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The Detection of Doping in Sport and the Role of Forensic Science

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Abstract

The detection of doping in sport is a vital component to creating a fair competitive environment for athletes. Educating athletes on the process of doping detection from start to finish may help them make better decisions when they are faced with doping, either intentionally or unintentionally. The doping detection process starts with the World Anti-Doping Agency and other Anti-Doping Organizations deciding what athletes should be tested and what they should be tested for, and ends with either a positive or negative doping test. Athletes' knowledge on the doping detection process often ends when their sample is collected, unless they are accused of doping and face consequences of suspension or even being banned from the sport. However, athletes should be more knowledgeable on the testing procedures used once their samples are collected. Today, the most common tests used are gas chromatography-mass spectrometry, liquid-chromatography-mass spectrometry, and mass-spectrometry-mass spectrometry, among others. These techniques can be used to detect steroids, narcotics, stimulants, masking agents, contaminants of dietary supplements, and other substances on the WADA Prohibited List. Innocent athletes should be able to defend themselves if a test method gives a false-positive result, and understanding the accuracy of these test methods and how many different techniques there are should deter dishonest athletes from doping. Advancements are constantly being made to detect new substances and better detect substances that are commonly used. Informing athletes of the possible health risks and how certain drugs may even decrease their athletic performance should be deterrents as well. Educating the athletic community on the doping process from start to finish is the key to creating a doping free environment for competitive sports.

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CHAPTER ONE: INTRODUCTION

Doping is unfortunately a common occurrence in the sports industry today. It seems as if there is a new athlete under scrutiny for doping each day. Rather than simply looking to just punish these athletes to deter doping, the athletic community should work to make them better informed on not only the dangers of doping, but the entire process. Most athletes lack the background to understand exactly what doping is, how substances are tested for, and what the effects can be. A more informed athlete might make better choices if they are better educated. Educating athletes on doping and the methods utilized by anti-doping laboratories may stop athletes who are currently doping, and prevent new ones from starting.

1.1 History Of Doping

The act of doping dates back to before the start of organized sports. Dating back to the third century BC, Ancient Greek Olympic athletes used various alcoholic mixtures and ate hallucinogenic mushrooms and sesame seeds to enhance their performance in competition. Plants were used as well to improve athletes' speed and increase their endurance. They also doped to mask their pain so they could continue to compete. However, this does not mean that doping was not seen as cheating or unethical. Even though there was no committee to oversee the punishment of these dishonorable athletes, identified cheaters were often sold into slavery in ancient Greece (Reardon and Creado, 2014).

More modern doping began in the early 20th century. Until about 1920, higher-level or professional athletes commonly used combinations of strychnine, heroin, cocaine, and caffeine. By 1930, it was commonplace for performance-enhancing drugs (PEDs) to be used in competitions such as the Tour de France. Eventually, the rules changed because national teams

were going to be paid by the organizers of the events, and athletes were reminded that they would not be provided with drugs because the organizers did not want to be held responsible (Reardon and Creado, 2014). However, it was not difficult for athletes to gain access to these substances, and there were no routine tests for doping. From the 1920s until the end of World War II in 1945, programs aimed at individual athletes expanded doping and the athletes themselves, or their coaches controlled administration. These doping programs were mostly restricted to athletes at the elite level, but this has changed significantly over time. Post-World War II, systemic team doping programs emerged as well (Bird, et al., 2015). No longer were individual athletes targeted, rather entire teams, expanding the scope of PEDs in sports.

The Soviet Union was a major player in team doping programs. In the 1950s, the Soviet Olympic team wanted to increase their strength and power, so they started experimentation with testosterone supplementation. Surprisingly, this performance-enhancing program was government-sponsored. National team trainers and sports medicine doctors carried out the program without knowing what the possible short-term and long-term effects might be (Reardon and Creado, 2014). Because doping was expanding so rapidly, it became harder to ignore the role that PEDs were playing in sports. France was the first country to implement anti-doping legislation in 1963 (Reardon and Creado, 2014). But despite these efforts, the doping continued. In 1967, a 29-year-old English cyclist, Tom Simpson, collapsed during the Tour de France and died. His autopsy revealed high levels of methamphetamine in his system, and he had a vial of the substance on him at the time of his death. This was the first televised doping-related death and it had a large impact not only on those working within the athletic community, but the spectators who were affected by the actions of these athletes (Bird, et al., 2015). This led to an outcry from the public that something more had to be done to regulate doping in sports. When

the Berlin Wall fell in 1989, the East German government's doping program of giving PEDs to their elite athletes was exposed to the public, which led to even more concern. People had long questioned the success of the East German athletes in the Olympics, and this gave the public some answers (Reardon and Creado, 2014). However, it also raised more questions about doping in sport. Incidences of doping in sport over the past hundred years or so, have forced the athletic community and the public to ask, who is doping, what substances are they using, what are the health effects of these substances, and how can these substances be tested for?

CHAPTER TWO: THE WORLD ANTI-DOPING AGENCY (WADA)

As these questions are being raised and ways to identify and regulate doping are researched, the substances being used to illegally enhance performance have evolved and continue to evolve. As detection strategies improve, various outside parties have determined ways to avoid detection by developing more advanced doping techniques. Before the tests and instruments used to detect these substances can be discussed, it is important to understand who regulates doping in sport, who decides what substances are banned, and how these anti-doping committees were formed.

2.1 History Of The WADA

The World Anti-Doping Agency (WADA) was established in 1999 to create a collective movement around the world for doping-free sport. The WADA seeks to build a world where athletes can compete fairly in an environment without doping (Who We Are, 2013). The WADA got its start after the events that took place during the 1998 Tour de France. French customs police arrested Willy Voet, a cyclist of the Festina team, for transporting performance-enhancing

drugs during the competition. Voet's arrest led to an extensive investigation and a 30+-year history of doping in the sport of cycling was uncovered (Bird, et al., 2015). Due to these events, the International Olympic Committee (IOC) decided to hold a World Conference on Doping. On February 2-4, 1999, the First World Conference on Doping in Sport was held in Lausanne, Switzerland. At this conference, the Lausanne Declaration on Doping in Sport was created, which called for the creation of an independent international anti-doping agency that would be in operation for the Games of the XXVII Olympiad in Sydney in 2000. As a result of this declaration, the World Anti-Doping Agency (WADA) was established in Lausanne on November 10, 1999, with the goal to promote against doping in all sports. The WADA was established under the IOC with the support of governments, intergovernmental organizations, public authorities, and other public and private organizations who wanted to end doping in sport internationally. The fundamental undertakings of the WADA include scientific research, education, development of anti-doping capacities, and monitoring the World Anti-Doping Code (Who We Are, 2013).

2.2 The World Anti-Doping Code

The WADA wants to make its policies accessible to all of the athletes and coaches that their work applies to so there is no confusion over doping in sport. It is important for athletes to be provided with a clear guide on anti-doping and doping and making sure they understand what is expected of them, so they can be held accountable for their actions. One item of information available to athletes is the WADA's Athlete Guide. It gives a brief overview of the WADA, the Code, and the Prohibited List (At-A-Glance- About Anti-Doping, 2014). The World Anti-Doping Code, or more commonly referred to as the Code, along with the support of the World

Anti-Doping Program, works to protect athletes' rights to participate in sports that are free of doping, promoting health and leading to fairness and equality in competition worldwide. The Code also works to ensure that coordinated and effective anti-doping programs are available at both the national and international level. The main goals of the Code are to have effective detection, deterrence, and prevention of doping. The Code is the basic and universal document in which the World Anti-Doping Program is based on. The purpose of the Code is to help create a doping-free environment in sports by using standardized anti-doping elements. The Code can educate those involved in the athletic community on the definition of doping, anti-doping rule violations (ADRVs), the Prohibited List, the testing and analysis of samples, consequences for individual and team doping, research, and other applications of the Code, among many other topics. The goal is for the Code to be specific enough to create uniformity, but adaptable enough in other areas to allow flexibility with how the principles are employed. The Code works to protect "the spirit of the sport," which doping goes against (World Anti-Doping Code, 2014). This spirit involves fair play and honesty, a sense of community, health, and respect for rules and for other athletes, among many other aspects. Doping goes against all of these, and the WADA works to help keep "the spirit of the sport" alive.

2.3 Prohibited List

The WADA also keeps an updated list of the substances and methods that are banned in sport. This is known as the Prohibited List. It lists the substances that are banned both in- and out-of-competition, as well as listing substances that are banned in specific sports. The current list came into effect on January 1, 2016. The list is updated annually, and it is one of the five International Standards that are mandatory for all signatories of the WADA Code. The

Prohibited List is created and confirmed by the WADA's List Expert Group, the WADA's Health, Medical, and Research Committee (HMRC), and the WADA's Executive Committee (World Anti-Doping Code, 2014).

2.4 The Athlete Guide

The WADA raises the question of what doping actually is. The Athlete Guide provides a list of the anti-doping rule violations and explains that, "Doping is defined as the occurrence of one or more of the following anti-doping rule violations" (At-A-Glance- About Anti-Doping, 2014). The violations include: a prohibited substance being detected in an athlete's sample (urine or blood), the use or attempted use of a substance or method that is on the prohibited list, refusing to submit a sample of urine or blood to be collected after the athlete is notified it is needed, failure of an athlete to file their whereabouts information and missing tests to screen for doping, interfering with the doping control process during any point, possession of a method or substance that is prohibited, trafficking a method or substance that is prohibited, administering or attempting to administer a prohibited method or substance to another athlete, involvement in an ADRV, and having prohibited association with authorized Athlete Support Personnel (At-A-Glance- About Anti-Doping, 2014). If athletes are found to be in violation of one or more of these rules, they are considered to be doping and further action must be taken.

Part of the reason the WADA has been successful, is because it works with athletes and coaches to try and build the best, fair competition environment they can. This means that athletes have to be willing to participate in surveys and tests performed by the WADA so they can gain information. This also means that athletes need to be honest, otherwise research and studies will not be accurate. It is important for athletes to be honest about their whereabouts so they can be

contacted and located. Athletes who are part of the Registered Testing Pool (RTP) are expected to provide accurate information regarding their home address, training and competition schedules, venues, and regular personal activities such as work and school. Athletes identified in the RTP have to specify a location and 60-minute window each day, where they can be sought after for testing (At-A-Glance- About Anti-Doping, 2014). If they miss this window, it may be considered a missed test, and there could be serious consequences. Even athletes not included in the RTP can be requested to provide whereabouts information.

Urine and blood tests can be conducted in- and out-of-competition at anytime and at any place if an athlete competes at the international and/or national level. An athlete's International Federation (IF), National Anti-Doping Agency (NADO), or a Major Event Organizing Committee can perform the tests. Trained and accredited doping control personnel carry out all doping tests (At-A-Glance- About Anti-Doping, 2014). For in-competition testing, athletes can be chosen for testing at random, they may be chosen based on their finishing position, or they may be targeted for a particular reason, such as suspicious activity. Out-of-competition can be performed on any athlete at any time, and the athlete does not need to be given advanced notice (At-A-Glance- About Anti-Doping, 2014). This ensures that athletes do not have time to prepare for a drug test, such as trying to get the substance out of their system.

Violating the anti-doping rules can lead to a wide range of circumstances. Some athletes might just be reprimanded, while others may be banned from the sport and competition for life. The period of ineligibility also varies based on the type of violation, the substance or method used, the possibility of the repetition of the violation, and the circumstances surrounding each individual athlete's case. Athletes are also given the right to have their second sample (sample B) tested, and they are permitted to have a fair hearing. Athletes are also allowed to appeal any

decisions made regarding a positive test or penalty imposed on them following an anti-doping violation (At-A-Glance- About Anti-Doping, 2014).

2.5 The Doping Control Process

The WADA also breaks down the doping control process and clearly explains each of the eleven stages. Athletes being tested for doping are accompanied from the time they are notified about the test until the process is over. This ensures that the athlete cannot leave before taking the test, and that they cannot tamper with samples. Athletes have many rights and responsibilities during the doping control process. Athletes are allowed to have a representative or interpreter available if one is needed, and they are allowed to ask for supplemental information about the sample collection process. They are only allowed to delay their sample collection for an approved reason, otherwise they must report for testing immediately. Athletes are also allowed to request modifications to the sample collection procedure if they have disabilities. Along with having rights, athletes are also responsible for a number of items. They must remain in direct contact with the Doping Control Officer (DCO) at all times and they must be able to produce valid identification (At-A-Glance- The Doping Control Process, 2014).

Athletes should be aware of the complete doping control process so they can fully comply with the WADA and their testing procedures. The WADA provides this information in their Athlete Guide (At-A-Glance- About Anti-Doping, 2014). The first stage, is understanding that an athlete's sample can be taken anywhere and at anytime, depending on whether they are in- or out-of-competition. Second, DCO's will notify athletes when they are selected for doping control, and the athlete will have to sign a form stating that they understand their rights and responsibilities. The athlete will then report to the doping control station as soon as they possibly

can so sample collection can begin. The fourth stage begins when the athlete is given a selection of collection vessels to choose from. Since the athlete chooses their own collection vessel, they cannot claim that the vessel they were given was tampered with. The fifth and sixth stages involve the actual sample collection. A minimum of 90 mL of urine is required. The athlete uncovers from their knees to their navel, and from their hands to their elbows to provide a clear view of passing the sample. A DCO of the same gender observes the urine leaving the athlete's body. Again, this is another precaution to ensure the athlete cannot tamper with their sample, which they would have the opportunity to do if left alone with it. During the seventh stage, the athlete chooses a sample collection kit from a selection provided to transfer their urine sample into. The sample is split between A and B bottles so a second sample is available if further testing needs to be done. Stage eight requires the athlete to seal their samples. In stage nine, the DCO measures the specific gravity of the sample to ensure the urine is not too dilute to test. If the sample is too dilute, the athlete may have to provide an additional sample. This dilution may be natural, or caused by a substance such as a diuretic. Stages ten and eleven require the athlete to fill out the Doping Control Form by providing information about themselves, any substances they take, any concerns or comments they have about the doping control process performed, and confirming and signing the Doping Control Form. Samples are then sent to a WADA accredited laboratory following strict confidentiality guidelines and the samples are tracked to ensure security. Sample A is analyzed first, while sample B is securely stored for further testing if it is required. Test results are then returned to the Anti-Doping Organization (ADO) responsible for that athlete and the WADA (At-A-Glance- The Doping Control Process, 2014). The process for collecting blood samples will be discussed at a later point.

2.6 Athlete Biological Passport (ABP)

Another method used by the WADA to detect athletes who are doping is the Athlete Biological Passport (ABP). This method is not a drug test, rather it is used to track several biomarkers of doping over time which can indirectly expose the effects of doping, compared to directly detecting doping by analytical doping controls. There are currently two modules used that compose the ABP. These are the Haematological Module and the Steroidal Module. The Haematological Module targets enhancers of oxygen transport and any form of blood manipulation by monitoring the markers of blood doping, which can be measured in an athlete's blood sample. The Steroidal Module targets to identify endogenous anabolic agents by monitoring the biomarkers of steroid doping measured in an athlete's urine sample. The goal of incorporating the ABP into the anti-doping program is to identify athletes who are doping by intelligent and timely analysis of their passport data and to pursue ADRVs of the World Anti-Doping Code. For the Haematological Module, Erythropoiesis-Stimulating Agents (ESAs) and homologous blood transfusion (HBT) tests are included in the testing, and for the Steriodal Module, Isotope Ratio Mass Spectrometry (IRMS) is used to detect endogenous steroids that are administered exogenously (Athlete Biological Passport, 2014).

The WADA needs to constantly modify their techniques so they can detect new doping methods. One way they do this is by further developing the ABP. The WADA is currently working on an endocrine module, which will detect the abuse of growth hormones and other growth factors. Eventually, the goal of the ABP is to develop a panel of biomarkers of doping by utilizing the advances that have currently been made in analytical chemistry and gaining a better understanding of biological systems through the study of fields such as proteomics and metabolomics (Athlete Biological Passport, 2014).

The traditional doping control approach which is based on the detection of prohibited substances or their metabolites in an athlete's sample is generally effective, but limitations present themselves when an athlete uses substances sporadically or in low doses. Conventional means of detecting doping may not be able to detect new substances or modifications made to old substances, which can prevent their detection. However, the ABP may identify these substances because they were not present in the athlete's system before. The WADA requires consistency and uniformity in application of the ABP, but each ADO is free to implement the processes how they please. However, there are mandatory protocols in sample collection and analysis that must be followed to ensure legality, scientific certainty, and to share data between organizations. The ABP is not meant to replace traditional doping control, rather enhance it. Combining these doping control strategies makes the fight against doping more cost-efficient and effective (Athlete Biological Passport, 2014).

The WADA does not expect all ADOs to run both the Haematological Module and the Steroidal Module. The physiological risks of each specific sport should be assessed to decide which module(s) might be applicable. All routine urine tests are automatically subjected to the Steroidal Module so a "steroid profile" can be established regardless of whether or not a sport requires endurance or strength. However, it is up to the ADO to decide if the Haematological Module should be applied as well (Athlete Biological Passport, 2014).

2.6.1 The Haematological Module

The Haematological Module assesses variables with red blood cells. Red blood cells transport oxygen to other cells, so blood manipulation is more common in sports where increased endurance is beneficial to athletes. Blood manipulation includes the use of erythrocyte

stimulating agents and blood transfusions. However, just because blood manipulation is typically used to improve endurance, does not mean it is not used in sports that are not typically endurance events. Therefore, the Haematological Module can be applied to other sports with a large aerobic factor. In order for the Haematological Module to be used, athletes must be part of the ABP program because specific blood tests must be performed (Athlete Biological Passport, 2014). Biomarkers in the athlete's blood can be monitored and if there are changes in these biomarkers there may be grounds to believe blood manipulation or doping has occurred and a further investigation can follow.

2.6.2 The Steroidal Module

The Steroidal Module assesses for substances such as Anabolic Androgenic Steroids (AAS), which are more likely to be abused in sports that require power and strength. Some steroids also increase the production of red blood cells and decrease recovery time, so endurance athletes may abuse them as well. All urine samples sent for testing are analyzed for the Steroidal Module "steroid profile." This means that just about any athlete that has been tested is essentially part of a Passport style program. When an athlete has more than one urine sample analyzed, a more in depth steroid profile can be created in the Anti-Doping Administrative and Management Systems (ADAMS) (Athlete Biological Passport, 2014). Similar to how the Haematological Module operates, the Steroidal Module can track endogenous anabolic androgenic steroids (EAAS) in an athlete's system, and if these levels change an investigation can be pursued to determine if the athlete is administering them exogenously or if the change is natural. If no baseline is set for the athlete's natural levels, it can be difficult to determine if an athlete is doping, or they have naturally high levels.

