ISSN 0021-2571 (print) · 2384-8553 (online) | Coden: AISSAW 62 (No. 2) | 97-206 (2026)

ANNALI
DELL'ISTITUTO SUPERIORE DI SANITÀ

Volume **62** No. **2**
2026

A SCIENCE JOURNAL *for* PUBLIC HEALTH

ISTITUTO SUPERIORE DI SANITÀ
www.iss.it

Ann Ist Super Sanità 2026 | Vol. 62, No. 2

Annali
dell'Istituto Superiore di Sanità
A SCIENCE JOURNAL FOR PUBLIC HEALTH

Publication
Annali dell'Istituto Superiore di Sanità is published quarterly and in special issues.
Freely available online at www.iss.it/annali - https://annali.iss.it

Annali dell'Istituto Superiore di Sanità is indexed in
- CAB
- CHEMABS
- EMBASE/Excerpta Medica
- FSTA
- MEDLINE
- ProQuest
- SCOPUS
- WEB OF SCIENCE

The Journal Impact Factor is 1.7

Annali Editorial Office
Scientific Communication Service
Istituto Superiore di Sanità
Viale Regina Elena 299, 00161 Rome, Italy
Tel.: +39 06 49902945 Fax: +39 06 49902253
E-mail: annali@iss.it
www.iss.it/annali - https://annali.iss.it

Papers to be presented for publication should be submitted online to https://annali.iss.it

Instructions to Authors are available online at https://annali.iss.it

Publishing support
Il Pensiero Scientifico Editore, Rome
Via San Giovanni Valdarno 8, 00138 Rome, Italy
www.pensiero.it

Subscription information & terms
The journal is freely available online.
For printed copies of the journal contact:
Il Pensiero Scientifico Editore
Tel.: +39 06 86282324 Fax: 06 86282250
E-mail: abbonamenti@pensiero.it

Year 2026
Italy individual print subscription € 57,00 | Italy institutional print subscription € 67,00.
Other countries € 67,00
Each quarterly print issue € 21,00

ISSN 0021-2571 (print), 2384-8553 (online)
Coden: AISSAW 62 (No. 2)

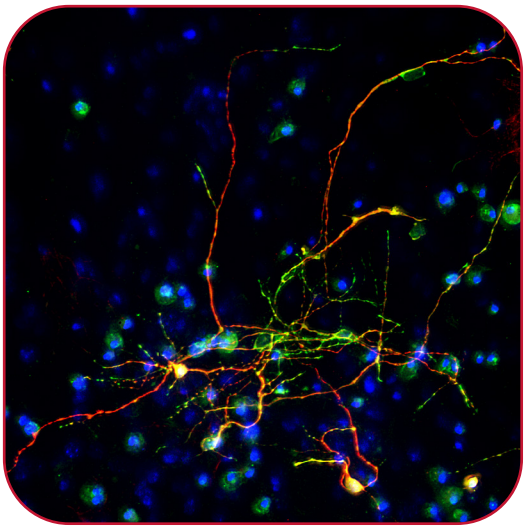
Reg. Stampa - Tribunale di Roma, n. 482 del 29 ottobre 1985 (cartaceo); n. 121 del 16 maggio 2014 (online)

Printed in June 2026 by Lion Brand srl, Rome

Ann Ist Super Sanità 2026 | Vol. 62, No. 2

Annali
dell'Istituto Superiore di Sanità
A SCIENCE JOURNAL FOR PUBLIC HEALTH

The cover image is a double immunofluorescence of "chemically induced neurons" (ciNs), derived trought chemical transdifferentiation of human dermal fibroblasts, stained with a neuronal marker Doublecortin, DCX (green) and Tubulin beta III (red). Nuclei are stained with DAPI (blue). Image is provided by Cristiana Mollinari and Daniela Merlo, Department of Neuroscience, Istituto Superiore di Sanità, Rome, Italy.



ANNALI
dell'Istituto Superiore di Sanità

Vol. 62, No. 2 2026

Contents

Commentary

Original articles and reviews

Ann Ist Super Sanità 2026 | Vol. 62, No. 2: 97-99
DOI: 10.4415/ANN_26_02_01

COMMENTARY
A call for neurovascular monitoring in an era of longer missions and broader spaceflight participation

Andrea Cusumano[1], Benedetto Falsini[2], Francesco Martelli[3] and Marco Lombardo[1]

[1]*Dipartimento di Medicina Sperimentale, Università degli Studi di Roma "Tor Vergata", Rome, Italy*
[2]*Macula & Genoma Foundation USA, New York, USA*
[3]*Dipartimento Malattie Cardiovascolari, Endocrino-Metaboliche e Invecchiamento, Istituto Superiore di Sanità, Rome, Italy*

Abstract
Spaceflight-Associated Neuro-ocular Syndrome (SANS) has emerged as a critical neuro-ophthalmic risk for human space exploration, particularly as mission duration increases and access to space expands. Current spaceflight ocular surveillance and research protocols have prioritized structural imaging and selected neuroimaging/physiological assessments. However, accumulating evidence suggests that SANS is not confined to the posterior pole as a purely structural optic nerve head phenomenon but may also involve vascular and hemodynamic alterations. At the same time, structural changes at the optic nerve head may not fully capture the functional integrity of the afferent visual pathway. We therefore propose to define a more targeted extension of current SANS surveillance protocols incorporating ultra-widefield swept-source optical coherence tomography angiography (UWF-SS-OCTA), visual evoked potentials (VEPs) and pattern electroretinogram (ERG) into standardized pre-flight, in-flight (when feasible), and post-flight assessments. Beyond its relevance to astronaut health, this topic may also be of translational interest to the broader scientific and clinical community.

Key words
- electroretinography
- retina
- space flight
- astronauts
- ophthalmic nerve

Spaceflight-Associated Neuro-ocular Syndrome (SANS) has emerged as a critical neuro-ophthalmic risk for human space exploration, particularly as mission duration increases and access to space expands [1].

SANS refers to a constellation of findings observed during and after long-duration spaceflight, including optic disc edema, posterior globe flattening, choroidal and/or chorioretinal folds, and hyperopic refractive shifts, with potential implications for visual performance and operational safety [1].

As national and commercial programs plan longer missions, greater cumulative exposure, and more diverse participant profiles, the need for sensitive, mechanism-informed ocular monitoring becomes increasingly urgent [2].

With the expansion of access to space, addressing the current lack of sex-specific evidence in SANS will become increasingly important, as will ensuring that future monitoring protocols explicitly incorporate sex-disaggregated analyses where feasible.

Current spaceflight ocular surveillance and research protocols have prioritized structural imaging and selected neuroimaging/physiological assessments [3]. A major step forward is the integrated NASA research framework, including the "Complement of Integrated Protocols for Human Exploration Research" (CIPHER) and its ocular study, "Investigating Structure and Function of the Eye" (iSAFE), which incorporates optical coherence tomography (OCT), standard OCT angiography (OCTA), visual field testing, electroretinography (ERG), ocular biometry, and intraocular pressure measurements (https://www.nasa.gov/reference/cipher/).

Nevertheless, important gaps remain in the vascular and functional neuro-ophthalmic domains, which may be particularly relevant to early detection, individual risk stratification, evaluation of countermeasures, and identification of subclinical sequelae not captured by standard assessments.

A similar principle has emerged in other ophthalmic settings, including drug-related retinal toxicity, where

Address for correspondence: Francesco Martelli, Dipartimento Malattie Cardiovascolari, Endocrino-Metaboliche e Invecchiamento, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy. E-mail: francesco.martelli@iss.it.

combining vascular imaging and electrophysiology has helped reveal persistent abnormalities not fully explained by structural findings alone [4, 5].

Beyond its relevance to astronaut health, this topic may also be of translational interest to the broader scientific and clinical community. Human research in space has repeatedly generated insights relevant to terrestrial medicine, including cardiovascular, neurovestibular, pulmonary, and immune physiology [6, 7]. In the specific case of SANS, spaceflight provides a unique human model of chronic fluid shift, altered venous and cerebrospinal fluid dynamics, and neuro-ocular adaptation under conditions that cannot be fully reproduced on Earth. In this sense, efforts to refine neurovascular monitoring in SANS may also contribute to the study of terrestrial disorders characterized by optic disc edema, disturbed cerebrospinal fluid dynamics, or neuro-ophthalmic involvement [8].

Therefore, our proposal is to define a more targeted extension of current SANS surveillance protocols to better capture neurovascular and functional changes across pre-flight, in-flight, and post-flight assessments. *Table 1* summarizes the main existing surveillance elements, along with the proposed additions, their rationale, and expected benefits.

First, accumulating evidence suggests that SANS is not confined to the posterior pole as a purely structural optic nerve head phenomenon but may also involve vascular and hemodynamic alterations associated with chronic headward fluid shifts. At present, these vascular changes should be regarded as plausible contributors to SANS pathophysiology rather than established primary drivers, and may, in some cases, also represent downstream manifestations of broader fluid-shift, venous outflow, and CSF-related disturbances [1, 3, 8]. Retinal vascular patterning analyses in astronauts have reported post-flight reductions in vascular density, with the most pronounced changes occurring in eyes showing clinical SANS features [9]. These observations support the rationale for vascular biomarkers that complement structural OCT. We therefore propose that the scientific community consider incorporating ultra-widefield swept-source OCTA (UWF-SS-OCTA) into standardized pre-flight, in-flight (when feasible), and post-flight assessments.

At present, the most immediate role of UWF-SS-OCTA would likely be in standardized pre- and post-flight surveillance, while in-flight implementation should be considered a feasible but forward-looking objective. This is conceptually consistent with the progressive adoption of portable ocular imaging in spaceflight, although dedicated validation would still be required to address portability, crew time, acquisition quality in microgravity, equipment handling, protocol standardization, and training requirements. Similar operational considerations would also apply to the broader integration of electrophysiological testing.

Compared with conventional spectral-domain OCTA, UWF-SS-OCTA can better visualize and quantify peripheral retinal perfusion and may provide a more robust investigation of the choriocapillaris and deeper choroidal circulation, tissues that are potentially sensitive to venous congestion, altered translaminar pressure gradients, and autoregulatory stress during microgravity exposure [10, 11]. These measurements could provide quantitative biomarkers for identifying subclinical vascular abnormalities, as well as for assessing the evolution or recovery of vascular changes associated with SANS.

Table 1
Current surveillance elements and proposed neurovascular-functional additions for Spaceflight-Associated Neuro-ocular Syndrome (SANS) monitoring

Spaceflight surveillance protocol element	Rationale	Expected benefit
Current protocol		
Optical coherence tomography (OCT)	Detects the main structural ocular changes associated with SANS (optic disc edema, chorioretinal folds)	Diagnosis and longitudinal structural assessment across the peri-mission timeline
Spectral-domain OCT-angiography (SD-OCTA)	Provides information on retinal microvasculature	Assessment of central retinal vascular changes
Visual field testing	Evaluates the functional visual consequences of neuro-ophthalmic changes	Detection of visual dysfunction relevant to mission performance and follow-up
Electroretinography (ERG)	Assesses retinal cellular function	Functional evaluation of retinal involvement
Ocular biometry	Helps document globe flattening and hyperopic shifts	Additional characterization of ocular structural remodeling
Intraocular pressure measurements	Provides a basic physiological parameter relevant to ocular status	Supportive physiological monitoring
Proposed additions		
Ultra-widefield swept-source OCTA (UWF-SS-OCTA)	Captures and quantifies retinal and choroidal vascular alterations over a wider and deeper field than standard OCTA	Improved microvascular characterization of SANS-related changes and possible subclinical sequelae
Visual evoked potentials (VEPs)	Provide an objective measure of afferent visual pathway function	Earlier identification of subclinical functional abnormalities and detection of dysfunction not fully explained by structural imaging or retinal functional assessment alone

Second, SANS is fundamentally a neuro-ophthalmic condition: structural changes at the optic nerve head may not fully capture the functional integrity of the afferent visual pathway [12]. Analogously, in other retinal and optic nerve conditions, structural recovery does not necessarily imply full functional normalization, underscoring the value of objective electrophysiological endpoints. Visual evoked potentials (VEPs) and pattern ERG provide an objective, quantitative measure of signal transmission from the retina/optic nerve to the visual cortex and may detect very early functional abnormalities in papilledema due to intracranial hypertension [13]. VEP acquisition has already been demonstrated in astronauts during weightlessness, with identifiable evoked components recorded in-flight and compared with pre- and post-flight sessions [14].

The broader rationale for integrating VEPs into SANS surveillance also draws on evidence from related neuro-ophthalmic conditions, where electrophysiology can reveal functional abnormalities not fully captured by structural imaging; in this sense, our recommendation remains a plausible, albeit prospective, extension of the protocol.

Integrating VEPs into current protocols, ideally with strict refractive correction, and using spatio-temporal stimuli that favor both magnocellular and parvocellular contributions could complement OCT/OCTA by detecting subclinical conduction changes, distinguishing optical blur from neuroaxonal dysfunction, and providing meaningful functional outcomes for countermeasure trials.

In summary, we urge the scientific community to view SANS surveillance as an evolving, multidisciplinary monitoring challenge and to help inform the assessment frameworks adopted by space agencies in an era of planned long-duration missions. The addition of UWF-SS-OCTA (to better characterize retinal and choroidal microvasculature across a wider field) and VEPs (to quantify visual pathway function) to existing protocols could provide a more complete neurovascular and functional characterization of SANS, thereby strengthening early detection, refining pathophysiological understanding, and supporting the development and evaluation of countermeasures across the full perimission timeline.

Conflict of interest statement

The Authors declare no conflict of interest.

Received on 23 February 2026.
Accepted on 31 March 2026.

REFERENCES

1. Nguyen T, Ong J, Brunstetter T, Gibson CR, Macias BR, Laurie S, et al. Spaceflight associated neuro-ocular syndrome (SANS) and its countermeasures. Prog Retin Eye Res. 2025;106:101340. doi: 10.1016/j.preteyeres.2025.101340
2. Meer EA, Church LE, Johnson BA, Rohde J, Sinclair AJ, Mollan SP, et al. Non invasive monitoring for spaceflight associated neuro ocular syndrome: responding to a need for in flight methodologies. NPJ Microgravity. 2025;11(1):61. doi: 10.1038/s41526-025-00502-y
3. Soares B, Ong J, Waisberg E, Sarker P, Zaman N, Tavakkoli A, et al. Imaging in spaceflight associated neuro-ocular syndrome (SANS): Current technology and future directions in modalities. Life Sci Space Res. 2024;42:40-6. doi: 10.1016/j.lssr.2024.04.004
4. Cusumano A, Pileri MA, Falsini B, Cesareo M, D'Ambrosio M, D'Apolito F, et al. Structural and functional insight into intracameral cefuroxime ocular toxic syndrome (ICOTS) in eyes with disrupted intraocular barrier. Ophthal Ther. 2025;14:1337-47. doi: 10.1007/s40123-025-01137-8
5. Vyas C, Jolia A, Tsang SH, Sharma T, Diaconita V. Drug-induced retinal toxicity. Adv Exp Med Biol. 2025;1467:253-62. doi: 10.1007/978-3-031-72230-1_50
6. Shelhamer M, Bloomberg J, LeBlanc A, Prisk GK, Sibonga J, Smith SM, et al. Selected discoveries from human research in space that are relevant to human health on Earth. NPJ Microgravity. 2020;6:5. doi: 10.1038/s41526-020-0095-y
7. Fullstone TL, Fischer LFJ, Bohmeier M, Frings-Meuthen P, Crucian BE, Rathert P. Epigenomic profiling of immune cell subtypes reveals H3K27ac-marked stress signatures after long-duration spaceflight. Sci Rep. 2025;15(1):32445. doi: 10.1038/s41598-025-17930-1
8. Paladugu P, Kumar R, Sporn K, Ong J, Song A, Sekhar T, et al. Shared mechanisms in neuromyelitis optica spectrum disorder and spaceflight-associated neuro-ocular syndrome: Insights into central nervous system fluid dynamics, glymphatic function, and astrocyte dysregulation. Life Sci Space Res. 2025;47:4356. doi: 10.1016/j.lssr.2025.05.006
9. Vyas RJ, Young M, Murray MC, Predovic M, Lim S, Jacobs NM, et al. Decreased vascular patterning in the retinas of astronaut crew members as new measure of ocular damage in spaceflight-associated neuro-ocular syndrome. Invest Ophthalmol Vis Sci. 2020;61:34. doi: 10.1167/iovs.61.14.34
10. Bateman GA, Bateman AR. A perspective on spaceflight associated neuro-ocular syndrome causation secondary to elevated venous sinus pressure. NPJ Microgravity. 2022;8(1):3. doi: 10.1038/s41526-022-00188-6
11. Mampre D, Spaide R, Mason S, Van Baalen M, Gibson CR, Mader TH, et al. Spaceflight associated neuro-ocular syndrome as a potential variant of venous overload choroidopathy. Aerosp Med Hum Perform. 2025;96:496-508. doi: 10.3357/amhp.6602.2025
12. Rodrigues GA, Russomano T, Santos Oliveira E. Understanding the relationship between intracranial pressure and spaceflight associated neuro-ocular syndrome (SANS): a systematic review. NPJ Microgravity. 2025;11(1):22. doi: 10.1038/s41526-025-00464-1
13. Falsini B, Tamburrelli C, Porciatti V, Anile C, Porrello G, Mangiola N. Pattern electroretinograms and visual evoked potentials in idiopathic intracranial hypertension. Ophthalmologica. 1992;205:194-203. doi: 10.1159/000310341
14. Cheron G, Leroy A, Palmero-Soler E, De Saedeleer C, Bengoetxea A, Cebolla AM, et al. Gravity influences top-down signals in visual processing. PLoS One. 2014;9:e82371. doi: 10.1371/journal.pone.0082371

Ann Ist Super Sanità 2026 | Vol. 62, No. 2: 100-114
DOI: 10.4415/ANN_26_02_02

Legal responses to new psychoactive substances in Europe: countries inside the REITOX network, Norway, and Türkiye

Valeria Aquilina[1,2], Francesco Paolo Busardò[1], Simona Pichini[2], Giulia Bambagiotti[1] and Silvia Graziano[2]

[1]*Dipartimento di Scienze Biomediche e Sanità Pubblica, Università Politecnica delle Marche, Ancona, Italy*
[2]*Centro Nazionale Dipendenze e Doping, Istituto Superiore di Sanità, Rome, Italy*

Abstract

Introduction. The rapid proliferation of new psychoactive substances (NPS) presents a major challenge to drug control systems. Unlike traditional controlled substances, NPS are synthetic compounds engineered to bypass existing regulations, raising serious public health concerns due to their high potency and unpredictable toxicity. To support a coordinated European response, the REITOX (Réseau européen d'information sur les drogues et les toxicomanies) network brings together national focal points from EU Member States, Norway, Türkiye and the European Commission.

Objective. To provide a comparative overview of legislative frameworks and regulatory responses to NPS across REITOX Member Countries within the European Union.

Methods. We conducted a cross-national analysis of legal texts, procedures for scheduling new substances, and alignment with international and EU legislation. The role of the Early Warning System (EWS), coordinated by the European Union Drugs Agency (EUDA) and supported by the REITOX network, was also examined.

Results. The analysis revealed substantial variation among REITOX member states in legal approaches, timelines, and integration with EU-level mechanisms. REITOX focal points play a central role in information exchange and in supporting the EWS for early detection and coordinated responses to NPS-related threats.

Key words

- new psychoactive substances
- national legislations
- European Union Drugs Agency
- REITOX network
- NPS scheduling

INTRODUCTION

The proliferation of new psychoactive substances (NPS) [1] over the past two decades has become one of the most significant challenges for drug policy, public health, and regulatory systems in European Union [2, 3]. NPS are predominantly synthetic or semi-synthetic compounds, frequently derived through minor chemical modifications of controlled substances. These structural changes, often designed to circumvent existing drug laws, can produce compounds of unpredictable toxicity and markedly increased potency compared to their parent molecules [4]. In parallel, the digital ecosystem has become a significant driver of NPS dissemination, with online supply channels, including semi restricted areas of the deep web and anonymised dark web marketplaces, facilitating rapid, transnational distribution and accelerating the emergence of novel compounds. As a result, NPS use has been associated with acute intoxications, overdose deaths, long-term neuropsychiatric sequelae, and substantial burdens on healthcare and forensic systems [5, 6].

According to the *European drug report 2025: trends and developments* [7], as of 2024, 1,000 NPS are currently under monitoring. A significant spike was observed in the years between 2012 and 2015, reflecting the initial surge in NPS emergence. Although the annual numbers have declined since then, the market remains active and dynamic. The largest group of NPS is represented by synthetic cannabinoids, which make up 28% of all monitored substances including new semi-synthetic derivatives with unpredictable pharmacological profiles. Synthetic cathinones, the second largest group of substances by number (18%), rank first in the EU in terms of quantity seized: 37 tonnes in 2023, up from 27 tonnes in 2022 and 4.5 tonnes in 2021. In 2024, one laboratory in Poland was dismantled, yield-

Address for correspondence: Valeria Aquilina, Dipartimento di Scienze Biomediche e Sanità Pubblica, Università Politecnica delle Marche, Via Tronto 10/a, 60020 Torrette di Ancona, Ancona, Italy. E-mail: v.aquilina@pm.univpm.it.

ing a seizure of about 800 kg. Of particular concern are new synthetic opioids, especially nitazenes: since 2019, at least 21 EU Member States have reported the presence of a nitazene in seizures as well as in acute intoxications and/or deaths. In Estonia and Latvia, for example, nitazenes made up 62 of 119, and 101 of 154, respectively, of drug-induced deaths in 2023. In 2024, seven new synthetic opioids were formally notified to the EU Early Warning System (EWS) [8], all of which were nitazenes, the highest number of nitazenes notified in a single year. These trends highlight a shift in the NPS phenomenon: the challenge is no longer characterised by the uncontrolled proliferation of numerous novel molecules, but by the emergence of fewer, highly potent and harmful compounds that present unprecedented risks to public health.

The global framework for drug control is established by the three United Nations international drug control conventions (1961, 1971, 1988), which provide a system of substance-by-substance scheduling. While these instruments guarantee legal certainty, their reliance on "positive lists" renders them structurally ill-suited to respond to the dynamic and adaptive nature of NPS. New analogues or derivatives not explicitly listed remain beyond control until formally scheduled, creating regulatory gaps that can be rapidly exploited by illicit producers and traffickers. Thus, although the UN treaties remain central to the international governance of narcotic and psychotropic substances, their mechanisms are insufficient to address the rapid evolution of the NPS market.

