Banned substances and their incidence: A retrospective view of the national laboratory of prevention and doping control of Mexico

Sustancias dopantes y su incidencia: una visión retrospectiva del laboratorio nacional de prevención y control del dopaje de México

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Abstract

The use of banned substances to increase athletes' performance has been a scourge in international sport. In this sense, the World Anti-Doping Agency (WADA-AMA) has implemented a series of standards that harmonize the fight against doping. In particular, accredited WADA-AMA laboratories play an important role in the eradication of sports doping. This report shows the data obtained in the National Laboratory of Prevention and Control of Doping (LNPCD-CONADE) according to the incidence of Adverse Analytical Findings (AAF) in the 2009-2015 interval, which were obtained from the analysis of a total of 18,085 biological doping-control samples. The distribution of samples was analyzed as a function of gender, type of sport either in competition or out of competition, as well as the prevalence of AAF during the period of time analyzed and the relation regarding group of doping substance and type of sport. The data presented here were compared with those reported worldwide by the WADA-AMA and it was observed that in the cases of substances of the S1 group, the percentage reported by the LNPCD-CONADE is higher than the one reported worldwide. The opposite was observed for AAF presented by some substance from Groups S6 and S8. Likewise, a higher prevalence in the use of doping substances by male athletes (75%) is observed compared to that observed in female athletes (25%). The sports with the highest number of AAF detected in the laboratory were baseball, cycling, and athletics.

Keywords: Anti-Doping Control; Banned substances; National Antidoping Laboratory-CONADE; ISO/IEC-17025.

Resumen

El uso de sustancias para incrementar el desarrollo deportivo de atletas ha sido un flagelo en el deporte internacional. En este sentido la Agencia Mundial Antidopaje (WADA-AMA) ha implementado una serie de estándares que permiten armonizar la lucha contra el dopaje desde diferentes aristas. Particularmente los laboratorios acreditados por la WADA-AMA forman parte importante en la erradicación de dopaje deportivo. En este informe se muestran los datos obtenidos en el Laboratorio Nacional de Prevención y Control del Dopaje (LNPCD-CONADE) de acuerdo a la incidencia de Resultados Analíticos Adversos (RAA) en el periodo 2009-2015, los cuales fueron obtenidos del análisis de un total de 18,085 muestras biológicas de control antidopaje. Se hace un análisis de la distribución de muestras de acuerdo al género, tipo de deporte ya sea en competición o fuera de competición, así como de la prevalencia de RAA durante el periodo de tiempo analizado y la relación respecto al grupo de sustancia dopante y tipo de deporte. Los datos aquí presentados se compararon con los disponibles en la página electrónica de la WADA-AMA y se observó que en los casos de sustancias del grupo S1 es más alto el porcentaje que se reporta por el LNPCD-CONADE que el reportado a nivel mundial, caso contrario se determinó para RAA que presentaron alguna sustancia del grupo S6 y S8. Así mismo se observa una mayor prevalencia en el uso de sustancias dopantes por atletas masculinos (75%) comparado con el 25 % observado en atletas femeninos. Los deportes con mayor número de RAA detectados en el laboratorio fueron béisbol, ciclismo y atletismo.

Palabras clave: Control antidopaje; Sustancias dopantes; Laboratorio Nacional de Prevención y Control del Dopaje-CONADE; ISO/IEC-17025.

Doping in sports is considered as the use of a substance or physical method that artificially increases an athletes' physical capacity. Accordingly, the World Anti-Doping Agency (WADA-AMA) was created at the end of 1999 in order to organize and manage the efforts aimed at the prevention of doping in sports. Since its inception, this agency published a document called the International Standard for Laboratories (ISL) (WADA-AMA, ISL, 2009, 2012, 2015) to harmonize the analysis of biological samples of doping control in its accredited laboratories. Before the creation of the WADA-AMA, several countries already possessed a laboratory acknowledged by the medical commission of the International Olympic Committee (IOC) to perform this type of analysis. Subsequently, these laboratories were accredited under the ISL issued by the WADA-AMA. Hemmersbach (2008) presented some historical data of the anti-doping laboratories to date. Other authors have described the impact and implications of sports doping in society and the sports community (Ramos, 1999; Catlin, Fitch & Ljungqvist, 2008; Dvorak, Saugy & Pitsiladis, 2014; Atienza, López & Pérez, 2014; Smith & Stewart, 2015). At this time, Mexico had a laboratory designed to meet the needs of analysis of biological samples for doping control of Mexican athletes, so the National Commission of Physical Culture and Sports (CONADE), the governing body of sports policies in Mexico, aimed its efforts at obtaining international accreditation of its laboratory under the standard of the WADA-AMA (WADA-AMA, ISL, 2009, 2012, 2015). In this sense, Mexico joined the international convention against the use of doping in sport, promoted by the UNESCO. In the year 2007, it reaffirmed its commitment to this cause, ratifying it through a decree published in 2007 in the Official Journal of the Federation (DOF, 2007), and conclusively supporting the accreditation of its laboratory.