2.6.3 Athlete Passport Management Unit (APMU)

The specific individuals assigned to administer an ABP make up the Athlete Passport Management Unit (APMU), and are designated by the ADO. The APMU is preferably associated with a WADA accredited laboratory and they are responsible for the administration and management of the ABPs, instructing the ADO on possible target testing, collecting and approving an ABP Documentation Package, and reporting Adverse Passport Findings (Athlete Biological Passport, 2014). Large ADOs may contain an APMU that operates in-house, while other ADOs work with WADA accredited laboratory-associate APMUs, which are only brought in when needed. The modules performed will also depend on the ADO and APMU used. Not every APMU operates both the Haematological Module and the Steroidal Module. The ADO will determine which module needs to be performed and contact the appropriate APMU. If an ADO does not already have an APMU in place and a steroidal Atypical Passport Finding (ATPF) is reported, the ADO should seek guidance from the laboratory that performed the test. An APMU would be beneficial in this case to handle further investigation into the athlete and further testing that might need to be performed. APMUs that are associated with WADA accredited laboratories have the most accessible expertise for the interpretation of data, however if the ADO does not run the Haematological ABP program and the risk of steroid doping is low, ATPF may be handled case by case and an APMU would not necessarily be required (Athlete Biological Passport, 2014).

CHAPTER THREE: INTERNATIONAL STANDARD FOR LABORATORIES

In order for a laboratory to be able to test for doping in sport, they must be WADA accredited. Utilizing WADA accredited laboratories ensures that testing will be kept fair and

procedures will be kept constant from laboratory to laboratory. As part of the World Anti-Doping Program, the World Anti-Doping Code International Standard for Laboratories (ISL) was developed as a mandatory International Standard (International Standard for Laboratories (ISL), 2016). The goal of the ISL is to ensure that laboratories are producing valid results and data, and to achieve consistent results and reporting from all laboratories. The ISL first came into effect in November 2002, and revisions have been continually made since then. The most recent version of ISL, version 9.0, came into effect on June 2, 2016 (International Standard for Laboratories (ISL), 2016).

3.1 WADA Laboratory Accreditation Process

The purpose of the ISL document is to explain the requirements for laboratories that want to show, "…they are technically competent, operate in an effective quality management system, and are able to produce forensically valid results" (International Standard for Laboratories (ISL), 2016). Laboratories are allowed to perform other types of analysis that are not under WADA accreditation, such as forensic testing, but this testing will not be covered by WADA and the laboratory will need to seek further accreditation.

A laboratory that wishes to seek accreditation by the WADA must officially contact the WADA and express their interest in becoming a candidate for accreditation. The candidate laboratory must first submit an initial application form and provide letter(s) of support from Signatory Anti-Doping Organization(s) that guarantee the laboratory will receive 3,000 samples annually from Code-compliant clients for a three year period within two years of when they receive accreditation. The candidate laboratory must also describe the conditions of their facilities, such as a staff list and their qualifications, instrumental resources, reference materials,

a business plan for the laboratory and how they will manage to test the required number of samples, and a list of the laboratory's sponsors. Once this information has been reviewed, the WADA conducts an initial visit to review the accreditation process and obtain more information about the laboratory. A final report will be issued and recommendations will be made to the laboratory on what they need to improve so they can receive accreditation. The laboratory will pay an initial accreditation fee, prove they can operate independently from ADOs, and show compliance with the Code of Ethics. The pre-probationary test requires the laboratory to test at least ten External Quality Assurance Scheme (EQAS) samples, which allows them to assess their competency at that time and compare their results with other laboratories for learning purposes. The candidate laboratory provides a test report for each sample, which the WADA uses to assess the laboratory's ability and provide them feedback on areas where they need improvement. After completion of the pre-probationary test, the laboratory will then enter a probationary period where it will become a WADA probationary laboratory, and prove it can handle the amount of samples to be tested and that it can test them properly. This period includes 20 EQAS samples, which are typically distributed over multiple EQAS rounds. The samples are given at different times to prepare the laboratory for when they will be given many samples at once. During this time, the laboratory must successfully analyze 18 of the 20 EQAS samples. To conclude the probationary period, the laboratory must complete a final proficiency test in which they analyze a minimum of 20 EQAS samples with WADA representatives present (International Standard for Laboratories (ISL), 2016). This test assesses the laboratory's scientific capabilities as well as their ability to work with multiple samples. The WADA wants to make sure they are giving accreditation to competent laboratories that can handle efficiently testing many samples at once.

During the probationary period, the laboratory must also create a plan for research and

development. WADA accredited laboratories do not only test samples, but also they must attempt to make developments in the field of doping control to better identify doping. The probationary laboratory must provide a three-year research plan along with a budget. The final step of the probationary period is a WADA accreditation assessment. Based on what the WADA has observed, they make a final decision regarding their recommendation for accreditation. The final report and recommendation are sent to the WADA Executive Committee for their approval. If the WADA recommends that the laboratory should not be accredited, the laboratory is given a maximum of six months to make improvements, at which time the WADA will make a further assessment. If the laboratory is to receive accreditation, a certificate signed by a duly authorized representative of the WADA will be given (International Standard for Laboratories (ISL), 2016).

3.2 Maintaining WADA Accreditation

The laboratory must follow several guidelines to maintain their accreditation. They must remain operationally independent from ADOs to ensure impartiality. They must provide an annual letter of compliance with the Code of Ethics to the WADA and maintain their insurance coverage. They are required to document all of their research and development undertakings and they must document that they are sharing this knowledge with other WADA-accredited laboratories. The laboratory must continually provide renewed letter(s) of support and demonstrate they are testing at least the minimum number of samples, along with a fee schedule for the tests being performed. The WADA also holds the right to inspect and assess the laboratory at any time, so they must participate in these re-assessments (International Standard for Laboratories (ISL), 2016). The WADA ensures their accredited laboratories are following all guidelines and that their work supports the goals of the WADA.

3.3 Suspension And Revocation of WADA Accreditation

Laboratories that cannot properly follow the WADA guidelines may have their accreditation suspended or revoked. This may occur whenever the WADA has a warranted reason to believe the loss of accreditation is in the best interest of the anti-doping community. Suspension of accreditation can occur for several reasons. If the laboratory fails to take appropriate corrective action after a re-assessment or they fail to comply with the requirements or standards of the WADA their accreditation may be suspended. It can also be suspended if they fail to cooperate with the WADA or fail to comply with the Code of Ethics. These non-compliances have to be assessed on a case-by-case basis to determine the severity of the noncompliance and the appropriate consequences. The laboratory can also have their accreditation suspended if they lose the support of Code-compliant clients (International Standard for Laboratories (ISL), 2016).

If non-compliance or other issue is not resolved during the initial suspension period, the suspension can be extended, or the laboratory's accreditation can be revoked. While the laboratory's accreditation is suspended they are ineligible to test doping control samples for any Testing Authority, unless the non-compliance is limited to a specific analysis procedure. Revocation of accreditation can occur if any of the above mentioned conditions are severe enough, or are not fixed. Revocation is also likely to occur if a laboratory is found to have reported a false Adverse Analytical Finding. This is a serious non-conformity because it could lead to negative consequences for an athlete who is innocent of doping. The WADA may require that the laboratory re-analyzes all relevant samples reported as Adverse Analytical Findings by the laboratory from the time of the false report to the previous 12 months, or the last satisfactory EQAS round. Laboratories who have had their accreditation revoked are not allowed to test

doping control samples so remaining samples should be sent to other accredited laboratories (International Standard for Laboratories (ISL), 2016). As long as WADA-accredited laboratories follow the guidelines to maintain accreditation they are permitted to test athletes' samples.

CHAPTER FOUR: INTERNATIONAL TESTING STANDARDS

As part of the WADA Program, the World- Anti-Doping Code International Standard for Testing and Investigation (ISTI) was developed as a mandatory standard (International Standard for Testing and Investigations (ISTI), 2014). The International Standard for Testing (IST) first came into effect on January 1, 2004 and it has been continually revised since then. The current version used was approved at the World Conference on Doping in Sports by the WADA Executive Committee on November 15, 2013 and went into effect on January 1, 2015. A new version was approved in May 2016, however it will not come into effect until January 2017. Therefore, this thesis will refer to the 2015 version. This text discusses how the WADA plans effective testing in- and out-of-competition, which is important for the sports industry to know so they understand how the WADA decides who and how they test. It also covers how samples are prepared for collection, how the collection process is conducted, and how the samples are transported and documented so the integrity and identity of the samples can be maintained. The ISTI also establishes standards for the efficient collecting and use of anti-doping information and for the effective handling of investigations into possible ADRVs (International Standard for Testing and Investigations (ISTI), 2014).

4.1 Test Distribution Plan

Different sports have different risks for doping and this must be taken into consideration when planning what tests for doping should be conducted. Each ADO with Testing Authority is required by the Code, "…to plan and implement intelligent Testing that is proportionate to the risk of doping among Athletes under its jurisdiction, and that it is effective to detect and deter such practices" (International Standard for Testing and Investigations (ISTI), 2014). Most ADOs have the authority to test their athletes for doping, or at least inform them of doping to prevent it from occurring. This requires having a plan that is appropriate for the sport at hand. The ISTI creates the steps that are necessary to produce a Test Distribution Plan that fulfills this requirement. This plan involves determining the population of athlete's within the ADOs anti-doping program, an assessment of which prohibited substances and prohibited methods are most likely to be abused in the sport(s) being observed, establishing between different types of athletes, deciding between different types of testing, and distinguishing between the types of samples collected and the types of sample analysis (International Standard for Testing and Investigations (ISTI), 2014). The ADO is required to file their Test Distribution Plan with the WADA to be sure that it meets the requirements of the Code. The two main objectives are risk assessment and prioritization. These assessments take into account several pieces of information such as the physical and mental demands of the sport(s), the possible effects that PEDs may have, the potential incentives for doping, the history of doping in the sport(s), research on doping trends, information previously gained on doping in the sport(s), and the outcome of earlier Test Distribution Plans for the sport(s) (International Standard for Testing and Investigations (ISTI), 2014). A Test Distribution Plan can then be created based on the risk assessment and prioritization and it can then be discussed with the WADA, implemented, and modified as

needed. This Plan is meant to target the athletes at risk for doping, determine which drugs are most likely to be abused, and determine the best way to test for and decrease doping.

4.2 Selecting The Athlete Pool

In order to have a successful Test Distribution Plan, an appropriate pool of athletes needs to be selected for testing. It would be impossible to test all athletes for doping, so the WADA must come up with a way to determine the population of athletes that should be tested. The Code allows National Anti-Doping Organizations (NADOs) to limit the number of athletes they have to test to those competing at the highest national level and to those who frequently compete at the international level. An ADO may decide to test athletes outside of this population range if they see fit, however they are not required to. National and International ADOs are free to set the criteria it will use to classify an athlete as a National-Level or International-Level Athlete, however they must do it in good faith and protect the integrity of the sport at that level. These organizations should also publish their criteria so their decisions can be clearly understood and their classification of athletes can be reviewed by other ADOs (International Standard for Testing and Investigations (ISTI), 2014). ADOs should review materials published by other ADOs to see what classification methods they use so more uniform decisions can be made.

ADOs also need to take into consideration if there are sports under their jurisdiction that take priority over other sports. This means that International Federations need to assess the risks of doping between the nations within its sport. If one nation seems more at risk than another for doping for a particular sport, that nation takes priority for testing. In reference to NADOs, they need to assess the relative risks of doping between the difference sports under their authority along with any national anti-doping policies that are imperative to help prioritize certain sports

over others. For example, some NADOs place priority on testing athletes who partake in sports involved in the Olympics, while others place priority on testing athletes that participate in other national sports. Prioritization is also important when taking Major Events into consideration. It is crucial to assess each of the sports that will be participating in the event and determine which sports are most at risk for doping. More resources should also be devoted to sports that contain larger numbers of athletes to try and prevent and detect more doping (International Standard for Testing and Investigations (ISTI), 2014). The work of International Federations and NADOs combined can be used to determine which sports have athletes most at risk for doping.

Once the athlete population and priority of sports and nations has been determined, Target Testing can be used by the Test Distribution Plan to focus on and prioritize specific athletes. This focuses resources on the most at risk athletes in a selected athlete pool. Random testing does not ensure that the most at risk athletes will be tested enough, or even at all. The WADA Code does not require there be suspicion for Target Testing. However, Target Testing should only be used for legitimate doping control. ADOs should consider conducting Target Testing on specific classifications of athletes. For example, International Federations should focus on athletes at the highest level of international competition, which can be determined by rankings and other criteria. NADOs should focus on athletes who participate in national Olympic or Paralympic sports, individuals who train individually, but compete at the Olympic/ Paralympic or Championship level, athletes who receive funding from the public, and high-level competition athletes who are nationals of other countries, but train or compete within a NADOs territory. Athletes who have been suspended or who have retired and come back to a sport should also be a part of Target Testing. The other factors used to determine athletes who should be made a part of Target Testing can vary from sport to sport. The WADA provides several factors that

are likely to point to at risk athletes. Some of these include prior ADRVs, sudden major advances in performance, failure to comply with whereabouts findings or refusing to file them, and absence from an expected competition (International Standard for Testing and Investigations (ISTI), 2014).

Random Selection can be used for testing that is not Target Testing. Athletes can be chosen completely at random, or a weighted random selection can be used. Athletes are ranked depending on a set list of criteria, which increases or decreases their chances of being selected for testing. This criterion ensures that a greater number of at risk athletes are chosen (International Standard for Testing and Investigations (ISTI), 2014). Using a Random Selection procedure to choose athletes for drug testing may be a greater deterrent against doping because athletes will not know when or if they will be chosen.

Depending on the risk assessment and prioritization process, the ADO must determine to what extent in- and out-of-competition testing, urine testing, blood testing, and ABPs are needed. The ADO must take into consideration what tests will be the best to detect and deter doping within the sport and the nation in question. Except for unique and justifiable circumstances, no advance notice will be given before testing. In order to ensure that an athlete is not given advanced notice about their testing, only the testing authority and those conducting the test receive notice of the athlete selection beforehand (International Standard for Testing and Investigations (ISTI), 2014).

4.3 Sample Collection

After it is determined what tests are necessary, and the testing process begins, samples are collected from the selected athletes. Samples are collected and analyzed based on the analysis

the Technical Document specifies. ADOs always hold the right to have a laboratory perform more extensive testing on a sample than the Technical Document satisfies, and they can also ask the laboratory to perform less extensive testing as long as all of the WADAs requirements are met. The WADA can allow for less extensive tests to be performed if it will lead to the most efficient use of the testing resources available. In its Test Distribution Plan, each ADO needs to outline its strategy for the retention of samples that may need to be tested again at a later date. Samples can be tested again at a later date due to laboratory recommendations, especially in case of the introduction of new detection methods (International Standard for Testing and Investigations (ISTI), 2014).

The authority that collects the sample is responsible for the overall conduct of the Sample Collection Session. The DCO is assigned specific responsibilities. It is the DCO that ensures the athlete is aware of his or her rights and responsibilities and who chaperones the athlete during the process. Once the athlete is made aware of the testing procedure, the Sample Collection Session begins. The collection of urine samples was previously examined during the discussion of the doping control process. The collection of blood samples differs from the collection of urine samples for several reasons. Unlike when collecting urine samples, local standards and regulatory requirements must be obeyed regarding precautions in the healthcare setting. If the sample is going to be used in conjunction with the ABP, only a single sample tube is needed. Samples not being used in connection with the ABP require both an A and a B sample tube. The laboratory will specify the other equipment used. The collection tubes are labeled with a unique sample code so it is clear whom they belong to. The type of equipment to be used and the volume of blood to be collected are specified in the WADAs Blood Collection Guidelines (International Standard for Testing and Investigations (ISTI), 2014). Before collection can begin,

there are several pieces of information that are needed, such as if the athlete participated in training or competition in the last two hours before the sample was collected, whether the athlete has trained or resided at an altitude greater than 1000 meters or utilized altitude simulation within the past two weeks, or whether the athlete has received a blood transfusion within the past three months. If all of the criteria are met, the athlete will select the collection equipment to be used, similar to urine collection. Once the athlete picks a kit and is satisfied it has not been tampered with, collection can begin. The amount of blood collected should be adequate for the tests required. If no on-site testing is required, the athlete observes the sample until it is sealed in a secure, tamper-evident kit. The athlete seals his or her sample in the sample collection kit, following the instructions of the DCO (International Standard for Testing and Investigations (ISTI), 2014). Once the sample is properly sealed, it will be appropriately stored for transport and sent to the relevant laboratory for testing. Once the laboratory receives the samples, analysis for doping can begin.

CHAPTER FIVE: TEST METHODS USED TO DETECT PERFORMANCE-ENHANCING DRUGS (PEDS)

Whenever an athlete is found to be using PEDs or other banned substances, either intentionally or unintentionally, markers of the drugs can be found in biological fluids such as biofluids, urine, blood, and saliva (Cadwallader and Murray, 2015). Many different types of tests and testing procedures have been created to try and detect this doping. As the substances abused and methods of doping change, the tests utilized must be adapted. Currently, there is no single test or method that can scan a sample for every banned substance. Creating a test that could do this would be nearly impossible considering doping methods and substances abused are

constantly changing. Rather than just focusing on getting better at detecting doping and improving detection methods, the athletic and scientific community should work together to better educate athletes on how doping is detected and what the health effects will be. Athletes should understand how their samples are being tested, because if they realize how extensive and accurate the tests are that are being used, they may be less inclined to dope. These testing procedures will be discussed throughout the rest of this thesis. When a sample arrives at the laboratory, a screening assay is used to determine if PEDs or their metabolites are present in the sample, usually urine. If the screening returns a positive result, which indicates the presence of a banned substance, a confirmatory test must be conducted. Screening tests tend to be qualitative; while confirmation tests are quantitative. The type of tests used in both the screening and confirmation procedures depends on the substances being assayed. Peptide hormones are typically screened and confirmed using immunoassay techniques, while the screening and confirmation of stimulants is typically done using gas chromatography (GC) or liquid chromatography (LC) and mass spectrometry (MS) procedures. Gas chromatography-mass spectrometry (GC-MS) is an analytical technique commonly used today, but it was first used as a screening-and-confirmation method at the 1976 Montreal Olympic Games. Since then, these instruments have been updated and improved to increase the capabilities of what they can test for. Simply stated, chromatography is used to separate the different compounds in the sample before it is injected in the MS where the compounds are identified and quantified (Cadwallader and Murray, 2015). If the confirmatory test comes back as positive, another aliquot of the sample may be tested to ensure accuracy. Based on these results, further action may or may not be required.

