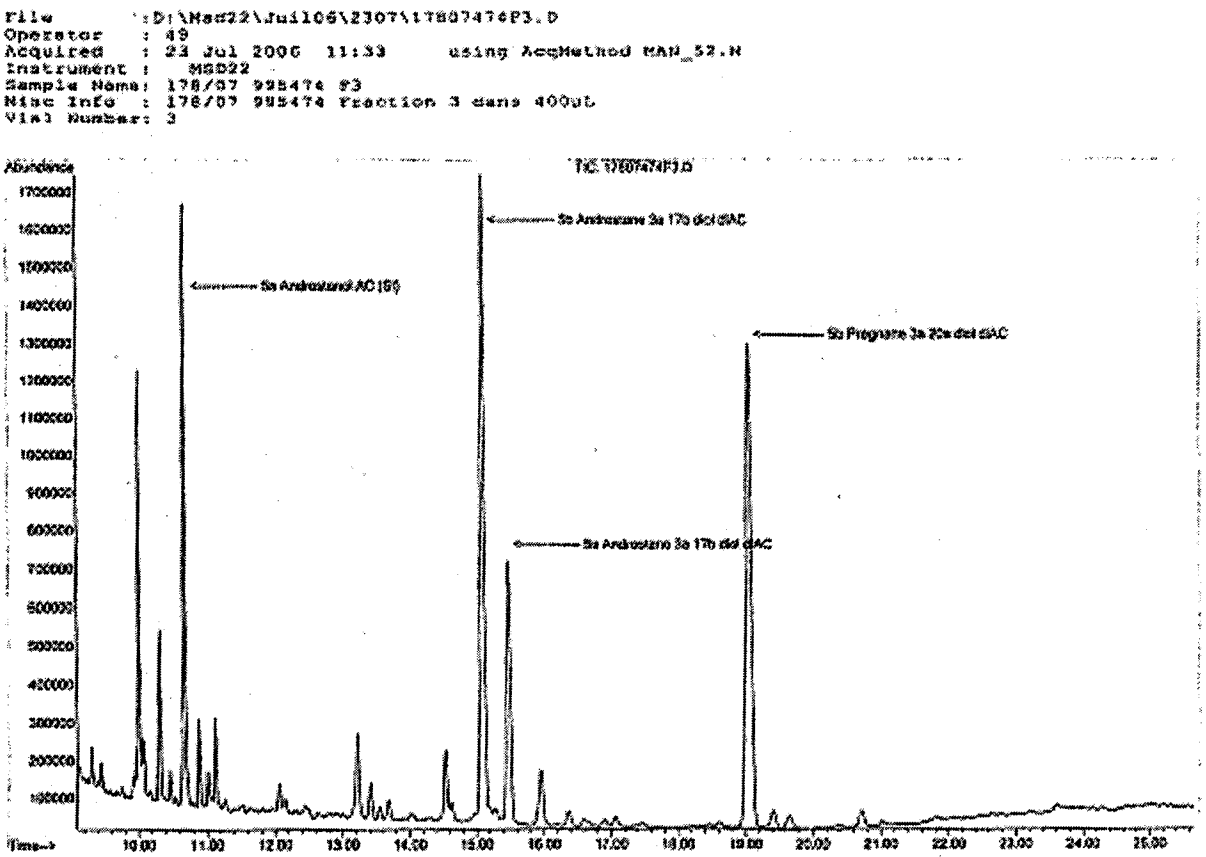
Chromatography Basics

Both GC/MS and GCC-IRMS require the use of data processing tools to extract information. A series of peaks is obtained from both instruments according to chemically distinct principles that, in this case, result in similar chromatograms. Before the widespread use

of computers to control instruments, GC and GC/MS data was collected photographically, or by a pen dragged across paper. In cases where the chemical amount was the desired measurement, scissors were used to physically cut out peaks from the paper, whereupon they were weighed on a balance. This manual method of peak integration was sufficiently accurate for many purposes. As computers were developed for data acquisition and analysis, tasks such as these were automated, and the need to manually process data has been driven to higher levels of output.

