EGAS MONIZ SCHOOL of HEALTH & SCIENCE | INSTITUTO UNIVERSITÁRIO EGAS MONIZ

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MESTRADO INTEGRADO EM CIÊNCIAS FARMACÊUTICAS

NEW HORIZONS IN DOPING CONTROL: TOXICOKINETICS, REGULATION AND THE EVOLUTION OF DETECTION STRATEGIES FOR PERFORMANCE-ENHANCING DRUGS

Trabalho submetido por
Candice Séverine Nathalie Vuillard
para a obtenção do grau de **Mestre** em Ciências Farmacêuticas

Julho de 2026

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Profª Doutora Maria Deolinda Auxtero

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Disponibilidade de dados	Modelo de declaração de disponibilidade de dados	Política de partilha	Assinale com "x"
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Dados disponíveis no artigo ou nos seus materiais suplementares	Os autores confirmam que os dados que suportam as conclusões deste estudo estão disponíveis no artigo [e/ou] nos seus materiais suplementares.	Básico, partilhar a pedido	
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Partilha de dados não aplicável - não foram gerados novos dados	A partilha de dados não é aplicável a este artigo, uma vez que não foram criados ou analisados novos dados neste estudo.	-	x
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Dados não disponíveis - consentimento do participante	Os participantes neste estudo não deram consentimento escrito para que os seus dados fossem partilhados publicamente, pelo que, devido à natureza sensível da investigação, os dados de apoio não estão disponíveis.	-	

O Orientando: Candice Séverine Nathalie Vuillard Data: 17/06/2026

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Resumo

Este trabalho analisa a capacidade do sistema antidopagem para acompanhar a evolução das práticas de dopagem, em particular o surgimento de novas substâncias psicoativas, integrando toxicocinética, estratégias de deteção e análise regulamentar. São abordados os princípios toxicocinéticos aplicados às substâncias dopantes, com especial atenção aos processos de absorção, distribuição, metabolismo e excreção, à variabilidade interindividual, aos modelos PBPK, à aprendizagem automática, ao Passaporte Biológico do Atleta e às abordagens ómicas, incluindo metabolómica, proteómica e transcriptómica.

A análise incide também sobre a evolução dos quadros regulamentares internacionais e europeus, considerando a resposta às novas substâncias psicoativas, o papel dos sistemas de alerta precoce e a transição de modelos predominantemente reativos para mecanismos mais preditivos. São discutidas abordagens baseadas em classes e em efeitos, bem como as suas limitações científicas e jurídicas. Por fim, são revistas estratégias analíticas convencionais e emergentes, incluindo GC-MS, LC-MS, perfilagem biológica, biomarcadores genéticos, ressonância magnética nuclear, polímeros de impressão molecular, solventes eutéticos profundos e técnicas de ionização inovadoras.

No conjunto, este trabalho propõe uma visão integrada de um controlo antidopagem mais preventivo, no qual o conhecimento toxicocinético, a inovação analítica e a adaptação regulamentar funcionam como ferramentas complementares para responder à diversificação das práticas de dopagem.

Palavras-chave: Dopagem desportiva; Toxicocinética; Métodos de deteção; Regulamentação

Abstract

This study examines the ability of the anti-doping system to keep pace with evolving doping practices, particularly the emergence of new psychoactive substances, by integrating toxicokinetics, detection strategies and regulatory analysis. It applies the principles of toxicokinetics to doping substances to investigate the impact of concepts based on PBPK methods, machine learning methods, the Athlete Biological Passport and the consideration of inter-individual variability. This aspect is explored in greater depth through an analysis of individualisation via the interface between PBPK, the Athlete Biological Passport and omics methods (metabolomics, proteomics, transcriptomics).

The analysis also examines the development of international and European frameworks. It considers the regulatory response to NPS, the role of early-warning systems, and the move away from reactive models towards more predictive mechanisms. It discusses class-based and effect-based approaches, as well as the scientific and legal debates they raise. Finally, the study examines a range of analytical strategies, from conventional methods and their limitations to technological advances (biological profiling, genetic biomarkers, nuclear magnetic resonance). It also looks at new approaches to sample preparation and ionisation, including molecularly imprinted polymers and supramolecular solvents.

The aim is to present a coherent vision of a more preventive approach to anti-doping, in which all elements are viewed as complementary tools for addressing the diversification of doping practices.

Keywords: Sports doping; Toxicokinetics; Detection methods; Regulation

Résumé

Ce travail examine la capacité du système antidopage à suivre l'évolution des pratiques, en particulier l'arrivée des nouvelles substances psychoactives en associant toxicocinétique, détection et réflexion réglementaire. Il utilise les principes de la toxicocinétique appliquée aux substances dopantes pour étudier l'impact des concepts basés sur les méthodes PBPK, de méthodes de machine learning, de l'Athlete Biological Passport et à la prise en compte de la variabilité interindividuelle. Cet aspect est approfondi par une analyse de l'individualisation à travers l'interface entre PBPK, ABP et méthodes omiques (métabolomique, protéomique, transcriptomique).

L'analyse porte également sur la progression des cadres internationaux et européens. En prenant en compte l'examination de la réponse réglementaire aux NPS, le rôle des systèmes d'alerte précoce et la tentative de passer de modèles réactifs à des dispositifs plus prédictifs. En ayant recours aux approches par classes, par effets et aux débats qu'elles suscitent. Pour finir, l'étude se penche sur un ensemble de stratégies analytiques, des méthodes conventionnelles et leurs limites jusqu'aux avancées technologiques (profilage biologique, biomarqueurs génétiques, résonance magnétique nucléaire). Également aux nouvelles approches de préparation et d'ionisation des échantillons, comprenant les polymères à empreinte moléculaire et les solvants supramoléculaires.

L'ensemble vise à proposer une vision cohérente d'un contrôle antidopage plus préventif, où tous les éléments sont pensés comme des leviers complémentaires pour répondre à la diversification des pratiques dopantes.

Mots-clés : Dopage sportif ; Toxicocinétique ; Méthode de détection ; Réglementation

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Abbreviations

ABP: Athlete Biological Passport

ADME: Absorption, Distribution, Metabolism and Excretion

AI: Artificial Intelligence

AUC: Area Under the Curve

CAS: Court of Arbitration for Sport

CRISPR: Cluster Regularly Interspaced Short Palindromic Repeats

DES: Deep Eutectic Solvents

DNA: Deoxyribonucleic Acid

EPO: Erythropoietin

EU: European Union

EUDA: European Union Drugs Agency

EWS: Early Warning System

GC-MS: Gas Chromatography–Mass Spectrometry

GH: Growth Hormone

GIT: Gastrointestinal Tract

HBA: Hydrogen Bond Acceptor

HBD: Hydrogen Bond Donor

HPLC-MS: High-Performance Liquid Chromatography-Mass Spectrometry

IOC: International Olympic Committee

ISO: International Organization for Standardization

LC-MS: Liquid Chromatography–Mass Spectrometry

MALDI-MS: Matrix-Assisted Laser Desorption/Ionization–Mass Spectrometry

MDPM: Methylenedioxyphenmetrazine

MIP: Molecularly Imprinted Polymer

NGS: Next-Generation Sequencing

NMR: Nuclear Magnetic Resonance

NPS: New psychoactive substance

PBPK: Physiologically Based Pharmacokinetic

PCR: Polymerase Chain Reaction

PSA: Psychoactive Substances Act

qPCR: quantative Polymerase Chain Reaction

rHuEPO: Recombinant Human Erythropoietin

RNA: Ribonucleic Acid

SERS: Surface-Enhanced Raman Scattering

UK: United Kingdom

UNESCO: United Nations Educational, Scientific and Cultural Organization

UNODC: United Nations Office on Drugs and Crime

WADA: World Antidoping Agency

WGR: Whole-Genome Resequencing

WISE: Western Australian Illicit Substance Evaluation

I. Introduction

1.1 Doping in sport: issues and developments

In sports, doping refers to the use of prohibited substances or techniques aimed at improving athletic performance. It is commonly viewed as a serious threat to both athletes' health and the integrity of sport, as well as to the principle of fair play. The reliability and efficiency of antidoping tests can be influenced by athletes' attitudes toward testing procedures. Studies also point out that athletes' views on the fairness and effectiveness of these controls, shaped by their own experiences, play a key role in how much trust and confidence they place in the overall testing system (1).

The use of performance-enhancing substances has likely existed for as long as organised sport itself. Ancient sources indicate that athletes in the early Olympic Games in Greece experimented with special diets and mixtures thought to improve strength or endurance. The term doping comes from the Dutch word dop, meaning a drink made from grape skins once used by Zulu warriors before battle and it entered common usage in sport during the nineteenth century. As contemporary athletics gained popularity, these practices continued, frequently with dire repercussions. Thomas Hicks is among the most notable instances from this period. After utilising a brandy and strychnine combination to win the marathon at the 1904 Olympic Games, he almost died. Sporting authorities were compelled to act as awareness of these risks grew. Despite the lack of reliable testing at the time, the International Association of Athletics Federations was the first organization to outlaw doping in 1928. Systematic testing was ultimately implemented in the 1960s after several stimulant-related deaths. As the first event where, doping controls were formally implemented, the 1968 Olympic Games were a watershed (2).

Even if it is not the focus of current discussions on doping, ethics in sports is an important subject. It seeks to uphold fairness, safeguard the rights of all athletes, and protect athletes' health. Antidoping policies provide ethical issues in this situation. Regulations and research focus more on identifying and stopping

behaviours that undermine sports fairness than on curing the illness itself. As a result, they may put athletes at unnecessary risk (3).

Doping practices have changed significantly over time, now include experimental chemicals, veterinary medications, excessive dosages, and the abuse of both legal and illicit narcotics as well as medicinal pharmaceuticals. The World Antidoping Agency's (WADA) Prohibited List, which is updated every year, makes a distinction between substances whose usage is limited to certain times and those that are outright prohibited. Among recent developments are novel psychoactive substances (NPS): newly synthesised chemicals, revived older drugs, or innovative uses of known molecules like cathinones, phenethylamines, synthetic cannabinoids and opioids (3).

