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Unravelling the threat of contamination in elite sports: Exploring diverse sources impacting adverse analytical findings and the risk of inadvertent exposure to prohibited substances

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ABSTRACT

In recent years, increasing concerns have emerged regarding athletes being exposed to various sources of contamination that could result in an adverse analytical finding (AAF), which is considered a positive doping test and may lead to the athlete's sanction. This review aims to examine the potential sources of contamination. Firstly, exogenous sources such as food, water, supplements, and medications will be described, along with endogenous sources, primarily arising from the athlete's physiological condition via the biotransformation of Medications. Finally, other hypothetical contaminations arising from sample collection procedures, poor transport or storage, and laboratory conditions will be discussed. Despite some legislative efforts to regulate the production of food and supplements, contamination remains a significant concern in the context of anti-doping, necessitating athletes to stay vigilant against the risks of inadvertent uptake of illicit products. Increased knowledge of the potential sources of contamination is essential for all parties involved in the fight against doping, including athletes, support personnel, legitimate supplement product manufacturers, and the anti-doping and scientific community. Such insights can contribute to developing the most effective strategy for preventing contamination and, most importantly, reducing the risk of inadvertent AAFs.

1. Introduction

Doping is generally understood as the act of using performance-enhancing drugs (PEDs), often illicit substances, to gain an advantage over others in sporting competitions. It may include the abuse of anabolic steroids, human growth hormones, stimulants, Endogenous Erythropoietin (EPO), and diuretics [1]. The use of drugs by athletes to improve performance dates back to the ancient Olympic Games; however, it was in the early 20th century, with the advent of modern medicine, when the first doping cases were documented. Anti-doping control began in 1928 with the introduction of the List of Prohibited Substances by the International Association of Athletics Federation (IAAF) [2]. Then, in 1960, during the Summer Olympic Games in Rome, the Danish cyclist Knud Enemark Jensen died suddenly. His death prompted the formation of a medical committee by the International Olympic Committee, leading to the establishment of The Institute for Drug Testing [3]. Ultimately, to harmonize rules and promote anti-doping activities, the World Anti-Doping Agency (WADA) was established in 1999, in the aftermath of the so-called "Festina doping scandal" in cycling during the 1998 Tour de France [4]. Currently, the World Anti-Doping Code (WADC) has extended its definition of a doping violation to include, amongst others, the use of prohibited substances, attempts to evade testing, trafficking in substances, complicity in the possession or distribution of prohibited substances, and efforts to tamper with samples [5]. A fundamental aspect of the WADC is the principle of "strict liability." This principle stipulates that any detection of a prohibited substance or its metabolites in an athlete's sample constitutes a doping violation, regardless of the athlete's intent or knowledge. In other words, under the principle of strict liability, an athlete is held responsible for any prohibited substances found in his bodily specimen, whether the substance was intentionally, unintentionally, or due to negligence. This means that the presence of a prohibited substance automatically violates

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anti-doping rules without considering whether the athlete was at fault or aware of the substance [6]. Additionally, WADA establishes standards to ensure the integrity and security of the testing process, which encompasses everything from athlete notification to laboratory analysis and reporting.

The primary motivations behind athletes doping are, for example, performance enhancement, winning, and meeting the immense sporting pressure they face after injury or during team selection. Athletes who use prohibited substances are sometimes aware of the associated health risks, yet they persist. In Bob Goldman's survey, a surprisingly high number of athletes indicated that they would take a performance-enhancing substance if it guaranteed victory and freedom from detection, even if it meant they would die within five years. This revelation underscored the significant ethical dilemmas (the Goldman dilemma) surrounding doping in sports and raised questions about the lengths some athletes are willing to go to achieve success. Its findings remain relevant because they highlight a fundamental motivation in sports today: the intense desire to win, even at a high personal cost. Despite the evolution of anti-doping regulations, such as the introduction of the World Anti-Doping Code (WADC), the underlying pressures faced by athletes, whether to perform at their peak, recover quickly from injuries, or secure a spot on a team, continue to drive some to risk their health by using performance-enhancing substances.

This is especially true for athletes with fewer resources and less access to comprehensive sports health education. These athletes are often more vulnerable, seeking out new substances in their search for an advantage over the competition [7] [8]. Additionally, commercialising top-level competitive sports has led to significant economic profits. Pursuing maximum profit during their limited sporting careers is fundamental to understanding the constant pressure athletes face to perform at their best and ensure future stability. An important study involving interviews with 1800 athletes at the 2011 World Championships in South Korea revealed that one-third of athletes were most likely using prohibited substances to enhance performance [9]. While general studies have explored broad psychological factors behind doping, more specific research has investigated concrete motivations across different sports, identifying factors like the quest for personal significance. This study analyzed phone calls from 115 cyclists, 203 bodybuilders, and 40 footballers to a French national anti-doping service to identify their doping motivations. The results revealed sport-specific motives: cyclists primarily doped to preserve health and manage the physical demands of their sport, bodybuilders focused on increasing muscular strength, and footballers used substances mainly for recreation and relaxation. Contrary to common assumptions, group influence was minimal, while health concerns were significant, especially among cyclists. The findings suggest that prevention campaigns must be adapted to each sport's specific motives and contexts to be effective [10]. In more recent research, authors delve into the psychological factors that motivate young non-professional athletes to consider using doping substances, applying the Quest for Significance Theory. The study finds that athletes who experience a loss of personal significance are more likely to develop an obsessive passion for their sport. This obsession can lead to moral disengagement, where athletes justify unethical behavior, such as doping, to regain their sense of worth. The influence of social networks, which may tacitly or explicitly approve doping, further exacerbates this behavior. The research highlights that doping is not solely about performance enhancement but also about fulfilling deeper psychological needs, such as the desire for recognition, self-worth, and significance, especially when other life aspects feel lacking [11].

Doping remains a significant issue across various sports, with prevalence rates varying widely depending on the sport and level of competition. On a broader scale, doping prevalence in competitive sports ranges from 0 % to 10 % of Adverse Atypical Findings (AAFs), though most sports have a prevalence below 5 %. The percentage of AAFs has decreased over the years from 1.32 % in 2016 in all Sports to 0.65 % in 2021. There has been an increase in the total number of AAFs during 2022 0.77 % of total AAFS. This highlights despite the improvement over the years reducing doping; there are still many challenges to face in eradicating doping from elite sport [12].

The use of prohibited drugs to enhance performance is impacting both the health of athletes and the integrity of sports. As such, correctly identifying an adverse analytical finding (AAF, i.e., the presence of a prohibited substance and/or its metabolite in a doping control sample) is integral to a successful anti-doping program. However, a significant challenge for anti-doping agencies and athletes subject to doping controls is the potential risk of an AAF being reported as the result of exposure to prohibited substances where the risk of exposure to the banned substances or testing positive is unforeseen. These cases occur when an athlete is unaware of having a prohibited substance in their urine or blood at the time of doping control or could not have suspected it. Whether due to contamination (presence of undesired substances, particles, or microorganisms), sabotage, or accidental transfer, inadvertent exposure that could result in an AAF is a reality. Due to the highly sensitive methods used in anti-doping laboratories and the presence of prohibited substances in various sources, these inadvertent exposure cases are increasing annually [13]. A shared goal of the anti-doping stakeholders is to catch and sanction those who cheat; the anti-doping stakeholders are conscious of the issue of accidental doping and, as will be demonstrated, endeavour to weed out the circumstances where the origin of the prohibited substance triggering the AAF are entirely unrelated to sport performance enhancement.

Understanding the problem from various perspectives, including the potential sources, current case studies and research, and the available laboratory detection techniques, is essential to reduce the risk of inadvertent exposure and better elucidate intentional doping cases. This review will also highlight various instances of contamination and how scientific research resolved them. Finally, this review will discuss how inadvertent exposure may be prevented and how to distinguish these from confirmed cases of intentional doping.

2. Instances and causes of inadvertent exposure

Whether in or out of competition, athletes are subject to anti-doping testing. Under the strict liability principle set forth by the WADC, they will be liable for a doping violation whenever a prohibited substance (markers or metabolites) is found in their bodily specimen. When athletes are positive, they face sanctions, such as a ban from competitions, regardless of whether they intentionally or unintentionally used a prohibited substance or were negligent or otherwise at fault. Athletes are burdened to establish the origin of the banned substance detected in their sample. If successful, they can see the sanction decreased or lifted upon the proof that the doping violation was not intentional or that they were not at fault.

Thus, while going about their regular training routines, competing, and travelling, athletes may be inadvertently exposed to various sources of contamination, potentially resulting in an AAF. These sources may range from food, water, and Medications to endogenous factors producing prohibited substances. Furthermore, the journey of a sample from collection to analysis is fraught with hypothetical pitfalls, each presenting an opportunity for inadvertent exposure. Table 1 summarizes the potential contamination sources, molecules and literature review described in this article. Fig. 1 illustrates the intricate journey of a sample through various stages, highlighting the possible points of vulnerability where contamination can occur and lead to an inadvertent AAF.

2.1. Food contamination

2.1.1. Growth promoters in livestock

One of the primary challenges in anti-doping involves the contamination of food with substances referenced in the Prohibited List maintained by WADA. This ongoing issue stems from various sources,

Table 1
Summary of the potential sources of contamination.

Sources	*Substances*	*Bibliography*
Food	Clenbuterol, zilpaterol, ractopamine, boldenone, zeranol, poppy seeds, cocaine, phthalates, Letrozole and Clomiphene.	[11–31]
Water	Hydrochlorothiazide, testosterone	[31–35]
Medications	Capromorelin, Letrozole, clostebol, hydrochlorothiazide, trimetazidine, codeine, Chlorazanil, Flutamide, Proguanil, Oxethazaine, Methylnaltrexone, Bicalutamide, Bupropion.	[36–61]
Supplements	Higenamine, SARMS.	[62–78]
Creams	Clostebol, Meclofenoxate, clorphenesin.	[79–82]
Sabotage	Dianabol, EPO.	[83–85]
In situ biotransformation	androst−4-ene−3,17-dione, 5α−5β-androstanediones, Δ1 steroids, 19-norsteroids, boldenone, boldione, prednisolone.	[86–90]
Bacteria	testosterone, androsterone, etiocholanolone, dehydrotestosterone, dehydroepiandrosterone, 5α−5β-androstanediones.	[91–99]

including the indiscriminate use of anabolic agents in livestock aimed at rapidly fattening animals for increased profits in some areas of the world or the illegal trafficking of contaminated meats and challenges with traceability. Even legitimate use of anabolic agents may present challenges, with farmers exceeding permitted dosages or allowing an inadequate wash-out period before slaughtering. Among the most prevalent anabolic androgenic steroids in meat is clenbuterol. Clenbuterol is referred to as the 'lean meat essence,' a broad term that also encompasses adrenal nerve stimulants such as ractopamine, salbutamol, salbutamol sulfate, and terbutaline. Other common anabolic agents found in contaminated meats include zeranol, a synthetic non-steroidal estrogen, and zilpaterol, a β2 adrenergic agonist [14].