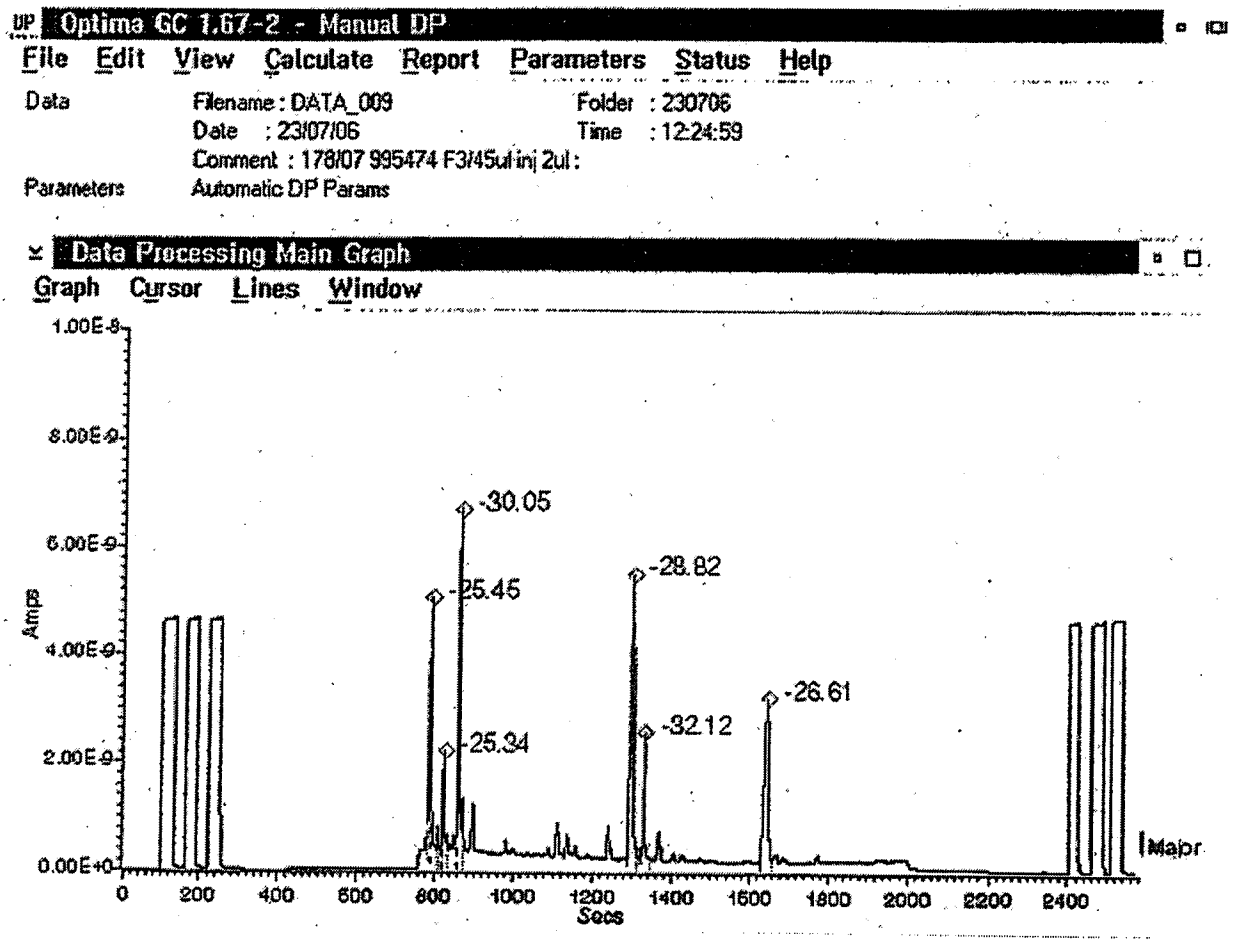
An example of a chromatogram produced using GC/MS is below (from Exhibit 24, USADA0171). Note that the internal standard and metabolites of interest are labeled.

Figure 1



An example of a chromatogram produced using GCC-IRMS is below (from Exhibit 24, USADA0173). (Note that the delta values of the peaks (which are the numbers to the right of each identified peak) are not related to peak size.)