1.2 Doping substances and toxicokinetic issues

Table 1 shows the WADA's annual Prohibited List. It is based on the World Antidoping Code's criteria, which state that a substance or technique is prohibited if it meets at least two of three requirements: ability to improve performance, real or potential health harm, and breach of the spirit of sport. The first two of these rely on pharmacological and scientific data, while the final one is still primarily an ethical issue. This highlights how important biomedical research is in developing antidoping regulations, but it also raises concerns about the quality and strength of the data used to support the inclusion of specific compounds (4).

Table 1: The 2026 prohibited list world antidoping code. Created by the author based on the WADA 2026 Prohibited List (5)

Substances & methods prohibited at all times	
S0	Non-approved substances
S1	Anabolic agents
S2	Peptide hormones, growth factors, related substances, and mimetics
S3	Beta-2 agonists
S4	Hormone and metabolic modulators
S5	Diuretics and masking agents
M1-M2-M3	Prohibited Methods
Substances & methods prohibited in-competition	
S6	Stimulants
S7	Narcotics
S8	Cannabinoids
S9	Glucocorticoids
Substances prohibited in particular sports	
P1	Beta-blockers

Toxicokinetics covers absorption, distribution, metabolism and excretion (ADME). Within the context of sports doping, it plays a key role in the scientific evidence base, governing both the physiological impact of prohibited substances and their traceability in antidoping tests, with particular emphasis on performance enhancement, detection windows and analytical interpretation (6).

These toxicokinetic characteristics vary greatly, even within the same chemical classes, as several revisions of the Prohibited List highlight. This has an impact on detection timeframes and the most effective markers to target analytically. Since the original substances frequently decompose rapidly, determining their metabolites becomes crucial for accurate doping controls. In summary, this type of information influences the analytical techniques and how they are interpreted in actual antidoping tests in addition to supporting the reasons why particular compounds are prohibited (7).

1.3 Regulatory and analytical challenges

According to Willick et al. (2), the World Antidoping Agency (WADA) defines doping as any behaviour violating antidoping rules, ranging from using or having traces of banned drugs/techniques, refusing tests and tampering with controls, to associating with sanctioned individuals. The Prohibited List is amended annually but athletes and their teams frequently stay ahead of the game as new doping techniques continue to emerge quicker than authorities can keep up. Furthermore, it is a difficult task to get all sports federations and nations to consistently apply the same standards, particularly given the stark differences in financial and regulatory frameworks.

Traditional antidoping tests aim to identify the presence of prohibited substances or processes in biological samples. Analytical chemistry is therefore essential for doping control. However, modern antidoping procedures increasingly leverage advances in pharmaceutical research, particularly investigational drugs that have not yet received marketing authorization. Consequently, antidoping laboratories must adopt proactive measures, such as the early integration of new drugs and their metabolites into screening tests, often in the absence of complete toxicological or toxicokinetic data. Analytical detection must therefore be able to predict new substances and structurally related analogues, in addition to identifying known compounds (8).

Analytical data are essential for examining unusual test results, complementing standard screening tests. Examples of this include atypical metabolic profiles, the deliberate manipulation of samples, or accidental exposure to contaminated food or dietary supplements. Addressing these issues requires specialized analytical methods and in-depth investigation; otherwise, it may be impossible to distinguish intentional doping from accidental contamination (9).

II. Aim and Objectives

The main objective of this dissertation is to assess the current relevance of toxicokinetics in issues related to antidoping control, particularly in terms of the detection, interpretation, and regulation of substances involved in performance enhancement, as well as emerging psychoactive compounds. Having this in mind, the underlying question of this work is:

To what extent is it possible to improve the detection and interpretation of the latest doping practices through a deeper understanding of toxicokinetics, the development of innovative analytical methods, and regulation?

In addition, the following specific objectives will be explored:

1. To evaluate toxicokinetic processes, which include the absorption, distribution, metabolism, and excretion of illicit substances, as well as the consequences these may have on detection limits, the manifestation of effects, and the interpretation of analytical results.

2. To analyse the impact of physiological, genetic, metabolic, and environmental factors on toxicokinetic variability and their impact on interpretation of doping monitoring.

3. To understand the diversity of new psychoactive substances and molecules with an impact on performance, as well as the consequences of the scarce information about them, particularly their structural diversity and reduced toxicokinetic data.

4. To analyse current legislation in the fight against doping, with particular emphasis on the WADA List of Prohibited Substances, as well as the limitations of regulating rapidly evolving substances.

5. To revisit the main analytical methods, both conventional and innovative, used in doping control.

6. To present indirect detection strategies, such as the Athlete Biological Passport and interventions based on "omics," for identifying the biological effects caused by doping.

7. To appreciate the potential of PBPK modelling and Machine Learning in optimizing detection and managing targeted tests.

8. To reflect on the ethical, regulatory, and scientific aspects and their impact on antidoping control.

In short, this study seeks to establish an integrated perspective on toxicokinetics, analytical innovation, and regulatory aspects, considering their potential role in improving the ability to detect and interpret doping practices in modern sports.

III. Methods

This study comprises a comprehensive literature review. The search was conducted in the PubMed, ScienceDirect, Web of Science, and Google Scholar databases and was supplemented by official documents from WADA, UNODC, EUDA, UNESCO, and the Court of Arbitration for Sport, whenever relevant. The study focused on works published between 2016 and 2026, although earlier references were also included when regarded as pertinent. Search terms included “doping control”, “toxicokinetics”, “ADME”, “new psychoactive substances”, “athlete biological passport”, “PBPK modelling”, “machine learning”, “omics”, “metabolomics”, “proteomics”, “transcriptomics”, “GC-MS”, “LC-MS”, “MALDI-MS”, “NMR”, “molecularly imprinted polymers” and “deep eutectic solvents”. Articles were selected according to their relevance to antidoping science, analytical toxicology, pharmacokinetics, regulatory frameworks and emerging detection strategies. Priority was given to peer-reviewed reviews, original research articles, international guidelines and official regulatory documents. Non-peer-reviewed sources were used only when they provided official or contextual information that was not available elsewhere.

IV. Toxicokinetics of doping substances

4.1 Fundamental principles of toxicokinetics applied to doping

Kinetics refers to the processes of ADME that determine how the body acts on a drug over time (10). Figure 1 depicts how the ADME processes govern the temporal progression of a chemical within the body.

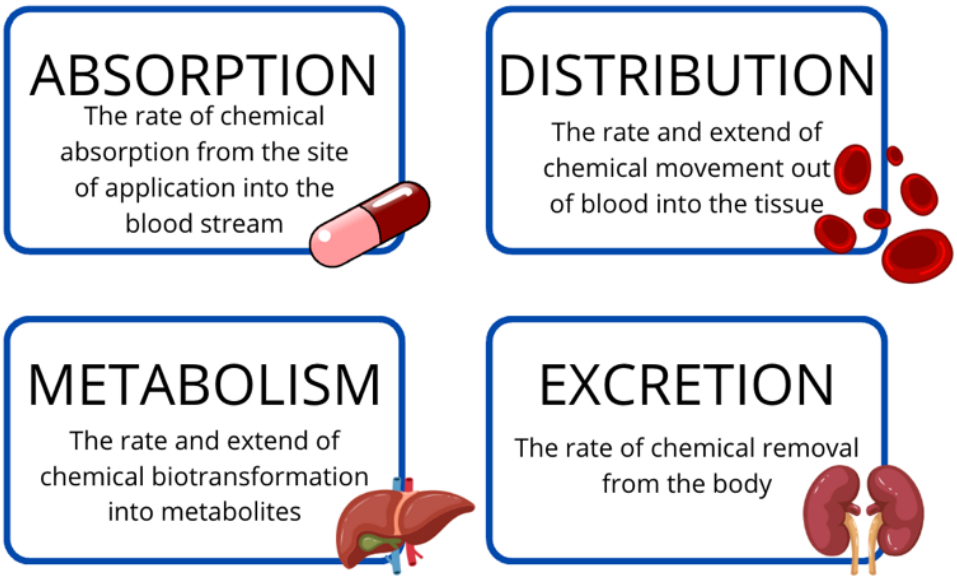

Figure 1: Overview of ADME processes. Created by the author based on Thomas-Brown et al. (11).

In toxicity studies, toxicokinetics refers to the production and analysis of pharmacokinetic data applied to xenobiotics, based on ADME parameters. It describes the dynamic activities of these external substances as they move between different compartments of the body and makes it possible to quantify the maximum plasma concentration and total exposure over time. Integrated into non-clinical studies, toxicokinetics is recognised as an essential element in comparing exposure to xenobiotics between species and rigorously interpreting similarities or differences in observed toxicological responses (12).

This toxicity may be due to the accumulation of a xenobiotic in the body. If excretion does not occur properly, the substance accumulates with repeated

administration, leading to a gradual increase in concentration and, ultimately, an increased risk of toxic effects (13).

Toxicokinetics is a crucial tool for linking an individual's actual exposure to doping agents with the toxic effects they cause and their potential detectable levels.

The study by Badawy et al. (14) focuses on androstenedione, a dietary supplement that has been banned by WADA. This hormone, naturally produced in both men and women, serves as a precursor for testosterone and oestrogen. As shown in Table 2*,* its toxic effects may differ between males and females.

Table 2: Toxic effects of androstenedione drug supplementation in males and females. Created by the author based on Badawy et al. (14).

Females	Males
• Hirsutism	• Hepatotoxicity
• Clitomegaly	• Shrunken testicles
• Male-pattern baldness	• Behavioural changes
• Abnormal menstrual periods	• Breast development
• Development of masculine traits	• Decrease sperm production
• Increase the risk for breast and endometrial cancer	• Increase the risk for prostate cancer

4.2 NPS as a disruptive case

NPS is a broad category of synthetic substances created to mimic the effects of traditional recreational drugs while momentarily evading current laws. These drugs are linked to the emergence of dependency syndromes and severe toxic effects (15).