In 2009, the National Laboratory of Prevention and Control of Doping of Mexico (LNPCD-CONADE), in collaboration with the Catalonian Anti-doping Laboratory Fundació Institut Mar D'Investigacions Mèdiques (IMIM) of Barcelona, Spain, a laboratory accredited by the WADA-AMA, began, in an organized fashion, the implementation of a quality management system based on ISO/EC-17025 standard, an indispensable requirement for the accreditation of the WADA-AMA at that moment. The preparations for the celebration of the 16th Pan American Games 2011, which would be held in October in the city of Guadalajara, Jalisco, Mexico, had already begun. Thus, the LNPCD-CONADE along with the Catalonian Anti-doping Laboratory, were responsible for the analysis of the biological samples of doping control of this important event in the form of satellite laboratory referred to in the ISL (WADA-AMA, ISL, 2009, 2012, 2015).

Later, in November, 2012, the LNPCD-CONADE obtained the accreditation of its standard quality management system under the norm NMX-EC-17025-IMNC-2006 (IMNC, 2006), by the Mexican Accreditation Entity, A.C. (EMA). Finally, in June 2013, after passing all the technical exams, having an organizational chart according to international standards and complying with all the requirements of the WADA-AMA, the LNPCD-CONADE received international accreditation. Therefore, the purpose of this descriptive study is to present the incidence of the doping agents used by athletes from the results generated by the analysis of biological samples of doping control delivered to the LNPCD-CONADE during the period of 2009 to 2015. The athletes' names are not presented because the delivered formats for doping control lack this data. Thus, reference is made only to the sport type (Olympic or non-Olympic), gender, and detected doping substance according to the classification used by the WADA-AMA (WADA-AMA, PL, 2009, 2010, 2011, 2012, 2013, 2015), data were compared with the available information in the electronic portal of the WADA-AMA during the same time interval (WADA-AMA, WLS, 2009, 2011, 2012, 2013, 2014, 2015). Importantly, the doping controls were randomly selected, so the entire universe of athletes is not included in every year for evaluation.

Methods

In this study, we only considered the analysis results obtained from 18,085 biological samples of urine received during the interval between 2009 and 2015. These samples, according to the ISL published by the WADA-AMA (WADA-AMA, ISL, 2009, 2012, 2015), were classified as samples in competition (SIC) or samples out of competition (SOC) (WADA-AMA, SCP 2006).

The urine samples were collected by doping control officers (DCO), staff external to the laboratory, who are responsible for the collection, transfer, and custody of the samples until their delivery to the laboratory as indicated by the WADA-AMA in its SCP document (WADA-AMA, SCP, 2006). Samples were collected throughout the year and are not dependent on the seasonal period, gender, age, sport or sports federation.

All the samples were received and registered for distribution and initial screening analysis using the internal operation analytical methods, which are validated in accordance with applicable international standards, as well as with the scientific information available at the time (Arnedo, Ricarte, Martínez & Salvador, 1998; Mareck, Geyer, Opfermann, Thevis & Schänzer, 2008; Kickman & Cowan, 2009; Sottas, Robinson, Rabin & Saugy, 2011; Botrè, De la Torre & Mazzarino, 2016). In addition, the indications referred to in different technical documents and guides issued by the WADA-AMA, (WADA-AMA, TD DL, 2010, 2012, 2013, 2014; WADA-AMA, EAAS, 2004-2014; WADA AMA, IRMS, 2014; WADA-AMA, IDCR, 2010, 2013, 2015;

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WADA-AMA, D-MRPL, 2009, 2010, 2012, 2014, 2015; WADA-AMA, NAND, 2004; WADA-AMA, TD-EPO, 2009, 2013, 2014; WADA-AMA, LDOC, 2009; WADA-AMA, ICOC, 2009) were followed in order to detect doping substances or their metabolites or markers as described in the list of banned substances and methods, which the WADA-AMA publishes at the beginning of every year (WADA-AMA, PL, 2009, 2010, 2011, 2012, 2013, 2015). Initial screening for detection of luteinizing hormone (LH) and of chorionic gonadotrophin (hCG) was performed using the analytical equipment Axym and COBAS e411. The confirmation of a suspicious sample, that is, initial screening detects the possible presence of a doping substance or its metabolites or markers according to the above mentioned list, was performed using specific analytical methods developed for each type of substance.