In the anti-doping context, the first investigation into the possibility of contaminated meat was prompted in 2011 by five AAFs for clenbuterol during an out-of-competition control of the Mexican national football team, competing in the U-17 World Cup in Mexico. The Fédération Internationale de Football Association (FIFA) initiated an inquiry into potential food contamination impacting sports drug testing in Mexico. The subsequent research included the analysis of 208 doping control samples collected in Mexico, of which 52 % yielded clenbuterol findings ranging from 1 to 1556 pg/mL. Notably, only 5 out of the 24 football teams involved in the study provided urine samples without traces of clenbuterol. Extensive evidence strongly indicated meat contamination as the most plausible cause for the unusually high prevalence of clenbuterol findings. As a result, none of the football players of the U-17 Mexican National team were sanctioned [15].

In the 2010 Tour de France, Alberto Contador tested positive for clenbuterol in blood (1 pg/mL) and urine (50 pg/mL) samples collected at the same in-competition doping control. In his defense, the athlete suggested that the detected levels were consecutive to the recent ingestion of a contaminated beef tenderloin weighing 3.5 kg. In the same year, livestock organizations, the agri-food industry, and the European Chemicals Agency (ECHA) emphasized that clenbuterol was strictly prohibited in the European Union [16]. Additionally, despite numerous reported cases of illegal trafficking of bushmeat in the same year in Spain, no data on illegal imports of livestock from China or Mexico could be found [17].

To better understand the circumstances surrounding Contador's case, research at the Autonomous University of Madrid was conducted on pharmacokinetic simulations [18]. The objective was to determine whether Contador's results were more explained by doping or consuming contaminated meat. Researchers calculated the plasma concentrations of clenbuterol under two scenarios: first, after consuming 3.5 kg of meat with the minimum legal allowance of anabolics, and second, if the animal had been heavily treated with anabolics to fatten the cattle. The study has shown that the resulting concentrations observed on Contador's samples were more likely under a doping scenario than under the proposition of consumption of meat, legal or not. The Court of Arbitration for Sport (CAS) deemed it highly improbable that the athlete ingested meat contaminated with clenbuterol. After reviewing all the evidence, the positive test for clenbuterol was considered more likely to result from the intake of a dietary supplement containing clenbuterol rather than the consumption of contaminated

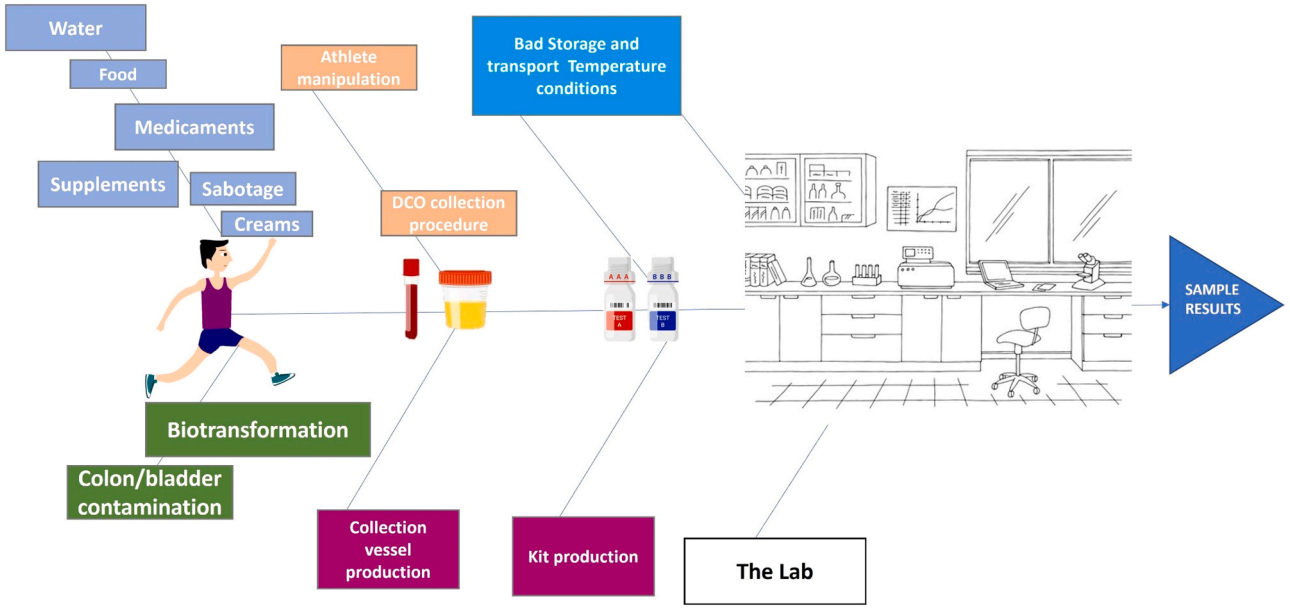

Fig. 1. The chronology of an athlete's activity and anti-doping sample collection, transport and analysis. The different grouped colours indicate various sources of potential contamination. Some may not cause an AAF, but the potential circumstances are identified as hypothetical contamination avenues. Blue represents exogenous molecules as sources of contamination found in food, water, medications, or sabotage. Green indicates endogenous sources primarily arising from the athlete's physiological condition. Orange is designated for any human manipulation during the sample collection. Light blue pertains to the transport and storage conditions. Purple relates to the sample container's material and cleanliness. Finally, the laboratory group is indicated in white.

meat [19,20].

Moreover, in 2012, researchers at the Institute of Biochemistry in Cologne, Germany, conducted a study to investigate the prevalence of clenbuterol contamination in meat in China. Urine samples were collected from 28 volunteers recently travelling to China and analyzed using liquid chromatography-tandem mass spectrometry. Results showed that 79 % of the samples contained clenbuterol. While the exact amount of consumed meat was unknown, considering the potential widespread misuse of clenbuterol, higher concentrations are anticipated in the population permanently residing in China [21].

Traveling can also pose a risk of contamination for athletes, especially concerning meat consumption in regions where certain anabolic agents are permitted in livestock or unregulated. In response to this concern and the growing phenomenon, the WADA Contaminants Working Group was established in 2019. Based on various studies and observations, this Group identified that clenbuterol is used as a growth promoter for cattle, lamb, poultry, and swine in China, Mexico, and Guatemala.

In addition to clenbuterol, other substances, including ractopamine, zeranol, and zilpaterol, are administrated to stimulate the growth of various animals, such as cattle, lambs, turkeys, pigs, and poultry. The WADA Contaminants Working Group has set residual levels in animal carcasses for the mentioned molecules that could lead to contamination. The Group has evaluated various scenarios, including cases where farmers adhere to withdrawal periods of anabolic agents before slaughtering animals, as well as scenarios where dosage and respect for elimination times may not be strictly followed due to occasional non-compliance with best practices. The WADA Contaminants Working Group also evaluated the concentrations of these substances expected to be present in the urine of athletes who consume meat from such livestock. The Group determined that the scientific evidence strongly suggests that the ingestion of edible tissue from animals fed with clenbuterol, ractopamine, zeranol, or zilpaterol is highly improbable to result in a urinary concentration exceeding (>) 5 ng/mL. Consequently, a sample with a concentration of less than 5 ng/mL will be considered an atypical finding ("ATF"), and the anti-doping organization must undertake investigative steps to understand whether the positive finding resulted from ingesting contaminated meat. If it is considered that the banned substance presence was due to inadvertent contamination from meat consumed by the athlete, the case will be closed without any sanction for the athlete. On the other hand, this means that a sample with a concentration of more than (>) 5 ng/mL of clenbuterol, ractopamine, zilpaterol, or zeranol or its metabolite(s) is considered sufficient to report an AAF [22].

Due to the lack of human excretion studies with zilpaterol, especially at food residue levels, a controlled elimination study was strategically designed and executed to reinforce anti-doping result management. Peak urinary concentrations of zilpaterol were detected in all participants between 1.5 and 12.5 hours post-ingestion. Maximum levels exceeding 5 ng/mL (considered an AAF in doping controls) were observed in one of the five study participants following ingesting 3 μg of zilpaterol on five consecutive days. This investigation underscores the complex interplay between meat contamination and its identification in athletes. Essential details such as the amount of meat consumed, the final concentration of anabolic agents in the meat, individual variations in the athlete's metabolism, and elimination kinetics for the molecule are frequently undisclosed [23].

Another potential contaminant, boldenone, is commercially available in ready-to-use anabolic preparations. It was formerly used for humans and is now primarily used in veterinary medicine, mainly for horses. However, it is also unlawfully used as a growth promoter in cattle farming in some Latin American countries, such as Colombia, Brazil or Argentina. Boldenone is permitted for veterinary purposes in the USA. Until recently, the detection of its illegal use was primarily based on identifying either 17β-boldenone or 17α-boldenone (its primary metabolite in cattle) in various matrices such as edible tissues, hair, feces, or urine. However, published data indicate the potential natural occurrence of these steroids in cattle, though not universally present. This introduces complexity to the analytical strategies for control measures. The research explored methods for uncovering the structures of the main metabolites (both phase I and phase II) in urine. The goal was to enhance the ability to compare boldenone urinary profiles between treated and non-treated animals. Most metabolites were identified as glucuro-conjugated, with sulfo-conjugated forms being infrequent, except for 17β-boldenone. Therefore, the investigation revealed that the absence of 17β-boldenone sulfoconjugate in non-treated animals offers a potential means to differentiate between treated and non-treated animals containing boldione, boldenone, and boldenone esters [24].

2.1.2. *Presence of contaminants in cattle's milk*

Some WADA-accredited anti-doping laboratories analyze plasticizers to support the allegation of athletes illicitly manipulating their blood values by using IV (plastic) blood bags. A plasticizer is a chemical used to improve the flexibility and durability of plastics. Phthalates, the most common plasticizers, are widely used in food processing and packaging. For example, milk is a significant source of phthalate contamination. Phthalates, being lipophilic, tend to concentrate in the lipid phase of milk. A 2012 survey in Belgium found Phthalate contamination at various stages in the milk chain, with potential sources including the mechanical milking process and the consumption of phthalate-containing feed by cattle [25]. Industry and retail levels also contributed to contamination, with packaging materials playing a role. For these reasons, whilst identifying plasticizers in athletes' samples may indicate intentional doping, identifying plasticizers in an anti-doping sample does not result directly in an AAF due to the high level of plasticizer contaminants in foods and the environment.

Returning to the case of Alberto Contador, during the 2010 Tour de France, the athlete not only tested positive for clenbuterol but also had an atypical level of phthalates detected in his in-competition urine sample. This finding prompted allegations of an illegal blood transfusion. The athlete's defense argued that in the environment, there is considerable uncertainty about how an athlete may have come into contact with it due to the ubiquitous presence of chemical residues, specifically the plasticizer bis(2-ethylhexyl) phthalate (DEHP). WADA and the Union Cycliste Internationale did not accept the rider's contamination scenario. Research conducted in 2012 demonstrated that the plasticizer concentration excreted in urine could indicate autologous blood transfusion up to 2 days post-reinfusion [23]. On the days before reinfusion, volunteers had DHEP levels described after everyday environmental exposure; however, a few hours after the reinfusion of one blood bag, a significant increase was observed in all metabolites in all volunteers. Additionally, concentrations of DEHP metabolites tended to be higher after longer storage times (14 days vs 28 days of storage, $p < 0.05$). Nevertheless, given the controversy surrounding the widespread presence of phthalates, the results from laboratory urine analyses for plasticizers are used solely as supportive evidence in cases of suspected autologous blood transfusion.