In regulatory practice, they are commonly organised along two complementary axes. From a structural perspective, NPS encompass a wide array of chemical families, including:

- Aminoindanes

- Benzodiazepines,
- Etomidate analogues
- Fentanyl analogues
- Lysergamides
- Nitazene
- Orphine analogues
- Phencyclidine-type substances
- Phenethylamines
- Phenidates
- Phenmetrazines
- Piperazines
- Plant-based substances
- Synthetic cannabinoids
- Synthetic cathinones
- Tryptamines
- Other substances *(16)*

In parallel, Figure 2 presents a functional or “effect-based” classification. Synthetic cannabinoid receptor agonists, traditional hallucinogens, stimulants, synthetic opioids, sedatives/hypnotics and dissociatives are the six major pharmacological classes that are distinguished based on how they primarily affect the central nervous system. When combined, these structural and effect-based classifications demonstrate the difficulty of incorporating such a diverse environment into current toxicokinetic models and antidoping frameworks. Together, these categories illustrate both the chemical diversity and the psychopharmacological breadth of NPS (16).

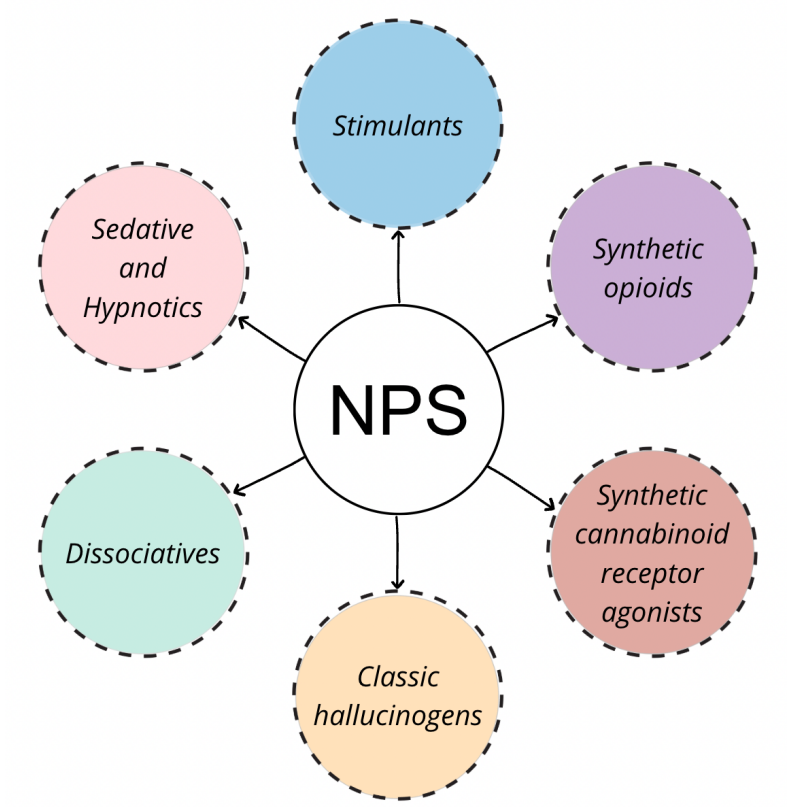

Figure 2: The main classes of NPS. Created by the author based on the United Nations Office on Drugs and Crime (UNODC) classification (17).

3,4-Methylenedioxyphenmetrazine (MDPM), a phenmetrazine-derived stimulant recently investigated *in vitro*, provides a useful example of the toxicokinetic challenges associated with NPS (18).

MDPM exhibits low plasma protein binding (32%, fu=0.68), moderate lipophilicity (logP=1.3) and an *in vitro* half-life higher than180 minutes in human microsomes and S9 liver fractions. Its metabolism is limited to exclusive demethylation by CYP2D6, producing M1 (nor-MDPM) and M2 (O-methylated), with strong inhibition of CYP2D6 and moderate inhibition of CYP1A2 (39%) and CYP3A4 (28%) (18).

In terms of NPS, this profile highlights the shortcomings in the antidoping system. Urinary detectability is ensured by the extended half-life and low binding. However, sensible criteria cannot be determined. The predominant involvement

of CYP2D6 may increase interindividual variability: extensive metabolisers may eliminate the substance more rapidly, whereas poor metabolisers may show prolonged exposure or different metabolite profiles. Any medication interactions are hidden by cross-inhibition. When low quantities, genetic diversity, or potential contamination situations are present, this profile may make it more difficult to interpret unfavourable test results. Overall, NPS challenge not only prohibited lists, but also the toxicokinetic assumptions used to predict exposure, metabolism and detectability (18).

NPS have transformed antidoping into a genuine analytical arms race. Gas Chromatography–Mass Spectrometry (GC–MS) screening of athletes' urine must now encompass hundreds of potentially psychoactive compounds, yet predicting the retention times of newly synthesized derivatives remains a major bottleneck. Each structural modification requires continuous updating of spectral libraries, increasing the risk of false negatives until retention behaviours are properly characterized. As a result, laboratories accredited by the World Antidoping Agency often operate in a reactive position, striving to keep pace with these rapidly evolving synthetic compounds (19).

4.3 Advanced modelling and individualisation

4.3.1 PBPK

Physiologically based pharmacokinetic (PBPK) models offer, in the antidoping context, a way to anticipate how a prohibited substance behaves in different athletes without exposing humans to deliberate doping experiments. By integrating the compound's physicochemical properties with detailed physiological parameters (organ sizes, blood flows, enzyme expression, renal function), these models simulate the full ADME profile *in silico* and generate concentration-time curves across a wide range of "virtual" individuals. PBPK models were originally developed to optimise dosing in populations in whom clinical studies may be difficult, such as paediatric, elderly or renally impaired patients. In antidoping, similar principles can be applied to estimate how long a

prohibited substance may remain detectable under different physiological and dosing scenarios. In this sense, PBPK modelling becomes a decision support tool both for regulators, who can more precisely calibrate thresholds and detection windows (20).

In practice, these principles generally take the form of multi-compartment models that incorporate the organs and tissues essential to the distribution and elimination processes. Figure 3 provides an overview of this type of 'whole-body' model, in which arterial blood transports the substance to various organs and venous blood takes it up following exchange or excretion. This diagram is primarily intended to illustrate the possible pathways of a doping substance, its accumulation and its elimination. It also shows how organ-specific processes influence the profiles detected in biological samples taken during antidoping tests (21).

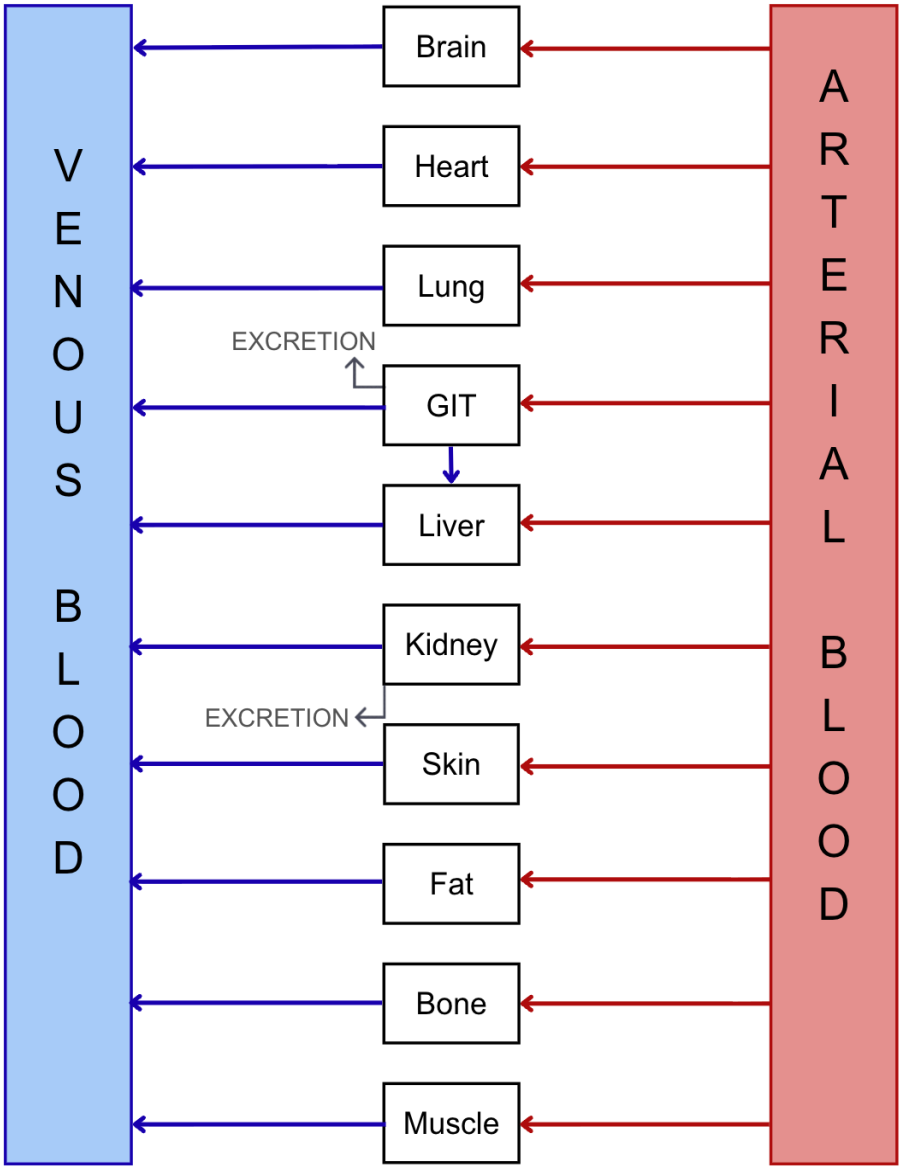

Figure 3: Structural overview of the whole-body PBPK model. Created by the author based on Yang et al. (21).