According to the technical documents of the WADA-AMA, substances classified with a cut-off threshold (WADA-AMA, TD-DL, 2010, 2012, 2013, 2014) were quantified and confirmed prior to the issuance of the analytical result. Regarding the rest of the substances that are not included in this category, their mere presence at a limit of acceptable detection in a urine sample constitutes an AAF. All samples, regardless of their outcome, were reported to the respective applicant agency, through the procedure implemented by the laboratory and, since 2013, they are reported through the Anti-Doping Administration and Management System (ADAMS) of the WADA-AMA.

Results

Table 1 presents a description of the analytical equipment currently possessed by the LNPCD-CONADE, as well as the group of doping substances that were detected in each case according to the list of banned substances and methods issued by the WADA-AMA (including parent substances, their metabolites or markers). These equipments were mostly acquired during the time interval analyzed herein.

As mentioned previously, 18,085 analytical results of urine samples were included in this study. Figure 1 shows the number of samples received per year in the period of 2009-2015. Urine samples from 48 different sports disciplines classified as Olympic and non-Olympic were analyzed. Since blood samples were not received before 2015, resultant data is not included in this report, nevertheless, some samples were analyzed upon request by the organizing committee of the 22nd Central American and Caribbean Games in 2014, when the LNPCD-CONADE performed the analysis of the samples obtained during this event. Figure 1 shows a continuous and substantial increment on the number of samples received since the accreditation granted by WADA-AMA.

Table 1. *LNPCD-CONADE analytical equipment.*

Analytical equipment	Number of instruments	Substance group detected
CG-EM (GC-MS)	10	S1, S3, S6, S7, S8
CG-DNF (GC-NPD)	3	S6, S7
CL-ES (LC-MS)	7	S1, S3, S4, S5, S6, S7, S9, P2
CLAP-ADD (HPLC-DAD)	1	S1
CG-C-EMRI (GC-C-IRMS)	2	S1
CG-EM 3Q (GC-MS 3Q)	2	S1
Luminometer tubes	1	S2
Flow cytometer	1	S2
Immunoassay (IMMULITE 1000, COBAS e411, Advia Centaur XP)	3	S2

Note. *S1: *S1: anabolic agents; S2: peptide hormones, growth factors, related and mimetic substances; S3: β-2 agonists; S4: hormonal and metabolic modulators; S5: diuretics and masking agents; S6: stimulants; S7: narcotics; S8: cannabinoids; S9: glucocorticosteroids; P2: β-blockers.

Figure 2 shows the total of AAF reported per year by the LNPCD-CONADE, regardless of the group of substances detected, for both Olympic and non-Olympic sports. We noted that the percentage of AAF was higher in non-Olympic sports in most of the years that were included in this study (*i.e.* American football, power lifting, body-building, and Ju-Jitsu among others).
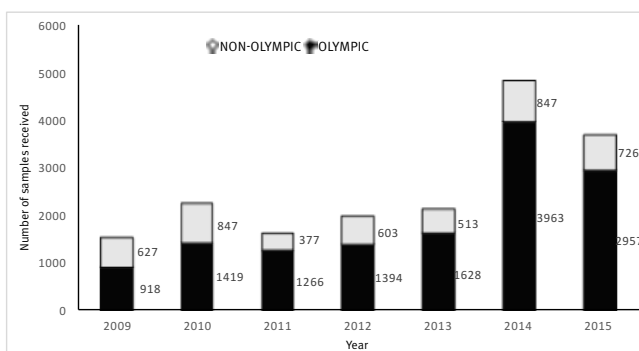

Figure 1. Total of urine specimens received during the 2009-2015 period, organized by type of sport.
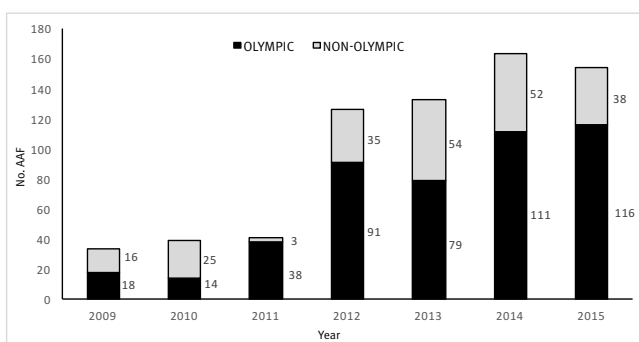