Other potential contaminants in cow's milk include aromatase inhibitors, specifically utilized in cattle to regulate the ovulation of female animals. Letrozole, a non-steroidal aromatase inhibitor, is among the compounds employed for this purpose. It is prohibited in sports because it is used together with anabolic androgenic steroids to reduce their adverse secondary effects. Recently, two Belgian cyclists, Toon Aerts and Shari Bossuyt, tested positive for Letrozole during a 2022 and 2023 cycling competition, respectively, in Normandy, France and claimed that their AAF may be attributed to the consumption of milk contaminated with Letrozole, proposing that milk from the area could contain trace amounts of the substance. While Letrozole is not prohibited in many countries and is frequently used to enhance ovulation induction in cattle, its use is currently not permitted in the European Union [26].

A study conducted at the University of Saskatchewan demonstrated

the excretion of Letrozole in milk, where the maximum Letrozole concentration observed was around 25 ng/mL. The research indicated that after a 2-day washout period, the Letrozole concentration falls below the limit of quantification (5 ng/mL), highlighting the rapid elimination of Letrozole. However, as with other substances used by livestock farmers, the recommended withdrawal times for these substances are not consistently adhered to [27].

Aerts also sought to prove his case through hair analysis for Letrozole. Hair analysis, depending on the nature of the analyte, is sometimes used to discriminate between repetitive use and occasional/inadvertent administration. To identify the dose capable of producing a positive hair test and apply these results to scenarios of inadvertent letrozole ingestion by an athlete, researchers investigated the urinary excretion and incorporation into the hair of single doses of Letrozole in seven volunteries. Hair collected after a single dosage showed concentrations of 16–60 pg/mg, while in women in chronic therapy, concentrations were higher than 160 pg/mg along the entire hair shaft [28].

In Aerts' case, hair analysis demonstrated that the athlete had been exposed to minute amounts of Letrozole months before the relevant urine sample collection. However, it did not indicate how Letrozole entered the cyclist's organism, which is the crux of the matter in doping cases. Alternatively, Aerts argued that a dietary supplement containing high amounts of milk derivatives could be the source of Letrozole contamination. After weighing the evidence adduced by the cyclist and the rebuttal evidence submitted by the Union Cycliste Internationale, the hearing panel rejected the "milk contamination scenarios", and the athlete was sanctioned [29]. Likewise, the contamination scenario did not convince the hearing panel in charge of the Shari Bossuyt case.

2.1.3. Other food sources of contamination

As mentioned previously, zeranol can be illicitly used in domestic livestock. Still, unintended contamination could also occur through the biotransformation of the mycotoxin zearalenone to zeranol, possibly ingested with contaminated food, primarily cereals. The following study describes that zearalenone in foods and feeds has been reported in infested wheat, barley, and corn in numerous countries worldwide over the last decade. The research is focused on differentiating whether the contamination comes from the misuse of zeranol as a growth-promoting agent in livestock or from the unintended ingestion of zearalenone. Four athletes (three females and one male) tested positive for zeranol, zearalanone, and taleranol during doping controls. Reanalysis of the samples also showed the presence of mycotoxins (zearalenone, a-zearalenol, b-zearalenone). The isomers zeranol, taleranol, and zearalanone constituted an adverse finding. Zearalenone, a-zearalenol, and b-zearalenone occurred consistently, prompting further investigation into the alternative sources of mycotoxin. Quantitative analysis showed notable differences in compound quantities between suspicious samples and those from oral zeranol administration. Suspicious samples exhibited a metabolic pattern consistent with literature descriptions of zearalenone metabolism, suggesting potential mycotoxin ingestion, as concentrations of zeranol, taleranol, and zearalenone remained consistently low [30]. In light of these findings, WADA has guided WADA-accredited laboratories on distinguishing whether zeranol results from mycotoxin-contaminated food consumption to avoid reporting AAFs stemming from food consumption [31].

Another observed source of food contamination is the ingestion of poppy seeds. In a study assessing the risk of positive doping results from consuming cakes containing poppy seeds, eight products were analysed for alkaloid content, including poppy seeds and baking mixtures. A batch of poppy seeds was used in a cake for an excretion study with nine volunteers. Urine specimens from the study showed morphine concentrations exceeding the 1 μg/mL cutoff set by WADA, with peak values reaching approximately 10.0 μg/mL. This suggests a potential risk for athletes to inadvertently test positive after consuming products with poppy seeds [32].

Coca tea, famous in parts of South America for its purported medicinal benefits, contains natural cocaine (COC) alkaloids. In a Peruvian anti-doping test, an athlete tested positive for benzoylecgonine (BZE), ecgonine methyl ester (EME), and COC in their urine, allegedly due to consuming coca tea before the competition. A study was conducted with similar tea bags to investigate the issue, collecting urine specimens over three days to monitor COC and metabolite elimination. Analysis revealed maximum COC detection times of 20 hours, with concentrations from 6 to 91 ng/mL, and BZE/EME detection times of 70/60 hours, with concentrations from 6 to 3730/1738 ng/mL, respectively. Profiles were consistent among volunteers, supporting the athlete's claim. Subsequent hair strand analysis showed negative results for COC, reinforcing the claim and highlighting the importance of hair analysis in distinguishing tea consumption from COC abuse [33].

Clomiphene, a selective estrogen receptor modulator (SERM), has been found in trace amounts in eggs, potentially due to its use in poultry farming to enhance egg production. Clomiphene is a banned substance in sports and has raised concerns within the anti-doping community due to its potential presence in food products, particularly eggs. Recent scientific studies have investigated the risk posed by clomiphene contamination in eggs and its possible impact on doping test results for athletes.

The first study aimed to determine whether consuming clomiphene-contaminated eggs could lead to detectable clomiphene metabolites in human urine. The researchers administered clomiphene-contaminated eggs to volunteers and subsequently measured the levels of clomiphene metabolites in their urine. The results revealed that even low levels of clomiphene contamination in eggs were sufficient to produce measurable metabolites in human urine [34].

The second study focused on understanding the persistence of clomiphene residues in eggs and muscle tissue following its administration to laying hens. The researchers administered clomiphene to a group of hens and then monitored the levels of the drug and its metabolites in the eggs and muscle tissue over time. Their findings demonstrated that clomiphene residues could remain in the eggs and muscles for an extended period, even after the cessation of drug administration [35].

Analyzing the frequency of food contaminated with a prohibited substance(s) provides valuable insights into the tangible risks of inadvertent doping. Moreover, it prompts a closer examination of the potential sources of contamination, considering the widespread nature of these molecules and their impact on athletes' test results. In unveiling the intricacies of anti-doping testing, Table 2 is a visual testament to the prevalence and impact of specific molecules. From 2019, WADA figures associate clenbuterol with the highest proportion of Atypical Findings (ATFs). An ATF finding would trigger an investigation into potential sources of contamination that contribute to the lab results.

Table 2
WADA testing figures results for Anabolic agents from 2019 to 2021. Adverse Analytical Finding (AAF), Atypical Finding (ATF).

Drug Class	AAF	ATF	Total
S1	**1712**	**322**	**2034**
clenbuterol	199	190	**389**
stanozolol	267	0	**267**
19-norandrosterone	162	29	**191**
boldenone	120	71	**191**
drostanolone	163	2	**165**
Other	147	0	**147**
oxandrolone	92	0	**92**
dehydrochloromethyl-testosterone	90	0	**90**
metenolone	80	0	**80**
enobosarm (ostarine)	74	0	**74**
trenbolone	69	0	**69**
LGD−4033 (ligandrol)	62	0	**62**
mesterolone	38	0	**38**
methasterone	31	0	**31**
clostebol	28	0	**28**
Inconclusive		27	**27**

2.2. Water

Various types of molecules, including medications like antibiotics, diuretics, painkillers, and recreational drugs, can be identified as contaminants in water. Typically, the concentrations of these compounds remain within acceptable toxicity limits. However, a study analyzing micropollutants in Lake Como (Italy) highlights the prolonged presence of diuretics [36]. Another study focusing on the surveillance of pharmaceutical and personal care products as contaminants in the Great Lakes (United States and Canada) water surface found testosterone at a concentration of 12.4 pg/mL [37].

Athletes have attempted to argue that their positive cases were due to water consumption. In 2015, The Court of Arbitration for Sport (CAS) suspended Australian kayaker Tate Smith. This decision came after Smith tested positive for stanozolol during an out-of-competition test conducted in Hungary. Despite Smith's claims that the substance might have entered his system through water at his training site, the CAS ruled against him, highlighting a lack of evidence to support his argument [38].

In some instances, the evidence was too speculative to convince the panel. However, in exceptional circumstances, at least one athlete could establish, on the balance of probabilities, that his AAF for hydrochlorothiazide ("HTCZ") came from healthy water consumption and was thus not sanctioned. Veronica Campbell-Brown tested positive for the diuretic HCT following her participation in a national meet in Kingston, Jamaica, on May 4, 2013. Despite both 'A' and 'B' samples returning positive, she denied intentionally taking a banned substance. Testimony from Peter Sever, a professor of clinical pharmacology, indicated that contamination through sweat or water containing HCT was the "most likely explanation" for Campbell-Brown's positive test. He pointed out similar positive cases among athletes tested in the Kingston stadium, suggesting a common environmental source [39].

2.3. Medications

Medications have emerged as inadvertent causes of positive doping tests for athletes, resulting from accidental contact or the presence of trace amounts of prohibited substances in the medication itself. Over recent years, numerous cases have highlighted the challenge of distinguishing between genuine doping behavior and contamination. In this section, we delve into some of the most prominent cases in anti-doping, exploring the specific molecules or drugs implicated.

2.3.1. Medication contamination by primary transfer

Primary transfer refers to the direct transfer of physical material from a source to a recipient during direct contact between two surfaces or objects [40]. In the context of medication contamination, this initial transfer occurs when the medication and the athlete come into contact, but without any intention or knowledge on the athlete's part.

In one of the most notable cases of medication primary transfer, Czech athlete Katerina Nash, known for her Olympic achievements in cycling and cross-country skiing, faced a potential four-year doping ban after receiving an AAF for capromorelin. However, a thorough USADA investigation revealed that the positive result stemmed from contact with her dog's medicine, which contained the prohibited substance. During the inquiry, Nash presented records of the prescription medicine for her ailing dog, Rubi. The medication, designed to stimulate Rubi's appetite, unintentionally landed on Nash's hands during administration. 7 pg/mL of capromorelin appeared in Nash's urine, triggering an AAF. Although capromorelin wasn't explicitly listed as a prohibited substance in the 2022 edition of the Prohibited List when the athlete provided her sample, it fell under the category of "other" prohibited substances related to human growth hormones. The lack of a capromorelin threshold and the minimal amount detected led to Nash's clearance, averting a four-year ban. Subsequently, USADA advocated for rule revisions to prevent the public disclosure of such cases, underscoring the need for a fair anti-doping system. Nash, whilst temporarily suspended and reflecting on the ordeal, expressed relief at the resolution while emphasizing the potential career impact of a simple oversight in hand hygiene [41].