Figure 2



Fundamentals of Retention Time

Retention time is the time required for an analyte injected into a GC to elute from the GC column and into the detector (either a MS or IRMS). In both of the chromatograms above, time is on the horizontal ("x") axis. The location of the peak top on the horizontal axis is the retention time; peaks appearing progressively to the right are said to have "longer" retention times. The retention time for any particular steroid is product of several factors: 1) the flow rate of carrier

gas through the column, 2) the length of the column, 3) the degree of interaction of the steroid with the column's particular stationary phase (a "separating film"), 4) the distance and flow rates through any paths subsequent to the GC column. Within a particular instrument, it is also useful to calculate the relative retention time (defined for GC conditions such as used by LNDD) as the ratio of retention times of each analyte to a single internal standard. The relative retention time is often more reproducible than the retention time because of normal small variation in flow rate within one instrument.

TD2003IDCR does not apply to relative retention times between two instruments

At the Malibu hearing and in the appeal brief, Appellant's experts, particularly Dr. Meier-Augenstein, argued that relative retention times must match for analysis conducted on GC/MS and GCC-IRMS machines, and that a WADA technical document sets out this requirement. I disagree with this conclusion for the reasons I discuss below.

TD2003IDCR is a standard established by WADA that applies to a same-GC analysis; that is, it refers to chromatograms run on a single instrument. The specification pertains to peak identification, and requires that the retention time of an analyte can differ by no more than 1% or ±0.2 min of the retention time of a standard added to a sample from a known administration study to enable identification of the analyte. (This requirement is set out in USADA Exhibit 12, page 1.) As an expert in GC/MS and GC analysis, I read TD2003IDCR as a quality specification that requires that the retention time must be within 1% as a necessary condition for identification of a peak as a particular compound. In other words, if the retention time of a peak is beyond 1% of the retention time of a standard for a particular instrument for a particular run, then that analysis cannot be used as evidence for the identity of the peak.

As an example of how this would apply to this case, in the case of the Fraction 3 analyses, the retention times reproducibility for the internal standard was ±0.07% for the A samples, and ±0.13% for the B samples, as shown in Exhibit 134. This reproducibility is well within the 1% specification established by WADA, meaning that the result can be used for identification of a peak as a particular compound.

Contrary to the arguments by Dr. Meier-Augenstein, the WADA requirement does not, and cannot, refer to two different instruments doing different analyses, such as for GC/MS and GCC-IRMS. The retention times, and relative retention times, for GC/MS and GCC-IRMS would not, and should not, be identical when two different instruments are used for analyses.

As noted in part in my rebuttal testimony (starting p1967, with "fused silica capillary"), there is an extra length of capillary tubing, as well as other tubes, that the analyte must traverse in the GCC-IRMS before reaching the detector, compared to the GC/MS. If the temperature programs and flow rates through the GC column were identical between the GC/MS and the GCC-IRMS, the retention times will not match for at least this reason. Beyond this, GC/MS can achieve superior peak separation in a shorter time than GCC-IRMS. Thus, in a working laboratory, it is reasonable that the GC/MS will be set to run samples as rapidly as possible, especially when there is no compelling reason to slow down the chromatography to match retention times. There is no compelling reason to slow down a working laboratory's productivity for the artificial purpose of matching retention times to the slower GCC-IRMS.

Moreover, the laboratory's protocols specifically call for the setting of flow rates to produce a retention time for the internal standard of approximately 10.7 minutes (642 seconds) and 870 seconds (14.5 minutes) for the GC/MS and GCC-IRMS, respectively. The difference in retention times and relative retention times are therefore required by LNDD's SOPs. Contrary to

Appellant's claims that this difference is a problem, the differences are acceptable and required by LNDDs SOPs.

While the relevant WADA Standard does not require any comparison of retention times between the GC/MS and GCC-IRMS instruments, I would note that based on my review of the data in this case, a comparison of retention time and relative retention time between the two instruments establishes that there is a reproducible and reliable relationship in retention times, even though the analysis conditions are non-identical. My analysis indicated that for the given set of measurement parameters in the two different instruments, there is a close correlation for the retention time and relative retention time measured for the same analyte mixture analyzed by each instrument.

1. The metabolite peaks were properly identified by LNDD

The GCC-IRMS detects only CO2 derived from steroids, and it is the GC/MS that yields information that reveals the structure of the steroid associated with each peak. In combination, the two sets of results provide reliable evidence of metabolite identity and delta values to support the finding of doping in this case.