In response, the European Union has progressively developed a more adaptive regulatory framework to supplement international instruments. Since 1997, the EWS has served as the EU's cornerstone mechanism for the detection, assessment, and control of emerging substances. Coordinated by the European Union Drugs Agency (EUDA) and supported by the REITOX (Réseau européen d'information sur les drogues et les toxicomanies) network of national focal points (30 national focal points of EU Member States, Norway, Türkiye, and the European Commission) [9], the EWS operates as a multi-layered surveillance and response system. It integrates toxicological data, forensic evidence, law enforcement information, and clinical case reports to enable a three-step process: early notification of new substances, scientific risk assessment, and, where appropriate, the adoption of EU-wide control measures.

The EU regulatory architecture has been further strengthened by recent legislative reforms. Regulation (EU) 2017/2101 [10] streamlined the risk assessment process, while Directive (EU) 2017/2103 [11] broadened the definition of "drug" within EU criminal law to explicitly include NPS. The most significant development, however, came with Regulation (EU) 2023/1322 [12], which expanded EUDA's mandate, consolidating and upgrading its role as the successor to the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA). Under this regulation, EUDA has been tasked with developing predictive and preventive tools with the aim to reconcile the traditional trade-off between legal certainty and regulatory timeliness.

Despite these advances, significant heterogeneity persists among national legal responses to NPS within the REITOX member countries. Some countries rely on highly formalised legislative procedures, requiring parliamentary approval for each new scheduling decision. While these approaches ensure legal robustness and compliance with the principle of legality enshrined in Article 7 of the European Convention on Human Rights [13], they often result in delays of several months or even years before a new substance is brought under control. Conversely, other countries have adopted more flexible approaches, such as generic scheduling (covering groups of substances based on shared chemical structures), analogue provisions (extending control to substances with similar pharmacological effects), or emergency measures allowing temporary bans. These mechanisms provide speed and adaptability but may introduce legal ambiguities, raising concerns about enforceability and proportionality [14-18].

Against this background, the aim of this article was twofold. First, it seeks to provide a comparative overview of national legislative frameworks for the control of NPS across REITOX member countries, focusing on the instruments and procedures employed, the timelines for the inclusion of new substances, and the degree of alignment with EU-level mechanisms. Second, it aims to evaluate how divergent legal architectures affect the timeliness, robustness, and coherence of policy interventions. In doing so, it exposes structural weaknesses and considers opportunities for harmonisation, with the ultimate goal of supporting evidence-based and forward-looking policies that protect public health and the safety of European citizens against the evolving NPS phenomenon.

METHODS

This study is based on a comparative legal analysis of national frameworks governing the control of new psychoactive substances (NPS) across the Member States of the European Union (EU) and additional countries participating in the REITOX network.

The primary sources of data consisted of official legal texts at both national and EU levels, including criminal codes, drug control laws, ministerial decrees, and emergency measures. Where available, information was retrieved directly from government databases, national gazettes, or parliamentary archives. These were supplemented by secondary sources such as reports, policy briefs, and databases published by the EUDA and the former EMCDDA.

Given the multilingual nature of the data, many of the legal documents were originally available only in the official language of the respective country. In such cases, documents were translated into English for analytical purposes, either using official translations where provided or, when necessary, by the authors to ensure accurate comprehension and comparability across jurisdictions.

To facilitate systematic comparison, national control mechanisms were classified into three main categories:

1. relevant national legislative instruments: identification of current legal sources governing the control of narcotic substances and NPS, including primary leg-

islation, implementing decrees, and relevant sections of national penal codes;
2. timelines and procedures for NPS scheduling: evaluation of legislative pathways and the temporal dynamics involved in updating national controlled substance lists;
3. substance classification systems: verification of the presence and structure of formal drug scheduling mechanisms.

This comparative approach enabled the identification of structural similarities and differences in national practices, as well as variations in the speed and flexibility of regulatory responses. Where available, informal data regarding risk assessment processes and institutional actors involved in decision-making were also incorporated to provide contextual depth.

RESULTS

Country-specific summaries of REITOX members, outlining the main legal instruments in force, the authorities responsible for scheduling decisions, and the modalities through which NPS are incorporated into national control lists, are presented in *Appendix A available online as Supplementary Material*. A comparative matrix on the national procedures is summarized in *Table 1* [19-110].

Data analysis has allowed to identify five major dimensions that shape the legal and operational landscape: the typology of national laws, the procedures and timelines for scheduling, the classification models used to define controlled substances, the scheduling systems and structures, and the mechanisms for transposing international standards and aligning with European Union legislation.

Typology of national law

A comparative analysis of the national legal framework reveals two main typologies for the control of NPS: integration into general drug legislation versus the adoption of NPS-specific legal instruments. In most REITOX member countries, NPS are regulated within broader narcotic and psychotropic drug control laws, often through periodic amendments to existing schedules or annexes (e.g., Italy, Poland, and Portugal). These frameworks typically do not distinguish NPS as a separate legal category but treat them under the same provisions as traditional controlled substances. In contrast, a limited number of jurisdictions (e.g., Germany) have established dedicated legislation for NPS, reflecting the unique challenges these substances pose. Such frameworks are designed to allow more flexible and timely responses through broader definitions, streamlined procedures, and mechanisms that can target entire groups of substances rather than single compounds.

Scheduling procedures

One of the central findings concerns the accuracy-speed trade-off. Countries relying on formal legislative scheduling, parliamentary processes or multi-step legal amendments, ensure high legal certainty and compliance with the principle of legality, but the extended timelines (often 6-12 months) hinder responsiveness to rapidly emerging substances. In contrast, administrative decrees and executive measures enable swifter interventions (sometimes within weeks), mitigating immediate health threats but providing weaker legal durability and greater susceptibility to procedural challenges.

A third regulatory pathway consists of temporary control measures, applied in countries such as Hungary, Latvia, Lithuania, and the Netherlands. These measures, often lasting up to one year, function as legal "bridges" that allow substances to be regulated quickly while a full risk assessment is ongoing. Risk assessments themselves vary widely: in some countries (e.g., Bulgaria, Estonia, France, Lithuania, Norway, Slovakia) they are formally mandated; in others (e.g., Croatia, Czech Republic, Luxembourg, Poland, Portugal, Slovenia) they are *ad hoc*; while in jurisdictions such as Cyprus, Greece, Ireland, and Spain they are not required at all. These divergences introduce asymmetries in the scientific grounding of scheduling decisions.

Classification models

The legal classification of NPS can be broadly grouped into three models. The individual listing model, used by most countries (e.g., Italy, France, Germany, Spain), requires explicit identification of each substance by chemical name. This ensures clarity but is inherently reactive and often too slow. To overcome this, some jurisdictions have adopted generic classification systems (e.g., Ireland, Türkiye, Belgium, Hungary), which define families of compounds by core molecular structures and allow automatic inclusion of analogues. A third approach is the analogue control model, applied in countries such as Norway and Latvia, where substances may be regulated if chemically and pharmacologically similar to already scheduled compounds. Each model reflects a trade-off between legal certainty and flexibility, and several countries (e.g., Belgium, Germany, Italy, Ireland) adopt mixed approaches to maximise coverage.

Scheduling models

Most national frameworks rely on three criteria for classification: public health risk, potential for abuse, and possible therapeutic utility. Their normative translation, however, differs significantly. Some countries (e.g., Italy, France, Germany, Poland) adopt multiple schedules, allowing differentiated regulation from strict bans to restricted medical use. Others (e.g., Portugal, Norway, the Netherlands) adopt simplified or binary schedules, distinguishing mainly between licit and illicit substances. This heterogeneity influences prescribing practices, pharmaceutical traceability, thresholds for enforcement, and prevention strategies.

Transposition of international standards and harmonisation

All Member States are formally bound by UN Conventions, but mechanisms for transposing international decisions differ. In some jurisdictions (e.g., Italy, Germany, Poland), substances are incorporated with minimal delay – Italy even applying automatic inclusion via Presidential Decree 309/1990. In others, such as France, international listings are subject to national evaluation, taking into account domestic priorities and

Table 1
National legal frameworks and drug scheduling systems for new psychoactive substances (NPS) within the REITOX network

Country	Legal text	Drug scheduling
Austria	• *Suchtmittelgesetz (SMG):* Federal Narcotic Substances Act, 1998. Main law regulating narcotic and psychotropic substances [19] • *Suchtgiftverordnung (SV):* Narcotic Substances Ordinance: lists controlled narcotics [20] • *Psychotropenverordnung (PV):* Psychotropic Substances Ordinance: covers controlled psychotropic drugs [21] • *Neue-Psychoaktive-Substanzen-Verordnung (NPSV):* NPS Regulation and legal status [22] • *Suchtgift-Grenzmengenverordnung (SGV):* Narcotic Threshold Quantities Ordinance [23] • *Psychotropen-Grenzmengenverordnung:* Psychotropic Threshold Quantities Ordinance [24]	• The Suchtgiftverordnung (SV) and Psychotropenverordnung (PV) are structured into annexes (Anlagen) categorizing substances by their abuse potential and medical utility • New substances are added to the SV, PV, or Neue-Psychoaktive-Substanzen-Verordnung (NPSV) through ordinance amendments • Threshold quantities determining criminal or administrative liability are defined in the SGV and Psychotropen-Grenzmengenverordnung • These lists are maintained and updated by the Federal Office for Safety in Health Care (BASG) and published in the RIS (Rechtsinformationssystem)
Belgium	• *Royal Decree of 22 January 1998:* establishes lists of controlled psychotropic substances, regularly updated [25] • *Law of 24 February 1921:* primary legal foundation on drug control; frequently amended to adapt to evolving drug challenges. Act of 7 February 2014 expanded its scope to include psychotropic substances [26] • *Royal Decree of 6 September 2017:* introduces a generic system allowing the classification of entire groups of NPS based on chemical structure, ensuring faster regulatory response [27]	• The scheduling framework legally binding through Royal Decrees (primarily the 1998 and 2017 instruments). These decrees are structured by: - specific listing (chemical name) - generic control (chemical group classification)
Bulgaria	• *Ordinance n. 1 of 1992:* classification and control measures for narcotic and psychotropic substances. Updated regularly via annexes • *Drugs and Precursors Control Act (1999):* core legal framework for narcotic substances and precursors [28] • *Medicinal Products in Human Medicine Act: governs medicines containing controlled substances* [29] • *Penal Code, Art. 354a-354c:* criminalizes production, possession, and trafficking of illicit drugs	• The controlled substances are listed in Annexes to Ordinance n. 1 of 1992 • Substances are classified in Schedules I-IV based on their medical use and risk profile • Updates are published in the State Gazette following proposal by the Ministry of Health
Croatia	• *Law on Combating Drug Abuse (Zakon o suzbijanju zlouporabe opojnih droga):* core law regulating narcotic and psychotropic substances [30] • *Criminal Code (Kazneni zakon):* penalizes production and trafficking. Possession for personal use is decriminalized (administrative sanction) [31] • *Health Protection Act:* addresses treatment and prevention [32]	• The controlled substances are listed in annexes to the Act on Drug Abuse, grouped into categories based on risk and medical use
Cyprus	• *Narcotic Drugs and Psychotropic Substances Law (cap. 154):* regulates narcotics and psychotropic substances and defines offenses related to trafficking and possession [33] • *The Suppression of Crime (Controlled Delivery and Other Special Provisions) (Amendment) Law of 1998:* provides mechanisms for controlled delivery of illegal drugs and other special provisions related to crime suppression [33] • *The Treatment of Convicted Users and Addicts Law, 2016:* focuses on treatment programs for individuals convicted of drug-related offenses [33] • *The Prevention and Suppression of Money Laundering Activities (Amendment) Law (n. 18(I) 2016):* targets money laundering related to drug trafficking and other illegal activities [33] • *The Cultivation and Trade of Industrial Hemp Law of 2016:* regulates the cultivation and trade of non-psychoactive industrial hemp [33] • *The Road Safety (Amendment) Law of 2016:* includes regulations on drug use and road safety, specifically addressing driving under the influence of drugs [33] • *The Prevention of the Use and Dissemination of Drugs and Other Addictive Substances Law 2017:* focuses on preventing drug use and the dissemination of addictive substances through public health campaigns and national efforts [33]	• The controlled substances are listed in annexes to the Narcotic Drugs and Psychotropic Substances Law, grouped into categories based on risk and medical use

Continues

Table 1
Continued

Country	Legal text	Drug scheduling
Czech Republic	• *Act n. 167/1998 Coll. on Addictive Substances:* main framework for drug scheduling and control of narcotic and psychotropic substances, including NPS. Amended in July 2023 to introduce new substance categories [34] • *Act n. 40/2009 Coll. (Criminal Code):* article 283 and 284 criminalize unauthorized possession, manufacturing, and trafficking [35] • *Act n. 373/2011 Coll. on Specific Health Services:* regulates therapeutic treatment, prevention, and substitution treatment for addictions [36] • *Government Regulation n. 463/2013 Coll.:* contains the schedules of controlled substances (updated regularly; last update: 2024) [37] • *Act n. 65/2017 Coll. on Health Protection from Addictive Substances:* governs sale and advertising of alcohol, tobacco, e-cigarettes and includes preventive measures Amended twice in 2023 [38] • *Government Regulation n. 52/2024 Sb.*: effective March 6, 2024 includes substances such as HHC, HHC-O, and THCP [39]	• The Czech Republic uses a five-group scheduling system under Government Regulation n. 463/2013 Coll. Substances are listed from Group I to V, based on scientific assessment of harm, dependence risk, and medical utility. While the law does not provide explicit definitions for each group, Group I typically includes substances without accepted medical use (e.g., heroin), whereas Groups II-V reflect decreasing levels of control and wider therapeutic application
Denmark	• *Consolidated Act n. 748/2008 on Euphoriant Substances:* establishes the legal basis for regulating psychoactive substances and empowers the Minister of Health to rapidly add new substances via executive orders [40] • *Executive Order n. 2446 of 12 December 2021 on Euphoriant Substances:* primary legal framework listing controlled substances in Denmark [41] • *Danish Penal Code (Straffeloven), Section 191:* addresses serious drug offenses, including large- scale trafficking and distribution [42] • *Danish Penal Code, Section 191a:* specifically targets serious doping offenses [42]	• Five-tier classification system (Schedules A-E) for controlled substances: - List A: substances prohibited entirely - Lists B, D, E: substances permitted solely for medical or scientific purposes - List C: substances controlled in their unprepared form but not generally regulated when included in pharmaceutical preparations
Estonia	• *Act on Narcotic Drugs and Psychotropic Substances and Precursors thereof:* primary legal framework regulating controlled substances in Estonia. It outlines procedures for handling, inspection, and identification of narcotic drugs, psychotropic substances, and their precursors [43] • *Regulation n. 73 of the Minister of Social Affairs:* establishes the conditions and procedures for handling narcotic drugs and psychotropic substances for medical and research purposes, including maintaining records and reporting [44]	• Estonia employs a scheduling system categorizing substances into six schedules, depending on their effect and misuse/dependence potential. This classification is detailed in Annex 1 of Regulation n. 73
Finland	• *Narcotics Act (373/2008):* establishes the legal framework for controlling narcotic substances, including provisions for their manufacture, distribution, and possession [45] • *Government Decree on Substances, Preparations and Plants Considered as Narcotics (543/2008):* lists substances classified as narcotics under Finnish law [46] • *Government Decree on Psychoactive Substances Prohibited on the Consumer Market (1130/2014):* prohibits the sale and marketing of certain psychoactive substances not classified as narcotics but deemed harmful [47] • *Criminal Code of Finland, Chapter 50:* defines drug offences and associated penalties [48]	• The scheduling system categorizing substances based on their potential for abuse and medical utility. The classification is detailed in the Government Decree on Substances, Preparations and Plants Considered as Narcotics (543/2008)
France	• *Law n. 70-1320 of 31 December 1970:* the first major drug law in France that established the prohibition of narcotic drugs [49] • *Code de la Santé Publique, specifically Book III, Title III, Chapter I:* establishes the legal framework for the classification, production, and control of narcotic drugs [50] • *Order of 22 February 1990* establishes the official list of controlled narcotic substances (regularly updated) [51] • *Law n. 2016-41 of 26 January 2016:* modifies the framework for penalties and enforcement concerning drug trafficking and use [52]	• The substances are classified under the Public Health Code (articles L. 5132-1 et seq.). For instance, substances are categorized into Category 1 (which includes the most dangerous substances) and Category 2 (which comprises substances with a lower potential for abuse, but which remain under control)

Continues

Table 1
Continued

Country	Legal text	Drug scheduling
Germany	• *Narcotic Drugs Act (BtMG):* governs the control of narcotic drugs, including licensing, prescription, import/export, and criminal penalties • *New Psychoactive Substances Act (NpSG):* enacted in December 2016, this law prohibits the acquisition, possession, and sale of certain NPS, introducing generic controls over groups like phenethylamines and synthetic cannabinoids [53] • *Cannabis Control Act (CanG):* effective from April 1, 2024, this act removed cannabis from the BtMG, regulating its use under a separate framework [54]	• Germany employs a scheduling system under the BtMG, categorizing substances into three schedules: - Schedule I: non-marketable narcotics (e.g., heroin, MDMA) with no recognized medical use - Schedule II: marketable but non-prescribable substances, primarily for manufacturing purposes - Schedule III: marketable and prescribable narcotics under strict regulation
Greece	• *Ministerial Decisions:* specific substances are added to the controlled substances list through ministerial decisions. For example, mephedrone was added to Table A of the list of controlled substances included in Law 3459/06 • *Law 4139/2013:* primary legislation regulating the use and possession of addictive substances, replacing the earlier Law 3459/2006 [55, 56]	• Greece employs a scheduling system categorizing substances into three tables: - Table A: substances with a high potential for abuse and no recognized medical use (e.g., heroin, LSD) - Table B: substances with a high potential for abuse but with recognized medical uses (e.g., morphine, methadone) - Table C: substances with a lower potential for abuse and recognized medical uses
Hungary	• *Act C of 2012 on the Criminal Code:* effective from 1 July 2013, this act covers offenses related to drug trafficking, possession, incitement of minors to use drugs, assisting production, precursors, NPS, and performance enhancement substances [57] • *Government Decree 66/2012 (IV. 2.):* introduced on 3 April 2012, this decree allows for the rapid control of NPS through a formalized rapid assessment process. It created Schedule C for NPS, listing both individual substances and groups of chemical compounds [58] • *Ministerial Decree 55/2014 of the Minister of Human Capacities:* facilitates the inclusion of NPS into the controlled substances list following the rapid assessment process established by Government Decree 66/2012 [59]	• Scheduling system include: - Schedule A: narcotic drugs with high abuse potential and no recognized medical use - Schedule B: psychotropic substances with recognized medical use but potential for abuse - Schedule C: new psychoactive substances (NPS), including both individual substances and groups of chemical compounds - Schedule D: precursors and other substances used in the production of narcotic drugs and psychotropic substances.
Ireland	• *Misuse of Drugs Acts 1977-2016:* this series of acts forms the primary legal framework for controlling drugs in Ireland. It regulates the importation, manufacture, trade, and possession of psychoactive substances [60] • *Misuse of Drugs Regulations 1988 (SI n. 328/1988):* these regulations categorize controlled drugs into five schedules and outline the requirements for their handling [61] • *Criminal Justice (Psychoactive Substances) Act 2010:* this Act prohibits the sale, importation, and advertisement of psychoactive substances not already controlled under the Misuse of Drugs Acts [62] • *Misuse of Drugs (Amendment) Act 2015:* bill entitled an act to amend the Misuse of Drugs Act 1977; to confirm certain statutory instruments; and to provide for related matters [63]	• The controlled drugs are categorized under five schedules in the Misuse of Drugs Regulations: - Schedule 1: substances with no recognized medical use and high potential for abuse - Schedule 2: substances with recognized medical use but high potential for abuse - Schedule 3: substances with less potential for abuse than Schedule 2 drugs - Schedule 4: substances with low potential for abuse - Schedule 5: preparations containing limited quantities of certain controlled drugs
Italy	• *Presidential Decree 309/1990 (Testo Unico delle leggi in materia di disciplina degli stupefacenti e sostanze psicotrope):* primary legal framework regulating narcotic drugs and psychotropic substances in Italy. It outlines the classification of substances, penalties for offenses, and provisions for prevention and rehabilitation [64] • *Ministerial Decrees:* the Ministry of Health issues decrees to update the schedules of controlled substances, including the addition of new psychoactive substances (NPS). These decrees are published in the Official Gazette (Gazzetta Ufficiale) • *Law 16 May 2014 n. 79 (Conversion into law, with amendments, of Decree-Law n. 36 of March 20, 2014):* urgent provisions concerning the regulation of narcotic drugs and psychotropic substances, prevention, treatment and rehabilitation of the relative states of drug addiction, as per Presidential Decree n. 309 of 9 October 1990, as well as the use of less onerous medicines by the National Healthcare Service [65]	• The scheduling system classifies substances into five Tables: - Table I: substances with high abuse potential and no recognized medical use (e.g., heroin, LSD) - Table II: substances with high abuse potential but with recognized medical use (e.g., morphine, methadone) - Table III: substances with lower abuse potential and recognized medical use (e.g., codeine) - Table IV: preparations containing substances from Tables II and III in combinations that reduce abuse potential - Table medicinal products: medicinal products containing narcotic or psychotropic substances in minimal quantities