2.3.2. Medication contamination by secondary transfer

Secondary transfer refers to the indirect transfer of physical material from a person or object to another surface, object, or individual [40]. Unlike primary transfer, which occurs directly during contact, secondary transfer involves an intermediate surface or object. There has been a growing concern and discussion about a rise in AAFs in doping controls, with a suspicion that such instances may be linked to intimate contact with body fluids, including ejaculation, which may facilitate the secondary transfer of prohibited substances [42]. One example of a secondary transfer of medication is Virginia Fuchs. In 2020, US Olympic team boxer Fuchs, a 32-year-old flyweight, tested positive for two prohibited substances, letrozole and GW1516, in an out-of-competition urine test. USADA stated that the low amounts of letrozole metabolite and GW1516 metabolites in her sample were consistent with recent exposure through sexual transmission. Fuchs' partner had indeed been using the substances, and Fuchs was found to bear no fault or negligence, resulting in no sanction.

The potential for prohibited substances to be present in ejaculate and transferred through sexual intercourse into an athlete's vagina and subsequently into doping control urine samples has also been previously examined. Samples collected post-sexual intercourse showed detectable levels of semenogelin for up to 55–72 h. The hypothesis that the introduction of ejaculate into a doping control urine sample can be confirmed or refuted by testing for the presence of semenogelin using lateral flow immunochromatographic and mass spectrometric tests [43].

Once the potential transmission is confirmed, an emerging issue is that semen samples may contain residual urine from ejaculation left in the urethra. The contamination may originate from the medication in the urine of the primary source, in this case, the male. The following research examined the possibility that residual urine contamination of ejaculated semen, as it passes through the urethra, could contribute to measurable drug concentrations in seminal plasma. The authors hypothesized that creatinine in urine is a convenient and easily measurable tracer for the urine content in seminal plasma. Thus, by employing a reliable enzymatic measurement of creatinine in seminal plasma, the continued presence of a small residual volume of urine (median 52 μl) was identified in the typical seminal ejaculate immediately after ejaculation [44].

The transmission can also occur from women to men secondary transfer. One athlete attributed his positive test result to contamination from sexual intercourse with a partner using clostebol-containing medication. To investigate further, the Brazilian LABDOP, a former Olympic anti-doping laboratory before the existence of the WADA-accredited laboratory system, investigated the excretion of clostebol metabolites in the urine of men exposed to intravaginal clostebol acetate during sexual activity. Participants engaged in sexual intercourse following intravaginal application of clostebol acetate. Using a gas chromatographic-mass spectrometric method, urine samples were meticulously analyzed for the presence of specific clostebol metabolites. Urine samples from male subjects contained clostebol in concentrations ranging from 0.9 to 3.5 μg/L, with a peak concentration observed at approximately 16 hours post-application. Notably, baseline urine samples collected before clostebol acetate exposure showed no traces of clostebol or its metabolites. These results provide compelling evidence of systemic absorption and subsequent excretion of clostebol metabolites following intravaginal and topical application [45].

Saliva can serve as another vector of secondary transfer for medications and prohibited substances. A highly publicized case involved the French tennis player Richard Gasquet, who avoided a lengthy doping ban when the CAS accepted that he inadvertently ingested cocaine by kissing a woman in a nightclub [46]. In contrast to cases of sexual transmission where the contamination originates from a legitimate

medication taken by the source (the male), this represents a scenario of secondary transmission of an illicit drug, potentially leading to an inadvertent positive doping result. Understanding the impacts of primary and secondary transfer is crucial in anti-doping investigations, as it assists in tracing the origin of evidence and establishing potential links between individuals and molecules. This aids in the reconstruction of events and the determination of accidental exposure.

2.3.3. Contamination by medications containing other prohibited substances

The risk of cross-contamination in pharmaceutical manufacturing represents a critical, yet often underappreciated, challenge in the context of anti-doping efforts. Cross-contamination can occur during the production process when trace amounts of one substance inadvertently contaminate another product. This can happen due to shared manufacturing equipment, inadequate cleaning procedures, or even airborne particulate transfer within facilities that produce prohibited substances and legitimate medications.

In the anti-doping context, the implications of cross-contamination are profound. Athletes who strictly adhere to their medication regimens and are vigilant about avoiding banned substances can still be at risk if the medications they consume have been inadvertently contaminated with a prohibited substance. Even minute quantities of anabolic steroids, stimulants, or other banned substances, which may be present as contaminants, can trigger an Adverse Analytical Finding (AAF) during doping control. Research has highlighted several instances where cross-contamination has been identified as the source of doping violations. For example, pharmaceutical products, particularly generics or those manufactured in facilities with less rigorous contamination controls, have shown a higher propensity for cross-contamination. Cases have been documented where medications intended for therapeutic use were contaminated with substances like amphetamines or methamphetamines compounds that are strictly prohibited in sports due to their performance-enhancing effects [47].

Another common substance found as a contaminant is some diuretics. The unauthorized use of diuretics is strictly forbidden in sports due to their masking properties of other prohibited substances or inducing artificial weight loss. However, in the scope of a doping case in 2014, it was discovered that a medication had been cross-contaminated with a diuretic at the manufacturing stage and led to an AAF. More precisely, the Swiss athlete provided a urine sample, which yielded an AAF for HCTZ. An investigation into an AAF for HCTZ was initiated due to the relatively low urinary concentration of the drug (approximately 5 ng/mL). The athlete claimed only the use of a non-steroidal anti-inflammatory drug (NSAID) before the competition and provided the drug (in coated tablet form) along with the manufacturer's retention sample. Both samples confirmed the presence of approximately 2 mg of HCTZ per tablet [48]. HTCZ was not listed as an ingredient in the medication.

USADA observed other examples of medication contamination. Between 2017 and 2020, there were nine instances in the results management process where AAFs were attributed to contamination in commercially manufactured, generic prescription medication. Upon notifying athletes of their AAF, tablets or capsules of the original prescription, in their original packaging, were collected from each athlete and sent to a WADA-accredited laboratory for independent testing. The presence of detected prohibited substances was independently verified and matched the athlete's declaration at the time of doping control [49]. Of the nine athletes none were sanctioned.

In light of these findings, in 2021, WADA, upon recommendation of the WADA Contaminants Working Group, introduced a threshold for reporting six specific diuretics (acetazolamide, bumetanide, furosemide, hydrochlorothiazide, torasemide, and triamterene) since those had been recognized as contaminants in numerous pharmaceutical products. The WADA Contaminants Working Group indicate that the consumption of pharmaceutical products tainted with a diuretic could result in the detection of diuretics in an athlete's urine sample at concentrations not exceeding 20 ng/mL. At these levels, the diuretic would be ineffective in concealing the presence of any other prohibited substances that might be present in the sample. Therefore, under the cutoff of 20 ng/mL, the detection of either one of those six diuretics is not reported as an AAF to avoid cases caused by pharmaceutical contamination. However, in weight-class sports, where the abuse of diuretics for artificially inducing weight loss to satisfy the weight-in, the reporting of these diuretics at or below the Minimum Reporting Level (MRL) of 20 ng/mL will be treated as an Atypical Finding (ATF) where the case must be investigated [50, 51].

The challenge in addressing cross-contamination lies in its often undetectable nature until after consumption and testing. Anti-doping agencies and WADA-accredited laboratories have responded to this risk by establishing Minimum Reporting Levels (MRLs) and developing Technical Letters to interpret findings that may result from such contamination.

However, the problem persists, particularly with the globalization of pharmaceutical manufacturing and outsourcing production to facilities in regions with varying quality control standards.

2.3.4. Contamination by the metabolic transformation of medications

Further to the direct contamination of a medication with a prohibited substance, cases have been reported where the metabolism of a medication can result in an AAF. As demonstrated below, research has identified many instances where the metabolization of a permitted substance could potentially lead to an AAF or did trigger an AAF, to which the athlete had to answer. However, the following examples show how WADA and anti-doping stakeholders continuously incorporate scientific developments into its processes by advising WADA-accredited laboratories on the various phenomena to reduce the risk of having those unwanted AAF reported.

For example, a Futsal Premier League player in Iran, aged 25, was selected for urine sampling in 2014, which resulted in an AAF for morphine, which is banned in sports. The player denied using narcotics but admitted to taking acetaminophen-codeine tablets the day before the match to manage tooth pain. The reported concentrations of morphine and codeine in his urine sample were consistent with post-codeine consumption levels. The positive morphine result was attributed to codeine intake, and the case was dismissed. Codeine can be metabolized to morphine in the liver by cytochrome P450 2D6 (CYP2D6). The morphine/codeine (Mor/Cod) ratio is used for differentiating between codeine and morphine consumption. A Mor/Cod ratio below 1 is indicative of exclusive codeine intake, while a ratio above 1 suggests the use of morphine or heroin. However, the value of 1 is not absolute when pinpointing the origin of morphine. The following study showed that individuals with ultra-rapid CYP2D6 metabolism may exhibit Mor/Cod ratios higher than 1, even with sole codeine consumption. The function of CYP2D6 is also influenced by ethnicity, where Northern African and Middle Eastern populations show a higher prevalence of ultra-rapid CYP2D6 metabolizers [52].

In other cases related to the intake of codeine, the analysis of urine samples for the presence of the prohibited substance Hydromorphone is significant. This examination serves to identify its source, whether it stems from the permissible use of Hydrocodone or the consumption of high doses of either Morphine (which surpasses the permitted threshold) or the authorized drug Codeine [53,54].

Another substance that can be the result of metabolic conversion is Trimetazidine, a medication typically administered to elderly individuals dealing with angina and other cardiac conditions. Trimetazidine was prohibited by WADA in 2014 as the medication aids fatty acid metabolism and positively impacts the body's oxygen utilization, consequently enhancing performance. Intriguingly, Trimetazidine can also be generated through the metabolic conversion of Lomerizine, a non-prohibited medication prescribed for migraines. In a doping control conducted in Tokyo, a urine sample was found to contain Trimetazidine, yet the athlete asserted the sole use of Lomerizine. Meticulous research

utilizing high-resolution Mass Spectrometry conclusively demonstrated that Trimetazidine indeed results from the in vivo transformation of Lomerizine following its ingestion [55,56].

Several other examples illustrate the transformation of permissible medications into prohibited substances. One such instance involves Chlorazanil, a prohibited substance, appearing in urine samples due to the use of Proguanil, an anti-malaria drug. While structural similarities between Chlorazanil and Proguanil exist, no direct metabolic link is documented. However, Proguanil metabolizes into N-(4-chlorophenyl)-biguanide, a precursor to Chlorazanil synthesis, particularly in the presence of specific compounds like formic acid [57,58].

Another case involves analyzing and reporting SARM S4 (Andarine) Metabolites O-dephenylandarine and O-dephenylandarine glucuronide. These metabolites may also arise from the anti-androgen Flutamide used in prostate cancer treatment. Given that Flutamide is not prohibited in sports, laboratories have been advised not report an AAF for Andarine based solely on the presence of O-dephenylandarine [59,60].

Furthermore, Phentermine and Mephentermine (prohibited substances) may be detected in urine samples as minor metabolites of the permitted drug Oxethazaine, a topical anesthetic. However, the primary metabolites of Oxethazaine are detected in significantly higher concentrations than Phentermine and Mephentermine [61].