4.3.2 Machine learning

Machine learning trains algorithms to autonomously extract doping signatures from vast mass spectrometry datasets, rapidly identifying patterns and classifying substances that would overwhelm human analysts. By increasing detecting

capacity and speeding up result processing, this automation responds to the increase in testing requirements brought on by more athletes and more sporting events (22).

Figure 4 illustrates how machine learning can be integrated into the doping detection workflow. Large and intricate datasets are produced during sample collection and GC-MS or Liquid Chromatography–Mass Spectrometry (LC-MS) processing. Traditionally, experts have analysed these data manually, which may be laborious and inconsistent depending on the analyst. Machine learning provides an option in this situation by making automated data analysis possible. It facilitates the faster identification of pertinent patterns and can increase detection sensitivity and efficiency (23).

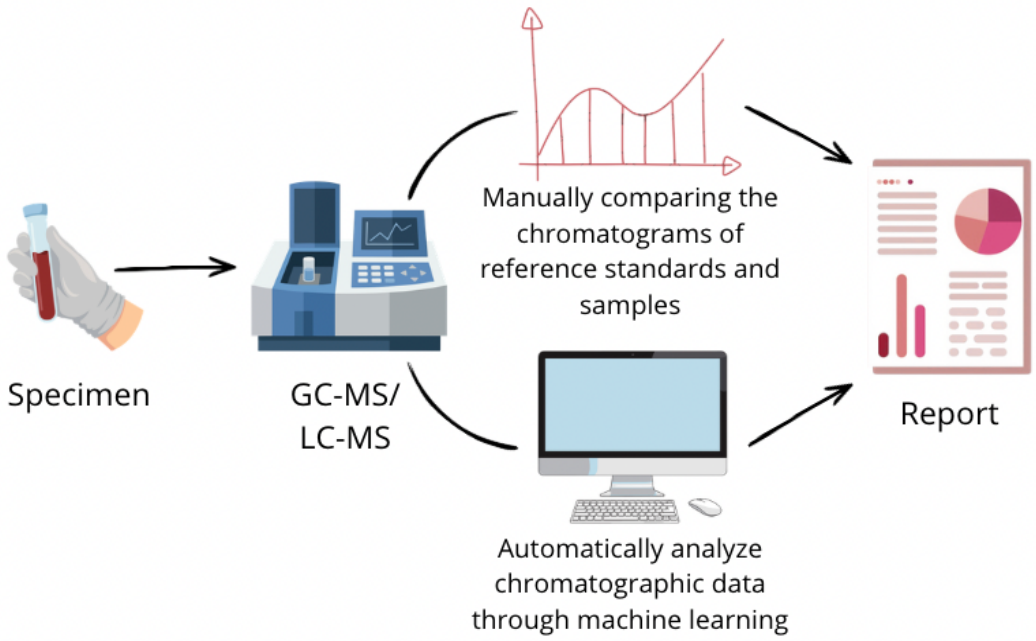

Figure 4: Proposed Integration of machine learning into the antidoping analytical workflow. Created by the author based on Yang et al. (22).

Figure 5 illustrates the use of machine learning for the first check of samples in doping control. A trained model is used to run the laboratory results and determine the probability that each sample is either suspicious or considered

normal. This allows for the rapid identification of outlying samples. The procedure may then be streamlined and analysis time shortened by prioritising these samples for confirmatory testing (22).

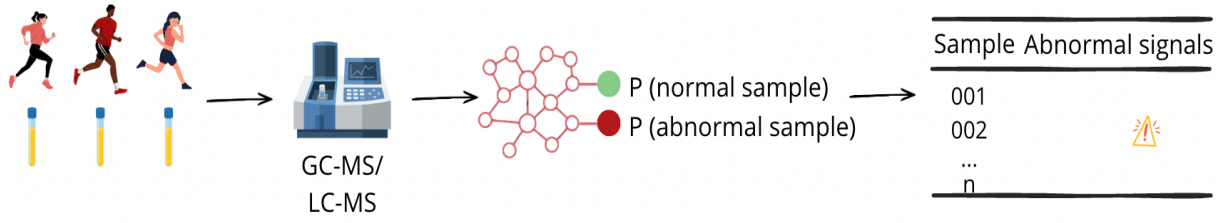

Figure 5: Use of machine learning for initial triage of doping control samples. Created by the author based on Yang et al. (22).

While machine learning holds great promise in the fight against doping, it also has significant drawbacks. For example, it can reproduce, or even amplify, biases present in training data or algorithm design. Fairness, as a complex human judgment, is difficult to translate into mathematical constraints. Furthermore, accuracy metrics mask the unequal consequences of false positives and false negatives. Finally, machine learning produces characteristic "non-human" errors that human expertise would naturally prevent (24).

The limitations of machine learning in this situation emphasise the need for supplementary methods that can capture the biological changes that occur over time in a person. The Athlete Biological Passport (ABP) exemplifies this by continuously tracking selected biological markers to detect atypical variations from an athlete's normal profile.

4.3.3 Athlete Biological Passport

The ABP was introduced in 2009 by the World Antidoping Agency. It works by tracking biological markers over time to detect indirect signs of doping. The system currently includes two modules: haematological and steroidal. A third one, focusing on endocrine markers, is still being developed. Instead of searching for

a specific banned substance, the ABP looks for unusual changes in an athlete's biological profile. However, natural variations in physiology can sometimes make interpretation difficult (25).

Haematological and steroid parameters are interpreted using an adaptive Bayesian model. This model updates the reference values with each new blood sample (Figure 6) (26).

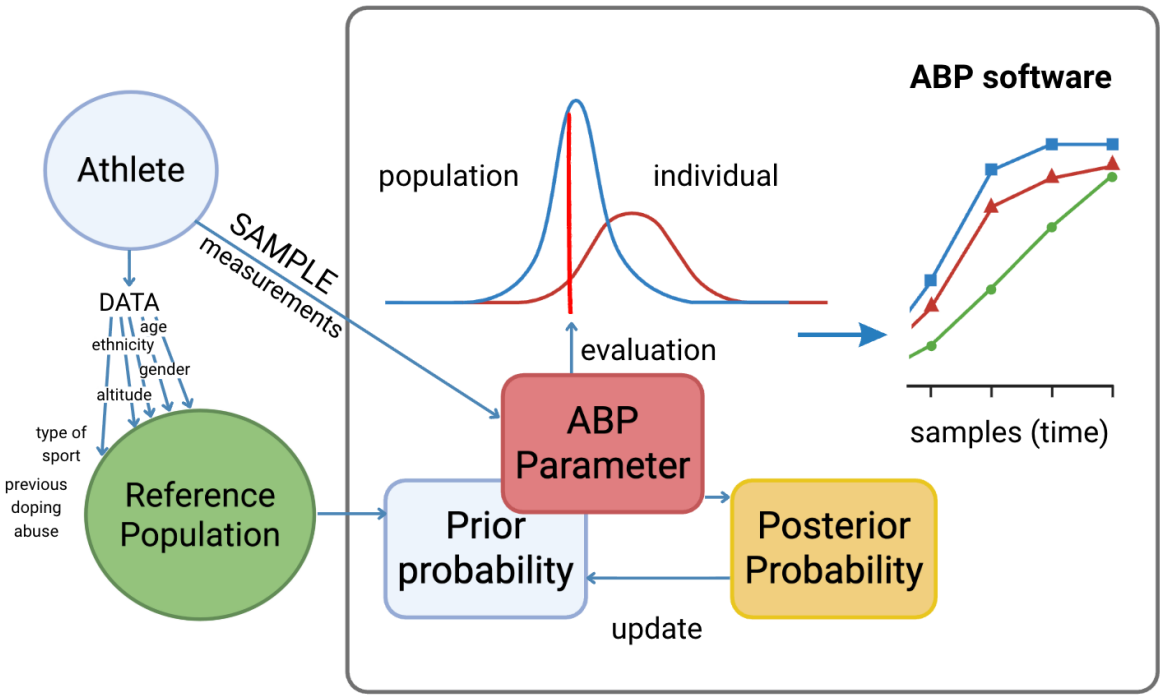

Figure 6: Schematic representation of the Athlete Biological Passport Bayesian model. Created by the author based on Dragčević et al. (26).

The ABP offers several advantages over traditional antidoping methods. Its longitudinal approach makes it possible to detect indirect markers of doping, even after prohibited substances have disappeared from the body. Hence, the sensitivity of antidoping approaches is increased and the detection window is broadened. The ABP also acts as a deterrent, since athletes are monitored continuously rather than through isolated tests. Furthermore, it enables a more objective and effective analytical approach, prioritizing athletes with biological profiles that may raise suspicions (27).

In fact, an athlete's biological condition is always somewhat unstable, even in the absence of doping. It is normal for daily fluctuations to occur in performance and endocrine biomarkers, and these can be quite significant. Consequently, minor changes noted throughout time may just reflect physiological variations, rather than the result of manipulation. This natural variability makes it difficult for the Antidoping Biological Monitoring Programme to distinguish genuine doping signals.

Physiological and performance-related variability poses a major challenge for any longitudinal approach such as the ABP. Among long-distance runners, the normal variability in competition performance has been estimated at between 1.0 and 1.4 per cent. Modest deviations from predicted results can occur without doping and already complicate the identification of 'abnormal' performances. High-level endurance triathletes exhibit significant intra-individual biological variation for the endocrine markers included in the ABP: coefficients of variation are around 8% for IGFBP-3 and can reach around 27% for insulin, whilst hGH itself does not exhibit a true steady-state profile. IGF-1, IGFBP-3 and P-III-NP also exhibit a high degree of individual variation and somewhat different distributions between men and women. Consequently, population-based reference ranges are of limited value, and athlete-specific data must be used to interpret changes over time (28,29).

