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Sports drug testing and the athletes' exposome

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Abstract

Similar to the general population, elite athletes are exposed to a complex set of environmental factors including chemicals and radiation and also biological and physical stressors, which constitute an exposome that is, unlike for the general population, subjected to specific scrutiny for athletes due to applicable antidoping regulations and associated (frequent) routine doping controls. Hence, investigations into the athlete's exposome and how to distinguish between deliberate drug use and different contamination scenarios has become a central topic of antidoping research, as a delicate balance is to be managed between the vital and continually evolving developments of sensitive analytical techniques on the one hand, and the risk of the athletes' exposome potentially causing adverse analytical findings on the other.

KEYWORDS
adverse analytical finding, contamination, doping, drug exposure, sport

1 | INTRODUCTION

The multifaceted and interconnected pathways, transmission routes, sources, and (bio)transformations of synthetic as well as natural substances has exponentially increased the complexity of the human exposome. Derived from "exposure," the term and concept of the exposome was introduced in 2005[1] and has since been the subject of numerous research projects and detailed considerations as to how the entirety of a human's lifetime exposure to environmental factors (including chemicals and radiation and also biological and physical stressors) can be monitored and conceptualized.[2–4] The exposome as such is of paramount importance in a broad context, for example, in acute and long-term toxicological evaluations,[5,6] medical and pathological considerations,[7–9] and, while from a different perspective, also in athlete doping controls. The latter features unique aspects with, among others, potential sanctions associated with adverse analytical findings (AAFs) of drug or drug metabolite residues at any detectable level (depending on the sample matrix, type of sample collection, i.e., in- vs. out-of-competition, and the identified drug/drug metabolite), combined with a comparably high testing frequency of the athletes. The exposome may also be considered a known or unknown confounder of direct detection of drugs or drug residues or indirect biomarkers that can be evidence of doping. Further, the analysis of athletes' urine and/or blood samples is conducted with comparably harmonized and standardized methods, targeting a constantly growing, diversifying, and comprehensive set of drugs and chemicals considered relevant in antidoping.[10] Consequently, in view of the exposome's extent that athletes are subjected to today, the possibility of AAFs through scenarios other than doping necessitates research, data, and strategies to support result management authorities in identifying and differentiating the inadvertent exposure to

prohibited substances from findings resulting from the intentional administration for performance-enhancing purposes.

Due to the plethora of potential situations of drug exposure, a main focus of preventive and proactive antidoping research has been the investigation and characterization of scenarios, which arguably (or evidently) exhibit risks of AAFs in routine doping controls, and ways to differentiate them from attempts of pharmacological manipulation of performance. Numerous cases of AAFs and resulting sanctions, when an antidoping rule violation (ADRV) was ascertained, have been the subject of judicial proceedings, where athletes argued that the source (and/or the time point of administration) of the prohibited substance found in their sample is unknown. Here, a main issue has been stated in court repeatedly with "the currency of such denial is devalued by the fact that it is the common coin of the guilty as well as of the innocent" and that an "oral testimony as to innocence, however impressively given, cannot trump scientific evidence as to guilt."[11,12] Consequently, analytical evidence providing corroborating information in support of either scenario is of utmost importance as summarized in the following and exemplified with selected situations.

2 | SCENARIOS OF POTENTIAL, SUSPECTED, AND/OR PROVEN EXPOSURE-CAUSED AAFS

One option to categorize the athlete's exposome, with particular focus on prohibited drugs, is illustrated in Figure 1 with four groups of scenarios potentially leading to unintentional AAFs: 1. Natural presence or residual drug content/contamination of food with prohibited substances, 2. dietary supplement contamination or adulteration, 3. metabolic conversion of legitimate drugs into, or contamination with, prohibited substances, and 4. drug transfer through (intimate) contact.

2.1 | Food containing prohibited substances

2.1.1 | Natural content or formation

The number of natural sources for substances prohibited in sports is substantial, and test methods and/or guidelines for decision-making processes have been optimized and installed for result interpretation and management purposes. Among those substances, plant alkaloids such as cocaine, ephedrine, morphine, related metabolites, and natural analogs,[13–19] but also steroidal compounds have been recognized for decades,[20–29] and further substances were more recently considered including, for example, zeranol[30–32] and higenamine.[33–36] It has been fundamental to antidoping to establish means to differentiate between the misuse of such substances and the occurrence of these compounds or their metabolites in routine doping controls due to other reasons, which has triggered numerous scientific investigations and resulted in a series of technical letters,[37] technical documents,[38] and guidance notices[39] issued and continuously updated by the World Anti-Doping Agency (WADA).