As detailed below, the method used by LNDD to identify peaks is reliable. Established chemical principles are used to connect the peaks in GC/MS to the peaks in GCC-IRMS, thereby establishing the chemical identity of the molecules from which the peaks detected in GCC-IRMS are derived. Dr. Meier-Augenstein and Dr. Davis testified that they believed that LNDD did not correctly identify the metabolites of interest in this case. I disagree with these conclusions for the reasons discussed below.

A. Method File Differences

Appellant argued in his appeal brief that the differences in retention times between the peaks in GC/MS and GCC-IRMS were very different, and by scientific reasoning and by the WADA criteria the steroid identifications are not reliable.[1] As established above, the WADA criteria do not refer to retention time correspondence between two instruments and thus does not apply. Here, the retention time differences are by design of the accredited and scientifically acceptable SOPs and result in highly correlated retention times, yielding reliable results.

As discussed above, among the parameters that must be adjusted prior to a GC analysis, is flow rate. Flow rate can be adjusted in a variety of ways, among which is the setting of flow of helium carrier gas through the GC column. Again, the GC column is a tube with helium flowing through it to sweep the steroids past the separating film coated on the walls. Like any tube that is directing flow, there is a set of plumbing fixtures at the front and back with various functions. Flow at the front of the column is forced onto the column by pressure, called the "head pressure", that is normally adjusted when a set of analyses is being set up. After the flow exits the column, it may pass through an electronic flow sensor that provides a digital readout of the flow. In many GCs still in use, there is no electronic flow sensor, and flow rate is measured by attaching a flow meter to additional plumbing. In either case, the ultimate goal of the flow setting is to make steroids elute at specific times that are known to result in acceptable separation. If the flow is too fast, there is too little time for separation, and if the flow is too slow, there is too much time for diffusional mixing (these concepts, among others, are embodied in the well-known "van Deempter equation").

By far the most robust, but also the most time-consuming, way to set flow rate is to actually measure the retention time of a specific steroid, then adjust the head pressure up or

[1] Appellant claims that one reason the retention times are so different is that there is, allegedly, a "dramatic" difference between the temperature programs. Based on the documents provided, this is not the case.

down, if necessary, depending on whether the elution time is too long or too short. The retention time of the specific steroid is then measured again, and process repeated iteratively until the retention time is within acceptable limits. LNDD follows this robust procedure. They set the flow rate for the GC/MS based on a retention time of about 10.7 min for 5aA, and for GCC-IRMS they set it to give 870 seconds. This is an optimal way to produce best day-to-day reproducibility in retention times within a single instrument.

On the issue of flow rate, in preparing this witness statement I noticed that there is an inaccuracy in my rebuttal testimony, caused by my initial misunderstanding regarding the degree of care with which LNDD adjusted flows. On page 1966, line 18, in response to a question from opposing counsel, I implicitly acknowledged "drift" of about 3 seconds in the elution of a standard. "Drift" in this context refers to an unintentional change in the flow rate through the column. I now realize that the average retention time for the B sample internal standard of 870.5 seconds was closer to the specified value of 870 seconds than for the A samples (866.6 seconds). While either is well within acceptable bounds, this is consistent with LNDD personnel having taken special care in the analysis of the B samples to match to specification, and not to random drift in the flow rate.

B. Identification of Peaks

In his brief, Appellant claims that LNDD's method of identification is nothing more than "eyeballing" the results. In fact, based on my extensive experience in this field and my thorough review of the documents, I conclude that the LNDD method for identification is based on inspection of the respective chromatograms with careful consideration of basic chemical principles involving the preparation of samples and standards, as well as the conditions and

detection mechanisms for the GC/MS and GCC-IRMS analyses. I find this method to be completely acceptable and scientifically sound.

In order to understand why this process is sound, it is important to understand all of the steps involved. Before analyzing the samples by GC/MS and GCC-IRMS, the samples undergo "clean up," the purpose of which is to remove all substances that would coelute and thus interfere with the analysis. This process also concentrates the analytes. It is also important to understand that the same analytes are present in both the GC/MS and GCC-IRMS analysis.