Continues

Table 1
Continued

Country	Legal text	Drug scheduling
Latvia	• *Law on the Procedures for the Coming into Force and Application of the Criminal Law (2002):* governs the addition of controlled substances and criminal liability related to narcotics and psychotropics [66] • *Law on Procedures for the Legal Trade of Narcotic and Psychotropic Substances and Medicinal Products (2005, with amendments):* establishes the legal framework for handling-controlled substances [66] • *Cabinet Regulation n. 428 (2009):* lists controlled narcotic, psychotropic substances and precursors in Latvia [67]	• Latvia classifies substances into four schedules: - Schedule I: particularly dangerous narcotic and psychotropic substances and associated plants - Schedule II: highly dangerous substances with medical and scientific uses - Schedule III: psychotropic substances with abuse potential - Schedule IV: precursors used in the manufacture of controlled substances
Lithuania	• *Law on the Control of Narcotic and Psychotropic Substances* (1998, with amendments): establishes the framework for the classification, control, and regulation of narcotic and psychotropic substances in Lithuania [68] • *Criminal Code of the Republic of Lithuania:* specifies criminal offenses and penalties related to the illegal handling of narcotic and psychotropic substances • *Lithuanian Government's Decision (2005):* regulates the control of precursors used in the illegal manufacture of drugs [69] • *Cabinet Resolution n. 1463 (2012):* establishes the rapid response mechanism for controlling new psychoactive substances (NPS) by grouping substances with similar structures	• Lithuania classifies substances into four schedules: - Schedule I: substances prohibited for medical use due to high potential for abuse and harm - Schedule II: substances used for medical purposes but considered highly dangerous - Schedule III: substances used for medical purposes with a lower potential for abuse - Schedule IV: substances used for medical or industrial purposes with the lowest potential for abuse
Luxembourg	• *Law of 19 February 1973:* governs the sale of medicinal substances and the fight against drug addiction [70] • *Law of 12 July 1996:* allows for an accelerated procedure to rapidly classify new substances deemed dangerous • *Grand Ducal Regulation of 19 February 1974:* details the classification of narcotic drugs and psychotropic substances [71] • *Grand Ducal Regulation of 30 July 2002:* implements measures for the control of drug precursors	• Luxembourg classifies substances into schedules based on the UN Single Convention on Narcotic Drugs (1961) and the Convention on Psychotropic Substances (1971). The schedules are: - Schedule I: substances with high potential for abuse and no recognized medical use - Schedule II: substances with high potential for abuse but with some accepted medical uses - Schedule III: substances with less potential for abuse and accepted medical uses - Schedule IV: substances with low potential for abuse and widespread medical use
Malta	• *Dangerous Drugs Ordinance (cap. 101):* governs control, import, export, sale, and possession of narcotic drugs [72] • *Medical and Kindred Professions Ordinance (cap. 31):* controls psychotropic substances and professional practices related to medical use [73] • *Drug Dependence (Treatment not Imprisonment) Act (cap. 537):* shifts focus from incarceration to treatment for drug-dependent individuals [74] • *Controlled Substances Regulations:* define registration, reporting, and licensing obligations for controlled substances	• The classification appears in Schedules A, B, C, and D of the Dangerous Drugs Ordinance and associated regulations
Netherlands	• *Opium Act (Opiumwet):* the foundational law governing drug control in the Netherlands. It distinguishes between Schedule I (hard drugs) and Schedule II (soft drugs) [75] • *Opium Act Directive:* provides guidelines for the enforcement of the Opium Act, including prosecutorial discretion and policy priorities [76]	• The Opium Act classifies substances into two schedules: - Schedule I: hard drugs (e.g., heroin, cocaine, MDMA, amphetamines) - Schedule II: soft drugs (e.g., cannabis, hallucinogenic mushrooms)
Norway	• *Medicinal Products Act (Legemiddelloven):* regulates the use and distribution of pharmaceuticals, including narcotic substances [77] • *Norwegian Penal Code (Straffeloven) Sections 231-232:* punishes the production, importation, exportation, possession, and trafficking of drugs [78] • *Regulation on Narcotic Substances (Narkotikaforskriften):* provides the list of controlled substances and outlines the classification [79] • *Drug Reform (2022):* the Norwegian Supreme Court decriminalized the possession of small amounts of drugs for personal use, emphasizing a health-based approach over criminal sanctions [80]	• Classification system for controlled substances based on the risk they pose: - Class A: high-risk narcotics with no or very limited medical use (e.g., heroin, cocaine, amphetamines - Class B: psychotropic substances and medicines with recognized medical use but significant abuse potential (e.g., benzodiazepines) - Class C: prescription medications with lower abuse potential • Substances not listed under these categories are regulated separately or remain uncontrolled unless specifically scheduled

Continues

Table 1
Continued

Country	Legal text	Drug scheduling
Poland	• *Act on Counteracting Drug Addiction (Ustawa o Przeciwdziałaniu Narkomanii, 2005):* primary legal framework regulating the production, distribution, and possession of narcotic substances in Poland [81] • Act *on Counteracting Drug Addiction, Amendment (2011):* amended provisions regarding the control and penalties for drug-related crimes [82] • *Regulation of the Minister of Health (2011):* details the classification of psychoactive substances and their management [83] • *Regulation of the Minister of Health of 16 March 2017:* defines controlled substances and establishes conditions for import and export of narcotics and psychotropic substances [84] • *Regulation of the Minister of Health of 21 August 2019:* updates the list of new psychoactive substances (NPS) [85]	• Poland classifies controlled substances under three categories based on their risk level: - Group I: narcotics with high potential for abuse (e.g., heroin, cocaine) - Group II: substances with moderate abuse potential (e.g., benzodiazepines, amphetamines) - Group III: prescription medications and substances with low abuse potential
Portugal	• *Decree-Law n. 15/93 (January 22, 1993):* establishes the legal framework for controlling narcotic substances, including provisions for their manufacture, distribution, and possession [86] • *Regulation n. 111/2005 (article 7):* Establishes the framework for the registration of operators in the controlled substances trade [87] • *Decree-Law n. 54/2013:* prohibits the production, export, advertisement, distribution, sale or simple dispensing of new psychoactive substances (NPS) named in the list accompanying the Decree Law and sets up a control mechanism for NPS [88] • *Ordinance n. 154/2013:* specifically addresses the control of NPS in Portugal, outlining which substances are prohibited [89] • *Decree-Law n. 254/2016 (October 16, 2016):* focuses on harm reduction and the regulation of certain psychoactive substances • *Law n. 9/2023 (March 3, 2023):* recent amendments to Decree-Law n. 15/93, incorporating new psychoactive substances (NPS) and bringing it into alignment with EU regulations [90]	• Portugal uses a system that classifies substances based on their risk and medical utility. These substances are categorized into classes A, B, and C
Romania	• *Law n. 143/2000 on Combating Illicit Drug Trafficking and Consumption:* establishes the legal framework for preventing and combating illicit drug trafficking and consumption • *Law n. 522/2004:* amends and supplements Law n. 143/2000, introducing definitions such as "dependent consumer" and establishing integrated assistance programs [91] • *Government Emergency Ordinance n. 121/2006:* regulates the legal regime of drug precursors. Law n. 186/2007: approves Government Emergency Ordinance n. 121/2006 • *Law n. 194/2011:* addresses operations with products suspected to have psychoactive effects, other than those stipulated by existing legislation • *Joint Ministerial Order:* establishing mixed teams to control new psychoactive substances (2011)	• Romania employs a scheduling system categorizing substances into three tables based on their potential for abuse and medical utility: - Table I: high-risk drugs with no medical use (e.g., heroin, cocaine) - Table II: risk drugs with limited medical use (e.g., cannabis, MDMA) - Table III: precursors and other substances medicinal products containing controlled substances
Slovakia	• *Act n. 139/1998 Coll.:* primary legislation regulating narcotic and psychotropic substances. It defines controlled substances and outlines penalties for drug-related offenses [92] • *Amendment (October 2013):* allows the Minister of Health to temporarily add new psychoactive substances (NPS) to a controlled list for up to 3 years if there's reasonable suspicion of abuse and harmful effects • *National Anti-Drug Strategy 2021-2025 (Horizon 2030)*: approved by the Ministry of Health in 2021, aligning national drug policy with the EU Drugs Strategy 2021-2025 [93]	• Five-tier classification system (Schedules A-E) for controlled substances: - List A: substances prohibited entirely - Lists B, D, E: substances permitted solely for medical or scientific purposes - List C: substances controlled in their unprepared form
Slovenia	• *Prevention of Illicit Drug Abuse and Treatment of Drug Addictions Act (1999):* establishes the legal framework for preventing illicit drug abuse and providing treatment for drug addictions [94] • *Act on the Production of and Trade in Illicit Drugs (ZPPPD):* governs the production and trade of illicit drugs, including penalties for violations [95] • *Decree on the Classification of Illicit Drugs (2000):* lists substances classified as illicit drugs under Slovenian law [94] • *Resolution on the National Programme on Illicit Drugs 2023-2030:* adopted in June 2023, this strategy outlines Slovenia's approach to reducing and containing the harm caused by illicit drug use	• Slovenia classifies controlled substances into three groups based on their risk and medical use: - Group I: extremely dangerous substances with no medical use (e.g., heroin, cocaine, THC/cannabis) - Group II: highly dangerous substances with recognized medical use (e.g., morphine, codeine) - Group III: moderately dangerous substances used medically (e.g., barbiturates, benzodiazepines)

Continues

Table 1
Continued

Country	Legal text	Drug scheduling
Spain	• *Law 17/1967:* modifies Law 25/1964 and establishes control over narcotic drugs [96] • *Royal Decree 2829/1977:* regulates the control of narcotic and psychotropic substances, aligning with international conventions [97] • *Criminal Code (Organic Law 10/1995):* establishes penalties for drug trafficking and related offenses [98] • *Law 3/1996:* addresses drug control and treatment of drug addictions [99] • *Royal Decree 1675/2012:* updates the legal framework to include new psychoactive substances (NPS) [100] • *Organic Law 4/2015 on the Protection of Citizen Security:* addresses public safety, including provisions related to drug consumption and possession in public places [101] • *Código Penal – article 368:* penalizes trafficking, cultivation, and possession of drugs intended for sale [102] • *National Strategy on Addictions 2017-2024:* provides the strategic framework for addressing addictions, including illicit drugs, in Spain [103] • *Addictions Action Plan 2021-2024:* operational document implementing the National Strategy [104]	• Spain uses a scheduling system that categorizes narcotic and psychotropic substances in accordance with UN conventions. These are published in official lists (not alphabetical or numerical classes) via Royal Decrees (RD), such as RD 2829/1977 and RD 1675/2012. The classification includes internationally controlled substances and emerging NPS, following recommendations from EU and international bodies
Sweden	• *Narcotic Drugs (Punishments) Act (SFS 1968:64):* defines offenses and penalties related to narcotic drugs [105] • *Act on the Control of Narcotic Drugs (SFS 1992:860):* establishes the framework for controlling narcotic drugs, including scheduling [106] • *Ordinance on the Control of Narcotic Drugs (SFS 1992:1554):* supplements the 1992 Act, detailing the list of controlled substances [107] • *Act on Penalties for Smuggling (SFS 2000:1225):* addresses penalties for smuggling narcotic drugs [108]	• Sweden maintains official lists of controlled substances, updated regularly by the Medical Products Agency (Läkemedelsverket). These lists align with international conventions
Türkiye	• *Law n. 3298 on Narcotic Drugs (1986):* specifically regulates opium and its derivatives, including cultivation, production, and distribution [109] • *Law n. 2313 on the Control of Narcotic Drugs:* establishes the framework for controlling narcotic drugs, including scheduling and classification [110] • *Turkish Penal Code (TPC):* articles 188-192 define offenses and penalties related to narcotic drugs, including production, trade, and facilitation of drug use. Penalties range from 2 to 30 years imprisonment, depending on the severity of the offense	• Türkiye maintains official lists of controlled substances, updated regularly by the Ministry of Health. These lists align with international conventions and are categorized without using alphabetical or numerical classes. The scheduling system includes both specific substances and generic groups to effectively control NPS

REITOX: Réseau européen d'information sur les drogues et les toxicomanies; HHC: hexahydrocannabinol; HHC-O: hexahydrocannabinol-o-acetate; THCP: tetrahydrocannabiphorol; MDMA: 3,4-methylenedioxymethamphetamine; LSD: lysergic acid diethylamide.

epidemiological trends. This discretion highlights the tension between regulatory harmonisation and national autonomy, often resulting in fragmented timelines for implementing international controls.

DISCUSSION

The comparative analysis reveals both areas of convergence and significant divergence among REITOX Member Countries, underscoring the complex interplay between national autonomy, international obligations, and supranational coordination. This heterogeneity forms the empirical basis for evaluating the regulatory trade-offs that shape both national and EU-level responses to the NPS phenomenon [111]. Recent developments in NPS markets highlight the urgency of addressing these regulatory disparities.

A substance swiftly scheduled in one country may remain legally accessible in another, creating "regulatory havens" that traffickers and online vendors can exploit. This patchwork of legal frameworks complicates cross-border enforcement and challenges the principle of equal health protection across Europe. Variations in scheduling speed and scope can result in uneven capacities among healthcare systems and public authorities to respond to NPS-related harms. Countries with slower legislative mechanisms may experience higher rates of acute intoxication and morbidity, while those with broader but less precise legal tools face challenges related to proportionality and legal certainty.

From a supranational perspective, these dynamics underscore the need for enhanced European coordination. While the EU Early Warning System provides a

structured platform for detection and risk assessment of NPS, its impact is limited by inconsistent national transposition. To address this, predictive and anticipatory tools – such as integrated forensic databases and real-time epidemiological monitoring – are urgently needed to align legal responses with the emergence of increasingly potent and toxic substances [112, 113].

The comparative findings presented suggest several policy directions. First, harmonisation between national frameworks and EU instruments is essential to reduce fragmentation. A stronger coordinating mandate for the EUDA could ensure consistent and timely translation of supranational risk assessments. Second, a multi-level governance strategy should be adopted, combining EU-level anticipatory instruments (e.g., generic definitions, analogue provisions), with national implementation tailored to local contexts. Third, integration of predictive tools (horizon scanning, predictive toxicology, algorithmic modelling) is critical to shift from reactive to proactive regulation.

Finally, reducing the exploitability of regulatory asymmetries by organised crime must remain a core priority, since criminal networks exploit jurisdictional gaps to market new compounds, undermining both public health and judicial coherence. Strengthening judicial cooperation, harmonising penalties, and enhancing cross-border enforcement are therefore indispensable.

Effectively regulating NPS in Europe requires a coherent, anticipatory, and multi-level governance framework. Building on comparative evidence, aligning national precision with EU-level agility, and embedding predictive tools into regulatory practice are essential steps to enhance resilience and safeguard public health against the evolving risks posed by NPS.

CONCLUSIONS

This comparative study demonstrates that regulatory responses to new psychoactive substances across Europe are shaped by deep divergences in legal instruments and implementation timelines. Such disparities result in uneven levels of protection for citizens, complicate law enforcement cooperation, and leave exploitable gaps for illicit markets. Although the European Union Drugs Agency, through the EU Early Warning System, provide important coordination platforms, their effectiveness remains inconsistent due to variable national transpositions.

Looking ahead, regulatory systems must evolve beyond reactive substance-by-substance listing mechanisms. The integration of predictive toxicology, artificial intelligence-assisted surveillance, and forensic-epidemiological databases would enable earlier identification of emerging threats and more rapid implementation of binding measures. At the same time, a multi-level governance model – combining the legal rigour of national legislatures with the operational agility of EU instruments – appears essential to reduce fragmentation and uphold both legality and proportionality in enforcement.

This study is not without limitations: the analysis has focused primarily on legislative frameworks, without systematically assessing clinical outcomes or enforcement effectiveness. Future research should therefore explore how regulatory divergences translate into measurable health impacts and judicial practices, and how predictive tools can be operationalised across REITOX countries.

In conclusion, enhancing supranational coordination, embedding predictive capacities, and aligning national discretion with common European standards are critical steps to strengthen resilience against NPS. Only by moving from a reactive to a truly anticipatory model can ensure consistent public health protection, reduce the exploitability of legal loopholes by organised crime, and keep pace with the rapidly evolving dynamics of synthetic drug markets.

Authors' contributions

VA: conceptualization, investigation, writing – original draft, writing – review and editing; FPB: formal analysis, supervision; SP: writing – review and editing; GB: conceptualization, supervision, validation; SG: conceptualization, resources, supervision, writing – review and editing.

Acknowledgments

The Author Valeria Aquilina wishes to express her sincere gratitude to the Centro Nazionale Dipendenze e Doping, Istituto Superiore di Sanità, Rome, Italy, for the research fellowship established in collaboration with the Università Politecnica delle Marche, Ancona, Italy.

Funding sources

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of interest statement

The Authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Received on 12 December 2025.
Accepted on 27 January 2026.

REFERENCES

1. European Union Drugs Agency. New psychoactive substances (NPS). Lisbon: EUDA. [cited 2026 Mar 02]. Available from: https://www.euda.europa.eu/topics/nps_en
2. van Amsterdam J, Burgess N, van den Brink W. Legal approaches to new psychoactive substances: first empirical findings. Eur Addict Res. 2023;29(5):363-72. doi: 10.1159/000531503
3. Reuter P, Pardo B. New psychoactive substances: are there any good options for regulating new psychoactive substances? Int J Drug Policy. 2017;40:117-22. doi: 10.1016/j.drugpo.2016.10.020
4. Santos IC, Maia D, Dinis-Oliveira RJ, Barbosa DJ. New

psychoactive substances: health and legal challenges. Psychoactives. 2024;3(2):285-302. doi: 10.3390/psychoactives3020018
5. Lo Faro AF, Di Trana A, La Maida N, Tagliabracci A, Giorgetti R, Busardò FP. Biomedical analysis of new psychoactive substances (NPS) of natural origin. J Pharm Biomed Anal. 2020;179:112945. doi: 10.1016/j.jpba.2019.112945
6. Seddon T. Drug policy and global regulatory capitalism: the case of new psychoactive substances (NPS). Int J Drug Policy. 2014;25(5):1019-24. doi: 10.1016/j.drugpo.2014.03.009
7. European Union Drugs Agency. European drug report 2025: trends and development. Lisbon: EUDA; 2025. Available from: https://data.europa.eu/doi/10.2810/3504283
8. European Union Drugs Agency. The EU early warning system on new psychoactive substances (NPS). Lisbon: EUDA. [cited 2026 Mar 06]. Available from: https://www.euda.europa.eu/activities/eu-early-warning-system-on-nps_en
9. European Union Drugs Agency. Reitox network of national focal points. Lisbon: EUDA. [cited 2026 Mar 06]. Available from: https://www.euda.europa.eu/about/partners/reitox_en
10. European Union. Regulation (EU) 2017/2101 of the European Parliament and of the Council of 15 November 2017 amending Regulation (EC) n. 1920/2006 as regards information exchange on, and an early warning system and risk assessment procedure for, new psychoactive substances. Official Journal of the European Union; 2017. Available from: https://eur-lex.europa.eu/eli/reg/2017/2101/oj/eng
11. European Union. Directive (EU) 2017/2103 of the European Parliament and of the Council of 15 November 2017 amending Council Framework Decision 2004/757/JHA in order to include new psychoactive substances in the definition of "drug" and repealing Council Decision 2005/387/JHA. Official Journal of the European Union; 2017. Available from: https://eur-lex.europa.eu/eli/dir/2017/2103/oj/eng
12. European Union. Regulation (EU) 2023/1322 of the European Parliament and of the Council of 27 June 2023 on the European Union Drugs Agency (EUDA) and repealing Regulation (EC) n. 1920/2006. Official Journal of the European Union; 2023. Available from: https://eur-lex.europa.eu/eli/reg/2023/1322/oj
13. European Union Agency for Fundamental Rights. European Convention on Human Rights – Article 7. FRA; 2018. Available from: https://fra.europa.eu/en/law-reference/european-convention-human-rights-article-7
14. Pirona A, Bo A, Hedrich D, Ferri M, Van Gelder N, Giraudon I, Montanari L, Simon R, Mounteney J. New psychoactive substances: current health-related practices and challenges in responding to use and harms in Europe. Int J Drug Policy. 2017;40:84-92. doi: 10.1016/j.drugpo.2016.10.004
15. Chatwin C. Assessing the "added value" of European policy on new psychoactive substances. Int J Drug Policy. 2017;40:111-6. doi: 10.1016/j.drugpo.2016.11.002
16. Santos-Toscano R, Guirguis A, Davidson C. How preclinical studies have influenced novel psychoactive substance legislation in the UK and Europe. Br J Clin Pharmacol. 2020;86(3):452-81. doi: 10.1111/bcp.14224. Epub 2020 Feb 23
17. Graziano S, Zaami S, Tittarelli R, Pichini S, Sciotti M, Berretta P, Busardò FP, Marinelli E, Tini A, Scaravaggi G, Busacaglia E, Brambilla E, Cavallo M, Locatelli C, Pacifici R. The complex and constantly evolving public health threat of new psychoactive substances in Italy: addressing the main functions of a national observatory of drugs. Ann Ist Super Sanità. 2021;57(2):144-50. doi: 10.4415/ANN_21_02_06
18. Mravčík V, Michailidu J, Pleva P, Lucký M, Kiššová L, Vobořil J. Psychomodulatory substances: new legislative framework for control of psychoactive substances in Czechia. Int J Drug Policy. 2024;133:104603. doi: 10.1016/j.drugpo.2024.104603
19. Rechtsinformationssystem des Bundes (RIS). Bundesrecht konsolidiert: Gesamte Rechtsvorschrift für Suchtmittelgesetz, Fassung vom 06.03.2026. Bundeskanzleramt der Republik Österreich; 2026. [cited 2026 Mar 06]. Available from: https://www.ris.bka.gv.at/GeltendeFassung.wxe?Abfrage=Bundesnormen&Gesetzesnummer=10011040
20. Rechtsinformationssystem des Bundes (RIS). Bundesrecht konsolidiert: Gesamte Rechtsvorschrift für Suchtgiftverordnung, Fassung vom 06.03.2026. Bundeskanzleramt der Republik Österreich; 2026. [cited 2026 Mar 06]. Available from: https://www.ris.bka.gv.at/GeltendeFassung.wxe?Abfrage=Bundesnormen&Gesetzesnummer=10011053
21. Rechtsinformationssystem des Bundes (RIS). Bundesrecht konsolidiert: Gesamte Rechtsvorschrift für Psychotropenverordnung, Fassung vom 06.03.2026. Bundeskanzleramt der Republik Österreich; 2026. [cited 2026 Mar 06]. Available from: https://www.ris.bka.gv.at/GeltendeFassung.wxe?Abfrage=Bundesnormen&Gesetzesnummer=10011054.
22. Rechtsinformationssystem des Bundes (RIS). Bundesrecht konsolidiert: Gesamte Rechtsvorschrift für Neue-Psychoaktive-Substanzen-Verordnung, Fassung vom 06.03.2026. Bundeskanzleramt der Republik Österreich; 2026. [cited 2026 Mar 06]. Available from: https://www.ris.bka.gv.at/GeltendeFassung.wxe?Abfrage=Bundesnormen&Gesetzesnummer=20007642
23. Rechtsinformationssystem des Bundes (RIS). Bundesrecht konsolidiert: Gesamte Rechtsvorschrift für Suchtgift-Grenzmengenverordnung, Fassung vom 06.03.2026. Bundeskanzleramt der Republik Österreich; 2026. [cited 2026 Mar 06]. Available from: https://www.ris.bka.gv.at/GeltendeFassung.wxe?Abfrage=Bundesnormen&Gesetzesnummer=10011056
24. Rechtsinformationssystem des Bundes (RIS). Bundesrecht konsolidiert: Gesamte Rechtsvorschrift für Psychotropen-Grenzmengenverordnung, Fassung vom 06.03.2026. Bundeskanzleramt der Republik Österreich; 2026. [cited 202 Mar 06]. Available from: https://www.ris.bka.gv.at/GeltendeFassung.wxe?Abfrage=Bundesnormen&Gesetzesnummer=10011024
25. United Nations Office on Drugs and Crime. Regulation on certain psychotropic substances and on the reduction of the risks and therapeutic advice (Royal Decree of January 22, 1998). UNODC Laboratory and Scientific Service Portals. Available from: https://www.unodc.org/LSS/Country/DetailsLegalSystem?code=DLIL&country=BE
26. Federale Overheidsdienst (FOD) Justitie. 24 fevrier 1921. Loi concernant le trafic des substances vénéneuses, soporifiques, stupéfiantes, psychotropes, désinfectantes ou antiseptiques et des substances pouvant servir à la fabrication illicite de substances stupéfiantes et psychotropes. Intitulé modifié par L 2003-05-03/46, art. 2, 007; en vigueur: 02-06-2003. (Note: Consultation des versions antérieures à partir du 21-10-1994 et mise à jour au 04-07-2024). Belgique FOD; 2024. Available from: https://www.ejustice.just.fgov.be/cgi_loi/change_lg.pl?language=fr&la=F&cn=1921022401&table_name=loi