Over a ten-year period as part of a national programme, the haematology module alone failed to detect any antidoping rule violations. This shows that its sensitivity may be low when dopers use microdoses or carefully timed transfusions that keep values within the normal range. Tests are often predictable, with most samples being taken on weekdays at specific times. Furthermore, if in-competition testing is infrequent or predictable, this makes it easier for athletes to adapt their doping practices. Implementing an ABP programme is costly and complex, requiring intensive sampling in high-risk endurance sports and specialist expertise, whilst many other sports and recreational levels remain less closely monitored. More generally, short detection windows, imperfect test

sensitivity and limited testing frequency mean that the overall probability of detecting doping remains relatively low, unless new methods extend detection windows and testing becomes less predictable (30,31).

4.3.4 Omics

4.3.4.1 Metabolomics

Metabolomics is a comprehensive analytical approach that maps the full range of small-molecule metabolites present in a biological system. It thus enables the detection of subtle metabolic changes, whether endogenous or exogenous in origin, that reflect an organism's physiological state or its exposure to drugs or other interventions (32).

One key benefit is the ability to identify persistent metabolic alterations. Following a course of EPO, testosterone or corticosteroids, lasting changes are observed in pathways such as fatty acid oxidation, amino acid metabolism and steroid metabolism. This is the case even though the doping substance itself is no longer detectable. These signatures are often multivariate and can extend the detection window compared with direct methods. They capture the functional trace left on the metabolism (33).

Metabolomics is also used to characterise an athlete's chemical environment (xenobiotics from supplements, medicines and diet). Serum profiles from elite athletes across different disciplines reveal distinct patterns of compounds such as caffeine, polyphenols and drug metabolites, reflecting sport-specific patterns of supplement and performance-enhancing substance use. This information can help distinguish doping-related abnormalities from simple differences in diet or supplementation (33).

4.3.4.2 Proteomics

Proteomics is the large-scale study of proteins within a biological system. It uses liquid chromatography coupled with mass spectrometry to separate, detect and characterise intact proteins or their peptides. The aim is to quantify changes in

protein abundance or structure that reflect exposure to a drug, toxicity or other physiological disturbances (34).

Proteomics shows that growth hormone (GH) administration significantly alters the serum protein profile. Dose- and time-dependent changes are observed not only in IGF-1 and IGFBP but also in chemokines, protease inhibitors and extracellular matrix proteins. These changes impact processes related to cell adhesion, glucose metabolism, and immunology. A sustained protein signature of GH doping is provided by several of these alterations, which last for several weeks after therapy has ended (35).

More targeted approaches confirm that GH leaves a specific imprint in plasma. A small group of proteins, including notably IGFBP-3, IGFBP-ALS, alpha-HS-glycoprotein, afamin and vitamin D-binding protein, show reproducible changes following several weeks of GH use. Taken together, these markers describe a 'GH-exposed' state that may complement current tests and potentially extend the detection window (36).

Finally, longitudinal monitoring via blood microsampling shows that many protein markers remain highly stable in the same athlete over time. Proteins associated with blood doping, such as SLC4A1, CA1, FTH1 or the transferrin receptor, can thus be monitored over weeks with low intra-individual variability. This opens the possibility of a proteomic module within the biological passport, where individual deviations in protein profiles induced by EPO, GH or transfusions would be monitored against a personal baseline (36).

4.3.4.3 Transcriptomics

Transcriptomics is the study of gene expression. It measures the messenger ribonucleic acid (RNA) present in a sample, such as whole blood or dried blood spots. It enables the identification of which genes are activated or suppressed in each situation (such as following an injection of recombinant human erythropoietin (rHuEPO) or a blood transfusion) and the detection of transcripts whose variations in level serve as specific markers of blood doping (37).

Transcriptomic studies carried out as part of the fight against doping have shown for the first time that certain genes respond very consistently to erythropoietin (EPO). In whole blood and dried blood samples, several transcripts undergo changes following the administration of both high and low doses of rHuEPO, and these changes persist beyond the short analytical window of the drug itself. Certain genes, such as ALAS2, remain detectable, suggesting that their altered expression reflects genuine stimulation of erythropoiesis rather than merely the presence of the molecule (38).

Other studies have focused on autologous blood transfusion and confirm that transfusion also leaves a transcriptomic signature. Genes expressed in reticulocytes, notably ALAS2, CA1 and SLC4A1, show a marked decrease in expression following reinfusion. This decline is more pronounced than the simple reduction in the percentage of reticulocytes. This indicates that the suppression of erythropoiesis following transfusion is detectable earlier and in greater detail at the RNA level (39).

Finally, these changes in expression can be reliably measured under conditions like those encountered during routine antidoping tests. The quantity and quality of RNA remain sufficient in blood samples stored without a specific stabiliser, paving the way for transcriptomic analysis of samples already in storage. Overall, these studies show that blood doping reproducibly alters the expression of groups of genes (37,40).

4.3.4.4 Omics and ABP

Omics-based approaches are not a separate system. They are designed to be integrated directly into the Athlete Biological Passport as new modules, on a par with the haematological and steroid modules. Transcriptomic, proteomic and, above all, metabolomic profiles can be monitored over time for each athlete, with individual reference ranges, exactly as is already done for haemoglobin or urinary steroids (27).

Several studies have already proposed metabolite panels for autotransfusion and the use of GH and testosterone. They suggest using these within a passport-style longitudinal monitoring framework. The aim is therefore not merely to add a few isolated biomarkers, but to build an ‘omics’ module for the passport that enriches existing profiles and strengthens the system’s ability to detect signs of doping despite confounding factors. Provided that clear rules are established for managing this new data (confidentiality, retention periods, medical information to be communicated to the athlete), omics-based approaches could represent the next stage in the evolution of the Athlete Biological Passport, remaining at the heart of the system rather than on the periphery (27,41).

Interpreting antidoping test results can be a particularly complex challenge, given the possibility of sample contamination, communication failures or delays, the vulnerability of the athletes involved, and legal issues. The widely publicised case of athlete Kamila Valieva is an example that illustrates how toxicokinetic, analytical, and regulatory factors can converge in antidoping monitoring (42).

4.4 Case Study: The Kamila Valieva Case and the Complexity of Antidoping Interpretation

The case of Kamila Valieva highlights the ethical, scientific, and legal complexities of modern-day antidoping testing. Valieva, a 15-year-old Russian figure skater, underwent a doping test during the Russian National Figure Skating Championships on December 25, 2021, when a urine sample was collected by the Russian Antidoping Agency. The sample was then sent to Stockholm for analysis at a WADA-accredited laboratory. The analysis revealed the presence of a prohibited metabolic modulator, trimetazidine (43). This result was not reported until February 2022, during the Beijing Winter Olympics and after the athlete had competed in the team event (44,45).

Trimetazidine is a drug used to treat ischemic heart disease; it increases the efficiency of glucose oxidation in the myocardium, even in the presence of low oxygen levels. It is this effect that the molecule can have on energy metabolism

that justifies its prohibition in sports, highlighting that antidoping control extends beyond classic stimulants or anabolic agents.

This case also demonstrates that the detection of a prohibited substance is only one part of the complex antidoping equation. In fact, while the laboratory result was unfavourable, it was insufficient on its own, as it was necessary to consider possible exposure to the compound, the chronology of events, and the athlete's explanation.

The athlete's defence claimed that this could have been a case of contamination, given that the drug was part of her grandfather's regular therapy. Nevertheless, the Court of Arbitration for Sport (CAS) found that the defence's arguments were insufficient and that it lacked evidence to prove that the situation was unintentional. Another important aspect was the fact that the athlete was a minor, classified as a "Protected Person" under antidoping rules. Nevertheless, the athlete was sentenced to a four-year period of ineligibility, and all her results from competitions held during that period were revoked (46). This outcome highlights the conflict between athlete protection and the principle of strict liability, according to which athletes are held responsible for prohibited substances detected in their samples. The long delay between the collection of the sample and the reporting of the result complicated the case, impacting the entire team, the awarding of medals, and public distrust of antidoping testing. This case highlights the need for timely laboratory analysis, efficient management of results, and clear coordination among national antidoping organizations, international federations, WADA, the International Olympic Committee (IOC), and the CAS. It should be remembered that the pharmacokinetic profile of trimetazidine depends on its binding to plasma proteins, among other biological factors.

This plasma protein binding affects the distribution of the molecule and may influence the results obtained, especially when plasma concentrations are low (47).

In summary, this case highlights the complexity of an antidoping system, which requires a high-sensitivity analytical system but also requires rigorous

pharmacokinetic interpretation, transparent proof rules, the protection of vulnerable athletes, and consistent legislation.

V. Evolution of antidoping regulations

5.1 International and European legal framework

Doping has brought attention to the necessity of a strong legal framework that goes beyond simple moral or medical conclusions to guarantee fair competition. With the establishment of WADA and the World Antidoping Code in 1999, the concept of a set of universal guidelines that are meant to be applicable globally while yet being compliant with national laws has gained traction (48).

Building on this basis, the Code progressively established a comprehensive antidoping regime. It defines what constitutes doping, harmonises sanctioning mechanisms and applies to a wide range of actors, including international federations, the IOC, national antidoping organisations and accredited laboratories. It operates together with International Standards on testing and investigations, laboratories, therapeutic use exemptions, the Prohibited List, data protection, compliance, education and results management. These instruments are designed to promote uniform procedures and a comparable level of control across sports and jurisdictions. The United Nations Educational, Scientific and Cultural Organization (UNESCO) International Convention against Doping in Sport further reinforces this framework by allowing States to formally commit to the principles of the Code, giving the antidoping system a stronger basis in public international law (49).

However, there are two significant barriers to this goal in Europe: the European Union's (EU) lack of universal sport-related competence and the continued existence of differing laws from country to country, each with its own different rules and sanctions regarding doping. To overcome this limitation, a redefinition of the international and European legal framework is being considered (48).