It is undisputed in this case that LNDD's GC/MS analysis correctly identified the four major peaks of interest found in Fraction 3: the internal standard; 5bdiol, 5adiol; and pdiol. There also can be no dispute that those same four peaks of interest elute in the same order in the Fraction 3 GCC-IRMS analysis. However, Appellant's experts claim that LNDD could not properly identify those same four peaks in the GCC-IRMS analysis. Appellant's experts are wrong for the reasons discussed below.

First, for the two analysis methods, LNDD uses a robust procedure for setting flow rates and therefore have very reproducible retention times in their results. They acquire GC/MS data for their test steroids in the samples and in their urine pools that are comparable to standard GC/MS data, thereby establishing the major peaks and their order of elution, as well as the purity of those peaks. Injection of a steroid mixture on the GCC-IRMS therefore produces a pattern that reveals the identity of the peaks.

Specifically, the Mix Cal Acetate, which LNDD runs in the GCC-IRMS analysis, provides known retention time markers for both the internal standard and 5beta. There is no dispute that LNDD correctly identified the internal standard and 5beta in the GCC-IRMS analysis of Appellant's sample. Appellant's claim rests on his argument that LNDD could not

properly identify the 5adiol and pdiol peaks in the IRMS chromatogram because those analytes are not present in the Mix Cal Acetate. I do not agree with this conclusion, because I believe any expert who is familiar with analyzing steroids in IRMS can review the sequence and pattern of peaks between the GC/MS and GCC-IRMS chromatograms and readily identify the 5adiol and pdiol peaks. This can be done by using the internal standard and 5bdiol as retention time markers. The relationship between retention times among the steroids common to the two standards can be used to locate the retention time of the remaining steroids.

Stated simply, if I know where the internal standard peak is and I know where the 5bdiol peak is, then I can use the pattern identified in the GC/MS analysis to find the 5adiol peak and the pdiol peak. Moreover, LNDD uses the blank urine sample as a further check, because the LNDD analysts are familiar with where 5adiol and pdiol elute in the blank urine based on the standard IRMS method. Accordingly, they know where they would expect to see the 5adiol and pdiol peaks to appear in any athletes sample and, as supported by the chromatograms in this case, the 5adiol and pdiol peaks in Appellant's IRMS chromatogram eluted at the expected retention times.

I also reject Appellant's claim that the peak heights do not provide relevant information. In fact the peak heights, along with the established sequence, provide further confirmation that LNDD correctly identified the relevant peaks.

It is important to note that the steroids in this case are very similar chemically and the detection mechanism for GC/MS and GCC-IRMS lead to a high degree of correspondence between the patterns of major peaks in two chromatograms. Collision cross-sections for steroids are very similar to one another, which means that the sensitivity of GC/MS total ion chromatograms are similar from steroid to steroid. GCC-IRMS responds to the total carbon in

each peak, as well as to the flow rate. The fraction 3 steroids have very similar carbon numbers (5bdiol and 5adiol are the same, pdiol has two more carbons). 5bdiol and 5adiol elute adjacent to one another so the flow rate is practically identical, and pdiol elutes a few minutes later, so peak intensities are close to their abundances in the samples. This means that, although GC/MS and GCC-IRMS are measuring different properties of the test steroids, the pattern of peak heights among major peaks for GC/MS is very similar to the pattern in GCC-IRMS. Therefore, I conclude, that because of the similarity of the carbon numbers of the steroids relevant to this case, as well as their GC/MS properties, the peak heights are similar enough to enable reliable comparisons to be made.

C. The Column Used in GC/MS and GCC-IRMS

The Appellant has argued, based on an entry in the document package, that two different column types were used in the GC/MS and GCC-IRMS, respectively. However, the performance of those two columns with respect to the target steroids unambiguously reveals that the columns were, in fact, the same. Examination of the chromatograms in the document package is consistent with this conclusion.[2]

The GC column is the technology that separates a mixture of chemicals from one another. They are a long (e.g. 30 meters), narrow internal diameter (e.g., 0.25 millimeters) glass tube with separating film coated on the walls. The nature of the film determines the time that any particular steroid will emerge (or "elute") from the column. In developing theories about how particular chemicals, such as steroids, behave in a GC column, it is customary to use the simple case where the analysis is conducted entirely at the same temperature, called "isothermally". A concept known as "relative retention time", which describes the time that a chemical elutes from

[2] I am aware from my review of the briefs that the factual testimony establishes that the two columns used were the same. However, as set forth herein, I can reach the same conclusion without the aid of the factual testimony.

the column relative to some reference compound, is generally developed under isothermal conditions. Proportionality of retention times is valid from compound to compound under isothermal conditions.