27. Federale Overheidsdienst (FOD) Justitie. 6 septembre 2017. Arrêté royal réglementant les substances stupéfiantes et psychotropes. Intitulé modifié par AR 2019-12-09/21, art. 1, 002; en vigueur: 16-01-2020. (Note: Consultation des versions antérieures à partir du 26-09-2017 et mise à jour au 16-01-2026). Service Public Fédéral Belge; 2024. Available from: https://www.ejustice.just.fgov.be/eli/arrete/2017/09/06/2017031231/justel
28. Bulgaria. Drugs and precursors control act. Sofia: Government of the Republic of Bulgaria; 1999. [cited 2026 Mar 06]. Available from: https://www.unodc.org/cld/document/bgr/1999/drugs_and_precursors_control_act.html
29. Bulgaria. Medicinal products in human medicine act. Sofia: Ministry of Health; 2021. [cited 2026 Mar 09]. Available from: https://www.bda.bg/images/stories/documents/legal_acts/20210208_ZLPHM_English.pdf
30. Croatia. Zakon o suzbijanju zlouporabe droga (Act on the suppression of drug abuse). Zagreb: Narodne Novine; 2001. [cited 2026 Mar 06]. Available from: https://www.zakon.hr/z/293/zakon-o-suzbijanju-zlouporabe-droga
31. Croatia. Kazneni zakon (Criminal law). Zagreb: Narodne Novine; 2011. [cited 2026 Mar 06]. Available from: https://www.zakon.hr/z/98/kazneni-zakon
32. Croatia. Zakon o zdravstvenoj zaštiti (Health care act). Zagreb: Narodne Novine; 2008. [cited 2026 Mar 09]. Available from: https://mvep.gov.hr/UserDocsImages/files/file/dokumenti/prevodenje/zakoni/26-Zakon-o-zdravstvenoj-za%C5%A1titi-NN-150-08,-71-10,-139-10...70-16-pro%C4%8Di%C5%A1%C4%87eni-tekst-ENG.pdf
33. Cyprus Police. Drug Law Enforcement Unit – Legal Framework. Nicosia: Cyprus Police. [cited 2026 Mar 07]. Available from: https://www.police.gov.cy/police/police.nsf/All/10BA4DF352643F79C2258447003090DB?OpenDocument
34. Czech Republic. Zákon č. 167/1998 Sb., o návykových látkách (Act n. 167/1998 Coll., on addictive substances). Prague: Sbírka Zákonů; 1998. [cited 2026 Mar 05]. Available from: https://www.zakonyprolidi.cz/cs/1998-167
35. Czech Republic. Zákon č. 40/2009 Sb., trestní zákoník (Act n. 40/2009 Coll., Criminal code). Prague: Sbírka Zákonů; 2009. [cited 2026 Mar 05]. Available from: https://www.zakonyprolidi.cz/cs/2009-40
36. Czech Republic. Zákon č. 373/2011 Sb., o specifických zdravotních službách (Act on specific health services). Prague: Sbírka Zákonů; 2011. [cited 2026 Mar 05]. Available from: https://www.zakonyprolidi.cz/translation/cs/2011-373?langid=1033
37. Czech Republic. Nařízení vlády č. 463/2013 Sb., o seznamech návykových látek (Government Order n. 463/2013 Coll., on lists of addictive substances). Prague: Sbírka Zákonů; 2013. [cited 2026 Mar 05]. Available from: https://mzd.gov.cz/wp-content/uploads/wepub/8942/19995/Order%20Gov%20463_2013.pdf
38. Czech Republic. Zákon č. 65/2017 Sb., o ochraně zdraví před škodlivými účinky návykových látek (Act on the protection of health from the harmful effects of addictive substances). Prague: Sbírka Zákonů; 2017. [cited 2026 Mar 05]. Available from: https://www.zakonyprolidi.cz/translation/cs/2017-65?langid=1033
39. Czech National Monitoring Centre for Drugs and Addiction. Government Regulation n. 52/2024 was officially published. Prague: Czech National Monitoring Centre for Drugs and Addiction. [cited 2026 Mar 05]. Available from: https://www.drogy-info.cz/en/article/news-czech-national-monitoring-centre/government-regulation-no.-52-2024-was-officialy-published/
40. Denmark. Bekendtgørelse n. 1329 af 18. december 2008 om ændring af bekendtgørelse om euforiserende stoffer (Decree n. 1329/2008 amending the Decree on Narcotics n. 748/2008). Copenhagen: Ministry of Health; 2008. [cited 2026 Mar 09]. Available from: https://www.unodc.org/LSS/Country/DetailsLegalSystem?code=DLIL&country=DK
41. Denmark. Bekendtgørelse n. 2446 af 12 december 2021 (Executive order on euphoriant drugs). Copenhagen: Ministry of Justice; 2021. [cited 2026 Mar 09]. Available from: https://www.retsinformation.dk/eli/lta/2021/2446
42. Denmark. Straffeloven (Danish Criminal Code). Copenhagen: Ministry of Justice. [cited 2026 Mar 09]. Available from: https://antislaverylaw.ac.uk/wp-content/uploads/2019/08/Denmark-Criminal-Code.pdf
43. Riigi Teataja. Act on narcotic drugs and psychotropic substances and precursors thereof. Tallinn: Riigi Teataja; 2013. [cited 2026 Mar 06]. Available from: https://www.riigiteataja.ee/en/eli/530102013024/consolide
44. Estonia State Agency of Medicines. Narcotic drugs and psychotropic substances and their legal handling. Tallinn: State Agency of Medicines. [cited 2026 Mar 09]. Available from: https://www.ravimiamet.ee/en/narcotic-drugs-and-psychotropic-substances-and-their-legal-handling
45. Finland. Laki huumausaineista 373/2008 (Act on narcotic drugs 373/2008). Helsinki: Ministry of Justice; 2008. [cited 2026 Mar 09]. Available from: https://www.finlex.fi/fi/lainsaadanto/saadoskaannokset/2008/eng/373
46. Finland. Valtioneuvoston asetus huumausaineista 543/2008 (Government Decree 543/2008 on narcotic substances, preparations and plants). Helsinki: Ministry of Social Affairs and Health; 2008. [cited 2026 Mar 09]. Available from: https://www.unodc.org/LSS/Country/DetailsLegalSystem?code=DLIL&country=FI
47. Finland. Laki 1130/2014 (Government Decree on psychoactive substances). Helsinki: Ministry of Justice; 2014. [cited 2026 Mar 09]. Available from: https://www.finlex.fi/fi/lainsaadanto/2014/1130
48. Finland. Rikoslaki, 50 luku (Penal Code, Chapter 50 – Narcotics offences). Helsinki: Ministry of Justice. [cited 2026 Mar 09]. Available from: https://www.unodc.org/cld/uploads/res/document/fin/penal-code-ch-50_html/Penal_code_ch_50_finland.pdf
49. France. Loi n. 70-1320 du 31 décembre 1970 relative aux mesures sanitaires de lutte contre la toxicomanie et à la répression du trafic et de l'usage illicite de substances vénéneuses. Paris: Journal Officiel de la République Française; 1970. [cited 2026 Mar 09]. Available from: https://bdoc.ofdt.fr/index.php?lvl=notice_display&id=15
50. France. Code de la santé publique. Paris: Journal Officiel de la République Française. [cited 2026 Mar 01]. Available from: https://www.legifrance.gouv.fr/codes/texte_lc/LEGITEXT000006072665/
51. France. Arrêté du 22 février 1990 fixant la liste des substances classées comme stupéfiants. Paris: Journal Officiel de la République Française; 1990. [cited 2026 Mar 09]. Available from: https://www.legifrance.gouv.fr/loda/id/JORFTEXT000000533085/
52. France. Loi n. 2016-41 du 26 janvier 2016 de modernisation de notre système de santé. Paris: Journal Officiel de la République Française; 2016. [cited 2026 Mar 08]. Available from: https://www.legifrance.gouv.fr/loda/id/JORFTEXT000031912641
53. Germany. Neue-psychoaktive-Stoffe-Gesetz (NpSG). Berlin: Bundesministerium der Justiz; 2016. [cited 2025 Jun 15]. Available from: https://www.buzer.de/gesetz/12250/index.htm
54. Germany. Cannabisgesetz (CanG). Berlin: Bundesministerium für Gesundheit; 2024. [cited 2025 Jun 23]. Avail-

able from: https://www.bundesgesundheitsministerium.de/service/gesetze-und-verordnungen/detail/cannabisgesetz.html
55. Greece. Νόμος 3459/2006 Κώδικας Ναρκωτικών (Law n. 3459/2006, Narcotics Code). Athens: Government Gazette of the Hellenic Republic; 2006. [cited 2026 Mar 02]. Available from: https://www.minagric.gr/images/stories/docs/agrotis/ktiniatrika_Farmaka/n3459_2006_narkotika.pdf
56. Greece. Νόμος 4139/2013 Περί εξαρτησιογόνων ουσιών και άλλες διατάξεις (Act on addictive substances and other provisions). Athens: Government Gazette of the Hellenic Republic; 2013. [cited 2026 Mar 02]. Available from: https://www.kodiko.gr/nomothesia/document/71221/nomos-4139-2013
57. Hungary. Act C of 2012 on the criminal code. Budapest: Hungarian Official Gazette; 2012. [cited 2026 Mar 09]. Available from: https://njt.hu/jogszabaly/en/2012-100-00-00
58. Hungary. Government Decree 66/2012. Budapest: Hungarian Official Gazette; 2012. [cited 2026 Mar 09]. Available from: https://www.unodc.org/LSS/Country/DetailsLegalSystem?code=TB&country=HU
59. Hungary. 55/2014. (XII. 30.) EMMI rendelet az új pszichoaktív anyaggá minősített anyagokról vagy vegyületcsoportokról (EMMI Decree on substances or groups of compounds classified as new psychoactive substances). Budapest: Hungarian Official Gazette; 2014. [cited 2026 Mar 09]. Available from: https://njt.hu/jogszabaly/2014-55-20-5H
60. Ireland. Misuse of drugs acts 1977 (n. 12 of 1977). Dublin: Government of Ireland; 1977. [cited 2025 Jun 15]. Available from: https://www.irishstatutebook.ie/eli/1977/act/12/enacted/en/html
61. Ireland. Misuse of drugs regulations 1988 (S.I. n. 328/1988). Dublin: Government of Ireland; 1988. [cited 2025 Jun 15]. Available from: https://www.irishstatutebook.ie/eli/1988/si/328/made/en/print
62. Ireland. Criminal justice (psychoactive substances) act 2010. Government of Ireland; 2010. [cited 2025 Jun 15]. Available from: https://www.irishstatutebook.ie/eli/2010/act/22
63. Ireland. Misuse of drugs (amendment) act 2015. Government of Ireland; 2015. [cited 2025 Jun 23]. Available from: https://www.irishstatutebook.ie/eli/2015/act/6/enacted/en/html
64. Presidenza del Consiglio dei Ministri. Decreto del Presidente della Repubblica 9 ottobre 1990, n. 309. Testo unico delle leggi in materia di disciplina degli stupefacenti e sostanze psicotrope, prevenzione, cura e riabilitazione dei relativi stati di tossicodipendenza. Gazzetta Ufficiale – Serie Generale n. 255, 31 ottobre 1990, Suppl. Ordinario n. 67. [cited 2025 Jun 15]. Available from: https://www.normattiva.it/uri-res/N2Ls?urn:nir:presidente.repubblica:decreto:1990-10-09;309!vig=
65. Presidenza del Consiglio dei Ministri. Legge 16 maggio 2014, n. 79. Conversione in legge, con modificazioni, del decreto-legge 20 marzo 2014, n. 36, recante disposizioni urgenti in materia di disciplina degli stupefacenti e sostanze psicotrope, prevenzione, cura e riabilitazione dei relativi stati di tossicodipendenza, di cui al decreto del Presidente della Repubblica 9 ottobre 1990, n. 309, nonché di impiego di medicinali meno onerosi da parte del Servizio Sanitario Nazionale. (14G00090). Gazzetta Ufficiale – Serie Generale n. 115, 20 maggio 2014. [cited 2025 Jun 15]. Available from: https://www.normattiva.it/uri-res/N2Ls?urn:nir:stato:legge:2014-05-16;79
66. Latvia. Par Krimināllikuma spēkā stāšanās un piemērošanas kārtību (Law on the procedure for the entry into force and application of the criminal law). Riga: LIKUMI.LV; 2002. [cited 2025 Dec 12]. Available from: https://likumi.lv/doc.php?id=50539
67. Latvia. Ministru kabineta noteikumi n. 428 Rīgā 2009. gada 12.maijā (prot. n. 31 40.§) (Cabinet of Ministers Regulation n. 428, Riga, 12 May 2009 – Minutes n. 31, § 40). Riga: Latvijas Vēstnesis; 2009. [cited 2025 Jul 17]. Available from: https://www.unodc.org/LSS/Country/DetailsLegalSystem?code=DLIL&country=LV
68. Lithuania. Law on the control of narcotic and psychotropic substances. Vilnius: Seimas of the Republic of Lithuania; 1998. [cited 2025 Dec 12]. Available from: https://e-seimas.lrs.lt/portal/legalActPrint/lt?jfwid=bkaxm23h&documentId=TAIS.90410&category=TAD
69. Lithuania. 2004 drug control and drug prevention: on the status of implementation in 2004 of the State Drug Control and Drug Prevention Program for 2004-2008 and on funding of its measures according to the information submitted to the Commission. Vilnius: Government of the Republic of Lithuania; 2005. [cited 2025 Dec 12]. Available from: https://www.lrs.lt/pls/inter/w5_show?p_r=3428&p_d=49009&p_k=1
70. Luxembourg. Loi du 19 février 1973 concernant la vente de substances médicamenteuses et la lutte contre la toxicomanie. Luxembourg: Journal Officiel du Grand-Duché de Luxembourg, Mémorial A; 1973. [cited 2025 Dec 12]. Available from: https://legilux.public.lu/eli/etat/leg/loi/1973/02/19/n1/jo
71. Luxembourg. Règlement grand-ducal du 19 février 1974 portant exécution de la loi du 19 février 1973 concernant la vente de substances médicamenteuses et la lutte contre la toxicomanie. Luxembourg: Journal Officiel du Grand-Duché de Luxembourg, Mémorial A; 1974. [cited 2025 Dec 12]. Available from: https://legilux.public.lu/eli/etat/leg/rgd/1974/02/19/n1/jo
72. Malta. Dangerous drugs ordinance (cap. 101). Valletta: Government of Malta; 1939. [cited 2025 Dec 12]. Available from: https://legislation.mt/eli/cap/101/eng/pdf
73. Malta. Medical and kindred professions ordinance (cap. 31). Valletta: Government of Malta; 1901. [cited 2025 Jul 17]. Available from: https://legislation.mt/eli/cap/31/eng/pdf
74. Malta. Drug dependence (treatment not imprisonment) act (cap. 537). Valletta: Government of Malta; 2014. [cited 2025 Dec 12]. Available from: https://legislation.mt/eli/cap/537/eng/pdf
75. Netherlands. Opiumwet. The Hague: Government of the Netherlands; 1928. [cited 2025 Jul 17]. Available from: https://wetten.overheid.nl/BWBR0001941/2024-04-16
76. Office of Medicinal Cannabis. Opium act. The Hague: Government of the Netherlands; 2024. [cited 2025 Jul 17]. Available from: https://english.cannabisbureau.nl/exemptions-from-the-opium-act/opium-act
77. Norway. Lov om legemidler m.v. (legemiddelloven) (Pharmaceuticals act). Oslo: Government of Norway; 1992. [cited 2025 Jul 17]. Available from: https://lovdata.no/dokument/NL/lov/1992-12-04-132
78. Norway. Lov om straff (straffeloven) LOV-2005-05-20-28 (Chapter 23. Protection of public health and the external environment. The Penal Code, Part II. Criminal acts). Oslo: Government of Norway; 2005. [cited 2026 Mar 09]. Available from: https://lovdata.no/dokument/NLE/lov/2005-05-20-28/KAPITTEL_2-8#%C2%A7232
79. Norway. Forskrift om narkotika (narkotikaforskriften) FOR-2013-02-14-199 (Regulation on narcotics). [cited 2025 Jul 17]. Oslo: Helse- og Omsorgsdepartementet; 2013. Available from: https://lovdata.no/dokument/SF/forskrift/2013-02-14-199
80. Olsen H, Lundeberg IR. Courts as policy actors: the blurring of punitiveness in Norwegian drug reform. Nordic J