Figure 2. Total of informed AAF during the 2009-2015 for Olympic and non-Olympic sports.
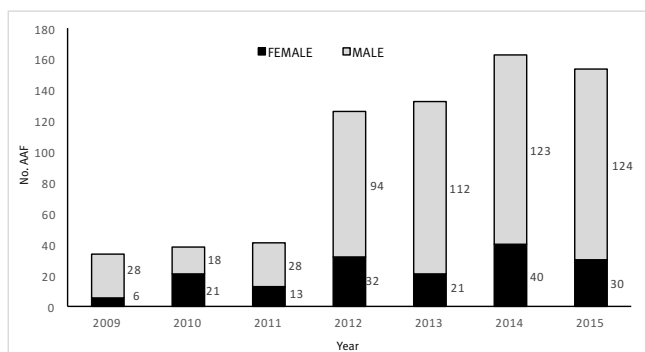
Figure 3. Total of informed AAF during the 2009-2015 as a function of male and female gender.

In the case of the total of AAF distributed by gender presented in Figure 3, we note that the incidence in males is greater than in females, we do not present a distribution by type of substance related to the athletes' gender.

Table 2 presents the total percentage of AAF per year reported by the LNPCD-CONADE as well as the annual percentage rate of AAF reported worldwide on the WADA-AMA website. In this regard, we noted that the percentage reported by the Mexican laboratory each year is above the percentage reported by the WADA-AMA.

From the conjoint analysis of the 18, 085 samples included, it was determined that the percentage of AAF repor-

Table 2. *Percentage of adverse analytical findings (AAF) reported by the LNPCD-CONADE during the 2009-2015 period.*

Type of sport	2009	2010	2011	2012	2013	2014	2015
Olympic	2.0	1.0	3.0	6.5	4.9	2.8	3.9
Non-Olympic	2.6	3.0	0.80	5.8	9.0	6.1	5.2
WADA-AMA*	0.90	0.90	1.0	0.99	0.97	0.77	0.83

Note.*Percentage of AAF, informed by the WADA-AMA on its website, only for Olympic sports.

Table 3. *Distribution of AAF by group of doping substances, during the 2009-2015 period.*

Group	2009	2010	2011	2012	2013	2014	2015	Cumulative total n (%)
S1	18	15	30	90	109	132	141	535 (75.54%)
S2	-	-	-	-	-	1	-	1 (0.14%)
S3	1	-	-	-	-	-	-	1 (0.14%)
S4	-	-	-	-	-	-	1	1 (0.14%)
S5	2	13	2	5	3	13	4	42 (6.09%)
S6	6	6	7	17	17	9	4	66 (9.57%)
S7	-	1	-	-	-	1	-	2 (0.29%)
S8	6	3	-	12	2	-	-	23 (3.33%)
S9	1	1	2	2	2	6	4	18 (2.61%)
P2	-	-	-	-	-	1	-	1 (0.14%)
TOTAL PER YEAR	34	39	41	126	133	163	154	690

ted by the LNPCD-CONADE was 3.8%. To provide more detailed information, Table 3 presents the data organized by year according to the group of substances detected. It is important to note that before July 2013 none of the released results by the laboratory had international validity. Nevertheless, they were recognized by the National Anti-Doping Committee of Mexico and the involved sports federation.

According to the list of banned substances and methods issued by the WADA-AMA, four groups of doping substances showed the highest incidence and they correspond to the following groups: S1 Anabolic agents (535 AAF), S5 Diuretics (42 AAF), S6 Stimulants (66 AAF), and S8 Cannabinoids (23 AAF). Each one will be discussed in detail in this document.