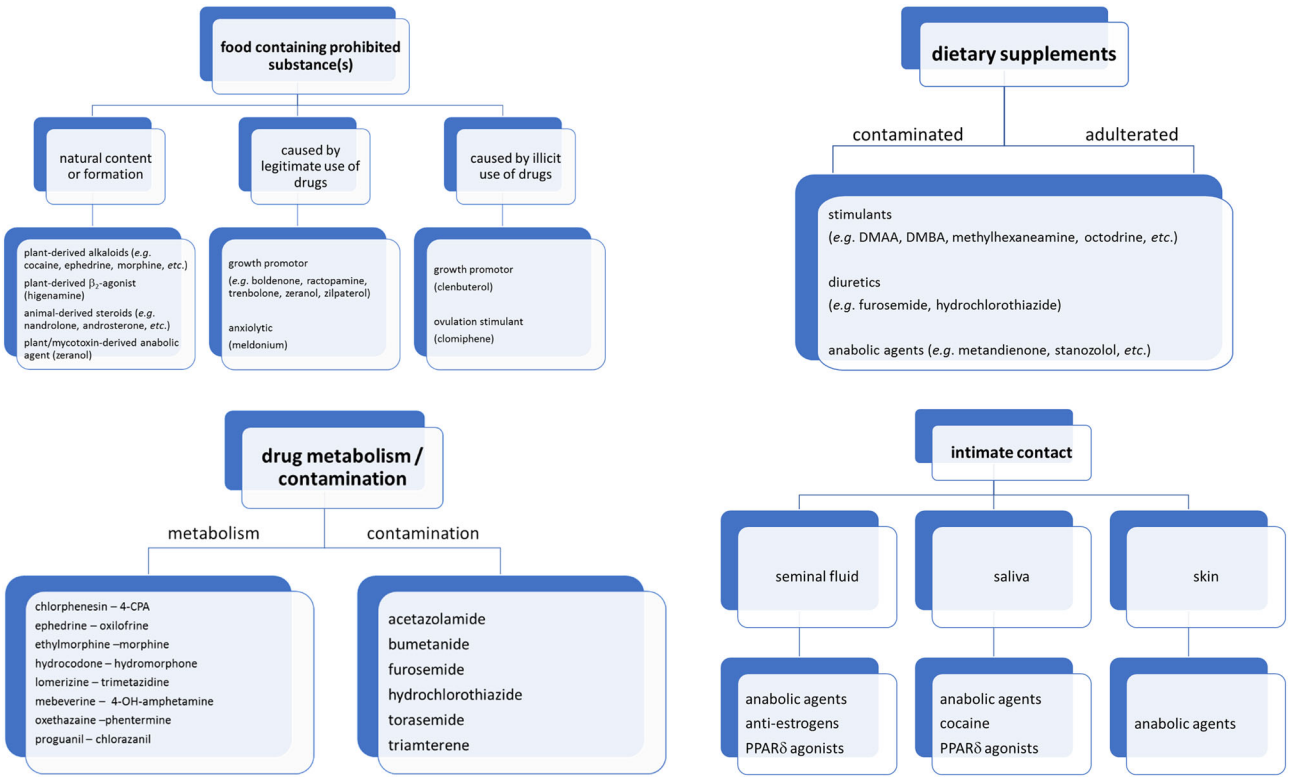

FIGURE 1 Selected situations that are suspected and/or proven to result in AAFs due to unknown/unexpected drug exposure or intake [Colour figure can be viewed at wileyonlinelibrary.com]

Strategies in support of distinguishing between drug abuse and diet-related exposure to prohibited substances that can naturally be present in food products include threshold (ephedrine, morphine, etc.) and specific reporting (cocaine, higenamine, octopamine, etc.) levels, but also the consideration of metabolite characteristics (e.g., zeranol), and, where possible, carbon isotope signatures, or combinations of the available analytical information (Figure 2). The consideration of metabolite ratios is required for instance in case of zeranol, a rarely detected compound in sports drug testing. In addition to illicit use, zeranol can originate from the consumption of mycotoxin-contaminated corn, sorghum, barley, or meat produced from animals that consumed forage consisting of the aforementioned contaminated components. The mycotoxin zearalenone converts mainly to α- and β-zearalenol, but also to a minor but measurable extent, to zeranol. Consequently, when zeranol findings occur, relative abundances of zeranol and the isomers of zearalenol suggesting zearalenone administrations are taken into account.[40]