5.1 Erythropoietin Test Methods

Erythropoietin test methods can be used to detect doping. Erythropoietin (EPO) is a glycoprotein produced by the kidneys, and it is included on the WADA prohibited list under the class of peptide hormones, growth factors, and their analogues (2016 Prohibited List, 2015). Recombinant human erythropoietin (rhEPO), a form of erythropoietin, is one of the most commonly abused substances in sport because it increases red blood cell mass which leads to enhanced aerobic strength, and maximum oxygen uptake and ventilatory threshold. The class of peptide hormones, growth factors, and their analogues was introduced by the IOC in 1989, and since that time there has been no definitive IOC-approved detection method for rhEPO. Immunoassay, which is currently the only direct routine test method, cannot detect abuse because blood and urine rhEPO cannot be distinguished immunologically from endogenous EPO. The current method of measuring the 'critical' haematocrit level is under scrutiny by researchers and may have unjustly damaged athletes by giving false-positive results that ruined their careers and reputations (Breymann, 2000). Until accurate direct methods, such as LC-MS, have been developed for this use, there needs to be indirect ways to test for substances that cannot be directly tested for. Testing the level of other components of the body can help show the possible abuse of substances even when they cannot be directly identified in the body.

Breymann (2000) discusses both direct methods and indirect methods to attempt and detect blood doping. Bioassays and immunoassays can be used to detect EPO in body fluids. Bioassays are not widely used because they are not sensitive and they are prone to interferences. Radioimmunoassays (RIAs) can be used, but they require the utilization of radioisotopes and require incubation, so it takes at least one day to get results, which is a long time by today's standards for a drug test. An enzyme-linked immunosorbent assay (ELISA) was developed

utilizing the same approach of immunological detection and it is now considered the standard measurement method. ELISA is quicker, less expensive, and can quantify low levels of EPO, which would be undetectable using a bioassay. However, despite the different techniques available to detect EPO, rhEPO is still indistinguishable from endogenous EPO because it has the same physiochemical, immunological, physiological, and pharmacological properties (Breymann, 2000).

The time that the peptides hormone EPO resides in the body is so short, that it is impossible to directly detect the recombinant product. Immunoassay is currently the direct method of measurement, however it is unreliable for detecting the abuse of rhEPO by athletes. In research performed by Breymann (2000) and others referenced in his work, the mean elimination half-life of rhEPO was only 42.0 (+/- 34.2) hours and EPO concentrations returned to normal within seven days of the last administration. Results showed that rhEPO doping was only detectable during, or within 4-7 days of ending administration. This means that, "…the erythropoietic effect only became evident when rhEPO was no longer detectable in the blood" (Breymann, 2000). This makes it difficult to prove doping occurred. Blood doping needs to be directly tested for, but if an athlete is tested after the effects of the doping becomes apparent, there will be no evidence left of the doping in their body unless they continually dope. This is one reason why the Haematological Module proposed by the WADA is useful. If ADOs conduct routine blood tests, this type of doping can be more easily identified because it may be tested for whether the athlete is suspected of tis type of doping or not. There are also indirect testing methods that can be utilized as well.

Because EPO exists endogenously and elevated EPO levels can only be detected several days after rhEPO is administered, Breymann (2000) discusses the indirect parameters that have

been introduced to detect doping. These parameters include not only the haematocrit level, but also hypochromic red blood cells and reticulocytes, serum transferrin receptors, and ferritin levels, and in the urine, fibrin degradation products, all of which are markers of functional iron deficiency (FID) during or after the rhEPO administration. EPO is responsible for the differentiation, survival and proliferation of erythroid cells, and rhEPO causes erythroid cells to uptake more iron. Eventually, the amount of iron present is not substantial enough for the numbers of erythroid cells, leading to a deficiency and the release of hypochromic reticulocytes and hypochromic red cells (HRC). FID caused by rhEPO doping is only avoidable if high amounts of iron are administered during the doping period (Breymann, 2000). This means that if an athlete is abusing rhEPO and does not take supplemental iron, FID can be an indicator of doping. The effects of rhEPO are dependent upon the dose, and the schedule and method of administration. Rather than testing for rhEPO directly, which can be unreliable, methods have been created to test for other factors that could indicate rhEPO doping. Currently, official sports organizations have only accepted and employed haemocrit testing as a method to detect rhEPO doping. Changes in haemocrit levels occur due to a change in red blood cell mass, which is ultimately the goal of rhEPO doping (Breymann, 2000). If ADOs look for an increase in red blood cell mass rather than rhEPO directly, which is nearly impossible to do, they could have reason to belief that doping has occurred. This does not count as proof, however it does give them reason to investigate further, which could lead to proof of doping.

Another indirect method that can be used is iron metabolism parameters. The number of transferrin receptors on erythroid cells relates to serum transferrin receptor (sTfR) levels. Periods of iron deficiency and the presence of extracellular iron for stimulated erythropoiesis cause these levels to increase (Breymann, 2000). Because these levels increase during and/or after rhEPO

doping, they can be used as markers of abuse. When rhEPO is abused, fibrin degradation products can be detected in urine due to the fibrinolytic activity of rhEPO. Breymann (2000) discusses a study which states that 10 of the 76 athletes used in the experiment had increased fibrinolytic activity due to rhEPO doping because these degradation products were not elevated in athletes who did not have rhEPO. Even though the indirect methods cannot precisely detect rhEPO doping, they can give results that suggest doping which can give an ADO enough of a reason to begin investigating an athlete if doping is suspected. Indirect test methods should continue to be researched and developed because certain drugs of abuse cannot be directly detected for either at all, or accurately, and there might be a better way to test for them indirectly.

While laboratories work to improve their detection methods, athletes work to improve their strategies to avoid detection. In Delanghe et al. (2014), the authors respond to the Lance Armstrong case, which was relatively recent at the time. Over 250 doping tests came back negative for Armstrong, yet he confessed to erythropoietin use, blood doping, steroid, and growth hormone abuse. This illustrates the restrictions of current laboratory tests that are used to confirm doping in sport. Despite the doping controls and indications of doping abuse among professional athletes in the past twenty years, the number of urine tests that are positive for rhEPO remains surprisingly low (Delanghe et al., 2014). Some of these reasons for this are discussed above, such as the lack of an official direct test for rhEPO. Along with this lack of adequate testing, athletes use various masking strategies, such as protease inhibitors, intravenous injections of rhEPO, and alternative erythropoiesis stimulating agents to avoid being detected by common drug tests. Mechanisms such as high altitude and low-oxygen training can be used to increase endogenous EPO production, and although this is currently considered an acceptable tool, this may cause problems in the future. If the WADA looks to ban this type of training, there

would be no way to test for this banned method because it only raises endogenous levels (Delanghe et al., 2014). There is also concern regarding the addition of protease to urine samples, which could mask EPO use. Adding protease inhibitors to the athletes' samples before they are supposed to be gathered for sampling could be used to prevent the destruction of peptide hormones. Protease activity could also be assayed in urine samples, and its presence could suggest potential masking of EPO doping (Delanghe et al., 2014).

The resemblance of so many substances, such as rhEPO, to endogenous factors is another reason the ABP can be useful. If endogenous levels of EPO are routinely monitored and they remain fairly constant, but then there is a sudden spike, it could be due to rhEPO doping. The ADO may want to monitor this athlete more closely and they will be more likely to catch them doping. However, if rhEPO is used in small dose and taken outside the time of normal testing hours, EPO values are likely to fall within the ABP range the next day. A solution to this could be to use an EPO assay (MAIIA diagnostics), which is currently used by the WADA. The WADA claims that a microdose injection administered the evening before the test and up to about 48 hours after the injection can be detected using this assay. The assay is a combination of an EPO sensitive immunoassay and chromatography into one device (Delanghe et al., 2014). Despite the advantages of using the ABP, discrepancies can be introduced because different parameters may exist between laboratories. Variations can be caused by seasonal effects, temperature, differences in sampling strategy, and variations in laboratory techniques among other factors. In order for the ABP to be effective, sampling conditions should be kept consistent (Delanghe et al., 2014). It is crucial that variations due to the laboratory are not mistaken for intentional doping.

Another performance-enhancing tactic being used is to return to older doping techniques,

such as autologous blood transfusions. Direct detection methods have yet to be established by the WADA, but indirect methods have been suggested that are based primarily on fluctuations in erythropoiesis-sensitive blood markers that are based on different red blood cell (RBC) indicators. One indirect strategy used for detecting blood doping is to detect transfusion-induced immune response, which results in specific changes in gene expression, related to leukocytes such as T lymphocytes (Delanghe et al., 2014). During the storage of the blood that is used for doping, plasticizers from the blood bags may leak into the blood. After the transfusion occurs, detection of these plasticizer metabolite levels can be detected in the urine (Delanghe et al., 2014). These metabolite levels would not be seen in a natural blood sample. This is another way to indirectly detect blood doping. Despite the advancements being made technologically to directly detect doping, indirect methods prove quite useful as well. Armstrong is not the only athlete who has manipulated the drug testing process to get away with doping, and he will not be the last. Some athletes use masking techniques to hide their doping, while others use low enough doses so they do not get caught. The testing procedures used in drug testing are not fool proof and they will not catch every cheating athlete. It takes the athletic community working alongside and cooperating with the ant-doping laboratories and those conducting research on doping in sports to try and fix this problem. Despite the efforts being made to improve testing, there will always be athletes who are willing to dope and try and cheat the system of fair competition. Because drug-testing procedures are not always 100% effective on their own, it is important that we look at other options to combine with drug testing to reduce doping in sport. Athletes need to understand the advancements being made in the field of doping detection so they know just how hard the athletic community is working to detect and deter doping.

5.2 Gas Chromatography And Mass Spectrometry (GC-MS)

One of the most commonly employed testing methods utilized today is GC-MS. GC-MS techniques can provide confirmatory evidence for the presence of drugs and their metabolites in forensic urine drug testing. When effectively trying to detect drugs in urine, analysis should involve an initial screening procedure to exclude negative samples, selection of presumptive positive samples, and a highly specific confirmatory test that can confirm presumptive results. GC-MS can be used as a sensitive confirmatory technique. Combining the separation versatility of GC with the specificity and sensitivity of MS makes it one of the most impressive techniques for identifying organic compounds. GC is used to perform the separation of complex mixtures. It is fast, sensitive, highly versatile, and hundreds of different compounds can be separated in s single analysis. MS is utilized to provide the identification of structural compounds (Lehrer, 1998). This is why it is important that GC effectively separate the different compounds. If the compounds are not separated when they reach the mass spectrometer, the mass spectrometer will not be able to yield a strong positive identification. After compounds are separated by GC, the sample is converted into ions, molecules, and molecular fragments by bombarding them with electrons and by colliding them with each other. These charged particles are then moved through an electric or magnetic field where they are separated from each other based on their mass-to-charge (m/z) ratios by a mass filter. A quadrupole filter is commonly employed, which produces an oscillating field that alternates between specific radiofrequencies. At specific radiofrequencies, the ions are separated based on their m/z ratio (Lehrer, 1998). The detector records the ions formed and their relative abundance to create a data display. Results are displayed with the m/z value on the x-axis and the relative intensity (%) on the y-axis. The peak with the highest intensity is known as the base peak and the peak with the highest m/z is the

molecular ion peak. The molecular ion peak represents the mass of the whole compound before it was separated. Each peak on the spectrum produced represents a different fragment and its abundance. The identity of these peaks can be determined by searching them in libraries uploaded into the system that contain reference spectra. If a prohibited substance is identified in a urine sample at a concentration that is above the acceptable limit, it can be confirmed that an athlete has doped.

5.2.1 Ion Trap Mass Spectrometer

Different detectors can be used based on the type of MS analysis being performed. An ion trap mass spectrometer combines the functions of an ion source and a mass analyzer. A heated filament releases electrons that are pulsed into the central cavity by a gate electrode. Here, they ionize sample molecules, which results in electron ionization (EI) fragmentation patterns characteristic of the present compound. What makes the ion trap mass spectrometers unique is that they trap and then store the produced ions over time in the ion source cavity. The trapped ions are then selected based on their m/z ratio onto the electron multiplier where they can be detected and a mass spectrum can be produced. Utilizing the ion trap detector (ITD) provides high sensitivity because trapping the ions allows for the accumulation of the ions of interest. This results in a greater concentration of the ions of interest and therefore greater specificity. The sensitivity of the ITD enables anti-doping laboratories to acquire full scan mass spectra, even when testing smaller quantity samples (Lehrer, 1998). This is extremely useful when testing urine samples that may contain substances of abuse. Doping may go undetected because the abused substance is present at such a low level. Utilizing ITD can identify these compounds that are present at low levels and prevent a false-negative test. Because of its sensitivity, ITD can also

help prevent false-positive results.

5.2.2 Full Scan Analysis In Electron Ionization (EI) Mode

One of the advantages to using GC-MS is the flexibility it provides. The analyst can use different modes of operation, instrumentation, and different analytical methods depending on what is being tested for. One method commonly used is full scan analysis in EI mode. This analytical technique can provide the definite identification of a drug or drug metabolites. When full scan mode is used, the mass range selected is repeatedly scanned and the mass spectra produced show the m/z ratios versus the relative intensity (%) of the ions (Lehrer, 1998). This method provides a high degree of specificity. EI works by bombarding vaporized samples with high-energy electrons. This produces molecular ions with different molecular weights. A great amount of fragmentation occurs, so again, the detector plots the ions based on their m/z ratios versus their relative intensity (%) (Lehrer, 1998). This fragmentation is unique for each compound and therefore will lead to positive identification of the compounds present. This combination of methods gives the best identification for a sample of high purity, such as most drugs abused in sports and their metabolites. It allows a wider range of drugs to be detected.

5.2.3 Selected Ion Monitoring (SIM) Mode

Another method that can be utilized is the selected ion monitoring (SIM) mode. The analyst selects a few intense masses that are characteristic of the compound they are looking for before the analysis is performed. This causes the mass spectrometer to only monitor the ion currents that are present at these preselected masses (Lehrer, 1998). This is useful if testing an athlete for a specific drug. The most common ions of the suspected drug are selected to be tested

for and by focusing on just a few ions, sensitivity can be greatly increased. However, because all of the data is not being taken into consideration, specificity is lost. SIM provides greater sensitivity, but a less specific identification than full scan mode (Lehrer, 1998). Analysts must take this into consideration when they deciding what type of method and mode they want to use for testing a sample. If an athlete were suspected of using high doses of a single prohibited substance, SIM would be a good mode to use. It would also be appropriate to use if testing for a drug that is commonly abused in a sport, such as steroids in baseball. However, if an athlete is suspected of doping, or a routine urine test is being performed, full scan mode may be more appropriate because it can better detect and identify a wider array of substances.

5.2.4 Chemical Ionization (CI)

Another instrumental option is to use chemical ionization (CI). This method helps to increase identification specificity. One of the most important pieces of information to be gained from mass spectra is the molecular weight. This information can be gained by looking at the molecular ion peak. When a sample is broken up into ions, some of the sample may not be broken up; therefore the peak is representative of the whole sample. This peak has the highest m/z value. When using EI, the sample tends to be completely bombarded and therefore there is no molecular ion peak and the molecular weight cannot be easily determined (Lehrer, 1998). CI is a softer technique that does not break the sample into as many ions, therefore maximizing the number of molecular ions. The mass spectra produced by this method contain very few peaks of a higher m/z. When using CI mode, electrons are emitted from the filament and ionize the reagent gas (eg. methane), as it is introduced into the ion source. As the reagent gas molecules collide with the sample, a charge is transferred from the reagent gas to the sample molecules

(Lehrer, 1998). The sample molecules are not bombarded with electrons like they are during the hard ionization process of EI. This allows the sample molecules to become charged without breaking them apart and therefore molecular ion peaks can be produced and the molecular weight can be determined.

5.2.5 Problems That Arise When Using GC-MS And Possible Solutions

Problems can arise when using these techniques because it is possible that incorrect identification and therefore false-positive tests may occur. For example, a protocol using SIM that was published by Hewlett-Packard suggests laboratories analyze amphetamines using m/z 44 as a quantification ion and m/z 58 as a qualifying ion (Lehrer, 1998). However, the ions chosen for this method cause problems that can affect identification. The m/z values chosen are low and they are subject to background interference. This can distort peaks and cause misidentification. Other significant identification problems can be caused because many common legal drugs, such as ephedrine, produce the same ion fragments as those that were selected to identify illegal amphetamines (Lehrer, 1998). An athlete who is taking a legal over-the-counter medication could potentially test positive for a prohibited substance, which could put their career and reputation at risk. Lehrer (1998) states, "In conclusion, it can be surmised that SIM techniques have specificity pitfalls, and that these hold the potential for serious errors." It is essential that athletes understand how important it is to document everything they put into their body. If an athlete documents that they are taking an over-the-counter medication, along with the dosage and how often they take it, it might help to save their career. It is also important for them to make others aware of any medication they may be taking, such as a trainer or coach, so they can have support if a drug test comes back as positive and is believed to be a false-positive. It is

critical for athletes to understand how these testing procedures work so they can understand not only how accurate and specific these tests can be, but also the downfalls of these tests as well to ensure they are not wrongfully punished.

A possible solution to this problem is to use CI data to supplement EI data. CI data may be more specific and yield greater accuracy of results. For example, methamphetamine and phentermine are structural isomers of each other, however methamphetamine is an illegal drug and phentermine is not. The EI mass spectra produced for these two compounds are almost identical, so identification based on full scan EI data or SIM is unable to distinguish between the two compounds. The GC retention times of these two compounds are similar as well, so this cannot be used to distinguish between them either (Lehrer, 1998). This makes the possibility of a false-positive result for methamphetamine very real. If an anti-doping laboratory were to only run this test on an athlete's urine sample and it came back as a positive result, the athlete could challenge the test method used. However, in order to challenge this and ask for their second sample to be tested with a different method, the athlete would have to be educated on the advantages and disadvantages of these different methods. In this case, an athlete could request that the CI spectra of the urine sample be reviewed as well. Methamphetamine and phentermine can be easily distinguished by looking at their CI spectra due to the presence of a significant ion peak at m/z 133 in phentermine, which is absent in the methamphetamine spectra (Lehrer, 1998). CI removes the possibility of a false-negative in this case. As important as it is for anti-doping laboratories to use proper techniques and appropriate methods depending on the sample being tested and what it is being tested for, it is just as important for athletes to understand it as well. Athletes should not blindly send samples off for drug testing without knowing what actually happens when a sample is tested. If an athlete understands the entire process of sample testing,

they may be less likely to dope because they understand how accurate tests can be, or they may be better able to defend themselves if a false-positive test does occur.