Most GC analyses are not conducted under isothermal conditions. Normally, they are conducted with "temperature programming", meaning that the GC oven containing the GC column starts out at a low temperature but then increases according to some specified temperature profile. This enables faster analysis of component mixtures with very different properties than would be possible with an isothermal run. Depending on the temperature program, the strict linear proportionality of elution times that enables relative retention times to be calculated may require unwieldy computation. Far more reliable when comparing retention times for two different temperature programs is to run a standard mixture with several of the components under the two sets of conditions to be compared, and then evaluate the correlation.

My review of the data in this case allows me to conclude that LNDD used the same GC column, a DB-17ms, in GC/MS and the GCC-IRMS instruments. The nature of the column determines elution order independent of temperature program. This means that the order of steroid elution, as well as any hypothetical contaminant, will be the same regardless of temperature program. This is relevant to the F3 analysis in at least two ways. First, the order of steroid elution established for the DB-17ms in one GC using any temperature program establishes the order of elution for all. Second, any hypothetical contaminant that might elute under a steroid peak of interest in the GCC-IRMS analysis, would also elute with the same steroid peak in the GC/MS analysis. The GC/MS full scan spectra, showing high correspondence between a standard mass spectrum for 5adiol and the athlete's 5adiol is strong evidence that there is no interference; were there a major unresolved contaminant, it would show

up as major peak in the athlete's 5adiol mass spectrum. Thus, there is no evidence of any interference.

In this case, the correlation between the retention times is very high using a quadratic fit. Because peaks elute in exactly the same order on an identical phase (column), the quadratic fit enables reliable prediction of RT for GCC-IRMS knowing the RT in GC/MS.

Overall, I find LNDD's operating procedures to be scientifically sound and without compromise. The LNDD method, which includes review of the sequence of analyte elution, consistency of results with the blank urine standard, and relative intensity of peaks is more than sufficient to make a positive identification.

2. <u>The IRMS instrument was in good working order</u>

I find that the various tests used by LNDD verify that the GCC-IRMS was in good working order as shown by various LNDD controls, and that Appellant's arguments that the instrument was out of order are without basis. I testified on these points at the Malibu hearing. In this statement, I specifically reject Appellant's claim that there was a problem with the linearity of LNDD's instrument.

Linearity in the case of IRMS is the ability of the instrument to give the same delta value for a particular compound whether the amount of that particular compound that is injected into the IRMS is small or large. It is routinely tested, and is tested based on peak height, not peak area.

Linearity considerations are important when the isotope ratios of small peaks are being compared with large peaks. There is no evidence that the linearity of the IRMS was significantly compromised when the measurements in question were made. I will address, however, the relevance of linearity to the measurements in question. In particular, I point out that linearity

would require careful evaluation if the difference in peak intensities between the metabolite (e.g. 5adiol) and the ERC pdiol were many-fold. In fact, they were not. When peak heights are similar, as they are in this case, linearity does not affect the delta, or delta-delta measurements.

In the F3 fraction, there are three peaks in question: 5bdiol, 5adiol, and pdiol, in the approximate ratio of 2:1:1. The quantities of interest are the delta-delta for 5bdiol-pdiol and 5adiol-pdiol, where the ratios of peak intensities are about 2:1, and 1:1, respectively. The results indicate that the 5bdiol-pdiol are of similar delta-delta, whereas those of 5adiol-pdiol are different by 6 permil and the AAF. Importantly, the two peak heights involved in the AAF are very similar, and thus linearity is not an issue. Linearity issues could, hypothetically, drive a biased measured difference between 5bdiol and pdiol because of their 2:1 ratio. However, because the measured difference was small, any non-linearity might be expected to work toward increasing the difference in isotope ratio. That does not apply here because of the similarity of peak heights between the 5adiol and the pdiol, and the instrument has been shown to be linear.

The simple conclusion is that linearity is not relevant to the 5adiol-pdiol difference because the peaks for these two compounds are very close to a 1:1 ratio.

In summary, the GCC-IRMS instrument was working properly as established by appropriate controls, the various signals inevitable in real samples do not degrade the analysis and the isotope ratio measurements in question are reliable, and the peaks are reliably identified.