2.1.2 | Drug residues from legitimate use

Zeranol is, in addition to other growth promoters, legitimately employed in selected countries (e.g., ractopamine, zilpaterol, boldenone, and trenbolone)[41,42] also a drug potentially ingested unknowingly, if drug residues exist in consumed meat. In consideration of that aspect, a urinary reporting level of 5 ng/ml was established, which is not expected to be exceeded through the ingestion of contaminated meat, based on drug residue levels known and reported by foodstuff inspections to date.[43] In a recent report, meldonium residues detected in milk and meat of meldonium-treated cows and chicken were described.[44] The determined amounts appear to represent a significant risk for athletes to unknowingly ingest meldonium at levels that are traceable in doping control urine samples and, further, could potentially exceed the currently enforced urinary reporting level of 100 ng/ml[45,46] in selected regions where meldonium is approved for animal treatment and in farming.

2.1.3 | Drug residues from illegitimate use

Similar to zeranol but illicitly used, clenbuterol has been employed as growth promoter in selected countries, and various AAFs were attributed to the consumption of clenbuterol-contaminated meat which, accordingly, were not considered as ADRVs.[47–52] A variety of analytical means has been assessed, aiming at providing data to differentiate AAFs caused by edible tissue-retained clenbuterol from preparations administered for doping purposes, for example, chiral chromatography for the separate consideration of clenbuterol's enantiomers[53–55] or hair analysis.[56] However, the differentiation of contaminated meat ingestion from doping scenarios has not been robustly accomplished, and the same urinary reporting limit of 5 ng/ml as utilized for zeranol, zilpaterol, and ractopamine has been implemented for clenbuterol. All findings of either drug residue below 5 ng/ml are interpreted as atypical findings (ATF) and subject of mandatory follow-up investigations by the relevant antidoping organizations.[43]

While the above scenario was corroborated in numerous cases, the introduction of clomiphene into an athlete's organism via contaminated produce (e.g., chicken meat) was argued concerning a recent AAF and follow-up investigations as to the plausibility were initiated.

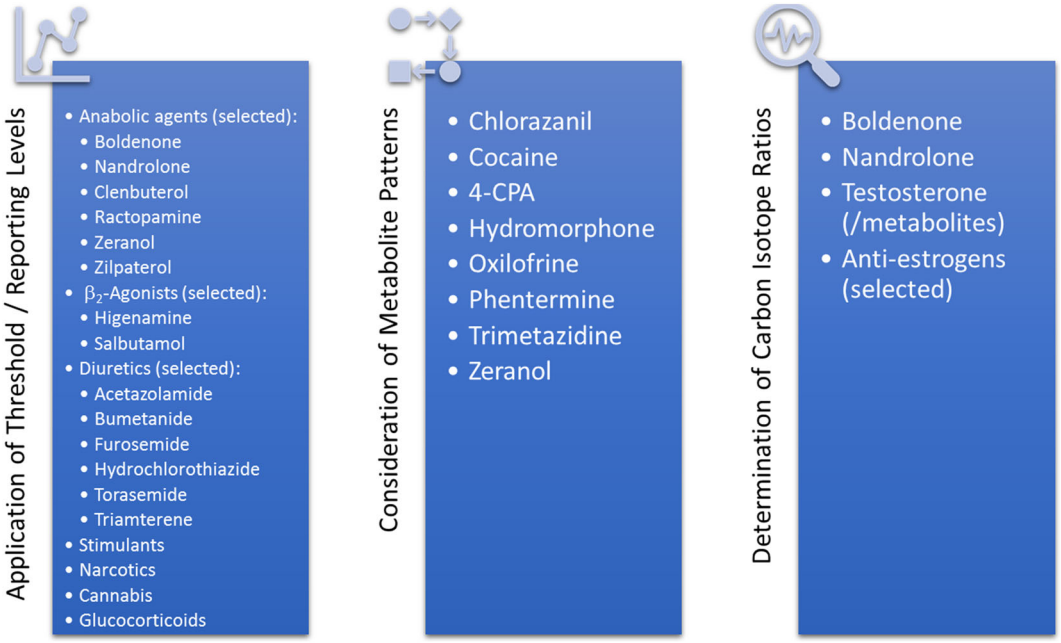

FIGURE 2 Selected strategies in support of distinguishing between drug abuse, perm issive drug use, in- versus out-of-competition use, and exposure to various prohibited substances or precursors thereof [Colour figure can be viewed at wileyonlinelibrary.com]