5.3 Detection Of Stimulants And Narcotics Using LC-MS And GC-MS

Anti-doping laboratories are required to test for and detect several classes of compounds that are prohibited by the WADA at all times. These include anabolic agents, peptide hormones, growth factors, beta-2 antagonists, hormones and metabolic modulators, and diuretics/masking agents. Other classes of compounds are only banned during competition, and these include stimulants, narcotics, cannabinoids, and glucorticoids (Ahrens, Kucherova, and Butch, 2016). A single class of compounds can contain many different prohibited substances, and Ahrens, Kucherova, and Butch (2016) feel that all of the stimulants and narcotics on the WADA prohibited list should be able to be tested for with one procedure. The authors describe a combined liquid chromatography-tandem mass spectrometry (LC-MS/MS) and GC-MS testing method that can detect all of these prohibited compounds (Ahrens, Kucherova, and Butch, 2016). This article is of extreme importance because it outlines the procedure for a method that can test for all of the stimulants and narcotics on the WADA prohibited list. The utilization of this procedure will help cut down on the time needed to test samples, since one test will be performed rather than several, and it will yield more accurate results since it is testing for a wider array of substances. Abuse of a prohibited substance in sport can go undetected if the right substance is not tested for. Typically, a sample is not tested for all possible prohibited substances, rather it is tested for specific substances that tend to be abused in a specific sport, or a specific substance that an athlete is suspected of using. However, this procedure would make it easier to detect doping since it can test for all substances that are a part of a class of compounds. Although this

article only outlines the use of LC-MS/MS and GC-MS for the detection of prohibited stimulants and narcotics, there is reason to believe it could be adapted to test for every class of compounds.

5.4 Detection Of Diuretics Using Metabolites

Diuretics and masking agents are another class of compounds that are on the WADA prohibited list. These compounds can be used to increase the excretion of other banned substances, and mask their use. If diuretics or masking agents are detected, there may be reason to believe that further doping has occurred, even if another substance cannot be detected. Diuretics and masking agents will be discussed in more detail at a later point. Tolvaptan is classified under class S-5 diuretics and masking agents on the WADA prohibited list. There is limited knowledge concerning the metabolism of tolvaptan and the excretion of its metabolites in humans, however, it is known that less than 1% of the administered dose is actually excreted in urine (Rzeppa and Viet, 2016). This can make its detection in urine samples quite difficult. Rzeppa and Viet (2016) performed a study aimed at developing a quick and simple method for detecting tolvaptan and its metabolites in urine samples using a high-performance liquid chromatography coupled to mass spectrometry (HPLC-MS/MS) approach. Their goal was to extend the detection window of tolvaptan by detecting and identifying specific metabolites (Figure I), which stay in the body longer, and to combine the study's results with routine doping analysis-screening methods. The experiment involved the analysis of ten doping-free samples, and ten samples spiked with varying concentrations of tolvaptan (0.2, 5, 100, 200, and 500 ng/mL) by HPLC-MS/MS. The blank samples showed no presence of tolvaptan, while all of the spiked samples showed peaks characteristic of tolvaptan. These samples were used for validation. In order to detect and identify metabolites, a male subject was administered a 15mg

dose of tolvaptan and urine samples were collected for analysis. Tolvaptan was identified in the urine by comparing the data to reference material. Samples of the excreted urine were analyzed for the presence of known metabolites (Figure I) and product ion scans of the calculated molecular ions of the metabolites and precursor ions scans of the most abundant fragment m/z 252 were performed for identification. Precursor ions are ions of a specific m/z ratio that are selected to be used to compare to product ions, or the resulting fragment ions, to identify them. At least two monohydroxylated metabolites that differed in the position of the hydroxyl group (metabolite group 1) and one carboxyl metabolite (metabolite 3) were identified. The signals for metabolite 2 showed only low intensities and therefore it would be of little relevance for the detection of tolvaptan for doping control reasons.

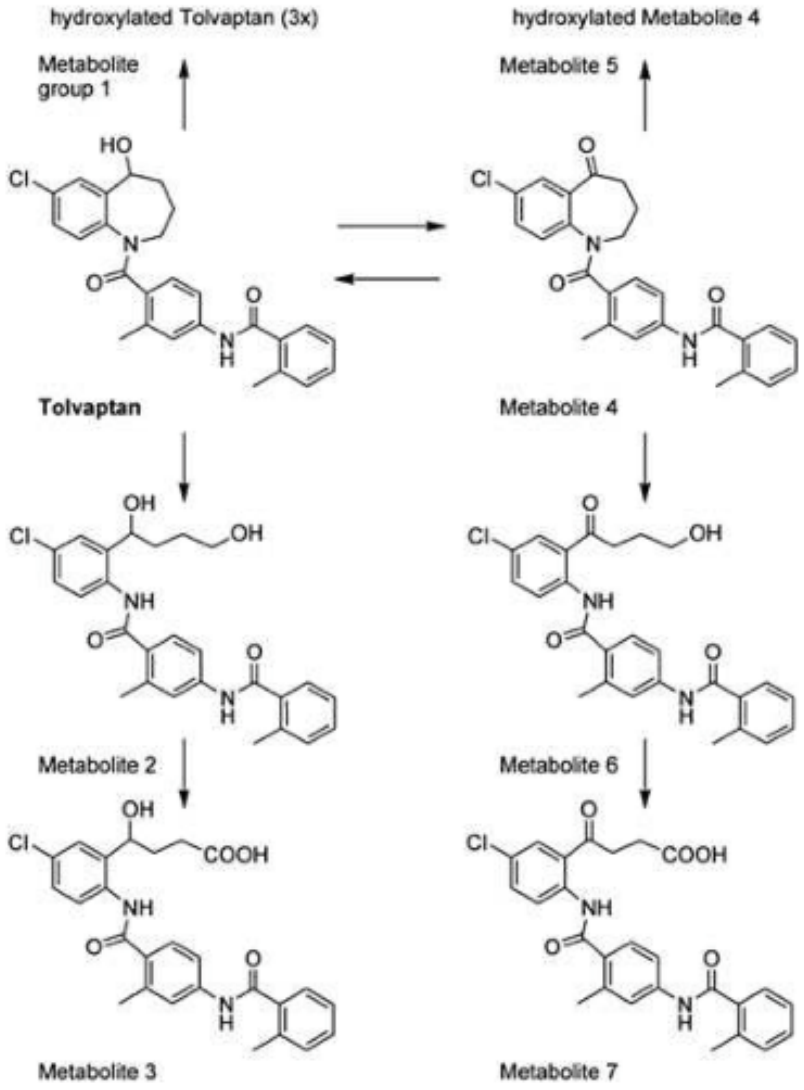

Figure I- Metabolites of tolvaptan (Rzeppa and Viet, 2016)

Metabolites 4,5,6, and 7 contain a keto function rather than a hydroxyl function, and they could

either not be identified or were only found in trace amounts. Figure II shows the HPLC-MS/MS chromatograms of tolvaptan and its metabolites in the human urine sample before the administration of tolvaptan and three and 120 hours after administration. Tolvaptan itself cannot be detected at 120 hours, however two of the selected metabolites can be. Figure III shows that tolvaptan can be detected in excreted urine samples up to 24 hours after administration, metabolite group 1 up to 120 hours, and metabolite 3 up to 150 hours (Rzeppa and Viet, 2016). If anti-doping laboratories were not able to test for metabolites, there would be a very small detection window for a substantial number of prohibited substances, such as tolvaptan.

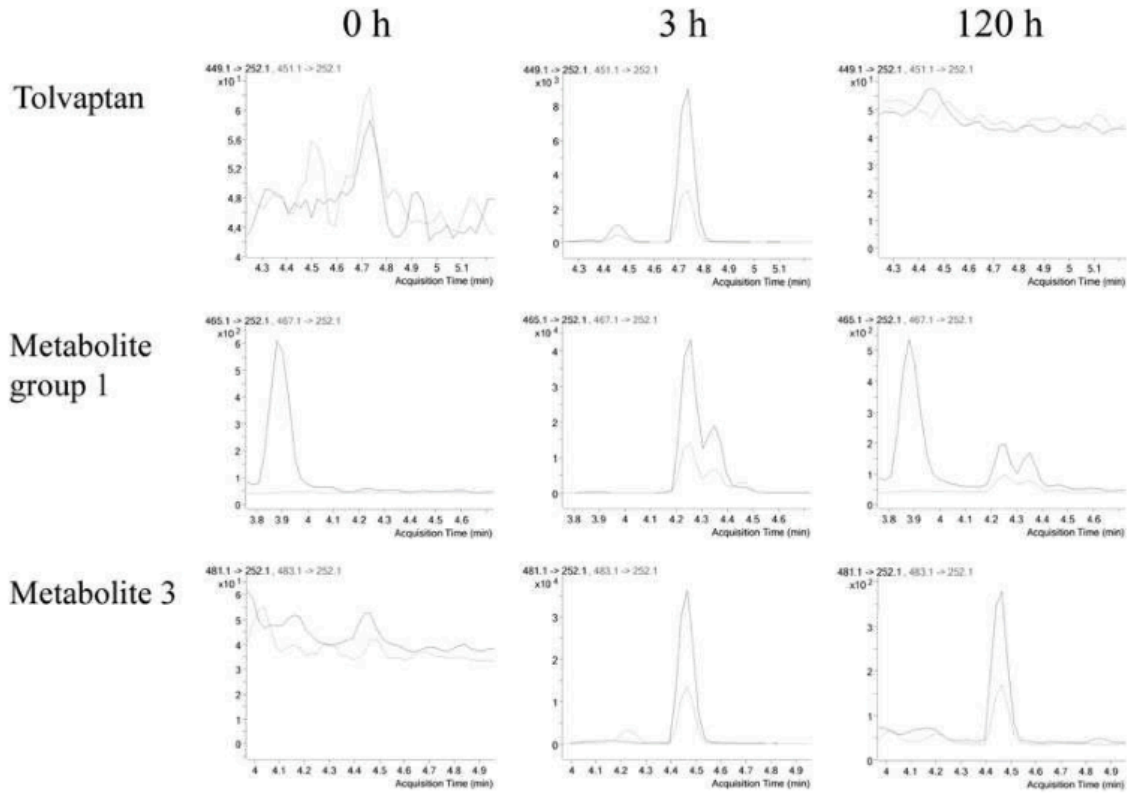

Figure II- HPLC-MS/MS chromatograms of tolvaptan and two of its metabolites, metabolite group 1 and metabolite 3, before administration, and 3 and 120 hours after administration of one single oral dose. Transitions for ^{35}Cl isotope (black) and ^{37}Cl isotope (gray) are present (Rzeppa and Viet, 2016)

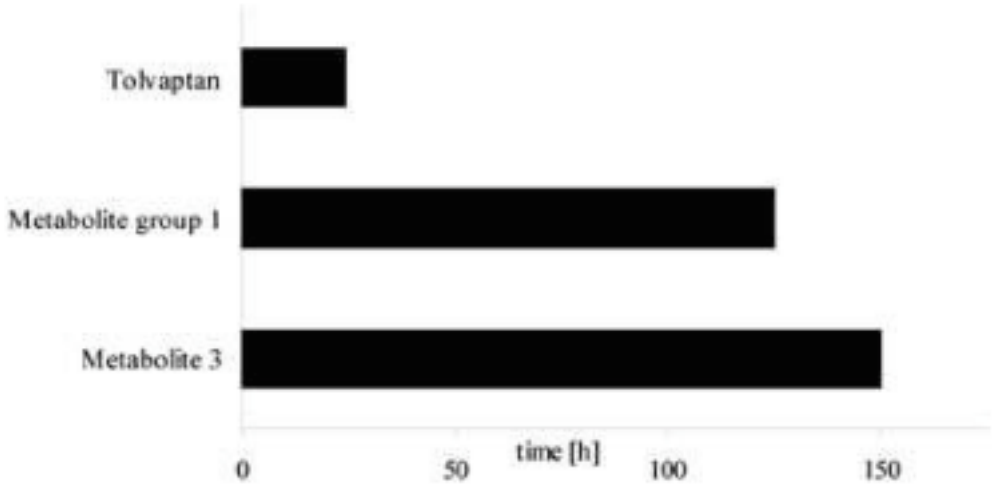

Figure III- Detection times of tolvaptan and its metabolites in human urine after administration of a 15mg dose to a male subject (Rzeppa and Viet, 2016)

Identifying metabolites and learning how long they can be detected in excreted urine gives anti-doping laboratories a great advantage. Diuretics like tolvaptan tend to have no enhancing effect on performance, however they can mask the administration of other drugs. Therefore, long-term detection methods can be of great value. Identifying the metabolites of other drugs could be useful as well because this may extend their detection time. This would give anti-doping laboratories more time to catch athletes who are doping since the metabolites stay in the athlete's system longer. It is crucial for athletes to know that proof of doping can be verified hours and even days after they have taken a prohibited substance, even if it was only a single dose, due to methods that can detect metabolites and identify which substances they came from.

5.5 Characterization Of Selection Androgen Receptor Modulators (SARMs) Using MS

The WADA has ranked anabolic agents at the top among statistics of adverse analytical findings for years now. Besides the conventional anabolic-androgenic steroids (AAS), alternative substances that have similar effects in regard to bone and muscle anabolism have been sought after. A prominent developing class of drugs is the chemically heterogeneous group of selective

androgen receptor modulators (SARMs) (Thevis et al., 2013). Some of these have been detected in doping control samples over recent years. Thevis et al. (2013) highlight the importance of expanding the proactive and preventative measures among anti-doping laboratories. It is important to analytically characterize substances that may potentially be misused, especially since adverse analytical findings have reported the abuse of SARMs in professional sports in recent years. In the study presented, the SARM candidates RAD140, a benzonitrile-oxadiozole-based substance, and ACP-105, a tropanol-derived SARM drug candidate, were reviewed in regards to their mass spectrometric behavior under tandem mass spectrometry with electrospray ionization (ESI-MS/MS) or electron ionization (EI-MS/MS) (Thevis et al., 2013). Both of these methods are commonly employed by anti-doping laboratories, and the analytical data provided supports the identification of the SARM candidates and related structures, such as metabolites and designer analytes, in specimens being tested for prohibited substances. The study provides proposed dissociation pathways of both ACP-105 and RAD 140 under both positive ESI-CID and EI conditions (Thevis et al., 2013). Providing the dissociation pathways of these SARMs can help support future drug testing methods and aid in the identification of related compounds and/or metabolites since their structures may be related to a structure of one of the analytes in the dissociation pathway. Understanding and being able to identify known substances, their metabolites, and substances with related structures will help anti-doping laboratories be better able to identify newly synthesized or less common substances. This will make them better equipped to catch doping athletes.

5.6 Detection Of Non-Prohibited Drugs In Human Urine Using LC-MS

Although it is crucial for anti-doping laboratories to be able to identify banned

substances, it is also important for them to be able to detect commonly used non-banned substances because this may indicate that the substance has performance-enhancing effects. The WADA prohibited list is referred to as an open list, meaning that although specific examples of substances are given for each class, other substances with similar chemical structure or pharmacological activity are banned as well. Anti-doping laboratories should have a method to quickly and easily identify these related compounds and what drugs they may be in so athletes can avoid taking them. It is also important to detect these types of substances because they may be illegally produced and distributed by non-approved laboratories and not even be approved for therapeutic human use (Mazzarino et al., 2016). In this case, an athlete could be taking potentially harmful drugs without knowing, or taking drugs that could have negative health effects but not give the athlete any of the desired performance-enhancing effects. WADA-accredited anti-doping laboratories are constantly on the lookout for new substances or classes of substances to include on the prohibited list. When the newly banned compound(s) is added, there is an immediate need to develop and validate procedures that can detect the illicit use of the newly prohibited substance. Not only do methods have to follow strict accreditation procedures, but also the metabolism and rate and route of elimination of the new compound need to be established so the appropriate biological fluid can be selected for testing, the optimal time of testing with respect to competition (in or out), and diagnostic markers for its administration (Mazzarino et al., 2016).

The main reasons to consider non-banned substance for inclusion on the prohibited list are that according to information provided by doping control forms, their use in sports increased, scientific evidence was discovered that they had a direct or indirect effect on sports performances, and that they demonstrated the ability to interfere current anti-doping analytical

methods currently used by anti-doping laboratories. A procedure now exists and has been validated by ISO and meets WADA requirements for use by anti-doping laboratories to simultaneously detect 7 selective serotonin reuptake inhibitors, 4 azole antifungal drugs, 19 benzodiazepine-like substances, 5 inhibitors of the PDE type 4, and 3 inhibitors of PDE type 5 in the urinary matrix by use of a LC-ESI-MS/MS method (Mazzarino et al., 2016). The substance classes above contain the most common non-banned drugs currently used by athletes and drugs that are suspected to be performance-enhancing and/or act as masking agents when used in certain settings. The agents described can be included in traditional LC-MS/MS multi-analyte screening procedures to detect different classes of banned compounds: 30 diuretics, 17 glucocorticoids, 6 anti-oestrogenic agents, 4 SARMs, 7 synthetics cannabinoids, 2 beta-adrenergic agents, 5 designer steroids, 3 narcotics, 2 metabolic modulators, and 9 stimulants (Mazzarino et al., 2016). Utilizing this method would allow anti-doping laboratories to gain significant information on the abuse of the aforementioned classes of drugs by athletes. If information can be obtained on these substances that give laboratories reason to believe they may have performance-enhancing or masking capabilities, it is possible they should be banned. It would also be beneficial to see how many samples these substances were detected in, so it can be estimated how many athletes are using them. If they were not advantageous to athletes and benefiting their athletic performance, they would not be taking them. The athletic community should stay informed on what classes of substances and individual compounds may be likely to be banned so athletes can avoid them and similar drugs.

CHAPTER SIX: UNINTENTIONAL DOPING

When doping analyses are performed, we often expect to get a straightforward positive or

negative result. However, this is not always the case. The possibilities of unintentional doping, false-positive results, and false-negative results all have to be taken into consideration. Unintentional doping can occur because an athlete is passively exposed to a banned substance or because they unknowingly ingest a food or product containing a banned substance. False-positives can occur because certain foods or other products give a positive result for drug tests because they may be derived from the same plant or other source as a drug.