Another concern is the possible detection of Oxymorphone in urine samples due to the decomposition of Methylnaltrexone (MTNX), a permitted drug. Oxymorphone may form as a degradation artefact of MTNX under certain analysis conditions, necessitating careful interpretation of analytical results [62,63].

Similarly, Enobosarm, a Selective Androgen Receptor Modulator (SARM), may present challenges in analysis and reporting due to its metabolites' presence as contaminants or minor metabolites of permitted drugs like Bicalutamide [64,65].

Finally, Tulobuterol, listed as a beta-2 agonist on the Prohibited List, shares structural similarities with Bupropion, a non-prohibited substance. Depending on the chemical environment, Bupropion may undergo various modifications, potentially leading to the formation of degradation products [66].

2.4. Dietary supplements in sport

Dietary supplements are commercially produced preparations designed to enhance an individual's diet, typically taken as a pill, capsule, tablet, powder, or liquid. Unlike pharmaceutical drugs, dietary supplements are not subjected to safety and effectiveness evaluations by the Food and Drugs Administration (FDA), the European Medicines Agency (EMA) or similar bodies. Numerous supplements have been discovered to contain undisclosed drugs and chemicals, which may even have adverse effects on one's health. In a sports context, the lack of effective control and regulation of supplements in the market, coupled with inadequate labeling practices, where ingredients are often either not listed or misnamed, contributes significantly to the risk of testing positive for a prohibited substance due to supplement consumption [67,68].

Athletes frequently use dietary supplements to support their performance. A study from the 2019 European Games reported that 72 % of athletes declared supplement use on their doping control forms, while Anti-Doping Norway found that 51 % of athletes subject to doping controls between 2015 and 2019 (n = 10,418) reported the use of at least one supplement [69]. It is expected that elite athletes understand the elevated risks associated with supplement use and the possibility of consuming a banned substance unknowingly. Nevertheless, the enticing assurances offered by supplements may obscure the actual risks they pose. An increase in muscle mass, for instance, might be a disguise for anabolic substances, while an accelerated metabolism could conceal stimulants [70–73].

At the Minsk 2019 European Games, 4082 athletes from 50 countries participated in 15 sports. Of all the competing athletes, 24.5 % (n=999) underwent testing as part of the anti-doping program. Each athlete subjected to testing had to complete a declaration on the doping control form detailing the medications or supplements they had used in the preceding seven days. Regarding supplements, 71.6 % of athletes reported taking at least one or more in the previous seven days [74].

2.4.1. Dietary supplement composition

The following study investigated the prevalence of anabolic androgenic steroids in dietary supplements used in sports and assessed the associated health risks stemming from deviations in the quality and content of these supplements compared to recommended standards [75]. Using liquid chromatographic methods, the researchers scrutinized 23 samples of dietary supplements. Chromatograms and the use of reference substances determined the presence or absence of substances with a steroid structure. Analysis of the data uncovered that 11 out of the 23 samples were free from anabolic steroid substances, while 52.2 % contained varying amounts of anabolic androgenic steroids. The identified substances include methandienone, methyltestosterone, oxandrolone, stanozolol, methenolone, boldenone, and androsterone. Notably, one of the samples exhibited the presence of nine undisclosed substances with a steroid structure.

A recent literature review conducted across prominent databases such as PubMed, Science Direct, Google Scholar, and Web of Science yielded a comprehensive dataset from 50 studies, collectively examining 3132 dietary supplements. Alarmingly, 28 % of these supplements, totaling 875, were identified to contain undisclosed substances, with prevalent instances of sibutramine and anabolic-androgenic steroids. This underscores a substantial risk of inadvertent doping associated with a significant portion of dietary supplements available to elite athletes [76].

Several national anti-doping organizations, such as the US Anti-Doping Agency (USADA), Nationale Anti-Doping Agentur Deutschland (NADA), and the Cologne anti-doping laboratory, curate an annual list of high-risk dietary supplement brands because they contain prohibited substances. This list is regularly updated and expanded. Athletes and sports organizations can access this information online to make informed choices and mitigate the risk of related doping violations [77,78].

2.4.2. Higenamine in plant extracts and food supplements

Higenamine, also known as norcoclaurine, is a naturally occurring compound found in several plant species. It has gained attention recently due to its potential use in dietary supplements and sports performance products. Higenamine is classified as a beta-2 adrenergic agonist, similar to substances like clenbuterol and salbutamol. In the context of sports doping, higenamine has raised concerns because of its structural similarity to other banned substances and its potential to enhance athletic performance. It is believed to act as a bronchodilator and vasodilator, increasing heart rate and improving oxygen supply to muscles, which could enhance endurance and exercise capacity. One of the challenges with higenamine is its presence in many dietary supplements marketed for weight loss, energy enhancement, and pre-workout purposes [79].

Higenamine, is found in certain Annona fruits. The following study aimed to assess if consuming Annona fruit could result in higenamine being detected in urine above the allowed limit. Single-dose tests with three Annona species showed higenamine levels below the limit of detection. A multiple-dose study revealed cumulative effects but still stayed within the limit. Most higenamine was found in urine as its sulfated form. Synthetic higenamine sulfates were characterized, suggesting a specific sulfate predominance. The study also explored additional biomarkers from Annona consumption for doping control. Urinary patterns unique to Annona ingestion, not seen with supplements, were identified, offering potential markers to distinguish between natural and artificial higenamine sources in drug tests [80].

2.4.3. Selective Androgen Receptors used as dietary supplements

Selective Androgen Receptor Modulators (SARMs) constitute a class of drugs that mirror the anabolic properties of traditional steroids, including testosterone, stanozolol, or nandrolone, but with diminished androgenic effects. This reduction in androgenic impact is crucial for minimizing the common side effects associated with steroids. The distinctive feature of SARMs lies in their selective binding to androgen receptors in specific tissues [81]. In 2024 SARMS are still not authorised by the FDA or the EMA. SARMs are gaining popularity as performance-enhancing supplements, renowned for their benefits in building lean muscle mass, reducing fat, enhancing endurance, and aiding recovery [82].

While numerous compounds fall under the SARMs category, Ostarine and Ligandrol are the most commonly mentioned and studied. In 2008, the World Anti-Doping Agency banned SARMs from sports. In 2017, the FDA issued a public advisory highlighting the increased risks of heart attack, stroke, and liver damage associated with these compounds found in bodybuilding products.

Despite the illegality of including SARMs in supplements, there is an evident risk of their presence, whether intentionally added to the composition (but often fraudulently not disclosed) or due to cross-contamination during the manufacturing process. As previously mentioned, USADA's high-risk supplement list highlights the concern that more than 200 supplements contain SARMs. Specifically, 98 of these supplements contain Ostarine, and alarmingly, 19 fail to disclose Ostarine on the supplement label, disguising it behind indications of vitamins or caffeine. This situation significantly heightens the risk of inadvertent doping for athletes who may unknowingly consume these supplements.

As an example of doping cases caused by supplements which are nowadays commonplace, Ultimate Fighting Championship (UFC) athlete Jim Wallhead from the United Kingdom accepted a nine-month sanction from the USADA after testing positive for the prohibited substance ostarine during an out-of-competition test in 2017. Upon notification of his positive test, Wallhead provided information about the dietary supplement he used during the sample collection, which did not list any prohibited substances on the label. Independent testing confirmed the presence of ostarine in the supplement. According to the UFC Anti-Doping Policy and World Anti-Doping Code, a violation caused by a contaminated product may result in a reduced sanction. In Wallhead's case, USADA considered factors such as his failure to thoroughly research the supplement and recognize the risk of purchasing from a supplier associated with prohibited substances. Consequently, a nine-month ineligibility period was deemed an appropriate sanction for his violation.

Distinguishing between inadvertent contamination, where an athlete unknowingly consumes a supplement containing SARMs, and intentional use of the molecule at low doses designed to mimic contamination presents further challenges for anti-doping agencies. The line between exposure via supplement intake and deliberate usage in a manner that appears accidental is not always clear-cut. It highlights the need for thorough investigations and careful consideration of anti-doping protocols. For example, research on ostarine micro-dosing provides valuable information on the drug's metabolism and elimination behavior. This research involved administering ostarine at levels significantly below the intended therapeutic dosages, simulating scenarios of contamination and inadvertent drug intake. The aim was to investigate how the drug behaves in such situations and understand its metabolic processes at these lower amounts [83].

2.5. Creams contamination

Creams and topical formulations play a significant role in sports doping as they provide a convenient means for administering prohibited substances by aiding the delivery of an active drug [84]. Creams containing testosterone or other prohibited substances, such as anabolic steroids or growth hormone creams, may be misused to enhance muscle growth, strength, and overall performance, seeking localized effects while minimizing systemic exposure. In contrast, other creams may contain masking agents designed to interfere with detecting prohibited substances during anti-doping tests. Alternatively, honest athletes may lack detailed knowledge about the chemical composition of the creams they use. Athletes, particularly those self-administering over the counter or prescription creams, might unwillingly expose themselves to prohibited substances due to a lack of awareness about the exact composition of the creams, leading to doping violations.

2.5.1. Clostebol in creams

Clostebol is a synthetic anabolic androgenic steroid that has been utilized in sports and bodybuilding for its performance-enhancing properties. Clostebol is a derivative of testosterone. Athletes may use clostebol to gain muscle mass, enhance strength, and improve overall athletic performance. Clostebol acetate is a common formulation in various pharmaceutical products, such as Trofodermin®. While it is approved for medical use in humans, the permitted applications are limited to topical administration, primarily dermatological and ophthalmological preparations. However, the misuse of clostebol in sports has become a concern, with increasing instances of athletes testing positive for the substance during anti-doping controls. The availability of pharmaceutical formulations containing clostebol acetate, coupled with more sensitive testing methods employed by anti-doping laboratories, has contributed to the increased detection of clostebol misuse. However, some AAFs involve athletes claiming inadvertent exposure through contact with individuals who use clostebol-containing products. It was previously shown that following the therapeutic application of Trofodermin® cream on the skin, it is possible to detect the primary clostebol metabolite as a glucuronide conjugate in urine. This not only leads to an anti-doping offense but also reveals that occasional contact with the application area of individuals who have used the cream can also result in an AAF [85].

2.5.2. Cosmetics containing chlorphenesin

Meclofenoxate, a stimulant reported on the WADA's Prohibited List, rapidly degrades to 4-chlorophenoxyacetic acid (4-CPA) in biological fluids. However, the presence of 4-CPA in urine may not solely result from meclofenoxate use. According to a Technical Letter (TL01) issued by WADA, using herbicides and plant growth regulators containing 4-CPA can also lead to its detection in urine. Consequently, a reporting level of 1000 ng/mL was set, and only concentrations exceeding this threshold constitute an AAF. It is also observed that 4-CPA can result from permitted administrations, such as Chlorphenesin [3-(p-chlorophenoxy)-propane-1,2-diol], a non-prohibited substance used as a preservative in cosmetics and lotions. It is also approved in selected countries as Chlorphenesin carbamate to relieve muscle pain.