Anabolic agents (Group S1)

The presence of substances within Group S1 (WADA-AMA, WLS, 2009, 2011, 2012, 2013, 2014, 2015) were the most frequently detected by the LNPCD-CONADE during the period of time analyzed herein. Figure 4 shows a percentage of AAF in this group reported by the LNPCD-CONADE, during the 2009-2015 period compared with the total AAF reported worldwide by the WADA-AMA. Particularly, in 2015, 92% of all the AAF reported by the LNPCD-CONADE to clients corresponded to substances included in this group, of which several authors had already published analytical methods for its identification (Donike, 2011; Delgadillo et al., 2012; Saugy, Lundby & Robinson, 2014; Thevis, Kuuranne, Geyer & Schänzer, 2017; Avella & Medellín, 2012). This shows that anabolic agents are still the most frequently used substances by athletes to increase their athletic performance, at least in the universe of outcomes studied. This kind of substances are used to increase muscle mass, therefore, strength, and they are usually detected by gas chromatography coupled with mass spectrometry after derivatization of the molecule. Recent studies carried out by González-Martí et al. (González-Mar-
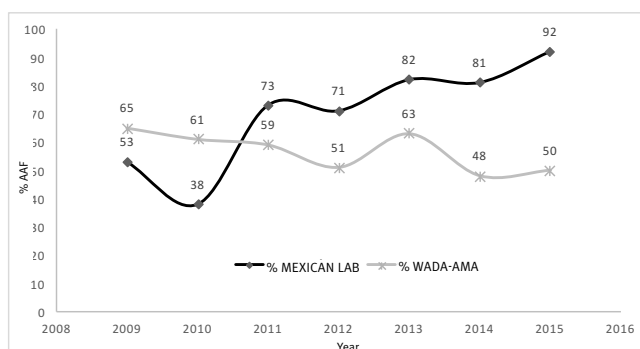

Figure 4. Percentage AAF reported by the LNPCD-CONADE during the 2009-2015 period due to the presence of substances of Group S1 anabolic agents vs. percentage of AAF reported by the WADA-AMA in the same period.

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tí, Fernández-Bustos, Contreras & Sokolova, 20185690) in a Spanish population of bodybuilders athletes and weight-lifters with muscle dysmorphia, showed that at least 50% of them usually consume anabolic androgenic steroids to treat this issue.

Of the 535 AAF, 11.03% corresponded to the presence of Nandrolone, meanwhile, Boldenone was detected in 2.62%; Stanozolol and its metabolites were identified in 4.67% of the samples and Clenbuterol was detected in 77.57%. Etiocolanolone, Epimetendiol, Drostanolone, Metandrolone, Danazol, Androsterone, Nandrosterone, Methandienone, Methyltestosterone, Oxandrolone, Gestrinone, Epitrenbolone, Mesterolone and Methenolone constituted 4.11% of the AAF altogether.

Figure 4 shows that the percentages of AAF for Group S1 substances reported by the LNPCD-CONADE are higher than those reported by the WADA-AMA.

Diuretics and masking agents (Group S5)

Figure 5 shows the percentage of AAF due to the presence of Group S5 substances, diuretics and masking agents, according to the classification of the WADA-AMA (WADA-AMA, WLS, 2009, 2011, 2012, 2013, 2014, 2015). Several studies reported in the scientific literature (Cadwallader, De la Torre, Tieri & Botrè, 2010; Thörngren, Östrvall & Garle, 2008; Koehler et al., 2011) refer to the analytical methods for the detection of this family of substances.

In general, the tendency of AAF due to diuretics reported by the LNPCD-CONADE remained below the global trend. Except for 2010, when the percentage reported by our laboratory was higher (33%) compared to the one reported by the WADA-AMA (7%) that same year. In general, this family of substances is detected using liquid chromatography coupled with mass spectrometry. According to Brunton et al. (Brunton, Chabner & Knollman, 2012) diuretics increase urine output, so they are used to increment the removal of exogenous substances present in the human body. It is not rare to detect this type of drugs in th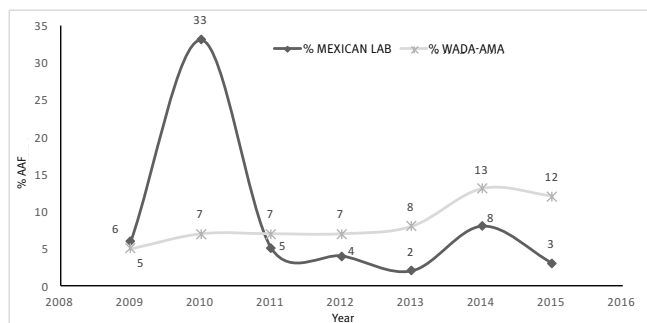e laboratory in combination with some other type of doping substance, which is considered sports doping. The percentages of these substances detected in the studied time period are: 47.62% Furosemide; 19.05% Chlorthalidone; 30.95% Hydrochlorothiazide; and 2.38% Bumetanide.