Professional athletes, and often athletes at lower competitive levels, are told they are responsible for what they put in their body whether they know it is illegal or not. But, if an athlete genuinely unintentionally dopes, is it really worth ending their career over? And if they claim unintentional doping, how can it be proven whether it was or not? In many cases a hearing can be held if an athlete claims to not know why a prohibited substance was found in their system, and in some cases, further tests can be performed to distinguish between the presence of banned substances, and substances that give false-positive results. However, it is much easier to educate athletes on the substances that are prohibited and how to protect themselves from unintentional doping to prevent the doping from happening in the first place. Jeffrey Anderson, MD (2011), evaluated the athlete's claim of an unintentional positive urine drug test and how this unintentional doping can occur. A very commonly abused prohibited substance is marijuana. There are cases of athletes having a positive urine test for marijuana, who claim to have not intentionally inhaled or ingested the drug. Anderson (2011) states that although the exposure must be dramatic and occur within close timing to the test being performed, it is possible for an athlete to give a weakly positive urine test for marijuana from passive exposure. However, in this case the athlete would most likely be aware that they were being exposed to the drug since they would to be in extremely close proximity to the source and inhale a high concentration. Athletes

should be extremely careful about putting themselves in a situation where passive exposure to a prohibited substance may occur.

6.1 Unintentional Doping Due To Poppy Seed Consumption

Another major cause of unintentional doping that Anderson (2011) discusses is the ingestion of food products that contain prohibited substances. The classic example of a false-positive and unintentional positive urine test for opiates is the ingestion of poppy seeds. Multiple studies have shown that the ingestion of poppy seeds can result in a positive urine drug test for morphine and codeine. The athlete may or may not be aware they are consuming poppy seeds and that consuming a large amount can result in a positive test for morphine or heroin. However, the ingestion of poppy seeds should not result in a positive test for more than several hours after ingestion. A way to distinguish between a positive test caused by the presence of morphine from poppy seeds and a positive test caused by opiates has been determined, but this test is not always performed. The test works by detecting the presence of thebaine, which is present in poppy seeds, but not in illicit drugs, such as heroin and morphine. The testing authority will take into consideration the variables surrounding the positive test and if unintentional doping is suspected, they can determine if further testing should be performed (Anderson, 2011).

In 1998, the IOC announced the cutoff limit for morphine would be 1 μg/mL. As stated above, concentrations of opiates, such as morphine and codeine, may be present in urine after the consumption of poppy seeds. This could cause an athlete to give a false-positive urine test for the presence of opiates. A quantitative analysis of morphine and codeine present in human urine after the ingestion of cakes that contained commercially available poppy seeds was performed in order to assess the possibility of positive doping results (Thevis, Opfermann, and Schanzer,

2003). Eight products were obtained from different manufacturers (Table I) and they were analyzed by gas chromatography-mass spectrometry (GC-MS) to determine the morphine content. A batch of poppy seeds with a high morphine content (number 3, Table I) was selected and used as an ingredient in a typical cake. Nine volunteers ingested the cake and were involved in an excretion study. The single pieces of cake were precisely prepared, so the amount of poppy seed intake was known, and therefore the quantity of orally administered morphine was known as well (Table II).

Table I. Products Investigated for Possible Morphine Contamination, Place of Acquisition, and Analyzed Morphine Content

No.	Trade name/ composition	Store	Origin	Morphine content (µg/g)
1	Neuform®	health food shop	Denmark	8.4
2	Neuform®	health food shop	Hungary	6.9
3	Müller´s Mühle®	supermarket	unknown	151.6
4	Insula®	supermarket	unknown	2.1
5	FJD®	supermarket	unknown	4.1
6	Rapunzel®	health food shop	Turkey	0.8
7	Davert®	health food shop	Turkey	0.9
8	baking mixture	bakery	unknown	0.6

Table I (Thevis, Opfermann, and Schanzer, 2003)

Table II. List of Volunteers with Details of Oral Intake of Poppy Seeds

Volunteer	Gender	Body weight (kg)	Poppy seed intake (g)	Morphine intake (mg)	Duration of application (min)
V1	female	62	12.5	1.6	20
V2	male	79	16.8	2.2	10
V3	female	68	16.3	2.1	20
V4	male	81	29.9	3.9	40
V5	male	77	31.9	4.2	60
V6	male	88	30.5	4.0	45
V7	male	83	36.4	4.8	30
V8	male	70	38.1	5.0	60
V9	male	67	52.3	6.8	120

Table II (Thevis, Opfermann, and Schanzer, 2003)

An HP 5890 gas chromatograph interfaced to an HP 5971 mass selective detector was used to perform the analyses of the urine or poppy seed samples (Thevis, Opfermann, and Schanzer, 2003). Table II shows that it would be possible for athletes to test positive for morphine in doping tests after the consumption of products containing commercial poppy seeds since the morphine concentration is above 1 μg/mL. In Table III, the concentration of morphine (μg/mL) is shown for every urine sample collected from each volunteer over an extensive time period. Some athletes claim the "poppy seed defense" when they test positive for morphine. However experiments done by others, such as by Cassella et al. (1997), investigate the presence of thebaine in urine samples of poppy seed eaters and true opiate abusers to distinguish between the two analytes and a false-positive or true positive result (Thevis, Opfermann, and Schanzer, 2003).

6.1.1 Utilizing Thebaine As A Marker For Poppy Seed Consumption

Thebaine, which is a natural constituent of poppy seeds, was investigated as a possible marker for poppy seed consumption by Cassella et al. (1997). Spice Time® Foods, Inc. poppy

seeds were obtained and a dozen poppy seed muffins were prepared using 132g of the poppy seeds and a boxed mix (Krusteaz® low-fat lemon poppy seed mix), resulting in 11g of poppy seed per muffin. Baseline urine samples were collected from nine volunteers before the consumption of any poppy seeds to rule out that the volunteers did not already have any drugs in their system that could affect the results. The volunteers then consumed 1-3 of the muffins containing poppy seeds. Urine samples were then collected from every subject at a range of times from 2 to 6 hours after consumption (Cassella et al., 1997). All of the urine samples were screened using the EMIT II immunoassay for opiates on a BM/Hitachi-717 analyzer. The samples that gave a positive result for opiates by immunoassay, and some of the negative samples, were assayed by GC-MS for the presence of thebaine. The EI mode was used for the MS, with mass-to-charge (m/z) data collected from 70 to 450 amu at a rate of 6.7 scans/sec.

Table III. Concentrations of Morphine in Urine Samples of Volunteers after Oral Intake of Poppy Seeds*

V1		V2		V3	
Morphine application: 1.8 mg		Morphine application: 2.4 mg		Morphine application: 2.5 mg	
Time after application (h)	Conc. (µg/mL)	Time after application (h)	Conc. (µg/mL)	Time after application (h)	Conc. (µg/mL)
2.00	0.06	2.00	**1.50**	2.50	**1.76**
4.00	0.79	4.25	**5.88**	4.50	**2.75**
6.00	0.93	6.25	**3.55**	6.25	**2.61**
8.25	**1.12**	8.00	**1.03**	9.50	0.78
11.00	**1.93**	9.50	0.37	10.25	**1.04**
12.75	0.83	12.00	**1.53**	13.50	0.19
21.25	0.50	21.00	0.74	21.50	0.34
24.00	0.40	24.00	0.31	24.50	0.70
46.00	0.23	46.00	0.23	46.00	0.34
V4		**V5**		**V6**	
Morphine application: 4.7 mg		Morphine application: 4.5 mg		Morphine application: 4.4 mg	
Time after application (h)	Conc. (µg/mL)	Time after application (h)	Conc. (µg/mL)	Time after application (h)	Conc. (µg/mL)
2.00	**1.40**	2.00	**2.96**	2.00	0.55
4.00	**3.23**	4.00	**5.76**	4.00	**3.46**
6.00	**3.91**	6.00	**1.98**	6.00	**3.53**
8.00	**3.08**	8.00	0.71	8.00	**2.44**
10.00	**2.49**	10.00	**1.72**	10.00	**1.73**
14.25	**1.95**	12.00	**1.60**	14.50	**1.14**
21.75	**1.50**	21.75	**1.46**	19.75	0.84
24.50	0.99	24.00	0.84	24.00	0.83
48.00	0.28	46.25	0.20	43.75	0.24
V7		**V8**		**V9**	
Morphine application: 5.3 mg		Morphine application: 5.6 mg		Morphine application: 7.7 mg	
Time after application (h)	Conc. (µg/mL)	Time after application (h)	Conc. (µg/mL)	Time after application (h)	Conc. (µg/mL)
2.00	**1.39**	2.25	**2.57**	2.00	**2.14**
4.00	**4.50**	4.00	**8.36**	4.00	**5.25**
6.00	**5.90**	6.00	**10.04**	6.00	**5.88**
8.00	**5.16**	8.25	**9.54**	8.00	**5.09**
10.00	**3.50**	12.00	**6.92**	10.00	**4.55**
12.00	**2.97**	21.00	**3.63**	20.00	**2.22**
14.75	**1.65**	24.00	**1.25**	24.00	**1.17**
21.00	0.79	47.50	**1.21**	44.00	0.25
24.00	0.43				
45.00	0.36				

* All values higher than 1 µg/mL are bolded and would represent positive test results according to the IOC rules.

Table III (Opfermann, Schanzer, and Wilhelm, 2003)

The Finnigan Magnum software program was used to reconstruct the ion chromatograms of thebaine (m/z 311), codeine (m/z 299), morphine (m/z285), cocaine (m/z 182), and 6-ECO (m/z 327) (Cassella et al., 1997). The results of the GC-MS analysis of the urine samples can be seen in Table IV.

Sample No.	Subject No.	Muffins consumed	Collection time (h)	Screen result	Codeine (ng/mL)	Thebaine (ng/mL)	Morphine (ng/mL)
1	1	2	8	positive	5.6	5.4	331
2	1	2	6	positive	6	8.7	318
3	1	1	8	negative	nt*	nt	nt
4	1	2	12	negative	nt	nt	nt
5	2	2	6	positive	9.4	12.1	nt
6	2	2	8	positive	0	0	nt
7	3	na	na	positive	4.5	3	471
8	3	1	na	negative	0	0	nt
9	4	na	na	positive	1	0	nt
10	4	1	na	negative	0	0	nt
11	5	na	na	positive	0	0	nt
12	5	1	na	positive	<1	0	129
13	6	3	2	positive	48.9	59.5	4776
14	6	3	4	positive	19.7	81.3	3252
15	6	3	6	positive	8.8	38.2	925
16	6	3	8	positive	7.8	36	855
17	6	3	10	positive	7.2	36	648
18	6	3	12	positive	1.6	3.5	508
19	6	3	24	negative	0	0	185
20	6	3	32	negative	0	0	196
21	6	1	na	negative	0	0	nt
22	7	1	6	positive	3	2.6	251
23	8	1	4	negative	0	0	nt
24	9	2	4	negative†	1.5	3	51

*Abbreviations: nt, not tested; na, not available.
†Result near the cutoff concentration.

Table IV-Results of GC-MS Analysis of Urine from Poppy Seed Consumption Study (Cassella et al., 1997).

It then needed to be determined if thebaine was present in heroin, morphine, and codeine samples. The Department of Consumer Protection, Drug Control Division, from Hartford, CT, gave seven crude heroin samples from the streets of CT to be used for the experiment. Seven urine samples from patients who had been admitted to the Hartford Hospital for heroin use, confirmed by both history and medical examination, were collected for testing as well. Codeine tablets were obtained from Roxane Labs, Inc. and morphine tablets were obtained from Purdue

Frederick. Standards of codeine, morphine, heroin, and thebaine were diluted to concentrations ranging from 1 to 300 ng/mL using drug-free urine (Cassella et al., 1997). GC-MS was used to qualitatively assay the samples of powdered street heroin, the pharmaceutical preparations of morphine and codeine, and the urine from unknown heroin users to determine if thebaine was present in any of these samples. If thebaine were determined to be present, it would not be a valuable marker for poppy seed use. Figure IV represents a gas chromatogram and partial mass spectrum of a sample of extracted crude heroin. The predominant peak shown is produced by heroin, as expected, with trace amounts of acetylcodeine and 6-MAM, the smaller peaks. According to Cassella et al. (1997), the samples of urine from heroin users, and the morphine and codeine tablets produced expected results as well. None of these samples showed peaks indicating the presence of thebaine. However, Figure V is a reconstructed gas chromatogram and partial mass spectra of a urine sample following the consumption of 11g of poppy seeds. The peak for thebaine is present in this sample (Cassella et al., 1997). Detecting the peak for thebaine in urine samples can be used as a marker for poppy seed consumption.

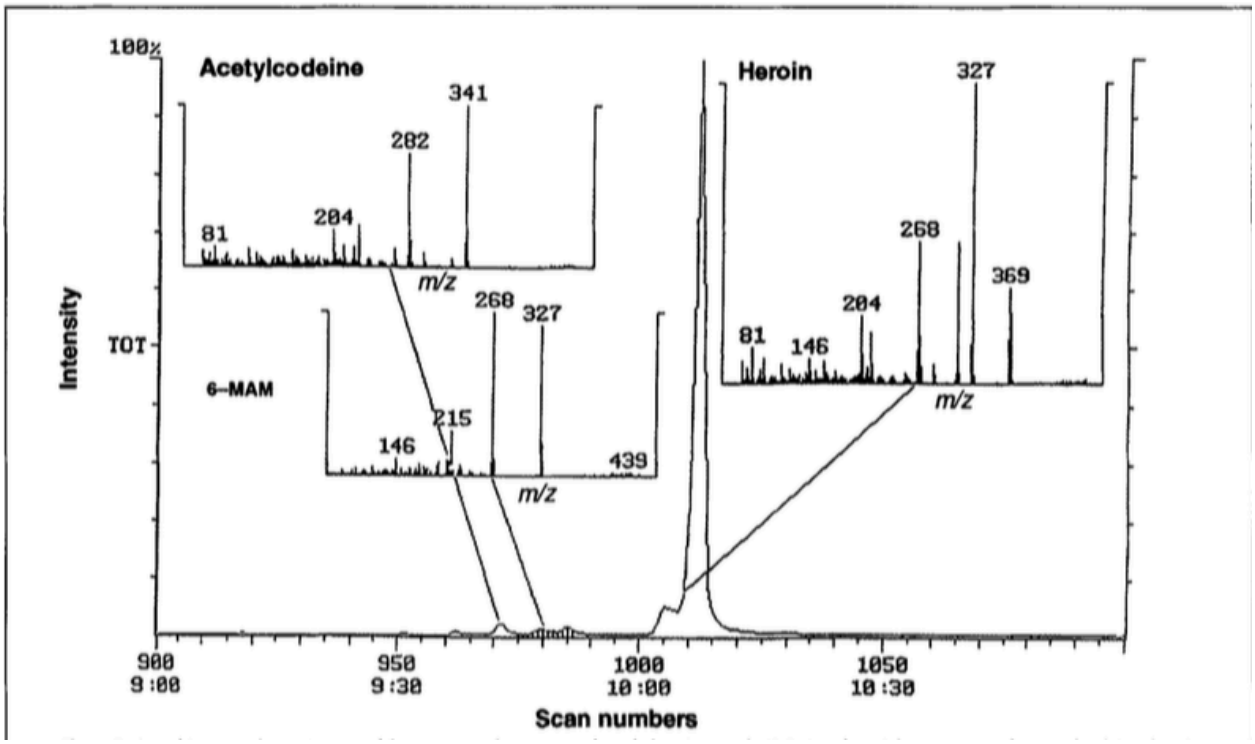

Figure IV- A total ion gas chromatogram of the contents of a crude heroin sample (0.5g) and partial mass spectra for acetylcodeine, heroin, and 6-MAM in the samples (Cassella et al., 1997)

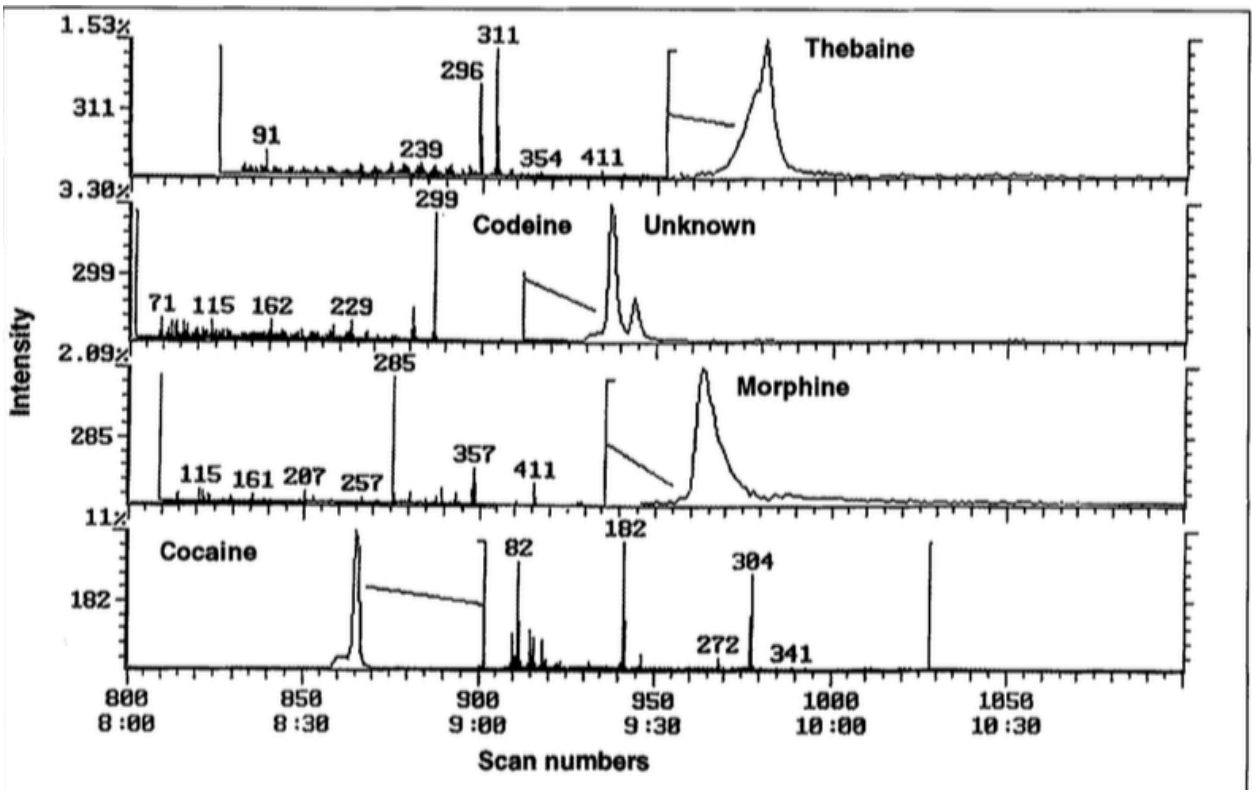

Figure V-A reconstructed ion gas chromatogram and partial mass spectra of components from urine following consumption of 11g of poppy seeds. Thebaine concentration, 59.5 ng/mL; codeine, 48.9 ng/mL; morphine, 4776 ng/mL. Underivatized cocaine was used as the internal standard (Cassella et al., 1997)

Although concentrations of codeine and morphine may be present in an athlete's urine after the consumption of poppy seeds, the presence of thebaine can be used to indicate a false-positive for the presence of opiates. However, "…the absence of thebaine in a urine sample screened positive for opiates does not exclude the possibility of poppy seed consumption as a cause of positive results, which occurred in four of the positive controlled cases" (Cassella et al., 1997). Similarly, a positive result for the presence of thebaine in an athlete's urine does not exclude the possibility of opiate use. An opiate drug abuser may try to carefully cover up drug abuse with the consumption of poppy seeds. When analyzing a sample for thebaine, several factors must be taken into consideration such as the amount of poppy seeds consumed, the thebaine content of the product, the time since the seeds were consumed, and the individual's rate of metabolism (Cassella et al., 1997). Analyzing a urine sample for thebaine to try and corroborate the claim of inadvertent doping is not fool proof, however it can help to reduce the frequency of positive results caused by the inadvertent consumption of opiates, such as morphine, through poppy seeds. This problem of false-positive results due to poppy seed consumption is not commonly seen anymore, but perhaps if other markers, like thebaine, can be found in other foods and products that cause false-positive doping results, we will be better able to distinguish between true drug addiction, inadvertent doping, and false-positive results.