5.2 Regulatory responses to NPS and emerging substances

New psychoactive substances (NPS) constitute a dynamic and diverse field that challenges traditional toxicokinetic paradigms, as explained in Part IV. They have already transformed the fight against doping into a veritable arms race in terms of analysis. Their structural variety, broad spectrum of action, and deliberately "innovative" approach make it difficult to predict their *in vivo* behaviour and integrate them into current testing procedures. Legislators and antidoping organizations are forced to modify not only the definition, but also the classification and sanctions applicable to these compounds due to the significant regulatory ambiguity their characteristics generate.

A few fundamental shortcomings in the existing legal systems are highlighted by the NPS legislation. Since NPS are intentionally designed to arise more quickly than control systems can be updated, their increasing chemical and pharmacological variety consistently outpaces the traditional framework of substance-by-substance classification. However, the underlying concepts of legislation are occasionally unclear or contested, creating legal loopholes that distributors and manufacturers might exploit. Chemicals that are classified and controlled as NPS in one jurisdiction may remain unregulated in another, promoting "regulatory shopping" and weakening consistent control efforts due to significant cross-border discrepancies. When combined, these elements result in regulatory responses to NPS that are often out of step with real consumption and damage patterns. This underscores the need for more proactive, flexible and globally coordinated strategies (50).

Due to these challenges, an institution at the EU level has been established to monitor and respond to NPS across Europe. An early warning system (EWS) run by the European Union Drugs Agency (EUDA) currently tracks hundreds of chemicals and transmits data from law enforcement, health services, and national focal points. Building on this network, Council Decision 2005/387/JHA established an EU-wide mechanism for the exchange of information, risk assessment and, where necessary, the subjection of certain NPS to control measures and criminal sanctions. Over time, it became apparent that this

framework was no longer rapid or flexible enough to cope with the rapid growth of the NPS market. This led the Commission and the Council to propose reforms aimed at shortening risk assessment times and enabling faster and better-coordinated decisions at EU level. This development illustrates how the EU has sought to move from a slow, substance-by-substance approach to a more responsive system that links early warning, scientific assessment and harmonised legal action across Member States (51).

Whilst the European Union has tended to refine mechanisms for information-sharing and risk assessment in relation to specific NPS.

Some national systems have experimented with far more radical approaches. The United Kingdom (UK) Psychoactive Substances Act (PSA) is a striking example of the shift from substance-by-substance control to a blanket ban model. This Act introduced a blanket ban on all psychoactive substances, with only a limited list of exceptions such as alcohol, tobacco and authorised medicines. A thorough legal and scientific analysis has shown that the apparent simplicity of the total ban is misleading. The key concepts on which the law is based prove to be conceptually complex and very difficult to implement in the courts. In practice, establishing a regime that is truly proportionate to the risks would require extensive and costly testing to characterise the risks associated with each compound, which would undermine the efficiency gains promised by this model. Overall, the UK experience suggests that total ban models, whilst rhetorically appealing, have inherent conceptual and practical weaknesses that limit their suitability as a long-term response to NPS (52).

Overall, current regulatory responses to NPS range from the gradual refinement of existing classification and information-sharing tools, as seen within the EU, to more radical approaches such as the blanket ban on psychoactive substances in the UK. Each of these models provides partial solutions, but also reveals significant shortcomings, whether in terms of speed, legal certainty, proportionality or enforceability.

Table 3 summarises the main regulatory levers that have been proposed to manage the growing spread of NPS and highlights how they can be combined rather than used in isolation (50).

Table 3: Summary of the main measures that can be proposed to control the growing spread of NPSs worldwide. Created by the author based on Santos et al. (50).

Future perspectives to control the growing spread of NPS
• Implement policies and cooperation systems among different countries
• Consider a multidisciplinary effort that takes into account the country's geopolitical and social matters
• Limit the industry's influence either in the political or regulation, or in medical prescription (relevant for opioids)
• Correct recognition of the risks and benefits for drug-approval purposes, and implementing methods for responsible prescription (relevant for opioids)
• Reinforcement of the substance abuse disorders' system and the stimulation of new responses for addiction
• Raise awareness and train healthcare professionals to address the abuse of NPS
• Bring public awareness to this problem (better information likely renders more conscious choice)
• Develop more sensitive laboratory tests to detect NPS

5.3 From reactive regulation to predictive governance

5.3.1 Early warning systems and anticipatory surveillance

Traditional regulatory approaches often come into play too late. They only emerge once a substance has been clearly identified, has already caused cases of poisoning, and is widely available on the market. This delay is problematic when new molecules emerge rapidly and give rise to serious concerns before any regulatory controls are put in place (53).

To reduce this gap, several regions have set up early warning systems. The EWS described in section 5.2 is a prime example, designed to quickly detect alert signals regarding emerging substances and provide useful information to decision-makers for them to use before a formal classification is even carried out. The challenge lies in predicting upcoming threats (54).

Early detection involves several data channels, such as the outcomes of analyses of confiscated substances, warnings from hospitals or forensic services, screening of online markets and social media, and reviews of the scientific literature. Specific clinical protocols, such as the Western Australian Illicit Substance Evaluation (WISE) study in emergency departments, show that the routine collection of biological samples from patients exhibiting atypical symptoms can serve as a monitoring tool to identify substances that are emerging (55).

Globally, the UNODC's EWS centralizes and shares information provided by member states and regional organizations. These early warning mechanisms illustrate the shift from purely reactive regulation to a more proactive form of governance, based on international cooperation and the joint analysis of analytical and toxicological indicators (56).

5.3.2 Class-based and effect-based prohibition

5.3.2.1 Class-based approaches

The traditional molecule-by-molecule approach is becoming increasingly outdated: new substances often appear in series of analogues derived from the same 'scaffold'. Several dozen NPS are detected each year. This constant stream of new substances makes it impossible to update lists in a timely and comprehensive manner, as demonstrated by recent reviews on NPS and national responses across Europe. This has led many countries to adopt class-based bans rather than targeting only a few high-profile compounds. This strategy clearly reduces legal loopholes and limits chemical substitution tactics to a minimum. However, it is not without its costs: overly broad definitions may

encompass molecules with potential therapeutic use or compounds with low activity, whilst overly narrow definitions leave illicit chemists the opportunity to circumvent the law through simple side-chain modifications. In the field of antidoping, the class-based approach of the WADA Prohibited List (anabolic agents, hormonal modulators, etc.) already reflects this tension between maximum coverage and legal certainty (57,58).

5.3.2.2 Effect-based approaches

So-called ‘effect-based’ laws seek to move away from a purely structural taxonomy by focusing on the mode of action or pharmacological effect, rather than the chemical formula. Under certain national laws, any compound with a specific psychoactive effect is considered a controlled substance, regardless of its specific structure. In the field of antidoping, this approach could make it possible to target ‘all substances that increase erythropoiesis’, ‘all agonists of a specific hormone receptor’ or ‘all agents that stimulate alertness’, bringing regulation closer to the concept of performance enhancement and the criteria of the World Antidoping Code. However, implementing this strategy remains complex: regarding emerging substances, the effects on performance and the mechanism of action are generally poorly documented, making any ban based on these factors precarious. Furthermore, overly broad terms could include common medicines or products posing minimal risk, raising issues of proportionality and clarity for healthcare professionals (59).

5.3.2.3 Critical discussion

Bans based on category and effect are precisely attempts to resolve this dilemma, but recent literature shows that they shift the problems as much as they resolve them. Empirical studies of legislation conclude that category-based bans have made the work of law enforcement agencies easier but have had a limited impact on the use of NPS and, in some cases, have been accompanied by an

increase in public health harms. The message is clear: broadening the scope of the ban does not guarantee a reduction in risks (57).

The early regulation of substances legally implies accepting a higher degree of uncertainty. In class-based legislation, this uncertainty lies at the boundary between what is and is not covered by the structural definition; slight modifications to the side chain may be enough to circumvent the text, whilst certain molecules that are not very active or that have therapeutic value are included without there being any proven risk. In effects-based approaches, the uncertainty lies in the evidence: for emerging substances, the mechanism of action, the risk profile and the potential effect on performance are rarely documented at the time their inclusion is being discussed (60).

Regulation introduced too early, without solid evidence or explicitly defined criteria, creates a risk of perceptions of unfairness and subsequent legal disputes. Legal action taken without sufficient evidence, or lacking clearly defined criteria, can undermine confidence in the system by contributing to a perception of arbitrariness and exposing management bodies to legal challenges. Consequently, preventive approaches must remain proportionate, selective, and evidence based. The expansion of prohibited categories or the adoption of effects-based definitions should only occur when supported by reliable empirical data and accompanied by transparent reassessment methods. Otherwise, antidoping policy risks evolving into a governance model centered on managing uncertainty, to the detriment of decision-making grounded in scientific evidence (56,59,60).

5.3.3 Predictive tools for regulatory decision-making

Currently, the ABP is the best example of a predictive tool already in place for antidoping rule enforcement. Rather than tracking a specific substance, the ABP monitors haematological and steroid indicators over time. It uses an adaptive Bayesian model to define individual reference thresholds and assess the probability that a profile is linked to doping. These profiles are used to guide

decisions regarding targeted testing and investigations, even without explicit identification of the doping agent. The inclusion of new (endocrine) modules and the incorporation of new biomarkers aim to enhance the system's sensitivity, although this results in more complex profiles to analyse. The ABP thus illustrates not only how longitudinal data and probabilistic models already support proactive regulatory decisions today (25,27).

From an evolutionary perspective, various methods offer the possibility of expanding this type of predictive use of data. Physiology-based pharmacokinetic modelling (PBPK) is highlighted as a potentially useful tool in the field of antidoping: it allows for the establishment of detection standards, the examination of microdosing models, and the assessment of the risk of false negatives based on various physical parameters. Simultaneously, these methods facilitate the identification of persistent biological doping agents, going beyond the mere detection of the drug in biological samples. Recent revisions have demonstrated that the application of artificial intelligence (AI) models to ABP data and performance passports can improve the identification of irregular performances and promote a better allocation of testing resources (61,62).