In the 2020 Summer Olympics scope, however, urine samples from nearly 80 athletes competing in Tokyo showed traces of 4-CPA. Before reporting those as AAF and triggering severe consequences for the athletes, the investigative work of anti-doping scientists in the United States and Germany assisted the WADA-accredited laboratory in discriminating those results. The scientists discovered that the stimulant could be found in a component of several over-the-counter sunscreens. Human administration studies were conducted with a commercially available sunscreen containing 0.25 % by weight of chlorphenesin. In one set, six study participants dermally applied 8 g of sunscreen, collecting urine samples before and up to 7 days after application. Another set of six participants applied 8 g of sunscreen on three consecutive days, with urine samples taken up to 5 days after the last dosing. Urine specimens were analyzed using liquid chromatography-high resolution (tandem) mass spectrometry. The results indicated that chlorphenesin produced characteristic urinary metabolites, including chlorphenesin glucuronide and the common metabolite 4-CPA. Urinary concentrations of 4-CPA reached up to 1500 and 2300 ng/mL after single and multiple

sunscreen applications, respectively [86]. This scientific advancement allowed the proper handling of those 80 cases (i.e. cases were not reported as AAFs), and WADA enshrined the scientific findings in its guidance for WADA-accredited laboratories [87].

2.6. Sabotage

Sabotage in the context of doping refers to intentional actions taken by individuals or parties to manipulate or contaminate an athlete's sample with prohibited substances without the athlete's knowledge or consent. This can be a malicious attempt to frame or discredit the athlete, undermining the integrity of anti-doping efforts. Per our understanding, sabotage cases are relatively rare, but they highlight the complexities and challenges in maintaining the integrity of anti-doping efforts in sports. Nevertheless, anti-doping organizations recognize the possibility of sabotage and have protocols to investigate such claims.

2.6.1. Spiking of drinks

In two separate incidents, notable athletes faced significant consequences due to sabotage involving banned substances. Japanese sprint canoeist Yasuhiro Suzuki received an eight-year ban from the Japan Anti-Doping Agency (JADA) for tampering with a fellow athlete's drink, leading to a failed doping test by Seiji Komatsu during the national canoe sprint championships in Komatsu. Suzuki's calculated actions aimed to eliminate Komatsu from Olympic team selection, and his sabotage efforts extended to thefts of equipment from other competitors. Suzuki admitted to spiking the drink with the prohibited steroid methandienone (Dianabol).

In another case, Azerbaijani powerlifter Gunduz Ismayilov saw his life ban from the Paralympics lifted after presenting evidence that his ex-girlfriend had spiked his drink with an anabolic steroid during the 2004 Games in Athens. The International Paralympic Committee (IPC) reevaluated Ismayilov's case, considering the "new and very relevant evidence" provided nearly nine years later. Ismayilov's former partner admitted in a Baku court of law that she had sabotaged his drink in the Athens Paralympic Village. This proven sabotage led to the reversal of Ismayilov's life ban, allowing him to resume competition.

2.6.2. Cream secondary transfer as sabotage

Cream secondary transfer involves the transmission of a substance, such as a medicated cream or ointment, from one person to another through contact. This phenomenon is not surprising, given that for transdermal testosterone replacement therapy, it is recommended to cover the applied gel's surface with clothing to prevent the direct transfer of testosterone through skin contact with family or relatives [88]. The potential for sabotage arises when an individual intentionally applies cream to their hands or other body parts, like arms and legs. Subsequently, this person may attempt to touch the targeted athlete, transferring the substance through the cream residues. The Trofodermin application study mentioned earlier highlights this risk, demonstrating that the individual applying the cream to someone else exhibited the presence of the clostebol primary metabolite in urine samples for up to 146 hours after the application [85].

In instances where athletes attribute inadvertent exposure to doping agents, possibly through covert methods, it's crucial to investigate the potential for substances to be administered via a handshake or brief contact with other body parts. This study aimed to delve into the transdermal absorption potential of doping substances. Twelve male participants underwent transdermal application of various anabolic androgenic steroids (AAS). Urine samples were collected and analyzed over 14 days. The experiments involved exposing individuals to oxandrolone or a mixture of oxandrolone, metandienone, clostebol, and DHCMT in different body locations to assess substance detectability, considering the administration site's influence. Surprisingly, the detectability of substances was evident as early as 1 h after transdermal application in some participants, as revealed by the presented data. Consequently, the possibility of unintended doping scenarios persists [89].

2.6.3. Deliberately mislabeled medication

In 2018, a doping control revealed recombinant erythropoietin (rEPO) in an athlete's blood and urine samples. The athlete claimed poisoning, attributing it to an anticoagulant, Clexane, received from a club member. The athlete self-injecting with these syringes during the November 2018 control. Suspecting sabotage, the athlete suggested that the AAF for rEPO resulted from the anticoagulant tainted with rEPO. To verify this, the athlete requested the French anti-doping organization to analyze the last sealed syringe. The analysis indicated a high EPO concentration, ruling out contamination. It suggested a complete anticoagulant replacement or a label switch to an EPO syringe, more likely due to distinct features. Another label, Eprex®, a genuine EPO drug, was found concealed in the syringe. Based on publicly available information, the case outcome is not available. Thus, the circumstances remain unclear. The athlete may have acquired counterfeit syringes or prepared the specific syringe for analysis. Alternatively, malicious individuals within the club might have sought to undermine the athlete's career [90].

2.7. Presence of exogenous compounds in urine by in situ biotransformation

WADA advises laboratories on potential issues arising from microbial enzymatic activities, resulting in the formation of prohibited steroids or metabolites from natural urine constituents. Examples of these transformations include the generation of androst-4-ene-3,17-dione, 5α- and 5β-androstanediones, Δ1 steroids, and 19-norsteroids from endogenous steroids demethylation. This highlights the complex enzymatic processes affecting urine samples [91].

The detection of 19 norandrosterone (19-NA) in an athlete's urine is commonly seen as solid evidence of the use of nandrolone or similar substances. However, a complication arises with norethisterone, a progestogen utilized in treating menstrual irregularities and birth control, as it also triggers the excretion of 19-NA, surpassing the reporting threshold of 2 ng/mL set by WADA. In studies involving female participants, the administration of norethisterone formulations resulted in notable concentrations of 19-NA in urine samples. This confirmed that norethisterone can convert to 19-NA, with the impurity making a minimal contribution to the observed levels. Furthermore, a crossover investigation comparing purified norethisterone capsules with norethisterone tablets containing the 19-norandrostenedione impurity highlighted the significant role of norethisterone itself in the urinary excretion of 19-NA [92].

In a separate study, researchers observed the formation of 19-norsteroids through demethylation of endogenous steroids in stored urine samples. Suspicious urine samples containing small traces of 19-norandrosterone and 19-noretiocholanolone were selected and mixed with deuterated analogues of androsterone and etiocholanolone at concentrations that mimic natural ones found in the body. Following incubation, the samples revealed the presence of corresponding 19-norsteroids (19-norandrosterone-d4 and 19-noretiocholanolone-d5) using high-resolution mass spectrometry. Furthermore, a notable temperature dependency was observed, with concentrations of 19-norandrosterone-d4 and 19-noretiocholanolone-d5 being 2.7 and 3.6 times higher, respectively, at an elevated temperature of 37°C compared to room temperature (23 ± 2°C). These experiments' applications may aid in elucidating AAF related to low levels of 19-norsteroid metabolites. None of the suspicious samples exhibited typical signs of microbial degradation [93].

Boldenone and boldione (ADD) are currently classified as anabolic steroids by the WADA. However, recent findings suggest that these substances might also be produced naturally within the body. While the potential endogenous origin of Boldenone is acknowledged, the same

recognition is not extended to ADD. A 2009 study aimed to investigate the natural production of ADD in human urine and assess the impact of consuming phytosterols, plant-based compounds found in certain foods. A 5-week trial was conducted involving both men and women, during which urine samples were analyzed for various steroids using gas chromatography coupled with mass spectrometry (GC-MS-MS). The findings revealed sporadic endogenous ADD production at concentrations ranging from 0.751 ng/mL to 1.73 ng/mL, while no evidence of endogenous Boldenone production was found. Additionally, the study examined the effect of daily consumption of a phytosterol-enriched yoghurt drink on the presence of these steroids in urine. Interestingly, correlations between ADD and other steroids were consistently stronger in participants consuming phytosterols compared to those who did not [94].

WADA monitors athletes for the illicit use of prednisolone, along with all corticosteroids, through in-competition controls. Prednisolone, a corticosteroid, has been detected in human, equine, and bovine urine. A study involving 34 human volunteers aged 22–62 utilized HPLC–MS3 to identify prednisolone in their urine. The results indicated prednisolone presence in all volunteers' urine, with concentrations about 100 times lower than cortisol, without gender dependence. This consistent ratio throughout the day suggests endogenous production of prednisolone, potentially stemming from microbial dehydrogenation of cortisol post-sample collection [95,96].

Today, IRMS analysis is a crucial tool for discerning molecules' exogenous provenance, particularly in suspected biotransformation [97].

2.7.1. Bacteria contamination

Bacterial contamination presents a significant challenge in doping analysis due to its potential to compromise the integrity of samples and the accuracy of test results. When samples intended for analysis are contaminated with bacteria, several issues arise. Firstly, the presence of bacteria can render samples invalid for analysis. The microbial activity may alter the sample's composition, making it unsuitable for accurate testing.

Moreover, bacteria can produce metabolites or enzymes that interfere with the detection methods used in doping analysis. These substances may mimic or mask the presence of prohibited substances, leading to false-negative results or inaccurate conclusions about an athlete's test result. In addition, bacterial contamination can hinder the application of IRMS, which is crucial for detecting the presence of exogenous substances. The interference from bacterial byproducts can obscure the isotopic signatures, making it challenging to obtain reliable results [98].

Contamination risks emerge at various testing stages, starting with urine collection, where bacteria from the environment, hands, skin, or collection equipment may compromise sample integrity. Additionally, inadequate storage or transportation conditions can foster bacterial growth and contamination [99].

2.7.2. Types of microbial contamination and steroidal alteration

Various studies have explored the potential impact of microbial contamination on urine analysis, particularly in the context of doping controls. Transportation of anti-doping urine samples under ambient conditions is a notable factor facilitating microbial growth and potentially leading to increased contamination levels. Subsequently, the urine condition must be considered when collecting and storing samples in the laboratory.

One study investigated 94 urine samples, including 60 from athletes undergoing routine doping control. Initially, sensory observations and pH levels were considered potential indicators of microbial contamination but were ultimately found to be unreliable. However, the analysis revealed a wide range of microbial levels in the urine samples. Transporting samples to doping laboratories under ambient conditions was identified as a potential factor facilitating microbial growth, potentially leading to increased contamination levels over time. Interestingly, females showed a higher prevalence of measurable microbial levels than males, indicating increased susceptibility to urinary infections. This gender difference was more pronounced in control samples, where 92 % of female samples exhibited microbial levels above the detection limit, compared to 36 % of male samples. Further examination of the microbial communities identified common genera such as Lactobacillus and Enterobacteriaceae in control and athlete samples. Notably, pseudomonads were found in significantly higher proportions in athlete samples than in control samples [100].