6.2 Unintentional Doping Due To Tainted Food Products

Chemically tainted meat and other animal products such as milk and offal can also result in unintentional doping (Anderson, 2011; Geyer, Schanzer, and Thevis, 2014). In this case the result would not be considered a false-positive because the athlete is actually ingesting a prohibited substance, they are just not aware of it. A common example of chemically tainted

meat is the presence of clenbuterol. This has been documented in some countries, such as China and Mexico. There are currently no laboratory tests to distinguish between the intentional ingestion of clenbuterol and its unintentional ingestion from tainted meat (Anderson, 2011). If an athlete can provide convincing evidence that the positive test was due to the unintentional digestion of tainted meat they may not face any consequences, such as Alberto Contador, the Spanish three-time Tour de France champion who tested positive for clenbuterol. However, if an athlete is in a country where tainted meat is common, they should be aware of this and avoid consuming the meat. Italian cyclist Alessandro Colo was denied a similar appeal because he was in Mexico and was told he should have been aware the beef is usually contaminated with clenbuterol (Anderson, 2011). Responsibility often falls on the athlete, and they must be prepared to face the consequences even if they do not believe they are responsible. Studies of pharmokinetics and the metabolism of clenbuterol are providing promising results that developments may be made to distinguish between clenbuterol from medication, and clenbuterol from contaminated meat (Geyer, Schanzer, and Thevis, 2014). If methods can be developed to distinguish between the two, it would be possible to identify who intentionally and who inadvertently doped. Anti-doping research and developments are not only meant to identify cheating athletes, but to protect athletes who compete fairly.

6.3 False-Positive Results Caused By Elevated Levels Of Physiologic Hormones

Elevated levels of physiologic hormones can lead to false-positive results for tests as well. In this case, the athlete does not unintentionally dope, rather they naturally have high levels of hormones in their body, which makes it appear as if they take extra hormones. Hormones such as testosterone, HCG, growth hormone, and erythropoietin occur in the body naturally, but they

also tend to be abused. Not all of these can be detected using urine tests, so challenges are faced when determining whom naturally produces elevated hormone levels, and who is administering the hormones exogenously. Testosterone is produced endogenously and administered exogenously, so being able to differentiate between the two using a urine test is extremely important for doping control (Anderson, 2011). One method currently used is the testosterone-to-epitestosterone (T/E) ratio. Testosterone and epitestosterone are typically present in human urine at a 1:1 ratio, so if an athlete is administering testosterone exogenously, their testosterone level will rise, but not their epitestosterone level. If the testosterone level is natural, repeated tests will also give a consistent ratio because the levels do not significantly change. The cut off used to identify doping is a T/E ratio of 6:1. Isotope ratio mass spectrometry (IRMS) can be beneficial as well. The WADA recommends that further investigations be carried out using gas chromatography, combustion, and IRMS (GC-C-IRMS) on samples that have a T/E value greater than 4.0 (Anderson, 2011). ABP is also very helpful in this case to monitor athletes' normal levels of hormones. If an athlete has a consistently documented high level of testosterone, it is more believable that it is endogenous. If an athlete has consistently normal levels of testosterone and then levels shoot up, there is reason to believe that doping has occurred and it is not a false-positive.

6.4 Unintentional Doping With Anabolic Agents And How To Detect Their Metabolites In Urine

Recently, inadvertent doping with anabolic agents has been identified as well. The WADA prohibited list notes anabolic agents as: exogenous anabolic androgenic steroids (AAS), endogenous AAS, and other agents such as clenbuterol and selective androgen receptor

modulators (SARMs) (2016 Prohibited List, 2015). The most common sources of unintentional doping with anabolic agents are nutritional supplements that have been tainted with AAS, contaminated meat products, and natural products containing endogenous AAS. When the WADA accredited laboratories reported their adverse and atypical findings in 2012, about 2,279 of the 4,500 cases (about 50%) were anabolic agents (Geyer, Schanzer, and Thevis, 2014). Anabolic agents are commonly abused because their affects are beneficial across an array of sports. They are commonly abused because they increase muscle growth, increase strength, and accelerate the recovery time for vigorous exercise. These factors are extremely beneficial to all different kinds of sports; therefore its abuse is wide spread.

Anti-doping laboratories face many challenges when trying to detect anabolic agents. Not only to methods of doping change, but substances are constantly being reformed as well. Major growing problems include, "…administration of unapproved and/or new substances, the evidently increasing use of endogenous substances, the constantly decreasing concentrations of the analytes detected in positive doping control samples, and genetic polymorphisms that lead to different metabolic patterns in the tested individuals" (Geyer, Schanzer, and Thevis, 2014). Not only do anti-doping laboratories have to deal with athletes who intentionally dope, but they have to identify those that inadvertently dope as well. The sources of inadvertent doping are important to identify to protect athletes from making the same mistakes as their peers.

Exogenous AAS are usually identified by the detection of their urinary phase-I and phase-II metabolites. AAS can have long lasting effects on athletic performance, which means its metabolites remain in the body for a significant time after administration. Current anti-doping research is mainly focused on searching for these long-term metabolites (LTMs) (Geyer, Schanzer, and Thevis, 2014). Screening for the LTMs rather than the immediate metabolites of

AAS prolongs the detection window. Utilizing methods such as LC-MS/MS, GC-MS/MS, and HRMS have also prolonged the detection window because they are highly sensitive techniques. They are able to detect low levels of LTMs. Employing these methods along with LTMs have led to an increase in adverse analytical findings (AAFs). Geyer, Schanzer, and Thevis (2014) report that the WADA accredited laboratory Cologne was the first to screen for the metabolite, 18-nor-17β-hydroxymethyl,17α-methyl-androst-1,4,13-trien-3-one, of metandienone in 2006. As a result, the number of AAFs for Cologne in 2006 was higher than the sum of AAFs for metandienone in all other WADA accredited laboratories. Similar cases have been observed by detecting new LTMs for other anabolic agents as well, such as dehydrochloromethyltestosterone and the exogenous AAS stanozolol (Geyer, Schanzer, and Thevis, 2014). If we are able to identify banned substances by testing for more than one metabolite, this will increase the chance of catching athletes who have doped.

CHAPTER SEVEN: DETECTION OF STEROIDS

7.1 Detection Of Designer Steroids

Another issue faced by anti-doping laboratories that pose a threat to the athletic community is designer steroids, or chemically modified steroids. These tailored agents started to become a leading issue in the early 2000s. These compounds are not approved as therapeutic, and they typically have not undergone clinical trials. These substances are most likely doctored to avoid being detected during doping tests. From 2002 to 2008, 22 designer steroidal compounds were identified by WADA-accredited laboratories (Geyer, Schanzer, and Thevis, 2014). However, because these compounds are constantly being modified to avoid detection, it is important for anti-doping laboratories to uncover methods that can detect these compounds

despite the changes being made. It is also important for athletes to understand that anti-doping laboratories are aware of these chemically modified compounds and they are creating methods to detect them. This may make athletes less inclined to dope with these substances. There is a need to complement target-oriented analytical methods with non-targeted methods. This would allow anti-doping laboratories to directly test for known metabolites, but also test for unknown targets that may also be of interest. Geyer, Schanzer, and Thevis (2014) discuss two strategies for combatting the abuse of designer steroids: a non-targeted approach and an indirect approach.

Anti-doping laboratories are familiar with and easily able to identify naturally endogenous androgens because they have been testing for them for so long. An approach to identify designer steroids is to use mass spectrometry to screen for commonalities of steroidal agents and to flag the peaks that are not common in doping control samples. This is called a non-targeted approach (Geyer, Schanzer, and Thevis, 2014). With this approach, laboratories look for peaks that are common for steroidal agents, but then they also look at the peaks that are not common. These peaks may be present because the substance in question is a steroid, but it is modified. These uncommon peaks can then be compared to one another and used to identify substances that have none of the common peaks. Unknown agents may be identified if their screening produces peaks that are also produced by other known unmodified/modified steroidal agents. However, this method is not as sensitive. Targeted analyses allow for detection limits as low as 5 pg/mL, but non-target approaches have detection limits of approximately 10-20 ng/mL (Geyer, Schanzer, and Thevis, 2014). So although a non-targeted approach may allow for a laboratory to have a better chance of detecting a designer steroid, that chance is reduced if the substance is present at a low concentration.

The indirect approach for detecting designer steroids works with the ABP. The indirect

approach works based on endocrinological feedback mechanisms and the effect that these steroids have on the level of urinary endogenous steroids. The use of AAS causes endogenous steroids to be suppressed during excretion. If a decreased level of endogenous steroid concentrations is observed, designer steroid use may be suspected (Geyer, Schanzer, and Thevis, 2014). The Steroidal Module of the ABP can be used to monitor endogenous steroid levels and if these levels suddenly decrease, there is reason to believe it could be due to the use of designer steroids and an investigation may be conducted.

Traditional detection methods utilized to detect steroids in human urine include immunoassays, GC-MS, and LC-MS. However, if compounds in a urine sample differ from previously identified compounds by as little as 1-2 Daltons, certain traditional methods will not detect them. If a suspicious urinary profile containing endogenous steroids is detected, additional urinary profiles can be created from precursor ion experiments. This strategy is also routinely used to identify metabolites. Thevis et al. (2005) offer a protocol that can be used as a complementary approach to analytical procedures that already exist. It provides compound identifications that are not provided by routine doping control analysis. An experimental procedure was performed that included two batches of six different blank urine samples each, obtained from three male and three female volunteers. Added to the samples were either 50 ng/mL of the synthetic steroids norbolethone, methyltestosterone, ethyltestosterone, 1-testosterone, and the internal standard d_4-THG; or with 50 ng/mL of gestrinone, dihydrogestrinone, THG, propyltrenbolone, and 300 ng of methyltestosterone as the internal standard (Thevis et al., 2005). Both sets of samples were prepared and analyzed using the suggested procedure, which allowed the estimation limits of the compounds to be identified. The product ions as m/z 109, 187, 189, 227, and 241 were selected and precursor ion experiments

were performed. The product ions are characteristic of testosterone and nandralone (m/z 109), 1-testosterone (m/z 187), androsterone (m/z 199), trenbolone (m/z 227), and gestrinone (m/z 241) and their corresponding analogues, and can be used to identify these compounds.

Determining these precursor ions of diagnostic fragment ions of particular steroids generates data that can be combined with traditional doping control screening methods. The product ions that are selected represent the principal nuclei of common steroids, such as testosterone and nandralone (m/z 109), 1-testosterone (m/z 187), and gestrinone (m/z 241). When steroids are chemically modified, their molecular weight is typically changed due to a change in structure. Because the molecular weight is generally used to identify compounds, this change of weight makes designer steroids difficult to identify. However, depending on the position of the modification made, several abundant product ions are still present. The presence of these product ions is the basis for assays that use precursor ion scan experiments (Thevis et al., 2005). Thevis et al. (2005) obtained commercially available steroids and synthesized steroids with similar structures. All of the steroids were analyzed and the results were compared. If a urine sample is analyzed and peaks that are known to be from steroids are detected in addition to the peaks that result from endogenous sources, there may be reason to believe that an unknown, structurally related, anabolic agent was administered. The generation of a product ion spectrum should be the initial step because the presence or absence of the steroid nucleus can be determined. The identification of additional peaks in a urine sample may indicate something suspicious in the sample. The sample can be scanned again and product ion scan experiments can be utilized to obtain more information on the analyte's structure. The unknown structures can be compared to known structures to determine the identity of the unknown compound. Despite the advantages of this complementary procedure, there are several limitations. Because hundreds of

other abundant compounds are present, it can be difficult to detect low concentrations of unknown compounds. However, this procedure does provide a way to identify compounds that would not be identified during routine doping control analysis (Thevis et al., 2005). With new drugs, like designer steroids, constantly being synthesized, there needs to be a way to test for them. The protocol previously described provides a complementary way to identify these designer steroids by comparing new results to previously recorded results. It is important for anti-doping laboratories to use all of their sources when detecting and identifying unknown compounds. By comparing spectra of unknown compounds to spectra of known compounds, similarities may be determined which can help laboratory analysts determine which common steroid was modified to create a new designer steroid.

7.2 Steroid Use In Major League Baseball

There are many possible situations in sports, where a positive drug test does not mean an athlete was intentionally trying to dope. However, in most scenarios, this does not excuse the athlete from the act of doping and they are still held responsible. One example of this is Jenry Mejia of the New York Mets. In April 2015, it was reported by Newsday that the baseball player had been suspended 80 games by the Major League Baseball after he tested positive for PEDs. Mejia stated that he was aware of the rules and that he accepted his punishment, but he claimed to have no idea how a banned substance ended up in his body. The MLB asserted that Mejia tested positive for stanozolol, or winstrol, an anabolic steroid. Around this time, pitchers David Rollins (Mariners), Arodys Vizcaino (Braves), and Ervin Santana (Twins) also tested positive for stanozolol (Carig, 2015). The manager of the team, Terry Collins, was asked how a player could be unaware of the substances he was putting into his body. Collins responded by stating, “I know

what goes into my body. I can't answer for everyone else" (Carig, 2015).

Unfortunately, Mejia did not learn from his mistakes. Whether he continued to unintentionally dope by being careless about what he put into his body, or he continued to intentionally dope, Mejia was determined to be doping not only one more, but two more times. After his first suspension in April 2015, Mejia returned to the MLB in July. After only seven appearances, Mejia tested positive again for doping and was suspended for 162 games. This time, he tested positive for not only stanozolol, but boldenone as well. This was the first time any player had been suspended twice within one season for the use of PEDs. Mejia was still serving his 162-game ban when he failed yet another doping test in February 2016. This time he tested positive for boldenone. The MLB has a three-strike policy on PEDs, resulting in Mejia's permanent ban from the game. Mejia is the first player to be permanently banned for this reason. Through all of this, Mejia continues to claim his innocence (Carig, 2016). It is important for athletes to understand just how important it is for them to know exactly what they are putting into their body and what the risks can be if doping occurs. Mejia's case should also bring attention to drug addiction and that although Mejia may have been doping to enhance his performance, he may have also had or even continue to have, a drug addiction. The sports community should be aware of these problems so they can help their athletes. It is possible Mejia was not aware he was taking steroids, and that they were hidden in a supplement he was taking or sports food he was eating, but one would think he would be more careful of what he was putting into his body after testing positive for steroids not only once, but twice. Mejia's case makes it seem unlikely that it was unintentional doping and this is why he has been banned. Because of doping, Mejia, just 26 years old, will never again be able to professionally play the sport he trained so long and hard for.

Another player of the MLB, Chris Colabello of the Toronto Blue Jays, faced an 80 game suspension in April 2016 after a urine test came back positive for a banned substance. Colabello tested positive for the anabolic steroid dehydrochlormethyltestosterone, which is commonly sold under the name turinabol. Like Mejia, Colabello claimed to have no idea how the drug entered his system. The suspension resulted in his ineligibility for postseason play, and it made him fall short of the service time he needed to become eligible for salary arbitration next winter (Harrison, 2016). Possibly worse than this however is Colabello's now tainted reputation. The general manager of the Blue Jays, Ross Atkins, fully believed Colabello's innocence and confirmed that attempts had been made to appeal the suspension, however they were denied. Kevin Pillar, an outfielder for the Blue Jays, held that he found it unbelievable that Colabello would knowingly use steroids. He stated, "A guy like him [Colabello] would never do that…I believe him wholeheartedly" (Harrison, 2016). Despite Colabello's coaches, managers, and teammates believing his innocence, Colabello still has to pay the consequences of using a banned substance. Unless it can be proven that an athlete doped unintentionally and there are reasons to believe they are truly innocent, they are held responsible and there will be consequences. However, because athletes are responsible for what goes into their body, whether they know it is banned or contains banned substances or not, they can be held responsible. Hopefully Colabello will learn from this mistake, whether that means he stops using banned substances or is more careful with what he puts into his body, so he does not end up like Mejia, permanently banned.

CHAPTER EIGHT: DIETARY SUPPLEMENTS

It could be possible that Mejia and Colabello both inadvertently doped due to contaminated nutritional supplements. Dietary supplements are one of the most controversial

categories used to improve health and enhance performance in modern athletics. Supplements include vitamins, minerals, protein powders, and botanical extracts, among other substances. Although nutritional supplements are often sold as a form of medicine, they are regulated as foods, which many people do not know (Cohen, Venhuis, and Brandt, 2016). According to a Harris Poll conducted in 2002, most consumers of dietary supplements believed that the products are approved by a government agency, and about two thirds believed that supplement labels were required to include warnings of possible side effects and other potential dangers (Cohen, 2009). This could lead not only to unintentional doping, but serious health problems. An individual may unknowingly ingest a pharmaceutical that are allergic to, or that reacts with another medication they are taking. Governmental agencies do not ensure the accuracy of supplement labels with respect to ingredients or health claims (Cohen, Venhuis, and Brandt, 2016). It is important for athletes who use nutritional supplements to understand the risks they are taking, and that the risks may outweigh the benefits. Athletes should take caution when approaching supplements and better understand them so they can be more safely used, and hopefully one day, better regulated by the athletic community.