Stimulants (Group S6)

The detection of Group S6 doping substances (WADA-AMA, WLS, 2009, 2011, 2012, 2013, 2014, 2015), which corresponds to stimulants, is only required in SIC, that, according literature (Brunton, Chabner & Knollman, 2012), exhibit an immediate and short term pharmacological effect, then, its detection is not considered relevant when athletes are out of competition. Several authors have reported interesting analytical proposals for the adequate detection of these substances in biological samples of urine, using liquid chromatography coupled with mass spectrometry (Deventer et al., 2009; Barroso et al., 2012; Beuck et al., 2012; Monfort, Martínez, Bergés, Segura & Ventura, 2015; O'Byrne, Kavanagh, McNamara & Stokes, 2013; Marclay, Grata, Perrenoud & Saugy, 2011; Strano, Abate, Bragano & Botrè, 2009).

Figure 6 shows the percentage of AAF in Mexican athletes' samples due to the presence of any substance in this group, we noted that the number of AAF due to Group S6 substances droped to 3% in recent years (2015), in contrast, worldwide data reported by WADA-AMA shows 15% of AAF in the same group. Twenty-one percent of AAF attributed to the presence of stimulants corresponded to samples of athletes in competition; the highest incidence is observed in non-Olympic sports. The substances of Group S6 with highest incidence were: Amphetamine (39.39%), Pseudoephedrine (4.55%), Metylhexaneamine (37.88%), Cocaine (7.58%), and Oxilofrine (3.03%). The remaining percentage is distributed between Methylphenidate, Isometheptene, Phentermine and Octopamine; in some cases, they do not exceed 1.5% on an individual basis. It is important to note that the percentages of AAF displayed by the LNPCD-CONADE were calculated from all the results reported per year.
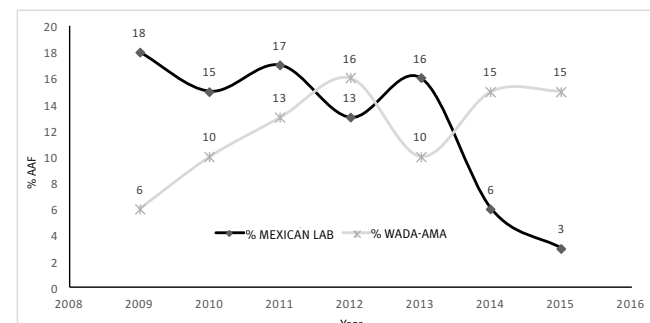

Figure 5. Percentage of AAF reported by the LNPCD-CONADE during the 2009-2015 period due to the presence of substances of Group S5 diuretics vs. percentage of AAF reported by the WADA-AMA in the same period.

Figure 6. Percentage of AAF reported by the LNPCD-CONADE during the 2009-2015 period due to the presence of substances of Group S6 stimulants vs. percentage of AAF reported by the WADA-AMA in the same period.

Cannabinoids (Group S8)

The last of the four groups that are considered with a higher incidence of AAF was Group S8 (WADA-AMA, WLS, 2009, 2011, 2012, 2013, 2014, 2015), due to the presence of cannabinoids as described by several authors like Mareck et al. (2009), Castaneto et al. (2015), Möller et al. (2011), Chbbah, Pozo, Deventer, Van Eenoo, and Delbeke, (2010), who described the analytical methods for the detection and confirmation of the presence of this kind of substances in urine samples. Figure 7 presents the percentage of AAF due to the presence of cannabinoids compared to the data reported by the WADA-AMA in the same period of time.
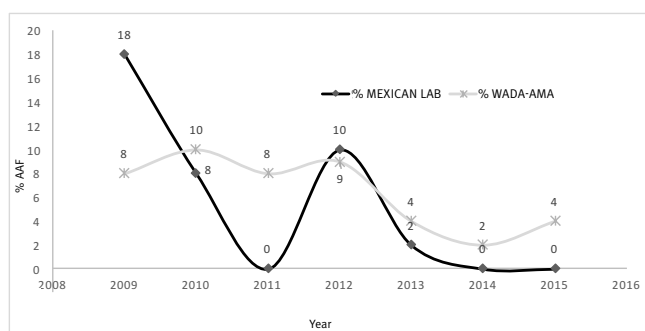

Figure 7. Percentage of AAF reported by the LNPCD-CONADE during the 2009-2015 period due to the presence of substances of Group S8-cannabinoids vs. percentage of AAF reported by the WADA-AMA in the same period.