However, the use of these tools as a basis for future regulatory decisions raises key questions. PBPK, AI and metabolomics models designed to combat doping are largely still at an exploratory stage, a fact which highlights the constraints relating to the quality of the data used. In a context of predictive governance, relying too hastily on obscure or insufficiently validated models can transform the issue of regulatory delay into one of legitimacy. In fact, rulings based on solid analytical evidence tend to enjoy greater acceptance among athletes and judicial authorities than decisions founded on scenarios of ambiguity or probabilistic risk indicators. For this reason, it is necessary to ensure review mechanisms that balance preventive objectives with requirements for scientific rigor and procedural guarantees. This issue might become increasingly important in a context where new forms of sports organization and alternative competitions challenge conventional antidoping models, requiring regulatory systems capable of transparently justifying their scientific, ethical, and legal basis.

VI. Analytical detection strategies

6.1 Conventional methods and their limitations

One of the most important steps in antidoping control is sample collection. At this stage, the sample is safeguarded and the entire process begins to be traceable. After being informed, the athlete is requested to submit the sample under observation. Samples are divided into two bottles, A and B, and sealed on-site. Its specific gravity is checked as well, to make sure the sample is valid for analysis. After completing all the forms, the sample is sent to the laboratory, where it enters the workflow shown in Figure 7: sample A undergoes an initial screening, followed by confirmatory tests on sample A and, if required, on sample B before any final report is issued in the event of a suspicious or positive result (63).

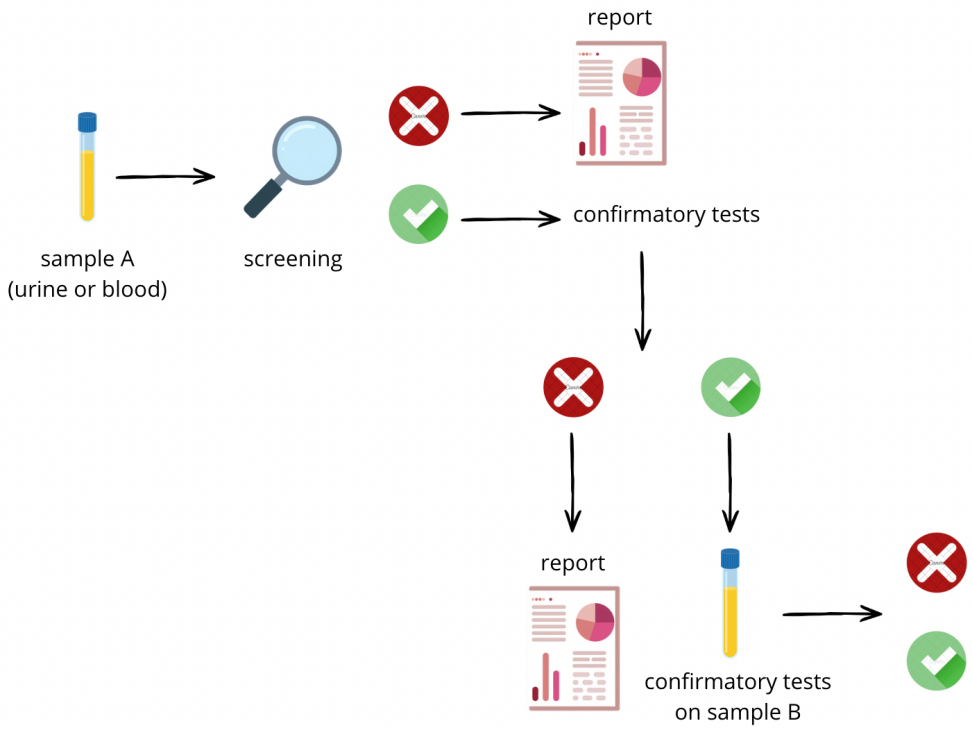

Figure 7: Simplified workflow of the antidoping testing process, from sample collection to screening, confirmation and reporting. Created by the author based on WADA guidance (64).

Since the introduction of antidoping regulations, mass spectrometry has become central to sports drug testing. GC–MS and LC–MS/MS are widely used because they are both sensitive and specific. They can detect prohibited substances even at very low levels (65).

GC-MS is one of the most established techniques in mass spectrometry. It is mainly used for compounds that can be vaporized or that can be made suitable for this by derivatization. In antidoping, it is especially useful for the detection of steroids and other small molecules. It offers good separation and solid identification, which makes it reliable in complex biological samples (66).

LC-MS is better suited to compounds that are not volatile, more polar, or unstable at high temperatures. For this reason, it is often more practical in antidoping analysis, where many prohibited substances do not fit well with gas chromatography. LC-MS, and especially LC-MS/MS, has become a very important tool because it can detect a wide range of substances with high sensitivity and flexibility (66).

As shown in Table 4, the two methods cover most of the categories included in the WADA Prohibited List.

Table 4: Main analytical approaches used to address selected categories of the WADA Prohibited List. Created by the author based on Thevis et al. (67).

LC-MS	GC-MS	Complementary (NGS; WGR; HPLC-MS; CRISPR-Cas based systems)
• S0. Non-approved substances • S1. Anabolic Agents • S2. Peptide hormones, growth factors, related substances, and mimetics • S3. Beta-2 agonists • S4. Hormone and metabolic modulators • S5. Diuretics and masking agents • S7. Narcotics • S8. Cannabinoids • S9. Glucocorticoids • M2. Chemical and physical manipulation • M3. Gene doping • P1. Beta-blockers	• S0. Non-approved substances • S1. Anabolic Agents • S6. Stimulants • S7. Narcotics	• M3. Gene doping

NOTE: This table provides a simplified overview of analytical approaches and does not replace WADA technical documents, International Standards or laboratory-specific validated methods.
Liquid Chromatography-Mass Spectrometry (LC-MS); Gas Chromatography–Mass Spectrometry (GC-MS); Next-Generation Sequencing (NGS); Whole-Genome Resequencing (WGR); High-Performance Liquid Chromatography-Mass Spectrometry (HPLC-MS); Cluster Regularly Interspaced Short Palindromic Repeats (CRISPR)

However, these methods are not without limits. The growing number of new and structurally complex doping agents makes detection more difficult and challenges their reliability (65).

6.2 Technological advances

In recent years, new detection techniques have emerged to address the growing complexity of doping practices. These technological advances aim to improve the sensitivity, selectivity and reliability of antidoping testing. The following sections will briefly outline three of the most significant advances in this field.

6.2.1 Biological profiling

As direct detection methods are increasingly challenged by the emergence of new substances, short detection windows and analytical limits, indirect approaches have taken on growing importance in the fight against doping. Among these, the Athlete Biological Passport is particularly important because it shifts the focus from isolated analytical results to longitudinal biological profiling, as discussed in section 4.3.3. (26).

The athlete's biological passport continues to evolve as new biomarkers are investigated. Omics-based approaches, particularly transcriptomics, proteomics and metabolomics, appear promising as they could help identify indirect signs of doping even when direct detection is difficult. Similarly, changes in red blood cell characteristics following a blood transfusion could provide additional clues, particularly in cases that are not easily detectable by conventional methods. More recently, endocrine biomarkers and artificial intelligence have also emerged as useful tools for improving longitudinal monitoring and better identifying abnormal patterns (68).

6.2.2 Genetic biomarkers

Beyond longitudinal profiling, a growing number of studies have focused on genetic biomarkers as direct indicators of genetic doping interventions. In this context, the biomarker is no longer a physiological response, but the presence of exogenous nucleic acid sequences. Such as deoxyribonucleic acid (DNA) derived from vectors or transgenic cassettes, which are not found in the athlete's

native genome. Targeting these foreign sequences offers a more specific means of detecting cases of genetic doping than monitoring indirect molecular or haematological alterations (69).

Recent advances in molecular biology have paved the way for genetic biomarkers that directly target exogenous nucleic acid sequences. In the context of genetic doping, these biomarkers correspond to the presence of transgenic constructs or vector-derived DNA in the athlete's bloodstream, which can be distinguished from endogenous genomic sequences. A set of real-time Polymerase Chain Reaction (PCR) assays has been developed using hydrolysis probes directed against four anabolic genes: follistatin, growth hormone 1, growth hormone-releasing hormone, and insulin-like growth factor 1 (70).

They demonstrated that they could detect as few as a handful of transgene copies amidst a significant excess of endogenous DNA, whilst maintaining high specificity. Accuracy and interlaboratory comparability were ensured using a synthetic reference compound, in accordance with International Organization for Standardization (ISO) 35 standards, which was validated in a plasma matrix. The use of genetic biomarkers can complement the ABP, making it possible to identify potential genetic interventions, such as the introduction, modification, or regulation of genes, intended to enhance an athlete's physical capabilities and improve sporting performance (70).

An example of a complementary strategy for detecting genetic doping is qPCR (quantitative Polymerase Chain Reaction) testing, which allows for the detection and quantification of specific DNA sequences and is used in the routine screening of certain anabolic transgenes. However, this technique can only detect what has already been identified as a target; in other words, it is a targeted, rapid, and effective method for detecting already known doping genes. On the other hand, exploratory genomics-based approaches involve more comprehensive techniques, such as next-generation sequencing (NGS). Rather than searching for just one specific sequence, these techniques analyse large portions of the athlete's genetic material, making it possible to identify modified genetic constructs that evade conventional tests and unexpected targets. The decision

to integrate these complementary tools into conventional testing requires a balance between test sensitivity, response time, and associated cost (69).

6.2.3 Nuclear magnetic resonance

It is important to take into account cutting-edge spectroscopic technologies like nuclear magnetic resonance (NMR) after examining molecular profiling methods and genetic markers. NMR allows for a direct and unmodified view of the structure of doping agents and their metabolites. It is therefore a useful addition to more specialised analytical systems. It provides a non-destructive, rich structural image of dopants (71).