Criminol. 2026;27(2):1-17. doi: 10.18261/njc.27.2.2
81. Poland. Ustawa z dnia 29 lipca 2005 r. o przeciwdziałaniu narkomanii (Act of 29 July 2005 on counteracting drug addiction). Warsaw: Government of Poland; 2005. [cited 2025 Jul 17]. Available from: https://isap.sejm.gov.pl/isap.nsf/DocDetails.xsp?id=wdu20051791485
82. Polish National Bureau for Drug Prevention. National Programme on Counteracting Drug Addiction (NPC-DA). Warsaw: Government of Poland; 2011. [cited 2025 Dec 12]. Available from: https://www.kbpn.gov.pl/portal?id=114114
83. Chief Pharmaceutical Inspectorate. Pharmaceutical Law – Act of 6 September 2001. Warsaw: Government of Poland; 2001. [cited 2025 Dec 12]. Available from: https://archiwum.gif.gov.pl/en/law/399,Pharmaceutical-Law.html.
84. Chief Pharmaceutical Inspectorate. Controlled substances. Warsaw: Government of Poland; 2017. [cited 2025 Dec 12]. Available from: https://www.gov.pl/web/chief-pharmaceutical-inspectorate/controlled-substances
85. Bureau for Chemical Substances. New psychoactive substances. Warsaw: Government of Poland; 2019. [cited 2025 Jul 17]. Available from: https://www.gov.pl/web/chemical/new-psychoactive-substances
86. Portugal. Legal system summary – including Law n. 47/2003 amending Decree-Law n. 15/1993. [cited 2026 Mar 09]. Available from: https://www.unodc.org/LSS/Country/DetailsLegalSystem?code=DLIL&country=PT
87. European Union. Council Regulation (EC) n. 111/2005 of 22 December 2004 laying down rules for the monitoring of trade between the Community and third countries in drug precursors. Official Journal of the European Union; 2005. [cited 2026 Mar 09]. Available from: http://data.europa.eu/eli/reg/2005/111/oj/eng
88. Portugal. Decreto-Lei n. 54/2013, de 17 de abril: prevenção e proteção contra a publicidade e comércio das novas substâncias psicoativas. Contém as seguintes alterações: DL n.º 9/2021, de 29/01. Lisbon: Ministério Público; 2021. [cited 2026 Mar 09]. Available from: https://www.pgdlisboa.pt/leis/lei_mostra_articulado.php?nid=1903&tabela=leis&nversao=
89. Portugal. Portaria n. 154/2013, de 17 de abril: aprova a lista de novas substâncias psicoativas. Lisbon: Polícia Judiciária; 2013. [cited 2026 Mar 09]. Available from: https://www.policiajudiciaria.pt/portaria-n-o-1542013-17-04-2013/
90. Portugal. Lei n. 9/2023, de 3 de março: inclui novas substâncias psicoativas na definição de droga e altera o Decreto-Lei n. 15/93. Diário da República n. 45/2023, Série I de 23-03-03. [cited 2025 Dec 12]. Available from: https://diariodarepublica.pt/dr/detalhe/lei/9-2023-208124551
91. Romania. Law n. 143/2000 on combating illicit drug trafficking and consumption. [cited 2025 Jul 17]. Available from: //sherloc.unodc.org/cld/en/legislation/rou/law_no._143_on_combatting_illicit_drugs_trafficking_and_consumption/chapter_ii/articles_2-18/law_no._143.html
92. Slovakia. Act n. 139/1998 Coll. on narcotic drugs, psychotropic substances and preparations. Bratislava: Government of the Slovak Republic; 1998. [cited 2025 Jul 17]. Available from: https://www.epi.sk/zz/1998-139
93. Ministry of Health of the Slovak Republic. Slovakia's National Drug Strategy 2021-2025, horizon 2030. Bratislava: Ministry of Health; 2021. [cited 2025 Jul 17]. Available from: https://www.euda.europa.eu/drugs-library/ministry-health-slovakia-2021-slovakias-national-drug-strategy-2021-25-horizon-2030_en
94. Agency for Medicinal Products and Medical Devices of the Republic of Slovenia (JAZMP). National Legislation. Ljubljana: JAZMP. [cited 2026 Mar 09]. Available from: https://www.jazmp.si/en/legislation/
95. Slovenia. Production of and trade in illicit drugs act (ZPPPD). Ljubljana: Government of the Republic of Slovenia; 1999. [cited 2025 Jul 17]. Available from: https://pisrs.si/pregledPredpisa?id=ZAKO1388
96. Spain. Ley 17/1967, de 8 de abril, por la que se actualizan las normas vigentes sobre estupefacientes y se adaptan al Convenio de 1961 de las Naciones Unidas. Boletín Oficial del Estado, n. 86 de 11/04/1967. p. 4806-9. [cited 2025 Jul 17]. Available from: https://www.boe.es/buscar/act.php?id=BOE-A-1967-5592
97. Spain. Real Decreto 2829/1977, de 6 de octubre, por el que se regulan las sustancias y preparados medicinales psicotrópicos, así como la fiscalización e inspección de su fabricación, distribución, prescripción y dispensación. Boletín Oficial del Estado, n. 274 de 16/11/1977. p. 24978-86. [cited 2025 Jul 17]. Available from: https://www.boe.es/buscar/act.php?id=BOE-A-1977-27160
98. Spain. Ley Orgánica 10/1995, de 23 de noviembre, del Código Penal. Boletín Oficial del Estado, n. 281 de 24/11/1995. [cited 2025 Jul 17]. Available from: https://www.boe.es/buscar/act.php?id=BOE-A-1995-25444
99. Spain. Ley 3/1996, de 10 de enero, sobre medidas de control de sustancias químicas catalogadas susceptibles de desvío para la fabricación ilícita de drogas. Boletín Oficial del Estado, n. 11, de 12/01/1996. p. 806-12. [cited 2026 Mar 09]. Available from: https://www.boe.es/eli/es/l/1996/01/10/3
100. Spain. Real Decreto 1675/2012, de 14 de diciembre, por el que se regulan las recetas oficiales y los requisitos especiales de prescripción y dispensación de estupefacientes para uso humano y veterinario. Boletín Oficial del Estado, n. 313 de 29/12/2012. p. 89272-98. [cited 2025 Jul 2]. Available from: http://cido.diba.cat/legislacio/1648023/real-decreto-16752012-de-14-de-diciembre-por-el-que-se-regulan-las-recetas-oficiales-y-los-requisitos-especiales-de-prescripcion-y-dispensacion-de-estupefacientes-para-uso-humano-y-veterinario-ministerio-de-la-presidenciaministerio-de-la-presidencia
101. Spain. Ley Orgánica 4/2015, de 30 de marzo, de protección de la seguridad ciudadana. Boletín Oficial del Estado, n. 77 de 31/03/2015. p. 27216-43. [cited 2026 Mar 09]. Available from: https://www.boe.es/eli/es/lo/2015/03/30/4
102. Spain. Código Penal: Artículo 368. Boletín Oficial del Estado, n. 281 de 24/11/1995. Consolidated version updated to 2024. [cited 2026 Mar 09]. Available from: https://www.conceptosjuridicos.com/codigo-penal-articulo-368/
103. Spain. Ministry of Health, Social Services and Equality. Spanish National Strategy on Addictions 2017-2024. European Union Drugs Agency; 2018. [cited 2025 Jul 17]. Available from: https://www.euda.europa.eu/drugs-library/spanish-national-strategy-addictions-2017-2024_en
104. Spain. Ministry of Health, Spain (2021), Addictions Action Plan 2021-24 (EN). [cited 2025 Jul 17]. Available from: https://www.euda.europa.eu/drugs-library/ministry-health-spain-2021-addictions-action-plan-2021-24-en_en
105. Government Offices of Sweden. Penal Law on Narcotics (1968:64). Stockholm: Government Offices; 2015. [cited 2025 Jul 17]. Available from: https://www.government.se/government-policy/judicial-system/penal-law-on-narcotics/
106. Sweden. Lag (1992:860) om kontroll av narkotika. Svensk författningssamling (SFS) (Act on control of narcotics, Swedish Code of Statutes). Socialdepartementet; 1992. Updated to SFS 2024:818. [cited 2025 Jul 17]. Available from: https://www.riksdagen.se/sv/dokument-och-lagar/dokument/svensk-forfattningssamling/lag-1992860-om-kontroll-av-narkotika_sfs-1992-860/
107. Sweden. Förordning (1992:1554) om kontroll av narkotika. Svensk författningssamling (SFS) (Ordinance

on the control of narcotics). Socialdepartementet; 1992. Updated to SFS 2025:1314. [cited 2025 Jul 17]. Available from: https://www.riksdagen.se/sv/dokument-och-lagar/dokument/svensk-forfattningssamling/forordning-19921554-om-kontroll-av-narkotika_sfs-1992-1554/
108. Sweden. Lag (2000:1225) om straff för smuggling. Svensk författningssamling (SFS) (Act on penalties for smuggling, Swedish Code of Statutes). Stockholm: Ministry of Finance; 2000. Updated to SFS 2025:645. [cited 2025 Jul 17]. Available from: https://www.lagboken.se/lagboken/start/straffratt/lag-20001225-om-straff-for-smuggling//d_49840-sfs-2000_1225-lag-om-straff-for-smuggling
109. Türkiye. Law n. 3298 on narcotic substances. 1986. [cited 2025 Jul 17]. Available from: //sherloc.unodc.org/cld/fr/legislation/tur/law_no._3298_on_narcotic_substances/article_4/law_no.3298.html
110. Türkiye. Drug laws/individual listing for Türkiye. Official Journal Karar Sayısı: 2011/1310. UNODC Legal Systems; 2011. [cited 2026 Mar 09]. Available from: https://www.unodc.org/LSS/Country/DetailsLegalSystem?code=DLIL&country=TR
111. Hughes B, Blidaru T. Legal responses to new psychoactive substances in Europe. European Union Drugs Agency; 2009. Available from: https://www.euda.europa.eu/publications/technical-reports/legal-responses-new-psychoactive-substances-europe_en
112. Varì MR, Mannocchi G, Tittarelli R, Campanozzi LL, Nittari G, Feola A, Umani Ronchi F, Ricci G. New psychoactive substances: evolution in the exchange of information and innovative legal responses in the European Union. Int J Environ Res Public Health. 2020;17(22):8704. doi: 10.3390/ijerph17228704
113. Rinaldi R, Bersani G, Marinelli E, Zaami S. The rise of new psychoactive substances and psychiatric implications: a wide-ranging, multifaceted challenge that needs far-reaching common legislative strategies. Hum Psychopharmacol. 2020;35(3):e2727. doi: 10.1002/hup.2727

Ann Ist Super Sanità 2026 | Vol. 62, No. 2: 115-120
DOI: 10.4415/ANN_26_02_03

Comparative analysis of the information reported on labels of medicinal products containing new active substances between Europe and the USA

Giuseppe Marano, Arianna Annunziata, Maria Cutillo, Roberto Da Cas, Francesca Menniti-Ippolito and Ilaria Ippoliti

Centro Nazionale per la Ricerca e la Valutazione Preclinica e Clinica dei Farmaci, Istituto Superiore di Sanità, Rome, Italy

Abstract

Introduction. Although notable efforts have been made to harmonise regulatory processes for the approval of new therapeutic drugs by the competent authorities, some discrepancies concerning risk-benefit assessments and regulatory actions remain.

Methods. This analysis compared the approaches of the European Medicines Agency (EMA) and the US Food and Drug Administration (FDA) in the approval of new active substances and identified potential clinical implications associated with these differences in "Indication", "Contraindications" and "Posology and administration of drugs" sections of drug summary of products characteristics (SmPC).

Results. The overall analysis showed major differences in 63.9% for the indications, in 75% for contraindications and in 13.9% for posology. Considering the more represented therapeutic areas (cancer and haematology/haemostaseology drugs), the percentage of major differences for the indications was 70% in cancer area and 50% in haematology/haemostaseology area. For contraindications major differences were observed in 90% of cancer drugs, and 60% in haematology/haemostaseology drugs.

Discussion. Our findings highlight the need of further efforts on harmonizing decision making driven by scientific data between regulatory authorities in the interest of patients in a public health perspective.

Key words

- drug approval
- drug evaluation
- indications
- contraindications
- posology

INTRODUCTION

The approval process of new therapeutic drugs is complex, and highly regulated. It comprises a series of well-defined and sequential phases, ranging from preclinical and clinical development to registration and marketing authorisation. Drug regulatory authorities, such as the European Medicines Agency (EMA) and the US Food and Drug Administration (FDA), are responsible for overseeing both the approval and post-marketing monitoring of medicinal products. They ensure the approval of high-quality, safe, and effective drugs to enable timely patient access to new medicines for the benefit of patients, using a wide range of regulatory mechanisms [1].

Over the past two decades, notable efforts have been made to harmonise regulatory processes, contributing to the globalisation of clinical trials through the adoption of shared standards and the development of common frameworks for evaluating the benefit-risk profile of medicinal products. As a result, the EMA and the FDA now assess largely overlapping datasets in most cases [2-5]. Nevertheless, despite progress toward aligning standards and requirements, some discrepancies concerning risk-benefit assessments and regulatory actions remain [6, 7]. Some studies have investigated these divergences, showing that differences in approved therapeutic indications between authorities of Europe and USA occur in more than 50% of cases [8-10], with clinically relevant discrepancies in about 10% of cases [10]. In assessing approved indications, differences between EMA and FDA, especially in the identified targeted population by age (e.g., inclusion or not of paediatric patients), presence of specific genetic or non-genetic mutations or biomarkers or stage of disease (e.g., first line of therapy, refractory, relapse), were found. Such divergences may have important implications for patients' access to appropriate therapeutic options and for clinical decision-making by healthcare professionals.

Address for correspondence: Giuseppe Marano, Centro Nazionale per la Ricerca e la Valutazione Preclinica e Clinica dei Farmaci, Istituto Superiore di Sanità, Via Giano della Bella 34, 00161 Rome, Italy. E-mail: g81.marano@iss.it.

Previous studies focused mainly on differences in the approved indications of use of anti-cancer medicinal products not considering potentially divergences in "Contraindications" and "Posology and administration of drugs" sections of drug labels.

The aims of this study were to compare the approaches of the two drug regulatory authorities (EMA and FDA) in the evaluation and approval of new active substances recommended by EMA in 2024 [11] for a European Union (EU)-wide marketing authorisation and to identify potential clinical implications associated with these differences.

METHODS

In 2024, EMA recommended 114 human medicines for marketing authorization [11]. In our analysis only the active substances (biosimilars and generics were excluded) recommended for marketing authorization in the EU in 2024 [11] and authorized by EMA in the same year and for which a label was available. To collect data about the authorised medicinal products, EMA online resources were checked at the webpage https://www.ema.europa.eu/en/medicines/download-medicine-data. A total of 46 medicinal products has been identified from EMA report [11]; of these, ten products were excluded: three vaccines, two diagnostic agents, and five not approved by FDA. For the remaining 36 products, we retrieved the "Annex I – Summary of Product Characteristics" (SmPC) and FDA Prescribing Information (label). The selection and identification of new active substances included in the analysis are reported in *Figure 1*.

All identified labels were extracted, tabulated, and individually cross-checked with the FDA repository of approved drugs consulting the webpage https://nctr-crs.fda.gov/fdalabel/ui/search. Then, the information reported in the "Annex 1 – SmPC", available from https://www.ema.europa.eu/en/medicines/human/EPAR/, was compared with the information specified in Section 1 (indications and usage) of the FDA label.

Our comparative analysis considered three specific domains: "Indications", "Contraindications", and "Posology". For the indications, Section "4.1 of Therapeutic indications" of the Annex 1 – SmPC and Section "1 Indications and usage" of the FDA label were compared. For the contraindications, Section "4.3 Contraindications" of the Annex 1 – SmPC and Section "4 Contraindications" of the FDA label were compared. Finally, for the posology, Section "4.2 Posology and method of administration" of the Annex 1 – SmPC and Section "2 Dosage and administration" of the FDA label were compared.

To standardise the comparative analysis a set of indicators have been identified for the analysed domain as follows:

- *indications*: treated disease(s) including stage or severity of disease, Type of therapy (e.g., prophylactic *vs* on-demand therapy; monotherapy *vs* combination with other drugs, limitations of use), eligible patients (characteristics of target population);
- *contraindications*: population at risk (e.g., pregnancy and breastfeeding), clinical conditions, comorbidities, and concomitant therapies;
- *posology*: standard dose for the target population, starting and maintenance doses (when provided), dosing frequency and treatment duration (when applicable).

In our analysis, minor and major differences were defined *a priori*. A major difference between EMA and FDA was defined as any relevant divergence involving

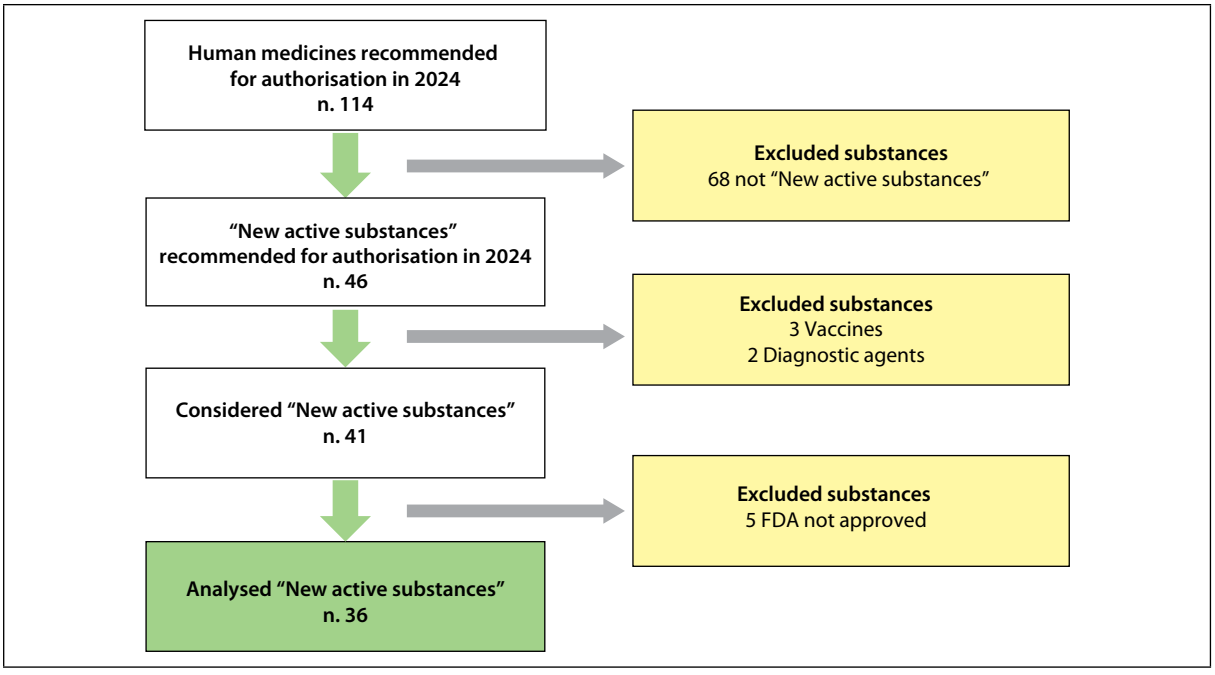


Figure 1
Flowchart of the selection and identification of new active substances.

the target population (e.g., age, gender, weight, etc.) and/or the indications (e.g., type of disease, stage of disease, line of therapy, biomarkers, etc.), contraindications (e.g., reported known *vs* not reported hypersensitivity to the active substance or to any of the excipients) or posology (e.g., differences in dose, frequency or treatment duration). For example, a major difference resulted in the rigorous application of inclusion criteria defined in the pre-registration studies from one agency, while the other restricted/broadened age, gender, or biomarker characteristics. On the other hand, a minor difference was defined as a wording variation that does not alter the meaning of the text or does not introduce any major divergence regarding the indications, contraindications, or posology of the medicinal product.

A panel of three assessors (RDC, II, and GM) independently reviewed the wording and content of the selected sections to determine their consistency, define discrepancies and evaluate the clinical relevance of any differences. In case of disagreement between the assessors, FMI was designated to function as a tiebreaker, although no situation required this intervention. Agreement for "major" and "minor" categories among three assessors was measured by the Fleiss's kappa index with 95% confidence interval (CI). Analysis was performed with the Statistical Software IBM® SPSS® Statistics 29.0.1.0 (https://www.ibm.com/products/spss-statistics).

Given the qualitative nature of the study, we present the results using descriptive statistics.

RESULTS

A total of 36 new drugs recommended for approval by the EMA in 2024 [11] were included in the study. Anti-cancer drugs and haematology/haemostaseology drugs were the dominant therapeutic areas, accounting for 55.5% of the total recommendations for approval (*Table S1 available online as Supplementary Material*). For the above-mentioned medicinal products, indications, contraindications, and posology have been compared between the two agencies.

The overall agreement for all raters on difference grades (major *vs* minor) was strong (κ, 0.889; 95% CI, 0.780-0.998; p<0.001). For "Indications" domain the agreement was high (κ, 0.766; 95% CI, 0.578-0.955; p<0.001) while for "Contraindications" and "Posology" domains was almost perfect (κ, 0.952; 95% CI, 0.763-1.140; p<0.001 and κ, 0.860; 95% CI, 0.672-1.049; p<0.001, respectively) [12].

With regard to the indications, major differences were shown in 23 SmPC. The differences concerned the treated disease in the 52.2%, the eligible patients in 26%, and the type of therapy in 21.8%. For example, we considered as major differences: i) toripalimab-tpzi has been authorised for two clinical conditions by EMA, while for only one by FDA; ii) iptacopan has been authorised for the treatment of subjects with paroxysmal nocturnal haemoglobinuria (PNH) and primary immunoglobulin A nephropathy by FDA and only for PNH by EMA; iii) EMA is more restrictive than FDA in indications for use of lecanemab and mirvetuximab soravtansine excluding apolipoprotein E ε4 (ApoE ε4) homozygotes and non-serous cancers, respectively. Examples of minor differences were: i) lazertinib for the first-line treatment of adult patients with advanced non-small cell lung cancer (NSCLC) with EGFR exon 19 deletions or exon 21 L858R substitution mutations; and ii) dasiglucagon for treatment of severe hypoglycaemia in adults, adolescents, and children aged 6 years and over with diabetes mellitus. For more details see *Table S1 available online as Supplementary Material*.

Analysing contraindications, major differences were shown in 75% of drugs (n=27). Among major differences, the most frequent (21 of 27 SmPC) was the presence of a statement in the EMA in section 4.3 of the SmPC about "hypersensitivity to the active substance or to any of the excipients" with respect to the "None" statement reported in FDA SmPC. Other major differences include: i) the contraindication of insulinoma in the FDA SmPC for dasiglucagon; ii) the exclusion of patients who are not currently vaccinated against Neisseria meningitidis in EMA SmPC for crovalimab and danicopan; iii) restriction for lecanemab in EMA SmPC in case of prior haemorrhagic events or ongoing anticoagulant therapy. For more details see *Table S1 available online as Supplementary Material*.

Regarding the reported posology, major differences were shown in 13.9% of drugs (n=5). No specifications regarding the recurrent nasopharyngeal carcinoma were provided for toripalimab-tpzi in the EMA SmPC while in the FDA SmPC is included a recommended dosage for this stage of the disease. For aprocitentan only EMA SmPC reported an increase of dose for patients tolerating the recommended dose and in need of tighter blood pressure control; moreover, a dose limit (30 grams per 2 weeks or 60 grams per month) for delgocitinib is reported only in the FDA SmPC. The FDA indicates that after 18 months of treatment with lecanemab a transition to the maintenance dosing regimen may be considered. For more details see *Table S1 available online as Supplementary Material*.

In *Table 1* the differences by therapeutic area are reported. Considering only the areas with ten drugs (cancer and haematology/haemostaseology drugs), the percentage of major differences for the indications was 70% in cancer area and 50% in haematology/haemostaseology area. For contraindications major differences were observed in 90% of cancer drugs, and 60% in haematology/haemostaseology drugs. Finally, one major difference in the posology was found in cancer drugs and none in haematology/haemostaseology drugs.

DISCUSSION

The decision-making processes within competent authorities (agencies) are designed to ensure that patients receive the greatest possible benefit, balanced against the risks, from the use of medicinal products. In this Policy Review, the divergences between EMA and FDA SmPC of 36 new active substances have been evaluated.

Major differences in the indications were found in 63.9% of the analysed drugs. The differences concerned the treatable disease in more than half of drugs. For example, toripalimab-tpzi (cancer), iptacopan (haematology), and nemolizumab (dermatology) have been authorised for a different number of clinical conditions

Table 1
Comparison between the indications, contraindications, and posology in the European Medicines Agency (EMA) summary of products characteristics and in the US Food and Drug Administration (FDA) labels stratified by therapeutic area

	Number of medicinal products (%)		
	Indications	**Contraindications**	**Posology**
Overall	**36 (100)**	**36 (100)**	**36 (100)**
Minor differences	13 (36.1)	9 (25)	31 (86.1)
Major differences	23 (63.9)	27 (75)	5 (13.9)
Haematology/haemostaseology	**10 (100)**	**10 (100)**	**10 (100)**
Minor differences	5 (50)	4 (40)	10 (100)
Major differences	5 (50)	6 (60)	0
Cancer	**10 (100)**	**9 (100)**	**9 (100)**
Minor differences	3 (30)	1 (10)	9 (90)
Major differences	7 (70)	9 (90)	1 (10)
Cardiovascular	**3 (100)**	**3 (100)**	**3 (100)**
Minor differences	2 (66.7)	0	2 (66.7)
Major differences	1 (33.3)	3 (100)	1 (33.3)
Neurology	**3 (100)**	**3 (100)**	**3 (100)**
Minor differences	1 (33.3)	0	2 (66.7)
Major differences	2 (66.7)	3 (100)	1 (33.3)
Gastroenterology/hepatology	**2 (100)**	**2 (100)**	**2 (100)**
Minor differences	0	0	2 (100)
Major differences	2 (100)	2 (100)	0
Uro-nephrology	**2 (100)**	**2 (100)**	**2 (100)**
Minor differences	0	2 (100)	2 (100)
Major differences	2 (100)	0	0
Dermatology	**2 (100)**	**2 (100)**	**2 (100)**
Minor differences	0	1 (50)	0
Major differences	2 (100)	1 (50)	2 (100)
Infections	**1 (100)**	**1 (100)**	**1 (100)**
Minor differences	0	1 (100)	0
Major differences	1 (100)	0	1 (100)
Endocrinology	**1 (100)**	**1 (100)**	**1 (100)**
Minor differences	1 (100)	0	1 (100)
Major differences	0	1 (100)	0
Pneumology/allergology	**1 (100)**	**1 (100)**	**1 (100)**
Minor differences	0	0	1 (100)
Major differences	1 (100)	1 (100)	0
Immunology/rheumatology/transplantation	**1 (100)**	**1 (100)**	**1 (100)**
Minor differences	1 (100)	0	1 (100)
Major differences	0	1 (100)	0

by the two regulatory agencies excluding a patient's subgroup from the treatment. Moreover, unlike EMA, FDA states clearly that the use of elafibranor and seladelpar – indicated for the treatment of primary biliary cholangitis in combination with ursodeoxycholic acid (UDCA) in adults with an inadequate response to UDCA, or as monotherapy in patients unable to tolerate UDCA – is not recommended in patients who have

or develop decompensated cirrhosis (e.g., ascites, variceal bleeding, hepatic encephalopathy).