Cohen, Venhuis, and Brandt (2016) discuss important contributions to the field of dietary supplements. Attempts were made throughout the 20th century to regulate vitamins and other supplements, however when the FDA tried to create forceful regulations, there was extreme backlash. The Dietary Supplement Health and Education Act (DSHEA) was eventually passed, which protected vitamins, minerals, and other supplements from being regulated by the government. This opened the door for manufacturers to design supplements comparable to medicine without the need to be regulated, which is dangerous for users. Although investigators lack the resources required to estimate a more accurate number, it was estimated in a 2015 study

that 23,000 emergency department visits each year in the USA are caused by supplements (Cohen, Venhuis, and Brandt, 2016). Aside from pharmaceutical ingredients, dietary supplements have been found to be contaminated with toxic plant material, heavy metals, and bacteria (Cohen, 2009). Not only do athletes have to be concerned with whether or not their supplements contain a prohibited substance, but they also have to be concerned with what other types of materials they may contain. Despite warnings and the adverse effects that have been reported, athletes continue to use these unregulated substances. The companies that manufacture dietary supplements do not have to prove their safety, rather it is up to regulatory authorities to demonstrate that a particular supplement is dangerous before it can be removed from the market (Bijl, 2014A). Not only might these supplements contain substances that can cause individuals to have serious health issues, but also because they are not closely regulated, it is possible for them to be laced with prohibited substances that could cause athletes to unintentionally dope. Dishonest supplement manufacturers have been deceiving the FDA and avoiding the detection of undeclared pharmaceutical ingredients in their products by utilizing pharmaceutical analogues. These analogues contain a different structure than the parent compound typically tested for, so they evade detection. Not only are these analogues dangerous because it is hard to regulate them, but because they have never been studied in humans, little is known about their health risks. The FDA has uncovered that more than 140 different products have been contaminated with active pharmaceutical ingredients, some of which are prohibited in sport (Cohen, 2009).

8.1 Unclear Labeling And Contamination Of Dietary Supplements May Cause Athletes To Unintentionally Dope

Dietary supplements have been used over the years to help athletes increase their strength

and enhance their performance, and due to their lack of regulation, this has caused some serious problems. More often than not, the pharmaceutical components of supplements are not clearly listed on package labels, either because they unintentionally entered the product, or because they are listed under another name. The concentrations of these substances may not even be high enough to cause health risks or enhance performance, but they could still lead to a positive doping test. In part I of his two-part review, Bijl (2014A) discusses how certain dietary supplements mention the presence of the natural sources of illegal stimulants, such as those prohibited by the WADA, but do not refer to the chemical entities themselves or their analogues. This can result in an athlete ingesting a prohibited substance without their knowledge, causing inadvertent doping.

A prohibited substance that has been found in dietary supplements is ephedrine and its analogues. Certain supplement labels list the natural sources of ephedrine, such as Ephedra sinica, rather than the chemical names of ephedrine and its analogues to make it more difficult to identify. This plant product has also been found in supplements labeled as 'ephedrine free.' Along with the consequences of a possible positive doping test, consuming products that contain ephedrine also poses health risks. Ephedrine has similar side-effects to amphetamine because they have a similar structure and therefore similar modes of action. Ephedrine can cause an increase in anxiety, increased agitation and other psychiatric symptoms, insomnia, tremors, and cardiac symptoms, such as heart palpitations (Bijl, 2014A). Ephedrine and pseudoephedrine have both been prohibited by the WADA. A clean athlete may unintentionally dope because of confusing labeling, or labels listing substances by a different name. Athletes should be aware of every ingredient in any supplements they take to avoid this kind of a problem. If they are unsure of an ingredient, they should get trustworthy clarification before consuming the product.

Dietary supplements on the market have also been adulterated with sibutramine, which is an anti-obesity agent. Sibutramine has been o the WADA prohibited list since 2006 and its only market approved as a prescription anti-obesity agent, however it has been found in products promoted as 'pure herbal' capsules and 'natural' tea. Sibutramine can cause elevated blood pressure, cardiac effects, like tachycardia, and severe systemic adverse effects. Patients using sibutramine should be monitored by a physician who is familiar with the agent (Bijl, 2014A). This shows how extreme the use of an agent such as sibutramine is, and it could be extremely dangerous for an athlete to accidentally take it. Not only is the athlete likely to fail a doping test, but also they are put at risk for serious health issues.

Another stimulant that has been detected in dietary supplements is methylhexaneamine, which was prohibited by the WADA in 2009. Methylhexaneamine was originally created to be used as a nasal decongestant. It was reported that two American soldiers who were taking commercially available dietary supplements that contained methylhexaneamine collapsed from cardiac arrest during physical exertion and died (Bijl, 2014A). This is another example of how an athlete, or any individual, puts himself or herself at serious risk when they put an unknown substance into their body. Methylhexaneamine can be found on package ingredient labels under many different chemical and non-chemical names, making it confusing for consumers to know exactly what they are ingesting. Methylhexaneamine and dimethylpentylamine are the only names that are listed on the WADA 2011 prohibited list, and this leads to even more confusion with product and ingredient identification (Bijl, 2014A). This reiterates that athletes and others in the athletic community should do extensive research before allowing any unknown substances or ingredients into an athlete's body.

In part II of his two-part review, Bijl (2014B) discusses classic and designer steroids,

clenbuterol, peptide hormones, and other newer compounds and their inadvertent ingestion due to contaminated supplements. In a previous study, it was demonstrated that about 15% of dietary supplements containing vitamins, minerals, proteins, and creatine, also contained AAS, which were not divulged. The presence of these prohormones could potentially be caused by contamination before or during the manufacturing process, however the level of prohormone detected in supplements during the aforementioned study could have been high enough to give a positive doping result (Bijl, 2014B). Contamination is a serious possibility that athletes need to consider. Even if they do extensive research on a product and are confident in the true identity of all of the ingredients, the presence of prohormone due to contamination would not be stated anywhere. Many athletes who consume prohormones view them as natural compounds that will give them strength and increased muscle mass, improve their body, and increase their overall feeling of well being without the adverse effects that testosterone or other synthetic androgenic steroids produce. Cholesterol is an example of a molecule that metabolizes into testosterone via numerous different intermediates. However, several different studies have shown that these intermediates do not produce any sort of anabolic or ergogenic effects, but they can cause health risks. LDL-cholesterol/HDL-cholesterol ratios were shown to increase by 11%, which can increase the chance for cardiovascular disease, and a decrease in luteinizing hormone levels, which can lower testicular and adrenal testosterone production (Bijl, 2014B). Athletes may take natural compounds with the idea that they are legal and will have the same performance-enhancing effect as other synthetic steroids, however prohormones are banned by the WADA and these substances can have negative health effects as well.

Steroid structures can be identified due to the presence of perhydrocyclopentanophenanthrene nuclei, which is comprised of four rings. Steroids can be

organized into six groups depending on the number of carbon atoms, i.e. gonanes, estranges, androstanes, pregnanes, cholanes, and cholestanes. Except for cholanes, steroids are natural hormones that can be classified as oestrogens, androgens, glucocorticoids, and mineralocorticoids depending on their function in the body. Steroid compounds have been found in high amounts in dietary supplements that are widely available to athletes and other individuals. Often, the steroidal ingredients are not listed on the package, or they are listed under a different chemical or non-approved name (Bijl, 2014B). Severe health problems can be caused by the use of steroids, especially if an individual is not taking proper care of their body because they are not aware of what they are actually taking. Ingestion of steroids can be extremely harmful to female and adolescent athletes as well. In men, steroids can produce acne, testicular atrophy, prostate enlargement, and infertility, among other complications. In females, some effects of steroid use may be clitoris enlargement, menstrual irregularities, and potentially irreversible masculinity. Psychiatric effects, such as aggression, psychosis, manic episodes, and depression have been documented in both sexes (Bijl, 2014B). Again, athletes are in charge of what is put into their bodies whether or not they know exactly what it is and what its side effects will be.

As previously discussed, designer steroids are manipulated versions of classic steroids, which can make them difficult to detect and it makes it harder to know what their health effects will be. Designer steroids tend to be produced solely for distribution on the black market. Examples of designer steroids that have been detected by anti-doping laboratories are prostanozol, mathasterone, and andostatrienedione, to name a few. Designer steroids are either listed under an unknown or unapproved chemical name, or they are not disclosed at all on the labels of dietary supplements (Bijl, 2014B). Even though little is known about some of these

compounds, it is still likely that if they were detected in an athlete's urine, they would face serious consequences.

A main reason athletes take dietary supplements is to improve their performance by increasing their muscle mass. Many over-the-counter supplements claim to increase the level of human growth hormone in the body, which increases lean body mass, however studies have shown that this has no effect on strength in athletes. Growth hormone-releasing peptide-2 (GHRP-2) has been detected in dietary supplements over the years. GHRP-2 itself is not explicitly banned by the WADA, however it belongs to a substance group that is on the prohibited list, so it should be avoided (Bijl, 2014B). Athletes who dope do it with the goal of improving their performance, yet, more often than not they are putting themselves at risk for serious health problems, and possible suspension from sport, without even getting the results they want. Athletes should take this possibility into consideration before doping.

8.2 Detecting Dietary Supplement Contamination

Merwe and Grobbelaar (2005) were interested in determining whether the ingestion of contaminated supplements could potentially cause an athlete to fail a dope test. An over-the-counter supplement was administered to five healthy male volunteers and their urine was collected at intervals and analyzed using GC-MS. The urine samples were identified as contaminated with 19-nor-4-androstenedione and 14-androsten-3,7-dione, meaning they were present in the supplement. However, neither of these two compounds was listed on the supplement's label. All of the urinary concentrations of 19-nor-4-androstenedione were above the WADA limit of 2 ng/mL up to two hours after administration. The 14-androsten-3,7-dione concentrations in two of the samples were above the WADA limit and could be detected up to 36

hours after administration. The recommended dosage of the supplement is four capsules, three times a day. The volunteers were only administered one capsule (Merwe and Grobbelaar, 2005). If the recommended dosage is consumed, athletes are likely to have levels way above the limit set by the WADA. This study shows that the ingestion of a dietary supplement that contains even minute amounts of a prohibited substance can cause an athlete to fail a dope test. The study shows that supplement labels and manufacturers' claims cannot be trusted. Athletes must decide for themselves whether or not they want to gamble with taking supplements. Even if an athlete unintentionally dopes because an ingredient was not included on the label, the athlete can still be at fault since the WADA has strict rules about athletes' responsibilities.

Other compounds not approved for clinical use have been found on the black market as well. This includes a SARM, which can produce anabolic effects, and agonists of the peroxisome proliferator-activated receptor δ, which can enhance endurance. GW501516 is another substance created to enhance endurance. Because this drug has not been approved for clinical use and it contains a serious toxicity profile, it has been withdrawn from future investigation by pharmaceutical companies (Bijl, 2014B). Molecules such as GW501516 are found in all different types of dietary supplements at varying concentrations. Many times, the full effects of dietary supplements are unknown to the consumer because they are not entirely sure what they are putting into their body due to mislabeling or the use of different chemical names. When taking supplements, athletes put themselves at risk for inadvertent doping and serious health complications. They cannot use the excuse that they did not know what an ingredient was, or even that an ingredient was not listed. Responsibility falls on the athlete. Athletes need to decide if the benefits of taking dietary supplements outweigh the risks.

CHAPTER NINE: MASKING AGENTS

9.1 Potential Use Of Liposomes As Masking Agents

Another problem that anti-doping laboratories face is the use of masking agents by athletes. Masking agents can work by altering the pharmacokinetics of prohibited drugs in vivo, and interfere with laboratory procedures ex vivo (Esposito et al., 2016). This can make it difficult for prohibited drugs to be detected during routine doping tests. A way to combat this problem is to detect the masking agents themselves, rather than the prohibited substance. If a masking agent, which has no performance-enhancing features, is found in an athlete's urine, there may be reason to suspect that they are doping and further investigating can be done. Liposomes may potentially be used as masking agents to hide doping in sport. Liposomes are composed of phospholipids (PLs), which are amphiphilic molecules made of a glycerol backbone, linked to one (monoglycerides) or two (diglycerides) fatty acids by an ester bond, and a hydrophilic head containing a phosphate moiety. Subclasses of PLs include glycerophocholines (PCs), lyso-glycerophocholines (lyso-PCs), sphingomyelins (SMs), glycerophosphatidylethanolamines (PEs), lyso-glycerophosphatidylethanolamines (lyso-PEs), glycerophosphatidylserines (PSs), glycerophosphatidic acids (Pas), glycerophosphatidylglycerols (PGs), and glycerophosphatidylinositols (PIs) (Esposito et al., 2016). SMs are liposomes that are an exception to the general structure rule. They contain a long-chain base of sphingosine with an amino-linked fatty acid. PLs and SMs are the main components of biological membranes, such as the phospholipid bilayer, and they can function as mediators of signal transduction. Many subclasses of PL are involved in other biochemical and physiological functions as well. SMs are a main component of the cell outer leaflet. These characteristics of PLs and SMs could allow them to be used as carriers for drug delivery systems. This would allow them to be used to

change the pharmokinetics of prohibited drugs making them harder to detect. Not only can liposomes potentially be used in vivo, but 'empty' liposomes can potentially be used ex vivo to interfere with the analytical laboratory procedures used to detect prohibited substances. They can affect the efficiency of the analytical procedures used for detecting AAS in urine by interfering with the extraction and derivatization steps that are used by anti-doping laboratories. Liposomes are not currently included on the WADA prohibited list, however other substances containing liposomes may be banned in the future under the Non Approved Substances section (Esposito et al., 2016).

Esposito et al. (2016) conducted a study to ensure the detection of liposomes in athletes' biological fluids, and determine how commonly they are used among athletes. Their study included the development of an analytical method that can detect (screen and confirm) nine classes of PLs in pharmaceutical formulations and biological compounds using normal-phase liquid chromatography coupled to electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS). Adequate chromatographic separation is a main issue that needs to be considering when using MS/MS, to make sure that an unequivocal identification is made, especially when complex matrices are being analyzed. Non-polar columns separate PL species based on their acyl chains length and saturation, therefore the different PL classes need to be separated even before chromatography can be performed. Polar columns, such as aminopropyl, which separate phospholipids based on differences on head-group polarity, separate PL and SM mixtures well, but there is poor reproducibility of retention times. The use of hydrophilic interaction (HILIC) stationary phases has also been proposed as an effective way to analyze phospholipids in recent years. Esposito et al.'s (2016) procedure utilizes a diol column to couple chromatographic separation to MS/MS analysis in different acquisition modes. A precursor ion scan or neutral loss

scan is usually selected to detect the main classes of PL and SM, and a product ion scan is chosen to confirm the chemical identity of each compound present. The method was used to analyze two products that are commercialized in Italy, Liposom® Forte and Tricortin® 1000. The method was also used to establish characteristic profiles of the PLs and SMs in biological fluid (plasma and urine), to make it possible to distinguish between endogenous compounds and pharmaceutical compounds containing phopsholipidic liposomes (Esposito et al., 2016). The ability to distinguish between endogenous compounds and pharmaceutical compounds is important because anti-doping laboratories are only concerned with samples containing pharmaceutical compounds, which could indicate doping. Liposomes are not currently prohibited, but because they may have characteristics common of masking agents, which are prohibited, it is possible they may be utilized to mask the use of prohibited substances. If liposomes are detected in an athlete's biological fluids, the ADO may want to consider investigating the athlete for drug use. If anti-doping laboratories could not distinguish between endogenous and pharmaceutical liposomes, it would be more difficult to argue that the athlete could be doping since the he or she could argue it is endogenous. However, since the method proposed can make the distinction, if pharmaceutical liposomes are present, this is a greater indicator of that doping may be masked, and the ADO may be more inclined to investigate.

The analytical procedure proposed by Esposito et al. (2016) was developed to characterize the phospholipid profiles in human biological fluids, such as urine and plasma, and two liposome pharmaceutical products. This was done to establish appropriate markers for the identification of the presence of non-endogenous components in biological fluids that could confirm the use of liposome-based drugs. This experiment detected 28 different PCs in precursor ion scans, and 16 of the more abundant molecular species were later identified; 8 lyso-PCs were

detected and 6 of them were identified; 24 SMs were detected and 14 of those were identified (Esposito et al., 2016). SMs, PCs, and lyso-PCs are constituents of biological fluids and cerebral tissues, and both the Liposom® Forte and Tricortin® 1000 are made using hypothalamic extracts. Since these are found in both endogenous and non-endogenous samples, they would not be good markers. 4 different product ions were detected, however none of them were found in the pharmaceutical preparations, so these would not be good to use as markers either. PSs and PEs were detected in the non-endogenous pharmaceutical preparations, and neither of them were found in the biological fluids. This finding makes the PS and PE classes of PLs idyllic markers to discriminate between the endogenous and exogenous source of phospholipids and phospholipid-based products (Esposito et al., 2016). This advantageous procedure not only allows for all of the chosen PLs and SMs classes to be screened for at once, but it also confirms the identity of each molecular species that is detected using the same procedure. If liposomes can be determined to be endogenous by identifying the markers above in a sample, there is little reason to suspect an athlete is doping. However, if the markers for exogenous sources of phospholipids and phospholipid-based products are identified in a sample, there is greater reason to suspect the athlete may be trying to mask doping, and further action can be taken.

9.2 Use Of Diuretics As PEDs And Masking Agents

Diuretics are another category of drugs that can be used as PEDs and masking agents. Diuretics are used to increase the rate of urine production and sodium excretion in order to regulate the volume and composition of body fluids or to eliminate excess fluids from tissues. Clinically, diuretics are used to treat diseases such as hypertension, heart failure, liver cirrhosis, renal failure, and kidney and lung diseases. Diuretics can be abused in sport in two different

manners. First, because diuretics can remove water from the body, they can be used to lose weight rapidly so an athlete can meet a certain weight requirement for a sporting event. Secondly, they can be used to mask other doping agents by increasing the urine volume and therefore reducing the concentration of the doping agent. Some diuretics are also able to alter urinary pH, which inhibits the passive excretion off acidic and basic drugs in urine, therefore masking them. It is the urine dilution effect that allows diuretics to be classified as masking agents and declared prohibited in sport both in and out-of-competition (Cadwallader et al., 2010). In recent years, the number positive findings of diuretics use have increased. However, this increase may be due to improved methods of detection, rather than an increase in doping. Diuretics are mainly used to enhance the renal excretion of salt and water, however they affect more than just sodium and chloride levels. Diuretics also play a role in the renal excretion and absorption of other cations (K^+, H^+, Ca^{2+}, Mg^{2+}), anions (Cl^-, HCO_3^-, $H_2PO_4^-$), and uric acid. Because there is a wide array of different diuretic compounds with different pharmacological and physiochemical properties, there are different ways to classify diuretics. The most common ways to classify diuretics are by their "…site of action in the nephron, relative efficacy, chemical structure, effects on potassium excretion, similarity to other diuretics, and mechanism of action" (Cadwallader et al., 2010).