This research specifically selected microorganisms commonly found in urine samples due to contamination during collection or storage. Aspergillus and Penicillium, prevalent in laboratory environments and cold storage rooms, were chosen. Additionally, all evaluated microorganisms are part of the normal human flora. *Döderlein bacilli* and *Gemella haemolysans* represent normal vaginal flora. In contrast, others like Staphylococcus epidermidis and Corynebacterium species are typically found on the skin and may cause clinical infections in immunocompromised individuals. Certain microorganisms, such as *Enterococcus faecalis*, *Escherichia coli*, *Proteus mirabilis*, and *Klebsiella pneumoniae*, associated with faecal contamination, are common causes of urinary tract infections. Microbial concentrations in the inoculum were adjusted to levels typically observed in clinical infections. Sterility controls confirmed the absence of microbial growth in urine aliquots before inoculation. Under experimental conditions, conjugated steroids were naturally deconjugated in samples incubated at 37°C for two weeks but not in non-inoculated samples and, to a lesser extent, in urine samples immediately frozen after sterilization. The hydrolysis rate of glucuronide metabolites of testosterone, epitestosterone, androsterone, and etiocholanolone was below 10 % (range 4–8 %) during the two-week study period. DHEA (Dehydroepiandrosterone) –sulfate exhibited greater susceptibility to hydrolysis than glucuronide compounds, with free DHEA present in the control urine on day 15. A deconjugation rate of over 5 % of total testosterone indicated contamination. Still, this criterion was not fully confirmed under the study's extreme conditions, as several microorganisms, including non-inoculated controls, showed deconjugation rates above 5 %. Additionally, unconjugated endogenous steroids above 10 % were reliable indicators of contamination or exposure to high temperatures [101,102].

In the following article, microorganisms selected for urine contamination represented species commonly found in sports urine samples, those associated with urinary tract infections (UTIs), components of normal human flora, and indoor environmental species. Notably, bacteria like *E. coli*, *P. mirabilis*, and *K. pneumoniae*, linked to UTIs in young females, were included. Other urinary pathogens, such as S. epidermidis, Enterococcus spp, and Pseudomonas spp, were also considered. *S. epidermidis*, abundant on human skin, is prevalent in lab tests. *N. simplex* extensively metabolizes testosterone. *E. faecalis*, a common commensal in the intestines, often proliferates in urine samples. Additionally, eukaryotic cells such as the yeast *C. albicans* and the fungus *A. flavus* were included for their capability to bio-transform steroids due to their expression of human enzyme homologs. *C. albicans*, part of typical vaginal flora, is a frequent fungal pathogen isolated from urine samples. *E. coli*-contaminated urine showed significant deconjugation rates for epitestosterone glucuronide, androsterone glucuronide, and etiocholanolone glucuronide, but these rates decreased considerably in preserved samples. *N. simplex* and *A. flavus* exhibited higher rates of microbial hydrolysis, leading to dihydrotestosterone (DHT) formation in free and/or glucuronide-conjugated fractions. *N. simplex* could convert unconjugated steroid substrates, producing boldenone and its metabolites. Samples stored at −20°C for one week showed the conversion of d3-testosterone to d3-boldenone by *N. simplex*. However, in samples incubated at 37°C for one week, the formation of d3-boldenone was not detected. Instead, the endogenous steroid profile was altered, with the formation of 5a/5b-androstane-3a,17b-diol, 5a/5b-androstane-3,17-dione, and DHT observed in both the free and glucuroconjugated

fractions [103].

2.7.3. Urine conservation studies

The transportation of urine samples to WADA-accredited laboratories poses challenges due to the potential exposure to high temperatures and storage conditions. During transit, these samples may encounter fluctuations in temperature, which can lead to microbial proliferation and compromise the integrity of the samples. Recognizing the critical need for preserving urine specimens before testing, research has been initiated to explore effective methods of urine stabilization in collection bottles. Investigations within this project have focused on evaluating both physical and chemical processes of microbial and enzymatic inactivation. These tests aim to determine the efficacy of various techniques in preserving urine samples and preventing microbial growth and enzymatic activity. Among the physical methods assessed, membrane filtration and ultraviolet (UV) exposure were examined. However, it was found that membrane filtration was impractical for large-volume doping-control specimens due to filter clogging, while UV exposure did not significantly reduce microbial concentrations. On the chemical front, experiments involving the application of a preservative mixture showed promising results. This mixture effectively inhibited microbial growth in urine samples, highlighting its potential as a viable method for urine sample preservation [104].

In a follow-up study, researchers aimed to refine the composition and application of the stabilization mixture. They coated pilot urine collection containers with the mixture and subjected them to various incubation cycles to assess effectiveness and minimize interferences. Three WADA-accredited laboratories (Athens, Ghent, and Rome) evaluated the coated containers. The spray-coated mixture effectively eliminated microorganisms and prevented the breakdown of steroid glucuronides, intact recombinant erythropoietin, and small peptides by proteolytic enzymes. Any observed analytical interferences were carefully documented using routine screening procedures [105].

Another article presented the Sysmex UF-500i, a urine particle analyzer, as a promising tool for assessing urine contamination levels and sample integrity in anti-doping laboratories. This device provides valuable insights into urine composition and contamination levels by examining parameters like red blood cells, white blood cells, epithelial cells, bacteria, and other particles. Statistical analyses assessed how gender, test type (in-competition vs. out-of-competition), and delivery time influence urine composition and contamination levels.

The findings revealed significant differences in various parameters among different sample groups, highlighting the impact of pre-analytical factors on urine quality. Establishing reference values for these parameters offers a standard for evaluating urine contamination levels and sample integrity, with the analyzers providing additional data on particle size, staining properties, and other variables. Establishing urine particle reference intervals could aid in evaluating contamination levels under uncontrolled pre-analytical conditions, facilitating the establishment of acceptance criteria for sample analysis. The study analyzed 501 urine samples, with gender and test type recorded from official doping control forms. The results suggest that sample quality upon delivery to accredited laboratories is suboptimal, emphasizing the need for improvements in urine collection, storage, transport, and temperature control [106].

3. Summary and discussion

In this review, we explore the diverse sources of contamination that may lead to AAF, although those sources may not be genuinely associated with illegal performance enhancement. Through a comprehensive review of existing literature, we aimed to elucidate the multifaceted nature of this challenge in anti-doping efforts. In the high-stakes world of competitive sports, athletes are pushing the boundaries of human performance and navigating a complex web of anti-doping regulations. While doping violations grab headlines and tarnish reputations, accidental doping can also pose a significant threat to athletes' careers and the integrity of their sport. Adopting proactive measures and remaining vigilant about the sources is an excellent way to minimize the risk of inadvertent AAF. Addressing these challenges requires a collaborative effort among healthcare providers, supplement manufacturers, sports organizations, and anti-doping authorities to establish precise guidelines, enhance education initiatives, and improve communication to mitigate the risk of inadvertent doping violations. Regular awareness programs for athletes are also essential to address these issues. Here, we describe several strategies that have been or can be implemented.

3.1. How to reduce the risk of an inadvertent analytical adverse result by contamination

3.1.1. WADA's strategic measures to differentiate doping from contamination: the role of minimum reporting levels and technical guidance

As described in the section on food contamination and other inadvertent exposure scenarios, the World Anti-Doping Agency (WADA) has developed specific protocols to distinguish between intentional doping and accidental ingestion.

One of the critical tools in this effort is the WADA Technical Document on Minimum Required Performance Levels (TD MRPL), which specifies Minimum Reporting Levels (MRLs) for various substances. The MRPL outlines the lowest concentration levels of certain prohibited substances that must be detected in an athlete's sample before they are reported as an Adverse Analytical Finding (AAF). This threshold-based approach is designed to prevent cases where trace amounts of a substance, possibly resulting from environmental contamination or consuming contaminated food, trigger an AAF that could unfairly lead to sanctions against the athlete. For instance, substances like clenbuterol and certain diuretics are known to potentially contaminate food sources, as discussed in our section on food contamination. By setting MRLs, WADA ensures that only those findings that exceed a scientifically justified threshold are flagged for further investigation, thereby reducing the likelihood of penalizing athletes for unintentional exposure.

In addition to the MRPL, WADA issues Technical Letters that provide more detailed guidance on handling specific compounds, particularly those that present contamination risks. These letters offer laboratories instructions on how to proceed when low levels of certain substances are detected, ensuring a consistent and fair approach across different testing scenarios. For example, as mentioned in our discussion on Medications, some Technical Letters address how to differentiate between contamination that might occur during the manufacturing process of pharmaceutical products and actual doping. This is particularly important for substances like hydrochlorothiazide, where trace contamination in a non-prohibited medication could lead to a positive doping test if not correctly accounted for.

These Technical Letters often specify actions that laboratories must take, such as conducting additional confirmatory tests, applying specific analytical techniques like isotope ratio mass spectrometry (IRMS), or considering environmental and contextual factors that might explain the presence of the substance.

By integrating these guidelines into the doping control process, WADA aims to uphold the integrity of sports while protecting athletes from the potential pitfalls of inadvertent contamination. The MRPL and Technical Letters are critical to this strategy, ensuring that anti-doping efforts are scientifically robust and fair.

3.1.2. Food contamination with doping substances: exercise caution and vigilance

Athletes must exercise caution to avoid inadvertently consuming prohibited substances through food and beverages. Be cautious when consuming meat products, particularly beef, pork, and poultry, as growth promoter contamination is most commonly associated with

these animal products. This risk is especially prevalent in countries where the use of growth-promoting substances in animal farming is widespread. When traveling to such regions, athletes must be particularly vigilant about the sources and safety of their food. Sometimes athletes cannot avoid contaminated food from countries with a higher risk of contamination due to illegal trafficking. Reducing the illicit meat trafficking involves addressing various factors, including enforcement measures, regulatory frameworks, and international cooperation. Implementing stricter regulations and oversight mechanisms within the meat industry can help prevent illegal or unregulated activities. This includes requiring comprehensive documentation and traceability throughout the supply chain to ensure that meat products are sourced from legal and reputable sources. Scientific knowledge about the composition of foods, the prevalence of contaminants, and strategies for sourcing safe and clean ingredients can help athletes make informed dietary choices while minimizing the risk of doping violations.

3.1.3. Medication: ensuring transparency and accountability

The issue of medications containing hidden doping substances poses a significant challenge to athletes and regulatory authorities alike. While athletes often rely on medications to manage health conditions and injuries, the presence of undisclosed doping substances in these products can lead to inadvertent doping violations. Promoting transparency and accountability in medication labels is crucial to addressing this issue effectively, empowering athletes to make informed decisions about their health and compliance with anti-doping regulations. Regulatory agencies responsible for overseeing medication manufacturing and labeling should implement robust measures to prevent the inclusion of doping substances in medications intended for human use. Medication labels should provide comprehensive information about all active and inactive ingredients. Manufacturers should disclose the presence of any substances, even if they are present in trace amounts or considered inactive ingredients. Transparent and standardized labeling practices can help athletes identify and avoid medications containing prohibited substances.