A higher percentage of major differences (75%) was found for the reported contraindications especially due to the presence of a statement in the EMA SmPC about hypersensitivity to the active substance or any of the excipients that was absent in the FDA SmPC. This divergence is related to the FDA recommendations to report only known hazards and not theoretical possibilities [13] and does not have any clinical impact or imply any real risk for patients. On the other hand, some differences on this domain could lead medical decisions. For example, only in the EMA label is clearly specified that treatment with lecanemab should not be initiated in patients receiving ongoing anticoagulant therapy. This statement has an impact on patients' management considering that patients with dementia due to Alzheimer's disease are often elderly with concomitant cardiovascular diseases requiring anticoagulation therapy.

As expected, a lower percentage (13.9%) was found for the posology considering that the optimal dose and regimen are defined in the pre-registrative clinical trials and adopted by the authorities for the clinical practice.

A recent article by Pierini *et al*. [8] compared 162 therapeutic indications of 80 medicinal products for solid tumours and blood cancers authorised by EMA between 2015 and 2022, with the corresponding labels approved by FDA. The authors reported clinically relevant discrepancies for about 52% of the evaluated indications [8]. A previous evaluation of oncology drugs approved by EMA between 1995 and 2008 reported 47% discrepancies in therapeutic indications between EMA and FDA that in 10% of the cases were considered clinically relevant [10]. These above-mentioned cases can have an impact on the clinical practice by excluding certain groups of patients (different place in therapy for the same anticancer drug and/or limitation to a specific subgroup).

More recently, a lower percentage difference in indications (about 20%) of new drug marketing applications for a new active substances, chemical entities, or therapeutic biologic products submitted to FDA and EMA in the period 2014-2016 was found [14]. Concerning contraindications, Alshammari TM *et al.* [15] found differences in the drug labels information in almost 70% of 100 drugs approved in the USA, the UK and Canada.

Although our findings are largely consistent with those reported in the literature [8-10, 14, 15], they should be interpreted in light of several limitations. First, we assumed that both agencies received a non-dissimilar Common Technical Document (CTD), which might not be always the case. For instance, the two agencies might have evaluated data at different timelines of the trials, such as different interim or final analyses of the same trial. Moreover, differences may be amplified by a different propensity of the two agencies to adhere to the population included in the pre-registrative studies when drafting the label and by the possibility to withdraw the approved indications in absence of a proven clinical benefit. Finally, the agency that approves the drug after the other one tends to be more restrictive in wording of the indications. Second, for most of the therapeutic areas, it was not possible to derive specific conclusions owing to the low number of analysed medicinal products (three or less products). Third, this is a single-year analysis (year 2024). In light of these limitations, the results may not reflect multi-year patterns particularly considering that product labels may be updated over time in response to emerging scientific evidence. To assess whether the observed differences between the two agencies persist over time, a three-year follow-up analysis could provide additional and valuable insights.

The main differences between the FDA and EMA drug approval processes reflected their health policies, regulatory structure, and review timelines. The FDA handles the US market with a single, faster approval pathway [16]. In contrast, EMA coordinates multiple national agencies for EU market approval, which could slower the authorization process and requires more comprehensive data for standard reviews. The agencies may reach different conclusions about a drug strength of evidence because they emphasize different findings from pre-registrative studies, leading to variations in approved indications or, in rare cases, despite a differing view on the overall benefit-risk assessment. In some cases, such differences could significantly affect patients access to relevant therapeutic options that could raise the uncertainty among patients and healthcare professional about the different statements of regulatory agencies.

In conclusion, a non-negligible number of differences were found in the labels of EMA and FDA licensed products, except for the posology domain. Further efforts on harmonizing decision making driven by scientific data between regulatory systems are needed for the patient benefit and public health.

Authors' contributions

GM, RDC: designed the study; AA, MC: identified and collected the information from EMA and FDA repository; GM, II, RDC: analysed the information from EMA and FDA repository; GM: drafted the first version of the manuscript; RDC, II: reviewed the manuscript and contributed to its content development; GM, FMI, RDC: supervised the overall development of the manuscript. All Authors read and approved the final version of the manuscript.

Funding information

This article did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

Conflict of interest statement

The Authors disclose no conflicts of interest.

Disclaimers

The views expressed in this article are the personal views of the Authors and may not be understood or quoted as being made on behalf of or reflecting the position of the respective Authors' organisations.

Received on 26 November 2025.
Accepted on 28 January 2026.

REFERENCES

1. Gomase VS, Sharma R, Dhamane SP. Global analysis of regulatory frameworks and drug safety standards in the drug approval process. Curr Drug Saf. 2025 Sep 4. doi: 10.2174/0115748863392869250827042742
2. Teixeira T, Kweder SL, Saint-Raymond A. Are the European Medicines Agency, US Food and Drug Administration, and other international regulators talking to each other? Clin Pharmacol Ther. 2020;107:507-13. doi: 10.1002/cpt.1617
3. Molzon JA, Giaquinto A, Lindstrom L, Tominaga T, Ward M, Doerr P, Hunt L, Rago L. The value and benefits of the International Conference on Harmonisation to drug regulatory authorities: advancing harmonization for better public health. Clin Pharmacol Ther. 2011;89(4):503-12. doi: 10.1038/clpt.2011.10
4. European Commission, European Medicines Agency, US Food and Drug Administration. Medicines regulators on both sides of the Atlantic confirm commitment to cooperation on medicines. London: EC, EMEA, FDA; 2008. (Doc. Ref. EMEA/515723/2008). Available from: https://www.ema.europa.eu/en/documents/press-release/medicines-regulators-both-sides-atlantic-confirm-commitment-cooperation-medicines_en.pdf
5. European Medicines Agency. European Medicines Agency and US Food and Drug Administration extend confidentiality arrangements indefinitely. London: EMA Press; 2010. (EMA/579151/2010). Available from: https://ipq.org/wp-content/uploads/2021/06/FDA-EMA-Confidentiality-Press-Release-09.15.10.pdf
6. Salcher-Konrad M, Naci H, Davis C. Approval of cancer drugs with uncertain therapeutic value: a comparison of regulatory decisions in Europe and the United States. Milbank Q. 2020;98(4):1219-56. doi: 10.1111/1468-0009.12476
7. Kühler TC, Bujar M, McAuslane N, Liberti L. To what degree are review outcomes aligned for new active substances (NASs) between the European Medicines Agency and the US Food and Drug Administration? A comparison based on publicly available information for NASs initially approved in the time period 2014 to 2016. BMJ Open. 2019;9(11):e028677. doi: 10.1136/bmjopen-2018-028677
8. Perini M, Castiglioni B, Galai E, Trapani D, Genazzani AA, Miglio G. Differences in the on-label cancer indications of medicinal products between Europe and the USA. Lancet Oncol. 2025;26(2):e103-10. doi: 10.1016/S1470-2045(24)00434-0
9. Rohr UP, Iovino M, Rudofsky L, Li Q, Juritz S, Gircys A, Wildner O, Bujar M, Bolte C, Dalla Torre di Sanguinetto S, Wolfer A. A decade comparison of regulatory decision patterns for oncology products to all other non-oncology products among Swissmedic, European Medicines Agency, and US Food and Drug Administration. Clin Transl Sci. 2023;16(9):1569-81. doi: 10.1111/cts.13567
10. Trotta F, Leufkens HGM, Schellens JHM, Laing R, Tafuri G. Evaluation of oncology drugs at the European Medicines Agency and US Food and Drug Administration: when differences have an impact on clinical practice. J Clin Oncol. 2011;29:2266-72. doi: 10.1200/JCO.2010.34.1248
11. European Medicines Agency. Human medicines in 2024. Luxembourg: Publications Office of the European Union; 2025. doi: 10.2809/6907594
12. Landis JR, Koch GG. The measurement of observer agreement for categorical data. Biometrics. 1977;33:159-74. doi: 10.2307/2529310
13. US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research, Center for Biologics Evaluation and Research. Guidance for industry. Warnings and precautions and boxed warning sections of labeling for human prescription drug and biological product – content and format. 2011. Available from: https://www.fda.gov/media/71866/download
14. Kashoki M, Hanaizi Z, Yordanova S, Veselý R, Bouygues C, Llinares J, Kweder SL. A Comparison of EMA and FDA decisions for new drug marketing applications 2014-2016: concordance, discordance, and why. Clin Pharmacol Ther. 2020;107(1):195-202. doi: 10.1002/cpt.1565
15. Alshammari TM, Devadasu VR, Rathnam RP. Comparison of the safety information on drug labels in three developed countries: the USA, UK and Canada. Saudi Pharm J. 2017;25(8):1103-7. doi: 10.1016/j.jsps.2017.07.006
16. Joppi R, Bertele V, Vannini T, Garattini S, Banzi R. Food and Drug Administration *vs* European Medicines Agency: review times and clinical evidence on novel drugs at the time of approval. Br J Clin Pharmacol. 2020;86(1):170-4. doi: 10.1111/bcp.14130

Ann Ist Super Sanità 2026 | Vol. 62, No. 2: 121-129
DOI: 10.4415/ANN_26_02_04

Occurrence of formaldehyde exposures in work activities and its diffusion in the Italian industrial context following the notification requirements

Alberto Scarselli, Monica Gherardi, Davide Di Marzio and Alessandro Marinaccio

Dipartimento di Medicina, Epidemiologia, Igiene del Lavoro ed Ambientale, Istituto Nazionale per l'Assicurazione contro gli Infortuni sul Lavoro, Rome, Italy

Abstract

Purpose. The aim of this study is to describe data on occupational exposure to formaldehyde in the context of Italian economic activities.

Methods. Measurement data of airborne concentrations (n=26,216) of formaldehyde in the period 1996-2024 were selected from the national register of occupational exposure to carcinogens (Sistema Informativo Registri di Esposizione Professionale, SIREP). Descriptive statistics were calculated for main exposure-related variables. The number of potentially exposed workers was estimated for the best-characterized sectors.

Results. The overall geometric mean was 0.028 mg/m^3. Most of the exposure was found in the wood (n=4,912), chemical (n=4,090), and plastic industries (n=3,925). A different distribution of exposure was found according to gender, as women exposures were more common in the healthcare sector. The estimated number of potentially exposed workers in the selected sectors was approximately 100,000.

Conclusions. The implementation of the new European Union (EU) regulation has enhanced the surveillance system, resulting in noteworthy improvements in the monitoring of occupational exposure to formaldehyde.

Key words

- formaldehyde
- exposure assessment
- occupational health
- surveillance system

INTRODUCTION

Formaldehyde, a well-known chemical, is the simplest of the aldehydes with a structural formula consisting of a carbonyl group (C=O) to which two hydrogen atoms are bonded, making the molecule highly reactive at ambient temperature. It occurs naturally as a colourless gas [1]. This substance is listed by the World Health Organization (WHO) as a pollutant of public health concern, known for its adverse effects [2]. In the environment formaldehyde is ubiquitous, both as a primary and secondary pollutant, released directly from combustion processes, including several natural sources, industrial emissions, and urban traffic, or formed in the atmosphere by photochemical reactions of other pollutants, such as volatile organic compounds [2]. It is a known respiratory and skin irritant, and can cause long-term damage, including eye irritation, allergies, and even genetic damage and cancer [1]. Exposure occurs primarily through inhalation into the respiratory tract or skin absorption. It is rapidly absorbed after inhalation and oral exposure, but it is poorly absorbed through the skin [1]. The International Agency for Research on Cancer (IARC) has classified it as a Group 1 carcinogen to human since 2012 [3]. The carcinogenicity of formaldehyde is mainly due to its ability to cause cross-links between DNA and proteins and cellular toxicity at the point of contact [3]. There is an ongoing debate about whether its carcinogenic mechanism is threshold (non-genotoxic) or non-threshold (genotoxic), but some leading scientific institutions currently consider it a non-threshold carcinogen, meaning that even low-level exposure may pose a risk, especially for nasopharyngeal cancer and some types of leukaemia [1-3]. Formaldehyde is widely used in different industrial and professional sectors due to its properties as a preservative, disinfectant and fixative agent. It is primarily used in the manufacture of resins, preservatives, lawn fertilizers, fixatives, cosmetics, and disinfectants, and workers involved in its production or use may be exposed to dangerous levels during their work activities [1, 2]. In 2016, with the entry into force of European Union (EU) Regulation n. 605/2014, the obligation to report occupational exposure to carcinogens to the national register (Sistema Informativo Registri di Esposizione

Address for correspondence: Alberto Scarselli, Dipartimento di Medicina, Epidemiologia, Igiene del lavoro ed Ambientale, Istituto Nazionale per l'Assicurazione contro gli Infortuni sul Lavoro, Viale Stefano Gradi 55, 00143 Rome, Italy. E-mail: a.scarselli@inail.it.

Professionale, SIREP) established by National Institute for Insurance against Accidents at Work (Istituto Nazionale per l'Assicurazione contro gli Infortuni sul Lavoro, INAIL) was extended to include formaldehyde, which is now among the substances classified in Group 1B of the EU classification pursuant to the Regulation n. 1272/2008. To prevent and control exposure through inhalation, the EU regulation requires, from 2020, an 8-hour occupational exposure limit value (OELV) of 0.37 mg/m^3 of formaldehyde in the workplace air. The OELV for formaldehyde has traditionally been based on both animal and human studies demonstrating its irritant properties and carcinogenic potential, highlighting significant risks at concentrations above certain levels. Given the evolving scientific evidence and new insights into genotoxicity and susceptible populations, there are compelling reasons to continue updating this limit value. However, there is no scientific evidence for the feasibility of assessing formaldehyde exposure through biological monitoring, and no biological indicators are available for this purpose.

The aim of this study is to describe the level of exposure to formaldehyde in different sectors of activity and occupational groups in Italy following the entry into force of the reporting obligation. An estimation of the number of workers potentially exposed to formaldehyde was also performed.

METHODS

Data collection

The measurement data on formaldehyde exposure were collected by SIREP and refer to the exposure period 1996-2024. SIREP is a relational database whose design and contents have been extensively described elsewhere [4]. In summary, according to Italian regulation on health surveillance in the workplace (Legislative Decree n. 81/2008), employers are required to record data on occupational exposure to carcinogens and notify them to the SIREP system every three years. Exposure levels must not exceed the OELVs established in Annex XLIII of Decree n. 81/2008 and exposure measurements must be aimed at managing exposure below this binding value (residual exposure). Exceeding these limits requires the employer to intervene immediately to reduce exposure and adopt appropriate prevention and protection measures. The reporting is mandatory for workers exposed to carcinogenic agents classified as Group 1A and 1B by the European Union (1A, substance known to be carcinogenic to humans; 1B, substances that should be considered carcinogenic to humans) under EU Regulation n. 1272/2008 on the classification, labelling and packaging of chemicals. Formaldehyde has been classified in Group 1B since 2016; prior to this year, data was notified to the system only on a voluntary basis. The management and transmission of data have to be completed exclusively electronically via a web application. The main information provided by employers includes the economic activity and size of the firm, the demographic and occupational data of the workers, and the levels of exposure in terms of intensity, frequency, and time duration. One or more exposure measurements are recorded for each worker and work period. Employers are responsible for exposure measurement procedures and air sampling methods, to be carried out in accordance with European standards which provide technical guidance on the implementation of air monitoring strategies [5].

Data selection and classification

A total of 26,216 measurements that refer to 19,242 exposure situations to formaldehyde were selected; 4,446 exposures of which were measured repeatedly over time. The term "exposure" refers to a specific job task of a worker that involves exposure to formaldehyde. Measurements (n=1,826) provided in parts per million (ppm) were converted to mg/m^3 using the standard conversion factor derived at 25 °C and 1 atmosphere of pressure (1 ppm=1.23 mg/m^3). The sample typology (*personal* or *environmental*) and the analytical method performed to collect the measurements were not always available (available percentage: 78% and 89% of cases respectively), while the sampling period was a typical 8-h working day (time-weighted average, TWA-8). The exposure-related variables selected for the descriptive analysis were the activity sector, the occupational group, the size and geographical location of the firms. The level of exposure was grouped into six value classes, according to the quantile distribution, to provide better readability of the data (<0.01, 0.01-0.025, 0.025-0.05, 0.05-0.1, 0.1-0.25, >0.25, unit in mg/m^3). The size of the firms was expressed in terms of workforce and divided into five classes based on the number of workers: <10, 10-20, 20-50, 50-100, >100. International standard classifications were used to code economic sectors (Nomenclature statistique des Activités économiques dans la Communauté Européenne, NACE, revision 2) and occupational groups (International standard classification of occupations, ISCO-08). Descriptive statistical analyses were carried out to estimate the geometric mean (GM) of exposure levels, the geometric standard deviation (GSD) and the 25th-75th interquartile range (IQR). A temporal trend analysis of the mean level (GM) of exposure was performed. A sample size of 50 measurements was selected as the minimum number required to perform reliable descriptive statistics. Statistical analysis was performed using R software v. 4.1.3 (R Foundation for Statistical Computing, Vienna, Austria).

Estimating potential exposed workers

The SIREP system does not include all firms where workers may be exposed to formaldehyde, as its coverage depends largely on each firm's risk assessment procedures and other factors that may affect the representativeness of the data. Nevertheless, firms that notify the register to SIREP are also required to declare the total number of employees (both exposed and not exposed). This allowed to determine the percentage of exposed workers within each firm. As a result, it was possible to estimate the number of workers potentially exposed to formaldehyde in each economic sector, including those who were not recorded in SIREP but were likely at risk based on the nature of their firm's economic activities. For this purpose, only the best-characterized sectors

in the database were included to ensure greater reliability of the estimates, given that representativeness across sectors in SIREP was not uniform. The activity sectors best-characterized in the database were those where the percentage of reported workforce (exposed plus non-exposed) was more or equal than 1% of the total sector workforce resulting from national statistics on industry of the Italian Institute for Statistics (ISTAT) (RW_i/W_i>=1%, where RW_i represents the reported workforce in SIREP, W_i represents the ISTAT total workforce, and *i* is the i-*th* activity sector) [6]. Moreover, only those sectors with a minimum of three firms reporting formaldehyde exposure in the SIREP database were included in the estimation. For the selected activity sectors, the number of workers potentially exposed to formaldehyde was reconstructed using the percentage of exposed workers in relation to both the workforce size of firms recorded in the SIREP database and the national statistics on workforce (i.e., $PE_i=W_i(E_i/RW_i)$, where PE_i=potentially exposed workers, W_i=ISTAT total workforce, E_i=SIREP exposed workers and RW_i=SIREP reported workforce). SIREP exposed workers (E_i) was the total number of workers having formaldehyde exposure measurements recorded for the i-*th* activity sector.

RESULTS

Overall, the GM of exposure to formaldehyde was 0.028 mg/m³, and was slightly higher in men (0.029 mg/m³) than in women (0.024 mg/m³). The distribution of exposure levels by activity sector (NACE codes), broken down by gender, is shown in *Table 1*. Considering men and women together, the wood, chemical and plastic industries accounted for about 50% of the total measurements. However, a different pattern for each gender is clear. The majority of exposures in men was found in the wood (20% of male exposure measurements), chemical industries (18%), and plastic (14%), while in women it was more concentrated in the healthcare sector (32% of female exposure measurements). The main analytical method used for sampling the exposure level was the one proposed by the National Institute for Occupational Safety and Health, NIOSH, (n. 2016) performed in 33% of the measurements, while the sample typology was *personal* in 49% of the cases. Among key industries (i.e., with a consistent number of exposure measurements), the sectors at highest risk were manufacture of wood and of products of wood for women (GM=0.057 mg/m³), and manufacture of rubber and plastic products (GM=0.040 mg/m³) for men. The distribution by occupational group reflects that by activity sector, showing the highest prevalence of exposures for woodworking-machine tool setters and operators in men (12% of male exposure measurements), and for medical and pathology laboratory technicians in women (13% of female exposure measurements). However, the occupational group with the highest level of formaldehyde exposure was manufacturing labourers (GM=0.083 mg/m³) in women, and pulp and papermaking plant operators in men (GM=0.120 mg/m³), even if the latter with a low number of measurements (n=147, 1% of male exposure measurements). Exposure levels by occupations are reported in *Table 2*, and in *Table 3* exposure measurements are described by firm size and Italian macro area. *Figure 1* shows the temporal trend of exposure levels (GM) by year of measurement. Levels of exposure to formaldehyde are significantly decreasing over time, showing a consistent increase in notifications since 2016, the year the obligation came into force. Over the past few years (2019-2024) the number of formaldehyde measurements reported to SIREP has become rather constant, averaging around 2,500 per year. Almost 21% of measurements exceeded the value of 0.1 mg/m³, but this percentage varied widely among activity sectors. The distribution of exposure measurements by value classes, for the most common activity sectors, is described in *Figure 2*. Workers in the wood industry had a prevalence (in percentage terms) of elevated exposure levels (>0.25 mg/m³) approximately twice as high as those in the furniture industry, while the lowest value was reported in the metalworking industry (3%). The highest percentage of elevated exposure level (>0.25 mg/m³) was found in the healthcare sector representing over 17% of the class total. About one-third of the exposure measurements in the rubber and plastic industry were >0.1 mg/m³, while in the wood industry almost half of the measurements were >0.05 mg/m³. Overall, 2.3% was found to be above the official OELV established by legislation over time. Regarding firm size, the highest GM (0.032 mg/m³) was found in the largest firms (i.e., with more than 100 workers), which also represent the highest number of measurements recorded (n=12,821, see *Table 3*). Most of the notifications on exposure levels came from firms located in the North-West and North-East regions of Italy (43% each), but the highest average levels were reported by firms located in the North-West area (GM=0.041 mg/m³, see *Table 3*). As regards the estimate of potentially exposed workers based on ISTAT census data, approximately one hundred thousand workers in the selected activity sectors (see methods) were found to be potentially at risk of exposure to formaldehyde, most of whom were employed in the furniture manufacturing sector (NACE rev. 2 code: 31, 33,227 exposed workers, 33% of total workers potentially exposed). The sector of health activities, however, was found to have the highest percentage of female workers potentially exposed to formaldehyde (63%). Detailed data for the selected activity sectors are shown in *Table 4*.