9.3 Pharmacology Of Diuretics

Cadwallader et al. (2010) discuss the pharmacology of several diuretics and how they are used in sports doping, as well to explain the analytical techniques that are currently used to detect and identify diuretics in urine. There are many different substances that are considered diuretics. Carbonic anhydrase (CA) inhibitors work to inhibit CA in the tubule cells of the

nephron. There are currently three CA inhibitors that can be used as diuretics: acetazolamide, dichlorphenamide, and methazolamide. All three of the substances show a half-life of 6-14 hours, which is a very brief detection window. The kidneys excrete both acetazolamide and dichlorphenamide as complete drugs, while methazolamide is significantly broken down (Cadwallader et al., 2010). Therapeutically, CA inhibitors have extensive uses. CA inhibitors are often used for glaucoma, to decrease the formation of aqueous humour and therefore intraocular pressure. They can also be used to treat pre-menstrual fluid retention. Acetazolamide can also be used to treat high-altitude mountain sickness by making blood more acidic by increasing bicarbonate excretion, which increases ventilation and allows the user to adjust to high altitude conditions. In 2008, acetazolamide was found responsible for 1.4% of the positive diuretic findings (Cadwallader et al., 2010). Athletes could use acetazolamide not only for diuretic purposes, but for training purposes as well. High-altitude training, which is not banned, can get athletes in better shape because they learn to work with less oxygen. However, if an athlete trains using a banned substance such as acetazolamide, they could face serious consequences.

Another class of diuretics is inhibitors of the $Na^+/K^+/2Cl^-$ symporter, which bind to the Cl^- binding site at the $Na^+/K^+/2Cl^-$ symporter at the loop of Henle, in the kidney (Cadwallader et al., 2010). A symporter is a membrane protein that allows different types of molecules to cross the plasma membrane at the same time. In this case, it allows for Na^+, K^+, and Cl^- to be transported together. Inhibiting this specific symporter would affect the kidneys' ability to concentrate urine. This would result in an increase in the concentration of Na^+ and Cl^- excreted. This would reduce the build up of other drugs in the urine, possibly prohibited substances, making them more difficult to test for, therefore masking them. Examples of these inhibitors are furosemide, bumetanide, and ethacrynic acid, among others. Most of these symport inhibitors

only undergo slight metabolism, so they are often excreted as intact drugs (Cadwallader et al., 2010). Excretion of intact drugs can be helpful, because it makes them easier to test for. Rather than having to detect metabolites, the drug itself can be detected. Again, if a diuretic such as this is detected, there may be reason to believe that further doping has occurred. These diuretics, referred to as loop diuretics, are used to treat pulmonary edema and chronic congestive heart failure. They can also lead to an increase in training ability. Loop diuretics also interact with other drugs, which produces a synergistic effect. When loop and thiazide diuretics are used together, there is an even greater increase in the amount of urine excreted, which could be used to mask other prohibited substances (Cadwallader et al., 2010). When the amount of urine produced increases, the athlete will excrete more urine, decreasing the concentration of any substances that may be in his or her body faster than if normal excretion occurred.

If an athlete is prescribed a diuretic for a medical condition, as long as the proper paperwork is filed and the reason is legitimate, they will be able to take the medicine with no further consequences, even if it is banned. If the athlete does not file the proper exemption forms, they can be held accountable for doping even if the medicine was prescribed. An athlete can also be held responsible for doping if the diuretic, even if it is approved, is detected in the urine with a threshold/sub-threshold level of another banned substance (Cadwallader et al., 2010). Diuretics are most commonly used before weigh-ins because they produce rapid weight loss, and before an anti-doping test because they can dilute the presence of prohibited substances in urine. They are most commonly abused for weight loss purposes in sports such as wrestling, weight-lifting, gymnastics, and swimming. Diuretics are either chronically abused, such as when weight loss is the goal, or in single doses, such as a few hours before a drug test. However, because they typically have a short half-life, it can be difficult to detect diuretics in urine 24-48 hours after

administration (Cadwallader et al., 2010). In sports where doping is likely to occur using diuretics, it may be beneficial to routinely test for them to prevent and more easily detect their use. This is also a reason why it is advantageous to not inform athletes of an anti-doping test too far in advance. If an athlete is notified of their test only a day, or a few hours prior, it is more likely that diuretic abuse will be detected because it will still be in their system.

9.4 Transition In The Methods Used To Test For Diuretics

The main mission of sports drug testing is to identify and quantify prohibited substances and/or their metabolites to determine if an athlete has been doping and if they have been, what the consequences should be. In the past, diuretics have been detected in biological samples using HPLC with ultraviolet-diode array detection (UV-DAD). However, this method cannot unequivocally identify substances, so it is not effective for the detection of drugs. Anti-doping laboratories have switched to using mass spectrometry methods, which can confirm the identity of substances. After proper sample preparation and derivatization, GC-MS can be used to detect and analyze diuretics in biological samples. Recently, anti-doping laboratories have started using LC-MS instead because the sample preparation is easier, and no derivatization is needed (Cadwallader et al., 2010). All of the techniques mentioned above, HPLC-UV-DAD, GC-MS, and LC-MS, along with LC-MS/MS, micellar electrokinetic chromatography, and capillary electrophoresis, can be used in the analysis of diuretics. However, regardless of the technique used, the WADA has set a minimum required performance level (MRPL) of 250 ng/mL for diuretics. This concentration is low enough to detect minor diuretic abuse in athletes. If the dosage is lower than this, it is likely that the diuretics are not causing a masking effect or resulting in the dramatic weight loss the abusers are pursuing. Cadwallader et al. (2010) writes

that GC-MS, LC-MS, and LC-MS/MS instrumentation works to detect parent compounds and/or their most indicative and abundant metabolites, however, the target analyte may not be the parent compound or the metabolites, but rather one or more of the degradation products that are formed after the diuretics are hydrolyzed in aqueous media. This situation is more common when there is a lapse between when the sample is collected and when it is tested. GC-MS was the most common analytical techniques used by anti-doping laboratories in the 1980s and 1990s to detect foreign chemical substances, xenobiotics, in biological fluids. This technique was also used to analyze diuretics. The shift to LC-MS has occurred for several different reasons: in recent years, there has been an increase in the number of target substances that need to be screened for by anti-doping laboratories, so more universal techniques are required; there is a need to simplify sample pretreatment; and there have been technological advances made with the instrumentation used, such as the production of bench top LC-MS and LC-MS/MS systems (Cadwallader et al., 2010). These reasons have caused a move from GC methods to LC methods. Anti-doping laboratories need to constantly evolve and adapt so they have the best instruments and techniques at their disposal to test for doping. The types of drugs being used are constantly changing and being modified, and anti-doping laboratories need to keep up with these changes. Athletes need to know that just because a drug is modified to avoid detection, does not mean they will not be caught doping. Research in the field of drug detection is a continuous process that will persist as long as new drugs are being produced. All athletes are at risk of getting caught if they make the choice to dope.

9.5 Health Risks Of Diuretic Use

Athletes should also be aware of the health risks that diuretic use can cause. Diuretic use

can cause severe dehydration, which can be harmful to the cardiovascular and thermoregulatory systems during exercise. This can lead to exhaustion, irregular heartbeat, heart attack, and even death. Diuretics can also preserve potassium levels in the body, which can lead to muscle cramps and cardiac arrhythmias. When diuretics interfere with uric acid metabolism, this can result in a gout attack. Certain diuretics can also cause a decrease in athletic ability, impair aerobic capacity, and decrease muscular strength (Cadwallader et al., 2010). An athlete may take diuretics to continuously lose weight because they think it will give them an advantage. However, the diuretic could cause a decrease in athletic performance due to dehydration, or other factors, resulting in the athlete performing more poorly. Now, not only has the athlete reduced their performance ability, but they have also put themselves at risk for disqualification and serious health problems. Often times when athletes dope they only think about how it will be advantageous to them and they do not consider the harmful effects it can have on their body. Diuretics are mostly used to increase urine excretions and produce rapid weight loss, which can seem harmless at the time, yet have serious health risks. Athletes tend to not consider that these substances could severely impair or even kill them.

CHAPTER TEN: CONSEQUENCES OF RECENT CHANGES MADE TO THE PROHIBITED LIST

Russia's five-time major tennis champion, Maria Sharapova, among many other athletes, has recently been affected by advancements made in the field of anti-doping and the changes it has produced in the athletic community. Sharapova faced suspension in early 2016 for using meldonium, or mildronate, which has commonly been used by Eastern European athletes in the past (Beacham, 2016). Before the new prohibited list was enacted in January 2016, meldonium,

also marketed as mildronate, was not a prohibited substance, and therefore anti-doping laboratories did not test it for. However, due to research, such as that done by Gorgens et al. (2015), it was added to the 2016 prohibited list for its similarities to other banned substances that are used in sport. Meldonium is an anti-ischemic drug that can result in increased endurance, improved recovery after exercise, protection against stress, and enhanced activations of the central nervous system. Outside of sport, meldonium is used for its cardioprotective properties, to treat neurodegenerative disorders and bronchopulmonary diseases, and it can be used as an immunomodulator (Gorgens et al., 2015). Due to its many different uses, meldonium is commonly taken for legitimate health reasons. However, its due to is performance-enhancing effects that it has been banned in sport. Before it was banned, many athletes taking meldonium claimed it was for health reasons, however researchers were interested in estimating the prevalence and magnitude of its misuse in professional sports. This data became very important in the decision-making process regarding if the drug should be banned (Gorgens et al., 2015). If researchers could show that a high volume of athletes were taking meldonium, either for health reasons or not, in high doses, it could be logical to think they were taking it to improve their performance.

10.1 Research On Meldonium That Emphasized Why It Should Be Banned

In 2015, meldonium was added to the WADA's Monitoring Program to determine the extent of its use and misuse in sport. This also meant that methods had to be created to measure and confirm the presence or absence of meldonium in urine samples. In their study, Gorgens et al. (2015) present two approaches for the detection of meldonium. One approach aimed to have the analyte implemented into existing routine doping control-screening methods so the anti-

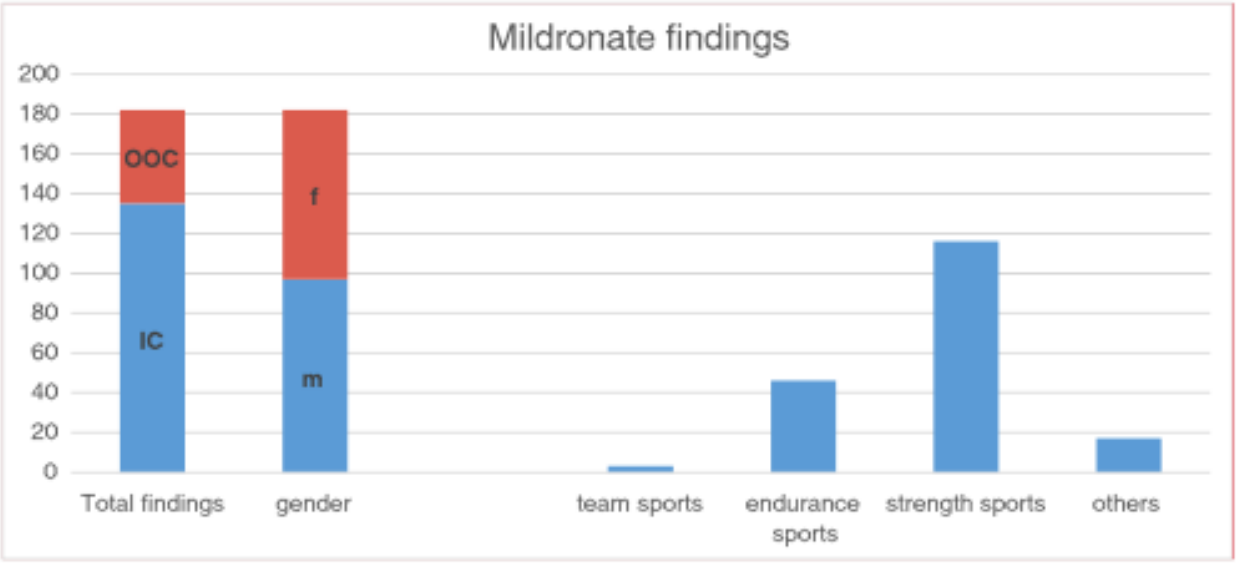

Figure VI- Mildronate findings in official doping control samples (n = 8320) and distribution between in- and out-of-competition samples (IOOC), gender (f = female; m = male) and type of sports (team sports, endurance sports, strength sports, others) (Gorgens et al., 2015)

doping laboratory could easily monitor its use, and the other approach was aimed at the specifics of the analyte so findings could be explicitly confirmed by hydrophilic interaction liquid chromatography-high resolution/high accuracy mass spectrometry (HILIC-HRMS). The experiment was used to analyze the urine samples of athletes from different classes of sports, both in- and out-of-competition (Gorgens et al., 2015). In order to suggest the substance be banned, the anti-doping laboratory must unequivocally prove that the substance is being widely used and in doses that could promote performance enhancement.

Figure VI shows the meldonium (mildronate) findings in the doping control samples. Of the 8320 random control urine samples used, 182 were confirmed for the presence of meldonium. It was determined to be used more in-competition, 74%, than out-of-competition, 26%. Meldonium was also found to be used in a wide range of sports, however it was used more in sports that require strength than sports that require endurance. No more information could be gathered on exactly why the substance is so widely used or abused, however the high

concentrations found in low-risk sports were alarming to the researchers (Gorgens et al., 2015). At the time this study was performed, meldonium was an approved drug that was suspected of being used to enhance performance (Gorgens et al., 2015). Meldonium was determined to effect humans in a way similar to the substance trimetazidine, which was included on the WADA prohibited list in 2015 because it can function as a metabolic modulator of cardiac metabolism. Both substances cause the inhibition of the β-oxidation of free fatty acids. Gorgens et al. (2015) present adequate test methods for the initial testing and confirmation of the presence of meldonium, and these methods can be included in existing screening methods of anti-doping laboratories. Because meldonium was determined to be so widely used and it was detected at urinary concentrations of more than 1 mg/mL, abuse of the substance was suspected. The authors of this study suggested, "…Under medical and pharmacological aspects as well as to preserve the integrity of sport the ban of mildronate [meldonium] from sport is deemed indicated" (Gorgens et al., 2015). Due to the findings of this research, and others like it, meldonium was included in the 2016 prohibited list. This caused problems for many athletes, such as Sharapova, who had been taking meldonium for years and failed to notice it was added to the prohibited list. New substances are continuously being researched and added to the WADA prohibited list due to their performance-enhancing effects. Athletes should be aware that just because a substance is not currently on the prohibited list, does not mean that it is safe to use or that it will not be included in the list one day.

10.2 A Failed Drug Test Does Not Always End An Athlete's Career

Sharapova tested positive for meldonium, causing her to fail her drug test in January 2016 while at the Australian Open. She admitted to taking the drug, and said she had been taking

it for 10 years for several different health issues under the care of a physician. However, it was argued that Sharapova's records with the doctor ended in 2013, and she continued to use meldonium anyway (Rovell, 2016). Although she says she was informed of the changes made to the prohibited list before it was enacted, she claimed to have not checked the list for changes since they did not apply to her in the past (Beacham, 2016). However, athletes are informed that they are responsible for whatever they put into their body, whether they know it is prohibited or not. Sharapova took full responsibility for her actions, but claimed they were unintentional and she takes pride in her integrity and would never want to risk it by doping. The International Tennis Federation (ITF) suspended Sharapova until the WADA could review the case and decide what her punishment would be. Sharapova originally faced the possibility of a penalty that could range from a multiyear ban, to an agreement that she would not be banned if it could be determined that she made an honest mistake (Beacham, 2016). However, this was not the case.

In June 2016, Sharapova was banned for two years by the ITF. The ITF panel claimed that although they believe Sharapova did not intend to cheat, she took responsibility for her actions that led to the positive doping test. Initially, the ITF wanted to ban Sharapova for four years, which is the required suspension for an intentional violation. However, intent could not be proven, so the rules state that an athlete cannot be suspended for more than two years if it is deemed that the drug use was unintentional (Rovell, 2016). Her lawyer, John Haggerty, believes the ITF gave Sharapova such a harsh sentence to make an example out of her. Sharapova claimed she would appeal the decision, however the Women's Tennis Association (WTA) issued a statement saying, "It is important for players to be aware of the rules and follow them" (Rovell, 2016). The athletic community does not want to punish innocent athletes, but they do not believe that ignorance should be allowed either. Even though it is believed that Sharapova did not know

the substance she was taking was banned, she was still aware of what she was putting into her body, and she was supplied with the list that stated meldonium was banned. If the governing bodies of the athletic community, such as the ITF and the WADA, do not punish all athletes who dope, regardless of their reason for doing it, it could become difficult to know whom to ban and who to let off with a warning.

Fortunately for Sharapova, her appeal to the Court of Arbitration for Sport (CAS) was a success. In October 2016, the CAS ruled that Sharapova did hold some degree of fault, but it was not significant enough to warrant a two-year ban, so her punishment was reduced to 15 months. The panel wanted to make it known that this case was about the degree of fault that could be attributed Sharapova for her failure to make sure that a drug she was taking for a long period of time remained in compliance with the updated anti-doping rules (Murphy, 2016). It is likely that if Sharapova had not been taking this drug for such a long time before it was banned that her punishment would not have been appealed. The governing bodies in the athletic community do not look for athletes to ban; rather they want to promote fair competition. The reason to ban athletes from a sport is to remove athletes who have an unfair advantage due to doping, and to prevent others from doing the same. However, if they decide a punishment is deemed to be unfair, they are willing to make changes so a proper punishment is given. Sharapova claimed that she learned how much better other federations were at notifying their athletes of the changes made to the prohibited list, especially in Eastern Europe where the use of meldonium is common (Murphy, 2016).

CHAPTER ELEVEN: CONCLUSION

Cases such as those discussed above show the importance of communication between the

governing federations and the rest of the athletic community. It also highlights the importance of having well informed athletes. Sharapova was fortunate enough that her case was heard and her punishment was reduced, however, other athletes such as Chris Colabello and Jenry Mejia, discussed earlier, have not been so lucky.

Athletes need to be well aware of the entire doping process from start to finish to ensure they are following the rules and to ensure that they are being treated, and their samples are being tested, fairly. Understanding the analytical procedures used to test their samples plays a vital role. Governing bodies of the athletic community, such as the WADA, have thousands of athletes to supervise worldwide, so it is essential that athletes stay well informed so they can ensure they are making the right decisions when it comes to their reputation, health, and athletic career.

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