It is important to address that, until 2013, a sample was considered an AAF if it had a concentration of THC and/or its metabolites above15 ng/mL. Later, in the mid-2014's, this value was modified and increased to a decision limit of 180 ng/ mL with an uncertainty of 10%; thus, the number of AAF due to the detection of cannabinoids showed a decrease in the LNPCD-CONADE until reaching zero, a trend that was observed until the year 2015. This type of substance is detected regularly by gas chromatography coupled with mass spectrometry.

Discussion

In 2013 and after LNPCD-CONADE obtained the international accreditation granted by the WADA-AMA the number of biological samples that were received in the laboratory increased, interestingly, not only Mexican federations sent their samples, but international sporting agencies (*i.e.* UCI, IAAF, FIFA, FINA, CONMEBOL) did as well.

Although our analytical methods from 2009 to 2011 were not accredited by ISO/IEC-17025 standard, they were developed and subsequently validated following a rigorous process according to the available technical documents and the applicable international technical standards for analytical methods. Hence, the results obtained by these methods are valid and can be compared with those available in the WADA-AMA website (Kioukia et al., 2014; Aguilar et al., 2017). We noted that the type of analytical method for the detection of the majority of the substances are freely chosen by each laboratory, as long as they reach the levels of detection and quantification required by the WADA-AMA. Regarding other methods such as, for example, those employed to determine the origin of endogenous substances that could have been consumed exogenously, there is a precise specification of the type of equipment and methodology to be used, which is isotopic relations mass spectrometry (IRMS), as well as a technical document indicating the steps to be followed in sample preparation, the method of analysis, and how to report the results (WADA-AMA, TD-IR-MS, 2014). The total percentage of AAF reported in the stu-died period was 3.8%. This value includes all the groups of doping substances considered by the WADA-AMA on its list of banned substances and methods.

As mentioned, the collection of urine samples is carried out by staff external to the laboratory; sampling was not done in a specific seasonal period, so the type of analysis and the time of collection depend on the season of the year. The WADA-AMA has not issued any Instructions concerning the treatment that should be applied to a sample collected in different seasonal periods of the year, so this is not a parameter that influences or determines the type of detected doping substance. It is important to mention that in the LNPCD-CONADE, most of the samples are received during May to September, possibly due to the fact that the majority of sports competitions are held during this period and the federations involved increase the amount of controls of their athletes prior to their participation. Additionally, during this period, the National Olympics is celebrated in Mexico, this event is local in nature and only involves national athletes, so, a number of biological samples are sent for analysis.

On the other hand, the detection and quantification of substances of the different groups considered by the WADA-AMA as doping are determined according to the technical documents it issues. Specifically, the "minimum required performance level" (MRPL) is a mandatory analytical parameter of technical performance, established by the WADA-AMA which the laboratory must comply whenever the confirmation of the presence of a particular banned substance or its metabolite(s) or marker(s) is required. This parameter is set to harmonize the analytical performance of the methods applied for the detection of non-threshold substances. The MRPL is the minimum concentration of a banned substance that laboratories must be able to reliably detect and identify in daily routine operations. In particular, these concentration values have decreased over the years, perhaps due to the availability of analytical equipment with higher detection power and sensitivity. Currently, in 2017, the values of the MRPL for each group of doping substances according to the requirements

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of the WADA-AMA are the following: S1=5 ng/mL, S2=2 ng/mL, S3=20 ng/mL, S4=20 ng/mL, S5=200 ng/mL, S6= 100 ng/mL, S7=50 ng/ mL, S8=1 ng/mL, S9=30 ng/mL and P2=100 ng/mL (WADA-AMA, MRPL-2017). Any substance that is included in the different groups and that the laboratory detects at these levels can lead to an AAF. It is also possible for a laboratory to detect any of them at levels below the MRPL with sufficient analytical reliability to report a sample with an AAF.