DISCUSSION

This study presents data on occupational exposure to formaldehyde recorded in the Italian national register (SIREP) from 1996 to 2024. Formaldehyde exposure was found to be widespread across various economic sectors, including the wood, plastic, and chemical industries. However, its presence was also remarkable in the healthcare sector, as well as in metallurgy and in the manufacturing of metal products. A similar study has already been carried out in the past on the same database, but before the obligation to report occupational exposures to the national register came into force [7]. The differences between this study and the previous one, conducted when reporting of occupational exposure to formaldehyde was voluntary, are clear. Only

Table 1
Distribution of mean levels of formaldehyde exposure, overall, by gender and activity sector (SIREP, 1996-2024)

Activity sector (NACE rev. 2 code)	N	GM	GSD	IQR
Overall	*26,216*	*0.028*	*7.05*	*0.013-0.091*
Women	*4,169*	*0.024*	*12.42*	*0.011-0.100*
Human health activities (86)	1,344	0.024	14.32	0.010-0.100
Manufacture of rubber and plastic products (22)	742	0.044	3.27	0.020-0.100
Manufacture of wood and of products of wood and cork, except furniture (16)	478	0.057	6.37	0.014-0.187
Manufacture of furniture (31)	288	0.012	6.90	0.005-0.038
Manufacture of chemicals and chemical products (20)	218	0.026	7.76	0.008-0.070
Scientific research and development (72)	179	0.032	3.67	0.010-0.120
Other manufacturing (32)	170	0.022	1.79	0.014-0.027
Manufacture of fabricated metal products, except machinery and equipment (25)	109	0.023	13.54	0.012-0.100
Men	*22,047*	*0.029*	*6.20*	*0.013-0.090*
Manufacture of wood and of products of wood and cork, except furniture (16)	4,434	0.035	5.65	0.018-0.089
Manufacture of chemicals and chemical products (20)	3,872	0.031	5.09	0.015-0.080
Manufacture of furniture (31)	3,296	0.015	7.74	0.010-0.040
Manufacture of rubber and plastic products (22)	3,183	0.039	5.41	0.015-0.111
Manufacture of fabricated metal products, except machinery and equipment (25)	1,019	0.021	5.90	0.008-0.050
Manufacture of basic metals (24)	870	0.042	4.43	0.022-0.114
Retail trade, except motor vehicles, motorcycles (47)	745	0.050	4.43	0.020-0.370
Human health activities (86)	549	0.031	10.57	0.010-0.105
Manufacture of paper and paper products (17)	481	0.050	6.00	0.010-0.200
Manufacture of textiles (13)	447	0.025	4.20	0.015-0.050
Manufacture of machinery and equipment nec (28)	382	0.029	4.49	0.010-0.120
Manufacture of other non-metallic mineral products (23)	236	0.049	4.85	0.020-0.086
Land transport and transport via pipelines (49)	229	0.062	4.26	0.034-0.034
Other manufacturing (32)	216	0.032	3.23	0.011-0.071
Manufacture of other transport equipment (30)	209	0.013	2.87	0.008-0.016
Manufacture of motor vehicles, trailers, semi-trailers (29)	206	0.026	3.45	0.010-0.094
Printing and reproduction of recorded media (18)	194	0.024	2.36	0.016-0.024
Waste collection, treatment and disposal activities; materials recovery (38)	175	0.007	16.46	0.005-0.033
Wholesale trade, except motor vehicles, motorcycles (46)	156	0.022	4.28	0.010-0.050
Scientific research and development (72)	151	0.022	3.94	0.009-0.064
Manufacture of electrical equipment (27)	135	0.036	3.60	0.011-0.111
Manufacture of basic pharmaceutical (21)	125	0.030	4.96	0.010-0.120
Warehousing and support activities for transportation (52)	104	0.033	24.79	0.001-0.220

SIREP: Sistema Informativo Registri di Esposizione Professionale; N: number of 8-h time-weighted average (TWA-8) exposure measurements (mg/m^3); NACE: Nomenclature statistique des Activités économiques dans la Communauté Européenne; GM: geometric mean; GSD: geometric standard deviation; IQR: interquartile range, 25th-75th percentile; nec: not elsewhere classified.

one sector appears to be regularly reporting exposures even before 2016: the healthcare sector. The distribution of measurements by exposure levels (e.g., <0.1 mg/m^3) from the previous study is quite confirmed [7], in analogy also to that performed within the CARcinogen Exposure (CAREX) Canada study [8]. Furthermore, a significant increase in notifications can be noted in 2016 (*Figure 1*), going from just over 150 in 2014 to more than 3,700 measurements recorded in 2016. In terms of prevalence of exposure, our results are also in line with those of the CAREX Canada study, which identified 2% of high exposure, 28% of moderate exposure, and 70% of low exposure in 2016 [9].

The exposure levels presented here, as well as the distribution by sector of activity and occupation, are consistent with those found in other similar studies, as reported in a recent broad review study [10]. In the plastics industry, formaldehyde is used in various contexts, such as in the production of thermosetting phenolic resins and amino resins [3]. A formaldehyde-

Table 2
Distribution of mean levels of formaldehyde exposure by gender and occupational group (SIREP, 1996-2024)

Occupational group (ISCO-08 code)	N	GM	GSD	IQR
Women				
Medical and pathology laboratory technicians (3212)	542	0.027	16.67	0.010-0.133
Specialist medical practitioners (2212)	451	0.035	6.78	0.012-0.120
Woodworking-machine tool setters and operators (7523)	295	0.031	5.88	0.010-0.165
Plastic products machine operators (8142)	291	0.041	5.69	0.013-0.100
Life science technicians (excluding medical) (3141)	248	0.011	24.74	0.007-0.073
Manufacturing labourers nec (9329)	221	0.084	2.12	0.050-0.143
Wood processing plant operators (8172)	220	0.064	4.30	0.028-0.150
Cabinet-makers and related workers (7522)	205	0.011	15.53	0.002-0.074
Chemical and physical science technicians (3111)	171	0.016	5.49	0.007-0.065
Precision-instrument makers and repairers (7311)	163	0.020	1.48	0.014-0.027
Nursing associate professionals (3221)	127	0.019	3.35	0.007-0.046
Metal moulders and coremakers (7211)	101	0.061	2.82	0.050-0.100
Men				
Woodworking-machine tool setters and operators (7523)	2,952	0.017	9.49	0.010-0.049
Chemical products plant and machine operators (8131)	2,669	0.029	5.26	0.015-0.075
Plastic products machine operators (8142)	1,959	0.030	7.10	0.015-0.100
Cabinet-makers and related workers (7522)	1,905	0.017	8.20	0.007-0.057
Wood processing plant operators (8172)	1,447	0.051	3.06	0.038-0.092
Toolmakers and related workers (7222)	1,229	0.071	2.69	0.062-0.129
Metal moulders and coremakers (7211)	818	0.048	3.69	0.029-0.116
Agricultural/industrial machinery mechanic/repairer (7233)	750	0.037	3.98	0.020-0.120
Shop sales assistants (5223)	712	0.050	4.20	0.020-0.370
Assemblers nec (8219)	661	0.017	2.47	0.010-0.017
Metal finishing, plating, coating machine operators (8122)	553	0.011	5.60	0.004-0.036
Manufacturing labourers not elsewhere classified (9329)	437	0.066	3.85	0.040-0.148
Lifting truck operators (8344)	400	0.037	1.92	0.040-0.050
Painters and related workers (7131)	375	0.028	4.02	0.020-0.050
Chemical and physical science technicians (3111)	351	0.016	4.61	0.007-0.070
Bleaching/dyeing/fabric cleaning machine operators (8154)	299	0.027	2.99	0.015-0.050
Specialist medical practitioners (2212)	298	0.037	6.76	0.010-0.122
Printers (7322)	274	0.084	3.27	0.019-0.200
Heavy truck and lorry drivers (8332)	233	0.060	4.24	0.034-0.034
Electrical line installers and repairers (7413)	233	0.043	2.55	0.027-0.090
Medical and pathology laboratory technicians (3212)	193	0.032	12.25	0.010-0.150
Freight handlers (9333)	182	0.045	8.10	0.020-0.162
Metal processing plant operators (8121)	160	0.051	2.83	0.019-0.110
Pulp and papermaking plant operators (8171)	147	0.120	1.70	0.108-0.200
Stock clerks (4321)	144	0.032	5.92	0.015-0.100
Incinerator and water treatment plant operators (3132)	136	0.010	2.53	0.005-0.031
Precision-instrument makers and repairers (7311)	122	0.015	1.68	0.011-0.025
Manufacturing supervisors (3122)	115	0.037	4.02	0.015-0.089
Process control technicians nec (3139)	105	0.052	3.08	0.020-0.116
Chemists (2113)	104	0.002	129.9	0.003-0.050

SIREP: Sistema Informativo Registri di Esposizione Professionale ISCO: International standard classification of occupations; N: number of 8-h time-weighted average (TWA-8) exposure measurements (mg/m^3); GM: geometric mean; GSD: geometric standard deviation; IQR: interquartile range, 25th-75th percentile; nec: not elsewhere classified.

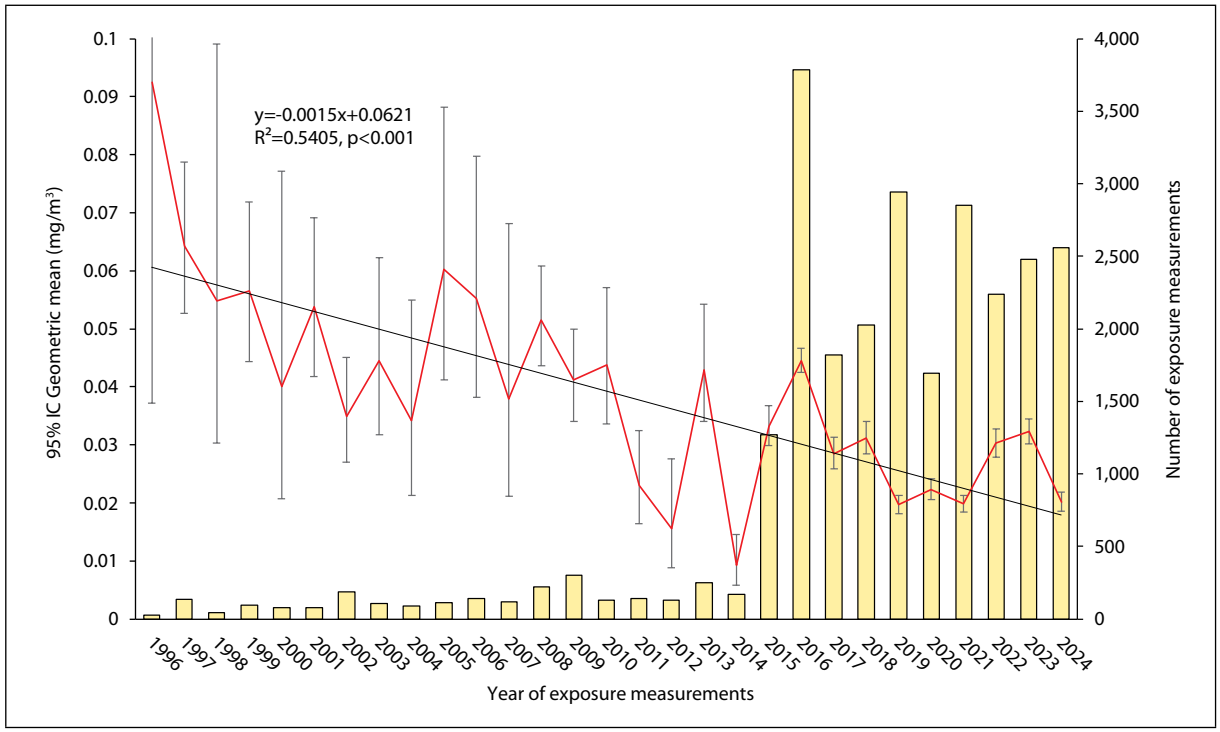


Figure 1
Temporal trend of formaldehyde exposure level and number of measurements by calendar year in Italy (Sistema Informativo Registri di Esposizione Professionale, SIREP 1996-2024); y: geometric mean; R2: coefficient of determination.

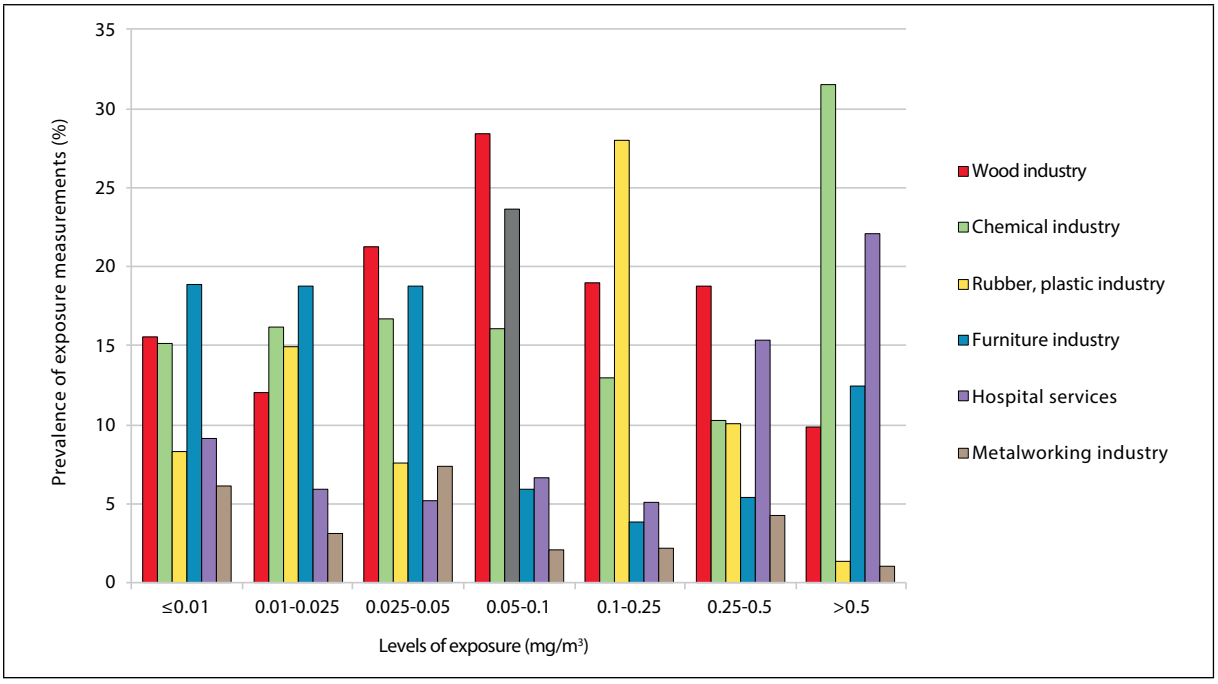


Figure 2
Distribution (%) of formaldehyde exposure measurements by value classes for the main activity sectors (Sistema Informativo Registri di Esposizione Professionale, SIREP 1996-2024).

induced toxicity study found significantly higher levels of exposure for workers in a plastic laminate industry compared to controls [11]. In the wood industry, formaldehyde finds wide application in the use of urea resins to produce particleboard, medium density fibreboard, and plywood [3]. In a cohort analysis of Finnish men exposed to wood dust and formaldehyde, workers exposed to formaldehyde had a relative risk of 1.18 (CI 95%=1.12-1.25) for lung cancer [12]. Woodworkers were exposed to significantly higher amounts of wood

Table 3
Distribution of mean levels of formaldehyde exposure by firm size and by Italian macro area (SIREP, 1996-2024)

Firm size/Macro area	N	GM	GSD	IQR
Firm size				
<10 workers	1,492	0.029	8.32	0.010-0.095
10-20 workers	2,474	0.018	10.47	0.007-0.060
20-50 workers	4,258	0.024	7.66	0.012-0.060
50-100 workers	5,171	0.029	6.96	0.011-0.092
>100 workers	12,821	0.032	6.07	0.016-0.100
Macro area of Italy				
North-West	11,186	0.041	5.26	0.020-0.106
North-East	11,170	0.021	8.62	0.010-0.063
Centre	2,480	0.026	4.58	0.010-0.064
South and Islands	1,380	0.023	12.75	0.014-0.091

SIREP: Sistema Informativo Registri di Esposizione Professionale.; N: number of 8-h time-weighted average (TWA-8) exposure measurements (mg/m^3); GM: geometric mean; GSD: geometric standard deviation; IQR: interquartile range, 25th-75th percentile.

dust and formaldehyde than controls in an oxidative stress induction study [13]. In the healthcare, formalin is used for its important preservative and cell-fixing properties, finding wide application in pathological anatomy [3]. However, our results showed lower mean (GM) values in this sector than in the other most affected industrial sectors (i.e., chemical, plastic and wood industries), in line with a health risk assessment study [14], probably due to a wider use of local exhaust ventilation [15]. A recent study on historical occupational exposure to formaldehyde in China found that most measurements came from the wood industry (e.g., production of veneer sheets and wood-based panels), and several occupational groups had elevated mean concentrations, including life sciences professionals, wood processing plant operators, and rubber and plastic-products machine operators [16]. A decreasing trend in mean exposure levels over time was also noted, and all of these findings are consistent with the main results of our study. The simultaneous increase in notifications and decrease in average exposure levels to formaldehyde observed in this study (*Figure 1*) could be attributed to several factors. Firstly, a reporting effect may be at play, where increased awareness and regulatory requirements encourage more frequent reporting of exposures, even as overall exposure levels decrease due to improved workplace practices. Methodological diversity in exposure measurement, including variations in sampling techniques and analytical methods over time, further complicates the interpretation of trends. Women represent a small but appreciable percentage of workers exposed to formaldehyde (about 16%), confirming the presence of a heavily gender segregation in the EU labor market across activity sectors and occupations [17]. In the healthcare sector, a prevalence of female workers was found (about 70% of total sector exposure measurements), while in other sectors a predominance of male workers was noted (a range between 80% and 95% of total sector exposure measurements). Noteworthy is the lack of data in the sector of other personal service activities (e.g., hairdressing and other beauty treatments), which is likely due to the ban on the direct use of formaldehyde in cosmetic products in the EU since 2019 [18]. Differences in distribution by geographical area of Italy may reflect the different distribution of industry types among Italian regions. The higher GM value for the North-West could be due to the high level of industrialization in that area of economic sectors at high risk for formaldehyde exposure resulting from our study, particularly in the industrial district between Milan, Turin, and Genoa [19]. The sectors identified as at high risk in this study may be the subject of information and training campaigns on prevention and protection measures aimed at raising awareness among workers and employers.

The estimated number of potentially exposed workers (about 100,000) is in line with the latest assessment under the CAREX Canada project (about 117,000 Canadians estimated to be exposed to formaldehyde at work in 2016), taking due account of the size and structure of the target population [9]. The difference between the estimate of this study and that of CAREX for Italy (about 115,000) could be due to the smaller number of sectors considered here and to the difference in the collection and estimation methods [20]. In the near future,

Table 4
Estimates of workers potentially exposed in the selected sector of economic activities (SIREP, 1996-2024)

Sector of economic activity (NACE rev. 2 code)	N. of firms[a]	% of firms[b]	N. of workers[c]	% of workers[d]	% of exposed[e]	N. of exposed[f]	% of men
Wood industry (16)	66	0.8	2,549	7.2	34.3	13,080	82.5
Chemical industry (20)	46	1.7	3,140	6.0	27.1	15,823	81.4
Rubber, plastic industry (22)	27	0.3	1,154	1.2	25.6	25,367	71.6
Metallurgical industry (24)	8	3.0	827	20.9	8.7	337	95.5
Metalworking industry (25)	34	0.8	1,108	3.1	28.1	10,161	77.0
Furniture industry (31)	85	0.6	3,507	3.1	29.8	33,227	73.5
Hospital services (86)	44	0.4	37,029	34.7	2.9	2,957	36.9

SIREP: Sistema Informativo Registri di Esposizione Professionale; NACE: Nomenclature statistique des activités économiques dans la Communauté européenne; [a]number of firms in SIREP; [b]percentage of firms in SIREP with respect to the latest industry census data; [c]number of workers reported by firms (exposed+non-exposed) in SIREP; [d]percentage of workers reported by firms in SIREP with respect to the latest industry census data; [e]percentage of exposed workers with respect to non-exposed workers reported by firms in SIREP; [f]estimated number of potentially exposed workers.

the information collected in SIREP will help us understand how the implementation of new EU regulations on the protection of workers' health from carcinogenic risks influences the application of primary prevention in the workplace, both in terms of reducing exposure levels and the number of exposed individuals.