3. The LNDD technicians followed the relevant SOPs when analyzing Appellant's samples

My visit to LNDD on 4-5 May 2007 and consideration of the information in the document package leads me to conclude that LNDD technicians followed the LNDD SOPs as accredited, and that those SOPs lead to reliable isotope ratios for the target steroids.

A. Chromatograms

I am aware of Appellant's claims regarding *poor chromatography*, *sloping baselines*, and *differences in baseline isotope ratios*. I disagree with these arguments. Having spent much of my professional career producing and reviewing GC/MS and GCC-IRMS chromatograms, I believe that the chromatograms are reliable and support LNDD's conclusion that there was an AAF in this case. The relevant chromatograms are those of the F3 fraction. As I described in my testimony at the Malibu hearing, the scientific data in this case refutes the claims made by Appellant alleging unacceptable chromatography.

Appellant's experts raised the concept of sloping baselines as issues in many of the chromatograms, but without any evidence or argument about why a sloping baseline would bias the analysis. Because the baseline is subtracted during data analysis, this is explicitly taken into account. The implication was made that measurements of the "baseline isotope ratio" showed it might be different by several delta units, and thus could drive the AAF. This is most clearly undermined by the data reprocessing that took place during the 4-5 May 2007 visit to LNDD. At that time, the raw data were recalculated in several ways, one of which would never be acceptable for reporting of isotope ratio results. This was to turn off the baseline correction during data analysis. This has the effect of adding the background carbon to the isotope ratio of the analytes as well as an arbitrary offset associated with the amplifiers. Even when doing so,

the sample was still positive for an AAF, with a delta-delta of about -5.6 permil. This increases confidence in the original finding of an AAF, but also is relevant to the claim that the baseline isotope ratio affected the results. Indeed this test is directly on point, and rebuts as forcefully as possible the assertion that it is the background isotope ratio that is driving the AAF.

I conclude that the data in this case firmly establishes that the analysis was not compromised by sloping baselines.

B. The chromatography peak resolution was consistent with high quality GCC-IRMS results.

The Appellant argued repeatedly that the chromatography peak resolution was insufficient to avoid contamination of target steroids by interferencing compounds. "Chromatography peak resolution" refers to separation of one peak from another in the chromatogram, and acceptable resolution for GCC-IRMS requires that the peaks be separated to within a few percent of baseline to avoid erroneous results. "Baseline" refers to the signal level when no steroids are being detected.

Appellant highlighted chromatography peak resolution in the athlete's F3 samples. My assessment of the chromatography peak resolution in the F3 samples is that they are consistent with high quality isotope analysis. The peaks for pdiol in both the A and B samples are well resolved from the adjacent very small peaks eluting later.

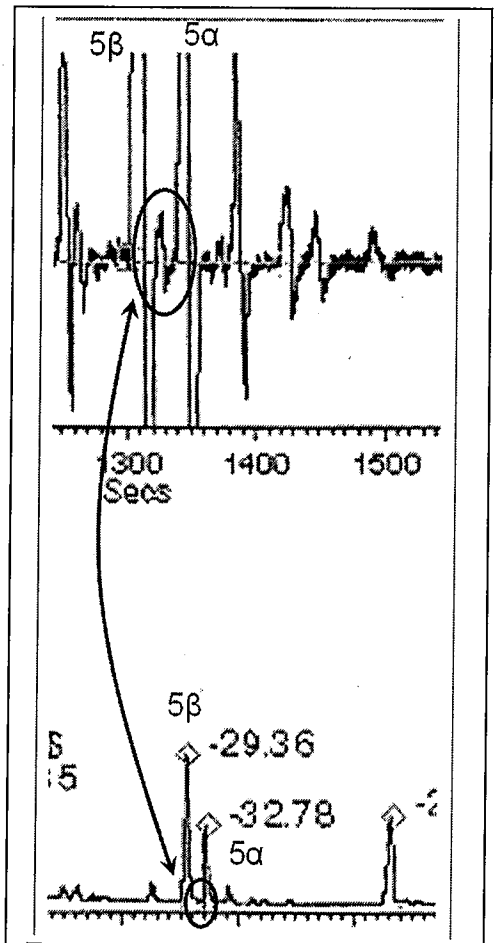
Appellant made specific reference to the small peak appearing between the 5bdiol and the 5adiol in the F3 sample. The relevant claim was that the small peak merged into the 5adiol peak and affected the reported isotope ratio. (Respondent's Supplemental Pre-trial Brief, pp 15-16). Although this claim is not supported by the chromatograms in the document packages, the results

of the 4-5 May 2007 data reprocessing definitively refute this contention after consideration of the 2/1 ratio trace.