According to the total AAFs reported by the laboratory, 75% corresponded to samples obtained from male athletes, and 25% to female athletes. This relationship has not changed over the time in any of the groups of doping substances. Further analysis by substance group, shows that, in Group S1, out of a total of 535 AAF, 76% are associated with male samples and 24% with females'. In the case of AAF corresponding to Group S5 (42 AAF), 45% were collected from female athletes and 55% to male athletes. Moreover, it was observed that, in the case of stimulants (Group S6), out of a total of 66 AAF, 80% (n = 53) were detected in samples of male athletes and only 20% (n =13) corresponded to female samples. On the other hand, in Mexico, since 2008, as mentioned in the Official Journal of the Federation (DOF, 2008), the use of pseudoephedrine and ephedrine for the manufacture of drugs is banned, as well as their importation, so, the diminution observed in the number of AAF due to the presence of such substances could be influenced by the restriction of their commercialization in the country. In this same line, out of a total of 23 AAF from Group S8, 91% corresponded to samples collected from males while the rest were from females. With regard to the athletes' gender, the rest of the groups of substances are not discussed since they do not represent more than 2% of the total of AAF. The described results allow us to observe that the number of AAF due to all the groups of doping substances is higher in male athletes.

Particularly, a noteworthy fact is the high incidence of AAF for the presence of Clenbuterol in urine samples. Clenbuterol is a non-steroid substance which, at certain concentrations, produces androgenic effects, and is detected in 77.57% of the samples. There is a history of a high probability of the presence of Clenbuterol in an athletes' biological sample being due to the unintentional consumption of beef contaminated with this substance, as evidenced by several authors (Guddat et al., 2012; Thevis et al., 2013). Likewise, other authors have studied the enantiomeric relationship of Clenbuterol detected in biological urine samples when this substance comes from the ingestion of drugs for commercial use (Thevis et al., 2013; Parr et al., 2017; Velasco-Bejarano et al., 2017). Regardless of the available scientific information, the laboratory must report any adverse result. The National Anti-Doping Committee and the involved sports federation will determine whether or not to sanction the athlete based on this analytical result. On the other hand, in general, the sports in which the most amount of AAF were detected is baseball and cycling, followed by athletics, American football, weight-lifting, body-building and soccer. In the case of baseball, the substances that were detected the most were associated with Groups S1, S6, and S8; the largest number of AAF was observed in the year 2009. In the case of athletics and cycling, most of the AAF of these sports corresponded to the presence of substances of Groups S1 and S6. In the case of urine samples from athletes practicing weight-lifting, body-building, and football, the presence of Group S1 substances was constant, and the presence of substances from other groups was almost zero. We noted that, of the aforementioned sports, soccer is the one wich performs more doping controls, therefore, the elevated number of analyzed samples could increase the number of AAF reported. It is important to note that the majority (79%) of the AAF corresponded to the presence of Clenbuterol.

Regarding the presence of Group S5 substances in biological samples for doping control, it is important to mention that we observed the presence of diuretics in combination with some other substances in urine samples only in 2012, 2013, and 2014 which corresponded to the following Groups: S1 (3 cases), S6 (1 case), and S8 (1 case), and S9 (1 case). Moreover, the percentage reported by the LNPCD-CONADE in 2015 was lower (3%) than the one reported worldwide (12%) by the rest of the laboratories accredited by the WADA-AMA.

Generally, the number of AAF attributed to samples from Olympic sports is twice the amount of those obtained from non-Olympic sports. This could be due to the increased number of samples collected from these sports that are sent to the laboratory. It was not possible to establish the existence of a dependency between the type of detected doping substance and the gender from whom the sample was obtained. We could only confirm a higher incidence of AAF in males (75%) than in females (25%). Figure 8 shows the distribution by type of doping substance regarding the athlete's gender.
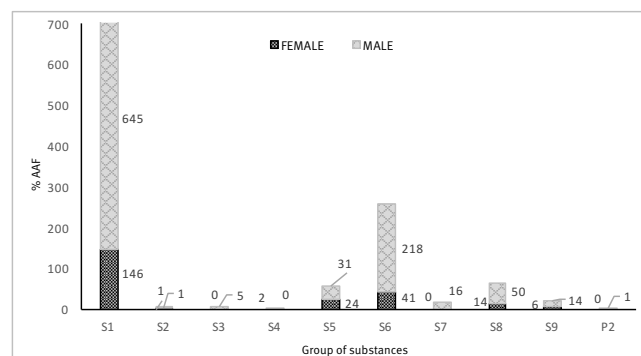

Figure 8. Number of AAF reported by the LNPCD-CONADE during the 2009-2015 gender vs. doping substance group.

However, in Group S5, the number of AAF was very similar in both genders; meanwhile, in the other groups of doping substances, a higher prevalence of AAF is observed in males. In that sense, it was noted that there is a clear trend of the presence of substances from Groups S1, S6, S7, and S8 in urine samples of male Mexican athletes.

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Conflict of interest

The authors declare they have no conflict of interest.

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