Mass-producing medications present a significant problem of cross-contamination, which, combined with inadequate labeling, can further exacerbate the risk of inadvertent doping violations. To effectively reduce the risk of cross-contamination in pharmaceutical manufacturing, it is essential to implement several critical practices. First, facilities should ensure that equipment used in producing multiple products is thoroughly cleaned and validated to prevent residual traces of substances from contaminating subsequent batches. This can be achieved by adopting rigorous cleaning protocols and regularly testing equipment for cleanliness. Air filtration systems should be enhanced to minimize the risk of airborne contaminants settling on surfaces or equipment involved in other manufacturing processes. Proper air control measures, including using high-efficiency particulate air (HEPA) filters and maintaining cleanroom standards, are critical in preventing cross-contamination. Moreover, personnel training programs are vital; hygiene, using protective clothing, and implementing strict procedural controls can significantly reduce the human factor in cross-contamination.

In addition to mass-produced medications, the role of compounding pharmacies presents another layer of complexity. These specialized pharmacies create custom medicines tailored to individual patient needs, often by altering a drug's form, dosage, or composition. While this personalization can benefit patients with specific medical requirements, it also introduces risks related to contamination or inadvertent inclusion of prohibited substances, particularly if stringent quality controls are not followed. Given the less rigorous oversight compared to large-scale pharmaceutical manufacturers, compounded medications may pose a higher risk of containing trace amounts of substances prohibited in competitive sports.

Athletes must be particularly cautious when using compounded medications and should consult with healthcare providers who are aware of the anti-doping regulations. They must understand that even small amounts of contamination can lead to an Adverse Analytical Finding (AAF). Therefore, it is essential to ensure that compounded medications are sourced from reputable pharmacies that adhere to the highest standards of practice.

3.1.4. Supplements: mitigating risks through quality assurance

Many athletes rely on dietary supplements to support training, recovery, and overall performance. However, these supplements may contain ingredients that lead to doping violations. Pharmacological research can help athletes identify potential risks and make informed decisions about supplement use. The production and distribution of dietary supplements present significant challenges in ensuring product safety and compliance with anti-doping regulations. Athletes are advised to stop using plant-based formulations and complements; many inadvertent exposures to prohibited substances come from these products. Athletes can mitigate the risk of inadvertent doping by choosing supplements manufactured according to rigorous quality assurance standards, including Good Manufacturing Practices (GMP) and third-party certification programs. Today's athletes are responsible for staying informed about the risks associated with supplements, particularly those that may contain prohibited substances. In the competitive world of sports, where performance-enhancing substances are strictly regulated, athletes must be diligent in their choices to avoid unintentional violations. This requires not only a thorough understanding of the types of substances that are banned but also a commitment to regularly visiting trusted websites and resources where lists of high-risk supplements are frequently updated. Athletes should be cautious and avoid taking any supplements without conducting comprehensive research into the brand's potential risks and reputability. By prioritizing their education on this topic and exercising caution, athletes can protect their careers and maintain the integrity of their sport.

3.1.5. Bacteria contamination: safeguarding the sanctity of samples

To safeguard the integrity of doping analysis, meticulous measures must be undertaken to prevent the intrusion of microbial agents or other contaminations. Qualitative training of sample collection staff is critical. Athletes themselves must diligently follow strict WADA protocols for sample collection. Temperature control emerges as another critical issue, and from collection to analysis, urine samples should be maintained at a stable and low temperature.

3.1.6. The crucial role of scientific research

Innocuous compounds may transform within the intricate pathways of metabolism, resulting in an unwanted AAF. Scientific research plays a pivotal role in validating and refining anti-doping protocols. By subjecting detection methods to rigorous scrutiny, researchers ensure their reliability and efficacy in identifying prohibited substances. The scientific community fortifies the foundations of anti-doping efforts through meticulous experimentation and validation studies.

The study of metabolites, the breakdown products of substances within the body, provides vital insights into how drugs and other compounds are processed after they are consumed. By understanding these pathways, researchers can identify unique biomarkers that signal the use of prohibited substances, even when the parent drug is no longer detectable. This research is crucial for developing reliable testing methods to detect doping long after administering the substance.

Metabolic ratios, which involve comparing the levels of specific metabolites, offer another layer of insight. These ratios can reveal whether a substance was naturally produced by the body or introduced externally. Research into these ratios helps establish baseline values and thresholds that distinguish between normal physiological processes and those altered by doping. Without ongoing research, these critical benchmarks could not be accurately defined, leading to false positives or undetected doping.

Research also focuses on the patterns of how these metabolites and

their ratios change over time. Understanding these patterns allows anti-doping organizations to better interpret test results, particularly in cases where the timing of substance administration is in question. Research in this area enhances the ability to differentiate between legitimate therapeutic use, accidental exposure, and intentional doping.

Moreover, as new substances and doping methods are developed, research must keep pace to ensure that testing protocols remain effective. This includes studying how new drugs are metabolized and how their presence can be detected through direct and indirect markers.

The findings from these studies are often incorporated into international standards, such as those provided by the World Anti-Doping Agency (WADA), ensuring consistency and reliability in anti-doping efforts across different regions and sports.

3.2. Differentiation between intended doping or inadvertent AAF

Distinguishing between inadvertent exposure and intended doping poses significant analytical challenges for anti-doping laboratories, less from an analytical perspective but more from the difficulty of putting the detected levels in the context of the alleged activities (inadvertent exposure versus doping). In cases of inadvertent exposure, identifying the source of contamination and differentiating between intentional and unintentional ingestion requires meticulous analysis of dietary habits, medication history and environmental exposures. This is also a matter of evidence and the circumstances of each case. Moreover, improving the detection of trace levels of banned substances with highly sensitive analytical methods capable of detecting picogram or nanogram concentrations has increased cases where differentiating contamination from doping is a real challenge. From a scientific point of view, assessing the plausibility of these factors requires a comprehensive understanding of pharmacokinetics, dose-response relationships, and the potential for substance accumulation in the body over time. By integrating clinical data, toxicological analyses, and athlete testimony, anti-doping authorities can effectively evaluate the validity of mitigating factors and their impact on the athlete's guilt. However, this is not always enough.

A recent proposal by anti-doping experts is the systematic use of dried blood spots during testing. Capillary blood sampled as dried blood spot (DBS) has shown substantial potential in sports drug testing, enabling the analysis of various drugs and/or metabolites. A novel advantage in the anti-doping context could be preserving a supplementary information source. Regularly collected DBS samples could be tested for specific compounds whenever follow-up investigations are needed, contributing to results management and decision-making. Athletes could undergo remotely supervised test missions, with samples collected regularly every 14 days and stored in WADA-accredited facilities. Only in the event of an AAF or ATF requiring further investigation would relevant DBS samples be analyzed. For example, here are two hypothetical scenarios: a) Contamination scenario: DBS samples collected between a negative and a positive doping control sample suggest no repeated use of pharmacologically relevant amounts of a doping agent, nothing is found in the in-between DBSs test analysis, and the positive one only present a meagre quantity; b) doping scenario: DBS samples collected between a negative and a positive doping control sample demonstrate the repeated use/exposure to pharmacologically relevant amounts of doping agents. Extra costs are minimized as analyses of these samples are performed only in response to specific testing outcomes. This contingency plan could contribute to selected compounds for anti-doping procedures, providing valuable information to support results management and decision-making processes [107].

3.3. Assessing Credibility in Contamination Defenses: Challenges and Considerations in CAS Rulings

In some instances, athletes have successfully argued that contamination caused their positive test results, leading to reduced sanctions or exoneration. CAS decisions have shown that when athletes can provide compelling evidence that the contamination was unintentional and beyond their control, the court is willing to consider this in its rulings. However, these cases require thorough and convincing documentation, often supported by expert testimony and scientific analysis.

The credibility of a contamination defense hinges on several factors. Cases that lack detailed evidence, such as clear documentation of the source of contamination or the chain of events leading to exposure, are often dismissed as speculative. For example, if an athlete cannot demonstrate a plausible and scientifically supported contamination pathway, their claim may not be deemed credible. Additionally, if the detected levels of the prohibited substance are inconsistent with the claimed source of contamination or if there are discrepancies in the athlete's account, the case is less likely to succeed.

On the other hand, cases where the athlete can provide precise and verifiable details, such as traceable contamination in food or supplements, supported by scientific tests or expert analysis, tend to be viewed more favorably. For instance, if the contamination can be linked to a specific product batch known to be tainted, corroborated by independent testing, the athlete's defense is strengthened. The court is more likely to consider the defense credible when the evidence aligns with known contamination scenarios, and the athlete's explanation is consistent and backed by reliable scientific data.

In recent years, there has been increasing concern within the anti-doping community that some athletes might exploit claims of contamination to disguise intentional doping. This trend complicates the already difficult task of distinguishing between genuine cases of inadvertent contamination and those strategically fabricated as defenses. With the growing prevalence of such claims, anti-doping authorities must carefully evaluate each case to ensure that innocent athletes are protected while those who intentionally cheat are held accountable.

The difficulty lies in the fact that contamination can occur under certain circumstances, making it crucial not to dismiss such claims outright. However, if the defense of contamination is too readily accepted, it could undermine the integrity of anti-doping efforts by providing a loophole for those seeking to evade sanctions. Therefore, the system must be designed to rigorously scrutinize the evidence presented, relying on scientific and legal expertise to uphold fairness and justice. By doing so, legitimate contamination cases can be recognized without allowing the defense to become a convenient excuse for those who seek to misuse it. Ultimately, the distinction between credible and non-credible cases often comes down to the strength and coherence of the evidence presented, as well as the athlete's ability to convincingly demonstrate that the contamination was both unintentional and unavoidable.

4. Conclusions

Addressing inadvertent exposure to substances banned by WADA requires a multifaceted approach informed by scientific principles and evidence-based strategies. Athletes must be informed about the importance of their anti-doping obligations and anti-doping authorities to ensure they comply with regulations.

Central to this endeavor is educating athletes, coaches, and support staff with the knowledge and awareness needed to navigate the complex anti-doping regulations and practices. Education is a powerful shield against inadvertent exposure, offering athletes invaluable insights into how contamination can occur and how to mitigate its risks. By fostering a culture of awareness and vigilance, education empowers athletes to make informed decisions about their dietary choices, medication usage, and supplement intake, thereby reducing the likelihood of ensuing doping violations.

Anti-doping agencies also promote education and awareness among athletes and support personnel. Through outreach programs, educational resources, and training seminars, they strive for a culture of compliance and integrity within the sporting community. By fostering open communication and transparency, anti-doping organisations

create an environment where athletes feel empowered to seek guidance and support when navigating the complex landscape of anti-doping regulations.

Finally, collaboration and coordination among various stakeholders involved in anti-doping efforts are essential. Scientists, manufacturers, doctors, coaches, and anti-doping organizations must work together to develop comprehensive strategies for preventing contamination and inadvertent doping. Through interdisciplinary collaboration, these stakeholders can leverage their expertise to identify emerging threats, develop effective detection methods, and implement targeted interventions to safeguard the integrity of sport.

CRediT authorship contribution statement

Neil Robinson: Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Conceptualization. **Christophe Champod:** Writing – review & editing, Supervision. **Thomas Piper:** Writing – review & editing, Writing – original draft, Supervision. **Louisa Lobigs:** Writing – review & editing, Writing – original draft, Supervision. **Ana Belén Moraleda Merlo:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Data curation, Conceptualization.

Declaration of Competing Interest

None.

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