The 2/1 ratio trace plots the instantaneous isotope ratio for the compound being analyzed. The 2/1 ratio shows relatively large fluctuations when only baseline and no analytes are being detected. When a peak is detected, the 2/1 trace first rises, and then falls. This is because the CO2 (derived by combustion of the steroid) that contains 13C is detected slightly ahead of the CO2 that contains 12C. It is common experience in GCC-IRMS that the 2/1 ratio trace tends to exaggerate peaks and baseline noise that are present in the GCC-IRMS chromatogram, and for this reason it is useful for quality control.

In direct testimony (p265 lines 1-17, especially line 15), I showed that the small peak appearing between the 5bdiol and the 5adiol in the GC/MS analysis had not disappeared in the GCC-IRMS analysis, and that it is distinctly visible, and resolved, in the ratio trace, as shown in the figure below. I conclude that this small peak did not contribute to the baseline, nor did it contribute to the 5adiol as argued by the Appellant, and that the 5adiol delta value is accurately measured.

Figure 3



From page 101, 4-5 May reprocessing documents.

C. Manual integration

I testified about the procedure called manual integration of data by the LNDD technicians. I described my observations of reprocessing starting p 272, line 12 and continuing for a few pages, and later on p 1979. The manual reprocessing is a quality control step that is clearly spelled out in the LNDD SOP. Exhibit 112, LNDD0603-0609. They first ensure that the baselines are calculated based on proper positioning of baseline anchor points, then they insure that the peak starts and stops are properly assigned. This procedure is required for the OS/2 software because of the state of the art in software at the time it was written. It is mostly automated, but required more attention to details than more highly developed software, and is more automated than software that came before it. This is not to say it is less reliable, but rather that more detailed quality control was required to ensure robust results.

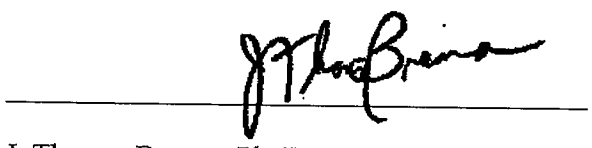
D. Electronic Data File Analysis

On 4 and 5 May 2007 I was present in LNDD during the electronic data file reprocessing analysis. At that time, the Respondent's representative requested that the electronic data files be reloaded into the OS/2 software and the results regenerated. LNDD technicians Cynthia Mongongu and Claire Frelat loaded the original data files from the A samples and B samples and reprocessed them according to LNDD standard operating procedures. For each file, data was loaded into a graphic user interface and the software set to find the background and peak start and stop. The technicians examined the software choices of background and peak start/stop for quality, made manual adjustments as necessary, and then calculated delta values. The procedure was repeated with the automatic computer-selected baseline and peak start-stop, without manual

quality control. Finally, data were transferred to a different computer running Masslynx for processing with that software. In all cases, the results of the test were positive. The Masslynx software performed without manual intervention, except for the B sample blank urine 5adiol (hearing transcript p288). Masslynx crashed when manual reprocessing was attempted as a quality control measure for a poorly placed point (see pp 183-184 of "Reprocessing of Electronic Datafiles" documentation). It was agreed by parties present in the lab at the time including Dr Davis that the Masslynx reprocessed data for this chromatogram would be disregarded, though Respondent did subsequently enter the data into evidence.

I conclude from these observations that the LNDD technicians followed relevant and appropriate SOPs for analysis of GCC-IRMS data, and that the results of the reprocessing on 4-5 May demonstrate the robustness of the results. I found that LNDD staff focused to produce accurate and unbiased results for all samples analyzed.

I declare under penalty of perjury of the laws of New York that the foregoing is true and correct and that the foregoing was executed on 7 March 2008 in Ithaca, New York.



J. Thomas Brenna, Ph. D.