This is not at all what mass spectrometry provides. When exposed to a strong magnetic field, certain nuclei, most often ^{1}H and ^{13}C, are excited and relax at unique resonance frequencies that reflect their local chemical environment. This results in spectra that provide details on atomic connectivity, functional groups, and even the three-dimensional structure of complex molecules. Examining whole molecules rather than fragments is one of NMR's main advantages from an antidoping perspective. This allows for the unambiguous structural elucidation of unknown compounds. It is particularly useful for characterising recently seized synthetic steroids, distinguishing closely related isomers, and confirming the structure and purity of reference materials (71).

In some cases, subtle differences in NMR signatures can even be used to distinguish endogenous steroids from exogenous steroids, which is essential when the prohibited substance has a physiological analogue. The main drawback of NMR in routine antidoping testing is its limited sensitivity compared to mass spectrometry. The latter generally allows substances to be detected at much lower concentrations. Requirements regarding sample volume, the cost of the instruments and acquisition time also limit its use for high-throughput screening of urine or blood. To overcome these issues, current strategies focus on combining NMR with chromatographic pre-concentration, optimising pulse sequences and using NMR primarily as a complementary tool: for structural

confirmation, the elucidation of new compounds and the certification of standards (72).

6.3 Emerging sample preparation and ionization strategies

6.3.1 Molecularly imprinted polymers (MIPs)

Molecularly imprinted polymers provide a highly selective recognition platform by creating binding cavities. These cavities correspond to the size, shape and functional groups of a target molecule, thereby faithfully mimicking ligand-receptor interactions in biological systems. This tailor-made selectivity is particularly valuable in complex biological matrices where conventional sorbents struggle to distinguish trace dopants from structurally similar interferents and endogenous components. Used as solid-phase extraction sorbents, MIPs enable high enrichment of analytes present at very low concentrations, thereby overcoming matrix effects that would otherwise lead to missed or erroneous detections of illicit drugs and performance-enhancing agents. In this context, MIPs have already been applied to the analysis of traces of β-agonists such as clenbuterol and its structural analogue, ractopamine, as well as ketamine and its main metabolite in urine and hair, demonstrating simultaneous pre-concentration of parent compounds, analogues and metabolites that can serve as indirect biomarkers of exposure. This approach is particularly useful for steroid analysis, where a single compound may disappear rapidly, whilst a set of long-lived metabolites and steroidomic disturbances bear the hallmark of doping (73).

More advanced architectures extend this principle to the detection of biomarkers rather than simply extraction and purification. Nanoscale MIPs doped with gold nanoparticles have been developed as optical receptors for Surface-Enhanced Raman Scattering (SERS) detection of Sudan IV, enabling highly selective rebinding relative to closely related Sudan derivatives and a significant enhancement of the Raman signal. In these nanoMIP systems, the imprinting process still governs molecular recognition, but the integration of plasmonic

nanoparticles converts binding events into amplified optical readouts, with reported detection limits as low as the femtomolar range in model systems. Together, these examples illustrate how MIPs can both enrich target analytes from complex matrices and act as selective receptors for steroid-type xenobiotics or their downstream biomarkers, making them a promising tool for future high-sensitivity screening in antidoping analysis (73,74).

MIP extraction proves useful when conventional GC-MS/LC-MS techniques struggle to detect trace analytes in highly complex matrices. By selectively enriching steroids and associated biomarkers prior to mass spectrometry analysis, these sorbents increase sensitivity and effectively broaden the detection window for low-dose or structurally diverse compounds.

6.3.2 Novel extraction solvents (DES/ionic liquids)

Innovative extraction solvents, such as ionic liquids and deep eutectic solvents (DES), offer a flexible alternative to conventional organic solvents. A DES is a eutectic mixture of a hydrogen bond acceptor and a hydrogen bond donor, often based on quaternary ammonium salts and acids or sugars, resulting in a low-volatility, low-toxicity liquid with adjustable polarity. Many “natural” DESs have demonstrated good yields for the extraction of bioactive compounds such as drugs, as well as improved stability of analytes thanks to a dense network of hydrogen bonds (75,76).

These properties can be exploited to extract dopants and their metabolites from complex biological matrices, where limited solubility and co-extractables pose problems with conventional solvents. By adjusting the Hydrogen Bond Acceptor (HBA)/ Hydrogen Bond Donor (HBD) pair and water content, ‘tailored’ DESs can be designed for specific families of molecules, thereby improving extraction selectivity and pre-concentration prior to LC-MS/MS (76,77).

However, high viscosity, sometimes limited compatibility with MS sources and incomplete characterisation of matrix effects still hinder their routine adoption,

even though they represent a promising avenue for overcoming certain limitations in recovery and durability associated with the GC-MS/LC-MS approaches presented in section 6.1 (75–77).

6.3.3 MALDI-MS and ambient ionization techniques

Matrix-Assisted Laser Desorption/Ionization–Mass Spectrometry (MALDI-MS) enables extremely rapid analysis, as the analytes are co-crystallised with a matrix and analysed directly from the target plate, without the need for chromatographic separation. This simple deposition protocol is compatible with high-density targets and makes MALDI particularly suitable for high-throughput screening of drugs in biological matrices, where hundreds of plasma or tissue samples can be processed in a single run. In addition to simple profiling, MALDI imaging enables direct two-dimensional mapping of drugs and metabolites in tissue sections. This provides label-free information on distribution that is not accessible with conventional LC-MS (78,79).

Recent advances show that, with appropriate methodological design, MALDI-MS can achieve quantitative performance levels that meet regulatory requirements. Optimisation of matrix selection and crystallisation, the use of ion mobility and MS/MS for post-acquisition filtering, as well as data processing like that used in LC, have made it possible to reduce the lower limits of quantification by up to two orders of magnitude. Parallel work on new matrices, notably custom-made small-molecule and supramolecular systems optimised for low-mass compounds. These aim to suppress background noise in the low m/z range and improve ionisation efficiency for small drugs and their metabolites, including those relevant to antidoping control. However, MALDI-MS for the analysis of small molecules still faces significant limitations. These include, for example, a strong dependence on the matrix/analyte combination, variable crystal homogeneity, ion suppression in complex biofluids, and the greater difficulty of achieving comprehensive validation across various biological matrices. It is therefore currently better positioned as a complementary tool for rapid screening and imaging rather than as a stand-alone confirmatory technique in antidoping laboratories (78–80).

MALDI-MS and ambient ionisation techniques enable very rapid on-plate assays, with increasingly robust quantitative performance. They therefore help to mitigate the throughput and method development challenges associated with the LC-MS/GC-MS approaches described in section 6.1, particularly when screening large panels of structurally diverse drugs and metabolites (79).

VII. Conclusion

This dissertation aimed to analyse the role of toxicokinetics in antidoping control, particularly regarding the detection, interpretation and regulation of performance-enhancing substances and emerging psychoactive compounds.

The evolution of doping control shows that antidoping science can no longer rely exclusively on the direct detection of known substances. The increasing diversity of performance-enhancing practices, the emergence of new psychoactive substances, the use of experimental compounds and the possibility of microdosing strategies have created a more complex analytical and regulatory landscape. In this context, toxicokinetics provides a central framework for understanding how prohibited substances are absorbed, distributed, metabolised and excreted, and how these processes influence both their biological effects and their detectability in blood, urine or other biological matrices.

This review emphasises that rather than relying just on one technical solution, the future of antidoping control will depend on the integration of alternative techniques. The sensitivity, specificity and forensic robustness of traditional analytical techniques like GC-MS and LC-MS/MS make them indispensable. However, when laboratories deal with chemicals that are changing quickly, narrow detection windows or compounds for which metabolic data and reference standards are few, their limits become apparent. High-resolution mass spectrometry, nuclear magnetic resonance, molecularly imprinted polymers, deep eutectic solvents, MALDI-MS and ambient ionisation techniques are examples of emerging strategies that could enhance sample preparation, screening capability and structural elucidation while also expanding analytical coverage.

Simultaneously, longitudinal and indirect techniques are becoming increasingly significant. A significant change from substance-centered detection to athlete-centered monitoring is represented by the ABP. It allows for the detection of unusual patterns that may indicate doping even after the illegal substance itself is no longer detectable by tracking biological variables over time. By capturing

the long-lasting biological impacts of doping, the possible incorporation of omics-based indicators, such as metabolomic, proteomic and transcriptomic signatures, might improve this model even further. However, these methods also bring up significant issues with biological variability, data interpretation, privacy, validation and the differentiation between disease, manipulation and physiological adaptability.

A more proactive antidoping system may also benefit from predictive techniques like machine learning and PBPK modelling. PBPK models may be used to predict detection windows under various physiological and dosage conditions, investigate inter-individual variability and simulate concentration-time profiles. Machine learning may help with sample triage, enhance focused testing techniques and facilitate the study of intricate analytical and biological information. These techniques must be used carefully, though. Predictive evidence in antidoping should supplement sound analytical and legal interpretation, not take its place.

The similar conflict between expectation and certainty may be seen in the regulatory reaction to NPS and other new drugs. While class-based or effect-based methods may increase coverage but create legal and scientific uncertainty, substance-by-substance ban is sometimes too sluggish to handle quickly changing markets. Thus, early warning systems, global collaboration, and adaptable regulatory frameworks are crucial. However, proactive governance must continue to be appropriate and supported by science, especially since antidoping rulings may have a significant impact on athletes' reputations and careers.

The established limits of antidoping regulation may potentially be challenged by future advancements. The rise of contests that publicly challenge accepted antidoping guidelines highlights the necessity of ongoing consideration of athlete autonomy, fairness, health protection and the function of pharmaceutical enhancement in sports. This problem highlights the significance of transparent, evidence-based, and morally grounded antidoping programs, even if it is outside the primary focus of this research.

In general, an integrated model that incorporates toxicokinetic information, sophisticated analytical chemistry, biological profiling, predictive modelling and adaptive regulation holds the key to doping control in the future. In addition to detecting banned drugs more successfully, such a model should be able to interpret results more precisely, foresee new risks, and safeguard athletes within a framework that is both morally and scientifically sound. In the upcoming years, striking a balance between innovation and legal certainty, sensitivity and specificity, and prevention and athletes' rights will be the main problem.

VIII. References

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