Clomiphene was shown to positively affect the fertility of animals[57,58] and significantly increased the egg laying productivity of hens.[59] Further, hydrophobic drugs are known to transfer into chicken eggs,[60] which was recently shown to apply also to clomiphene.[61] The amounts of clomiphene detected in eggs produced by clomiphene-treated laying hens reached 20 μg per egg, and the consumption of one or more of these is expected to result in AAFs in case of routine doping controls. Here, preliminary data suggest that diagnostic metabolites, identifying the "animal-processed" clomiphene in human urine, can be used to differentiate scenarios of drug use from food-related drug exposure. Conversely, chemical residues have been proposed as indirect markers of doping, particularly in demonstrating exposure to blood doping methods involving intravenous homologous and autologous blood infusions. Quantification of increased urinary concentrations of di(2-ethylhexyl) phthalate (DEHP) metabolites has been studied as a promising indirect approach to plasticizer exposure; however, the possible intra-individual variation of the metabolite concentrations combined with the increasing ubiquity of plasticizers in the environment make specificity a challenge with these indirect markers.[62–64]

2.2 | Dietary supplements—Contaminated or adulterated

One definition of dietary supplements was published in a recent consensus statement as "A food, food component, nutrient, or non-food compound that is purposefully ingested in addition to the habitually consumed diet with the aim of achieving a specific health and/or performance benefit."[65] Despite ongoing debates about the effectiveness of such supplements, an enormous and competitive market offering an extremely large variety of dietary supplements has developed, which compared with pharmaceuticals, is regulated in a postmarket fashion with products generally not required to undergo rigorous clinical evaluation for safety and efficacy. As comprehensively summarized in a recent review[18] and corroborated in a new study on risks of ADRVs associated with dietary supplements,[66] the issue of contaminated and adulterated supplements containing prohibited substances still exists. Contaminants or adulterants may or may not be explicitly listed on the product label, unconventional or generalized nomenclature may be used to conceal a prohibited ingredient (e.g., proprietary blends), and products themselves can be ambiguously viewed as relatively low risk (e.g., vitamins) to high risk (e.g., "testosterone boosters"). Contaminations, that is, drug amounts expected to have no pharmacological relevance, are unlikely to put the athletes at great health risk. However, due to the high performance of the analytical method's sensitivity, numerous cases exist where the athlete's career is at stake and the source of the drug that caused an AAF in doping controls was suspected and/or eventually identified to associate to a tainted dietary supplement. In addition, adulterated supplements, that is, those that contain pharmacologically relevant amounts of drugs or drug candidates without declaring these as components of the product, have frequently been reported. Issues arising from potential ADRVs resulting from the use of such supplements might be complemented by considerable health risks, caused by the effects of the drugs themselves or by drug–drug interactions. Examples include designer stimulants such as dimethylamylamine (DMAA), dimethylbutylamine (DMBA), commonly seen in pre-workout supplement products, and beta-methylphenylethylamine, or BMPEA, which was discussed in the context of supplements containing *Acacia rigidula*.[67] A further emerging area of concern is "dietary supplements" (and related foods and cosmetic products) containing the permitted substance cannabidiol (CBD). Some of these products have been demonstrated to have a high potential to contain prohibited cannabinoid impurities that may lead to an AAF.[68,69]

2.3 | Drug metabolism/drug contamination

2.3.1 | Biotransformation of drugs and health care products

While risks associated with the use of dietary supplements have been frequently discussed, AAFs resulting from the metabolism of permissive drugs or health care products are less often observed, and strategies exist to identify such scenarios at the initial testing or confirmation procedure level in antidoping laboratories. The conversion of permitted drugs such as ethylmorphine and codeine into morphine is well established, and the concentration of morphine only above the relevant threshold (or decision limit) results in an AAF, if the criteria involving the consideration of codeine or ethylmorphine and corresponding metabolites are met.[70,71] Similarly, characteristic metabolites of mebeverine or oxethazaine are to be monitored in cases of potential AAFs related to *p*-hydroxy-amphetamine or phentermine and mephentermine, respectively,[72,73] as the permitted use of mebeverine and oxethazaine is to be differentiated from the prohibited administrations of stimulants such as amphetamine, phentermine, and their derivatives. Further, in another case, the degradation products oxymorphone and naltrexone were identified as the degradation products of the permitted drug methylnaltrexone in urine and determine not to be from administration of oxymorphone.[74] While the metabolic pathways of these drugs have been thoroughly investigated and described in the literature, also unexpected sequential metabolic conversions leading to prohibited substances in doping control urine samples were recorded. Among those, the formation of the diuretic chlorazanil from the antimalaria chemoprophylactic proguanil was identified. Here, a main metabolite of proguanil, 4-chlorophenylbiguanide was shown to convert *in vesica* (i.e., in the bladder) in the presence of urinary formaldehyde to chlorazanil, which was detected in doping control samples of fencers in 2015.[75] More recently, the necessity to include characteristic metabolites of chlorphenesin (and its carbamate) into doping control analytical assays was noted.[76] Meclophenoxate is prohibited in sports in-competition, and an administration is detected by means of its metabolite 4-chlorophenoxy acetic acid; however, this metabolite is also a main metabolite of chlorphenesin and chlorphenesin carbamate, two (non-prohibited)

substances available as sun screen biocide and muscle relaxant, respectively.[77,78]

2.3.2 | Contamination of permitted therapeutics with banned substances

Further, critical situations can occur when legitimately obtained and permitted drugs are used, which however contain trace amounts of banned substances. Such cases were reported for instance with hydrochlorothiazide,[79,80] and by means of a recently issued technical letter,[81] also acetazolamide, bumetanide, furosemide, torasemide, and triamterene are taken into consideration as potential contaminants that warrant specific result management. The potential for pharmaceutical contamination has been heightened by the trend of increasingly complex generic pharmaceutical supply chains, where quality controls and good manufacturing practices may be compromised.

Also, blood products employed for legitimate transfusions (whole blood or plasma) were shown, while rarely, to contain drug residues potentially relevant for doping controls (including, e.g., stimulants and beta-blockers).[82,83] Thus, therapeutically indicated transfusions cannot be excluded as a source of minute amounts of drugs that an athlete is potentially exposed to.

2.4 | Intimate contact-transmitted drug (metabolite) residues

Exposure to drugs and drug metabolites that, if detected in an athlete's doping control sample, would constitute an AAF have been attributed to intimate contact-transmitted scenarios with increasing frequency, including saliva-, skin contact-, and seminal fluid-based analyte transfer scenarios. Already in 2004, the possibility of an AAF related to the anabolic-androgenic steroid clostebol as transferred by sexual intercourse was reported,[84] but also the application of clostebol-containing cream to a recipient of the therapeutic (as opposed to the own use) was shown to have the potential to result in an AAF,[85] and several additional cases, mostly related to saliva[86,87] and seminal fluid[88] followed. In the light of the plethora of therapeutics available and the growing body of knowledge regarding drug levels found in saliva[89] and seminal fluid,[90] the possibility of athletes having unknowingly contact to trace amounts of drug residues warrants in-depth consideration.

3 | CONCLUSION

The complexity and extent of today's drug contaminants and resulting routes of exposure was excellently summarized in 2011 by Daughton,[91] and numerous of the above discussed aspects were discussed in the context of forensic epidemiology and toxicology, including interpersonal dermal transfer of drugs.[92] In addition to these potential contributors to the athlete's exposome, also other factors could require consideration such as shared household equipment or jointly prepared food,[93] and the substantial intricacy appears to have further increased in the past decade as the exposure to drugs and chemicals by elite athletes that are subjected to routine doping controls adds another level of relevance concerning the potential consequences.

Protecting athletes from consequences due to inadvertent doping has become particularly challenging, especially when considering the required and continuously improving analytical sensitivity of antidoping testing procedures.[94] Investigations into the athlete's exposome and how to distinguish between deliberate drug use and different contamination scenarios has become a central topic of antidoping research, and although it might appear as counterintuitive at first glance, increasing the testing frequency of athletes seems to be a particularly useful approach in support of the differentiation of low (lowest) level drug exposure from applications of pharmacologically relevant doses of doping agents.[95] Further, "pharmacokinetic outliers," that is, drugs with for instance particularly long elimination periods and corresponding detection windows[96–104] and substances causing doping control urine samples to appear atypical or suspicious[105–110] necessitate continued consideration. It remains a delicate balance of needing to continually evolve to develop sensitive analytical techniques and new long-term analyte targets that extend the detection window of egregious doping substances and address the ever-changing and sophisticated techniques intentional dopers will take to cheat with the goal of achieving maximum detection and deterrence. While on the other hand, recognizing the risk of the athletes' exposome, which unfairly penalizes athletes for doping when alternative innocent explanations may be plausible. Often, uncovering these scenarios takes immense effort and cost, and in some cases, athletes proving their innocence may be virtually impossible due to the lack of identification of the original source.

Moving further toward the goal of identifying athletes committing intentionally ADRVs while excluding AAFs that result from inadvertent exposure to prohibited substances (or those that convert in the human organism to common metabolites) will require further contribution, cooperation, and investment by all stakeholders, including antidoping organizations, antidoping laboratories, and athletes.

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DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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