Limitations and strengths

The heterogeneous territorial coverage and the limited representativeness in some economic activities are the two main limitations of the SIREP database, as already underlined in the previous study [7]. Activity sectors and workers occupations are represented differently within the SIREP database. Exposure measurements in some sectors and/or occupations, known to be at risk of formaldehyde exposure, appear to be poorly represented (e.g., construction where it is used in form of resins or adhesives, manufacture of textiles where it is used as a fixing and anti-creasing agent) [3]. Moreover, potential underreporting in less regulated sectors and in small and medium-sized enterprises (SMEs) could also have an impact, as these settings often lack systematic monitoring and may not report exposures as rigorously as larger, more controlled industries. The possibility that firms that do not record or report exposure data have higher exposure levels may have influenced our estimates. Uncertainty may also have been introduced due to differences in air sampling, analytical procedures, sample collection methods (personal or stationary) and data classification. For these reasons, to increase the precision of estimates, only sectors and occupations having more than 50 measurements recorded were included in the statistical analysis. Finally, only the sectors better characterized in SIREP were considered to estimate the number of workers potentially exposed. As a consequence of this selection, certain activity sectors were excluded due to limited information on the size of the reported workforce (e.g., manufacture of textile). The robustness of the estimate for each selected sector depends on how many firms and workers registered in SIREP match those recorded in the ISTAT national census. Higher percentages of firms (column three of *Table 4*) and workers (column five of *Table 4*) in SIREP indicate greater reliability of the estimate. Lastly, the number of exposed workers in each sector was calculated assuming the same ratio of exposed to non-exposed workers in firms reporting and not reporting exposure data to SIREP, which could lead to a possible over- or under-rating of the estimated percentage of potentially exposed workers. The main challenges in accurately assessing formaldehyde exposure and risk arise from insufficient sector-specific data, particularly in textiles and construction, where widespread exposure is expected but systematic monitoring appears to be lacking. Inadequate reporting and inconsistent monitoring protocols further hinder reliable assessment of exposure risk. The uncertainties identified in this study are likely to contribute to a moderate or severe underestimation of actual exposure. Insufficient sector-specific data and incomplete reporting/notification are the most severe, while lack of standardized monitoring protocols and workplace practice variability have a moderate impact on the possible underestimation. To refine the evaluation of the data presented in this study, corrective actions could include harmonising measurement methodologies and increasing the frequency and coverage of monitoring, particularly in SMEs and sectors with historically lower oversight. It is also recommended to standardize reporting systems and provide targeted training for both employers and workers on exposure recognition and reporting procedures.

CONCLUSIONS

In summary, this study confirms that formaldehyde exposure remains a key occupational health issue in Italy, particularly in healthcare, laboratory, and manufacturing sectors. The results underscore the need for stronger preventive measures, improved monitoring and reporting, and consistent regulation. Addressing uncertainties, such as exposure variability and data underreporting, will require more detailed risk assessments and focused research to better safeguard workers' health and guide policy. The estimates here realized can be useful for conducting sector- and occupation-specific surveys, confirming the SIREP information system on occupational exposure as a precious source of data. The inclusion of formaldehyde under the Directive n. 2004/37/EC, on the protection of workers from the risks related to exposure to carcinogens, as expected, has significantly improved the reporting system, allowing for better mapping and identification of work situations most at risk of exposure.

Conflict of interest statement

The Authors declare that they have no competing interests.

Funding

This work was supported by the Italian National Institute for Insurance against Accidents at Work (Istituto Nazionale per l'Assicurazione contro gli Infortuni sul Lavoro, INAIL).

Received on 3 December 2025.
Accepted on 30 January 2026.

REFERENCES

1. National Toxicology Program. Report on carcinogens: formaldehyde (15th ed.). Durham, NC: Research Triangle Park – US Department of Health and Human Services; 2021. doi: https://doi.org/10.22427/NTP-OTHER-1003
2. World Health Organization. WHO guidelines for indoor air quality. Selected pollutants. Copenhagen: WHO Regional Office for Europe; 2010.
3. International Agency for Research on Cancer. Formaldeyde. Lyon: IARC; 2012. (IARC monographs on the evalua-

tion of carcinogenic risks to humans, 100F). p. 1-36.
4. Scarselli A, Montaruli C, Marinaccio A. The Italian information system on occupational exposure to carcinogens (SIREP): structure, contents and future perspectives. Ann Occup Hyg. 2007;51:471-8. doi: 10.1093/annhyg/mem022
5. European Committee for Standardization. Workplace atmospheres – Guidance for the assessment of exposure by inhalation to chemical agents for comparison with limit values and measurement strategy (EN 689). Brussels: CEN; 2018.
6. Istituto Nazionale di Statistica. 9th industry and services census. Rome: ISTAT; 2011. Available from: https://www.istat.it/statistiche-per-temi/censimenti/censimenti-storici/industria-e-servizi/imprese-2011/
7. Scarselli A, Corfiati M, Di Marzio D, Iavicoli S. National estimates of exposure to formaldehyde in Italian workplaces. Ann Work Expo Health. 2017;61:33-43. doi: 10.1093/annweh/wxw004
8. Peters CE, Calvin BG, Hall AL, Davies HW, Demers PA. CAREX Canada: an enhanced model for assessing occupational carcinogen exposure. Occup Environ Med. 2015;72:64-71. doi: 10.1136/oemed-2014-102286
9. CARcinogen EXposure Canada. Formaldehyde occupational exposures. Vancouver, BC: CAREX; 2025. Available from: https://www.carexcanada.ca/profile/formaldehyde-occupational-exposures
10. Cammalleri V, Pocino RN, Marotta D, Protano C, Sinibaldi F, Simonazzi S, Petyx M, Iavicoli S, Vitali M. Occupational scenarios and exposure assessment to formaldehyde: A systematic review. Indoor Air. 2022;32:e12949. doi: 10.1111/ina.12949
11. Bono R, Munnia A, Romanazzi V, Bellisario V, Cellai F, Peluso MEM. Formaldehyde-induced toxicity in the nasal epithelia of workers of a plastic laminate plant. Toxicol Res (Camb). 2016;5:752-60. doi: 10.1039/c5tx00478k
12. Siew SS, Kauppinen T, Kyyrönen P, Heikkilä P, Pukkala E. Occupational exposure to wood dust and formaldehyde and risk of nasal, nasopharyngeal, and lung cancer among Finnish men. Cancer Manag Res. 2012;4:223-32. doi: 10.2147/CMAR.S30684
13. Ghelli F, Bellisario V, Squillacioti G, Grignani E, Garzaro G, Buglisi M, Bergamaschi E, Bono R. Oxidative stress induction in woodworkers occupationally exposed to wood dust and formaldehyde. J Occup Med Toxicol. 2021;16:4. doi: 10.1186/s12995-021-00293-4
14. Soltanpour Z, Mohammadian Y, Fakhri Y. The exposure to formaldehyde in industries and health care centers: A systematic review and probabilistic health risk assessment. Environ Res. 2022;204(Pt B):112094. doi: 10.1016/j.envres.2021.112094
15. d'Ettorre G, Criscuolo M, Mazzotta M. Managing formaldehyde indoor pollution in anatomy pathology departments. Work. 2017;56:397-402. doi: 10.3233/WOR-172505
16. Nie J, Ge CB, Rothman N, Hu W, Lan Q, Vermeulen R, Peters S. Overview of historical formaldehyde occupational exposure in China. Ann Work Expo Health. 2025;69:808-19. doi: 10.1093/annweh/wxaf037
17. Eurofound and European Commission Joint Research Centre. European jobs monitor 2021: gender gaps and the employment structure. Luxembourg: Publications Office of the European Union; 2021. (European jobs monitor series).
18. European Commission. Commission Regulation (EU) n. 2022/1181 of the European Union, 8 July 2022 amending the preamble of Annex V to Regulation n. 1223/2009 of the European Parliament and of the Council on cosmetic products. Official Journal of the European Union L 184/3, 11 July 2022.
19. Istituto Nazionale di Statistica. Rapporto sul territorio 2020. Ambiente economia e società. Rome: ISTAT; 2020. (Letture statistiche, temi). doi: 10.1481/Istat.Rapporto-territorio.2020
20. Kauppinen T, Toikkanen J, Pedersen D, Young R, Ahrens W, Boffetta P, Hansen J, Kromhout H, Maqueda Blasco J, Mirabelli D, de la Orden-Rivera V, Pannett B, Plato N, Savela A, Vincent R, Kogevinas M. Occupational exposure to carcinogens in the European Union. Occup Environ Med. 2000;57:10-8. doi: 10.1136/oem.57.1.10

Ann Ist Super Sanità 2026 | Vol. 62, No. 2: 130-140
DOI: 10.4415/ANN_26_02_05

ORIGINAL ARTICLES AND REVIEWS

Pain prevalence and severity in an Italian university hospital: a cross-sectional study

Martino Bussa[1], Federico Ambrogi[2], Paola Rebora[1], Aurelia D'Acquisto[3] and Maria Teresa Cuppone[3]

[1]*Dipartimento di Medicina e Chirurgia, Università degli Studi di Milano-Bicocca, Milan, Italy*
[2]*Dipartimento di Scienze Cliniche e di Comunità, Università degli Studi di Milano La Statale, Milan, Italy*
[3]*Istituto di Ricovero e Cura a Carattere Scientifico Policlinico San Donato, San Donato Milanese, Milan, Italy*

Abstract

Background. Pain is highly prevalent among hospitalised adults, and in Italy Law 38/2010 mandates its assessment and relief. We aimed to estimate pain prevalence and severity among adult inpatients and identify factors associated with moderate-to-severe pain and satisfaction with pain management in a university hospital.

Methods. We conducted a cross-sectional survey in May 2023 at IRCCS Policlinico San Donato using a structured questionnaire in adult inpatients hospitalised for at least 24 hours. Multivariable logistic regression explored factors associated with pain severity and satisfaction.

Results. Among the 229 patients interviewed, 84% reported pain at the time of the interview, with 52.4% experiencing moderate pain and 9.6% severe pain. Pain in the previous 24 hours was associated with moderate-to-severe pain at the time of the interview and lower satisfaction with care. Thirty-two percent were aware of Law 38/2010, and awareness was associated with lower pain severity and higher satisfaction.

Conclusions. Pain remains highly prevalent among adult inpatients. Experiencing pain in the previous 24 hours was associated with greater severity and lower satisfaction, suggesting the importance of timely and consistent pain assessment and relief throughout hospitalisation.

Key words

- pain
- prevalence
- pain measurement
- patient satisfaction

INTRODUCTION

Pain is an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage [1]. Several studies have shown that pain is a common health issue across European countries [2-9]. The World Health Survey (WHS) [10] reported a pain prevalence of 34% in Europe. Persistent pain can lead to adverse health outcomes, including depression [11, 12], cognitive decline [13-15], premature death [16], and negatively impact quality of life [17].

Pain is also a common symptom among hospitalised patients, with up to 36% of adult patients reporting severe pain [18]. Beyond causing personal suffering, pain is associated with prolonged hospital stays, increasing the burden on healthcare systems [19-21]. A Swedish cross-sectional study by Wadenstern *et al*. [22] revealed that 65% (494/759) of hospitalised patients experienced pain during the previous 24 hours, with 42% rating the intensity as severe. Another cross-sectional study, conducted at two Swedish hospitals in 2014, found that 65.2% of 710 patients experienced pain, while 34% (243/710) reported both pain and fatigue distress with a score greater than 3 on an 11-point numerical rating scale (NRS) at the time of survey. More recently, a cross-sectional study conducted in a Spanish university hospital documented a pain prevalence of 52.9% (145/274) during the hospitalisation [23]. Furthermore, evidence from outside Europe indicates that pain is a pervasive issue in hospitals worldwide [24]. A recent retrospective study conducted in Taiwan reviewed the medical records of 73,814 inpatients, finding a pain prevalence of 48.1% during the hospitalisation period and of 46.5% on the day of discharge [25]. This underscores that pain is a global challenge in healthcare.

Despite its clinical relevance, few studies have evaluated the prevalence of pain among hospitalised patients in Italy, where pain assessment and management are

Address for correspondence: Martino Bussa, Dipartimento di Medicina e Chirurgia, Università degli Studi di Milano-Bicocca, Piazza dell'Ateneo Nuovo 1, 20126 Milan, Italy. E-mail: m.bussa@campus.unimib.it.

mandatory. Additionally, these studies were limited to specific geographical areas or particular patient groups [26, 27]. Visentin conducted the first national survey on 4,523 hospitalised patients in 20 hospitals predominantly located in Northern Italy, finding that 91% of patients (3,575/3,931) reported pain at interview, with 46.6% experiencing severe pain. A few years later, Costantini conducted a regional cross-sectional survey, reporting a prevalence of pain at 43.1% among adults (1,750/4,064) at the time of the interview, with 11.7% (477/4,064) reporting a pain score ≥7 on the NRS. However, even after the introduction of Law 38/2010 [28], which "guarantees citizens the right to access palliative care and pain management", few studies have assessed the prevalence and management of pain in Italian hospitals [29]. This lack of data limits the ability to assess the effectiveness of the law in improving pain control and management in hospitalised patients.

This study aimed to estimate the pain prevalence and severity, and to identify factors associated with moderate-to-severe pain, as well as patient satisfaction with pain management among hospitalised adults in an Italian university hospital, following the implementation of Law 38/2010.

MATERIAL AND METHODS

Study design and setting

This cross-sectional study was conducted at IRCCS Policlinico San Donato, a large high-specialisation teaching hospital in Northern Italy. Data collection was carried out on three non-consecutive days starting on 12 May 2023. The hospital's three main inpatient wards – surgery, medicine, and rehabilitation – encompassed over 270 beds and represented the target setting for data collection. All patients aged 18 years or older, hospitalised for at least 24 hours, and able to participate in an interview were considered eligible. Exclusion criteria included cognitive impairment, significant sensory-motor deficits, linguistic barriers, and admission to critical care units or the emergency department. Participation was voluntary, and all data were collected anonymously and treated confidentially. The study protocol was approved by the Ethics Committee of San Raffaele Hospital on 15 March 2023 (CE: 34/INT/2023). The research was carried out according to the Declaration of Helsinki.

Survey instrument

A structured questionnaire was developed to assess patients' pain experience, satisfaction with pain control, and to identify specific needs for pain relief during hospitalisation. The tool included 15 items organised into two sections. The first section collected socio-demographic information such as age group, gender, ethnicity, marital status (single, married, divorced, widowed), educational attainment (none, primary school, middle school, high school, university), area of residence (urban, rural), and awareness of Italian Law 38/2010 [28] on the right to palliative care and pain relief (yes/no). The second section assessed clinical characteristics including length of hospital stay (days), ward of admission, pain intensity at the time of the interview (NRS), presence of pain in the past 24 hours (yes/no), the primary reason for hospitalisation, and satisfaction with pain control (5-point Likert scale). A multiple-choice item also asked patients what they would need at that moment to find relief.

The questionnaire was developed pragmatically, based on routine clinical practice in hospital pain assessment, and included items already used in previous pain prevalence surveys [22, 30]. Items were selected to ensure clarity and feasibility of administration at the patient's bedside, while capturing key aspects of the pain experience relevant to care needs.

To ensure a standardised interview process, the survey was administered in person by trained physicians, who explained the study objectives and obtained verbal informed consent from all participants, and caregivers were involved only when necessary.

Outcome measures

The primary outcomes were the presence and the severity of pain at the time of the interview. Pain intensity was self-reported using the 11-point NRS [31-33], from 0 (no pain) to 10 (worst imaginable pain). Pain was categorised as mild (1-3), moderate (4-6), and severe (7-10). Secondary outcomes included pain reported in the past 24 hours, satisfaction with pain management, awareness of Law 38/2010, and patient-reported preferences for pain relief.

Statistical analysis

Descriptive statistics were reported as frequencies and percentages for categorial variables, and as medians with first and third quartiles (Q1, Q3) for numerical variables. Group differences were assessed using the Wilcoxon rank-sum test or the Kruskal-Wallis test for continuous variables, and the Chi-square test or Fisher's exact test for categorical variables, as appropriate.

To identify factors associated with pain, logistic regression models were applied dichotomising pain severity as moderate-to-severe (NRS≥4) *versus* none-to-mild (NRS≤3). Candidate predictors were selected based on evidence from the literature and clinical plausibility, including age, gender, ethnicity, educational attainment (categorised as lower: none, primary, or middle school; and higher: high school or university), clinical ward, length of stay, and pain in the previous 24 hours. In addition, knowledge of Law 38/2010, a novel predictor hypothesised to influence pain reporting and satisfaction, was also included. The clinically driven approach was complemented by sensitivity analyses using information criteria (Akaike Information Criterion, AIC) and penalized likelihood methods, specifically least absolute shrinkage and selection operator (LASSO) regression. A similar modelling approach was applied to investigate factors associated with satisfaction with pain management. Pain satisfaction was dichotomised as high satisfaction (including *very satisfied* and *extremely satisfied*) *versus* low or no satisfaction (including *not satisfied at all*, *slightly satisfied and satisfied*). Logistic regression models were used to identify factors independently associated with high satisfaction levels. Results were expressed as odds ratios (ORs) with corresponding 95% confidence intervals (CIs). A p-value <0.05 was considered signifi-

cant. All analyses were performed using R software version 4.1.2.

RESULTS

Demographic and clinical characteristics of patients

A total of 262 questionnaires were distributed to patients hospitalised in the Departments of Cardiology, Internal Medicine, Cardiac Surgery, Urology, Orthopaedics, and Rehabilitation. Twenty-eight patients were excluded due to sedation or language barriers. Of the remaining 234 patients, 229 completed the questionnaire, which yielded a response rate of 98% (229/234). Among respondents, 28.8% (66/229) completed the questionnaire independently, 64.6% (148/229) were assisted by healthcare professionals, and 6.6% (15/229) by family members. *Table 1* summarises the demographic and clinical characteristics, with further details in *Supplementary Table 1 available online*. Most respondents (59%, 136/229) were aged between 48 and 76 years, with a slight predominance of males (53.7%, 123/229). Over half were married (55.5%, 127/229), and 51% (117/229) had lower educational attainment. The sample was predominantly Caucasian (96%, 219/229). At the time of the interview, the median length of hospitalisation was 5 days (Q1-Q3: 3.0-8.0). Regarding ward distribution, 50.7% (116/229) of patients were admitted to surgical wards, 26.6% (61/229) to medical wards, and 23% (52/229) to rehabilitation wards. Cardiovascular diseases (42.9%) and musculoskeletal disorders (30.6%) were the most frequently reported primary diagnoses.

Pain prevalence, pain severity and factors associated with moderate-to-severe pain

Among the 229 patients included in the analysis, 84% (192/229) reported experiencing pain at the time of the interview (NRS>0), with 21.8% reporting mild pain, 52.4% moderate pain, and 9.6% severe pain. Conversely, 16% (37/229) reported no pain (NRS=0). Pain experienced in the past 24 hours was reported by 52% of inpatients (119/229).

Pain severity was higher among older patients (>76 years), with a median NRS score of 5 (Q1-Q3: 3-7) compared to those aged ≤76 years (median 5, Q1-Q3: 2-6; p=0.01). Similarly, patients hospitalised in surgery wards reported greater pain intensity (median 5, Q1-Q3: 3-7) than those in medical (median 4, Q1-Q3: 1-6) and rehabilitation wards (median 5, Q1-Q3: 2-6; p=0.01).

Pain levels were also higher among widowed or divorced individuals (median 6, Q1-Q3: 3-7) compared to unmarried individuals (median 4, Q1-Q3: 0-5; p=0.01), and among those with lower educational attainment (median 5, Q1-Q3: 3-7) compared to those with higher education (median 4, Q1-Q3: 1-6; p=0.03). Pain severity did not differ significantly by gender (*Table 2*). Women reported slightly higher NRS scores than men: median 5 (Q1-Q3: 2-7) *vs* 4 (1-6), p=0.11. No significant associations were found between pain severity and ethnicity or area of residence (*Table 2*).

Patients who had experienced pain in the past 24 hours reported higher pain levels (median 5, Q1-Q3: 3-7) than those who had not (median 4, Q1-Q3: 0-6; p=<0.01). Conversely, lower pain intensity was observed among patients aware of the Law 38/2010 (median 3, Q1-Q3: 1-6) compared to those unaware (median 5, Q1-Q3: 2-7; p=0.01).

Table 1
Demographic and clinical characteristics of patients

Patients' characteristics	Number of patients (N=229)	%	
Age			
18-47 years	27	12.0	
48≤76 years	136	59.0	
>76 years	66	29.0	
Gender			
Men	123	53.7	
Women	106	46.3	
Ethnicity			
Caucasian	219	95.6	
Non-Caucasian	10	4.4	
Marital Status			
Single	47	20.5	
Married	127	55.5	
Divorced/widowed	55	24.0	
Education[1]			
Higher	112	49.0	
Lower	117	51.0	
Residence			
Rural	70	30.6	
Urban	158	69.0	
Missing	1	0.40	
Wards			
Surgery	116	50.7	
Medicine	61	26.6	
Rehabilitation	52	22.7	
Disease for admission			
Cancer	9	3.90	
Cardiovascular	96	42.9	
Gastrointestinal	23	10.40	
Respiratory	6	2.60	
Genito-urinary	20	8.70	
Muscoloskeletal	70	30.6	
Other	4	1.70	
Missing	1	0.40	
Knowledge of the Law 38/2010			
No	155	67.6	
Yes	73	32.0	
Missing	1	0.40	
	Median	**Q1, Q3**	**Missing (%)**
Days from admission	5	3, 8	2 (0.9)

[1]Education was categorised as lower (none, primary, or middle school) and higher (high school or university); Q1: first quartile; Q3: third quartile.

Table 2
Pain severity (NRS) at the interview by demographic and clinical characteristics (N=229)

Patients' characteristics	NRS Median (Q1, Q3)	p-value[1]
Age		
≤76 years	5 (2, 6)	
>76 years	5 (3, 7)	0.01
Gender		
Men	4 (1, 6)	
Women	5 (2, 7)	0.11
Ethnicity		
Non-Caucasian	2 (0, 5)	
Caucasian	5 (2, 6)	0.06
Marital status[2]		
Unmarried	4 (0, 5)[a,b]	
Married	5 (2, 6)[c]	
Divorced/widowed	6 (3, 7)	0.01
Education		
Higher	4 (1, 6)	
Lower	5 (3, 7)	0.03
Residence		
Rural	5 (2, 7)	
Urban	5 (2, 6)	0.60
Wards[3]		
Surgery	5 (3, 7)[a,b]	
Medicine	4 (1, 6)[c]	
Rehabilitation	5 (2, 6)	0.01
Pain in the past 24 hours		
No	4 (0, 6)	
Yes	5 (3, 7)	<0.01
Knowledge of the Law 38/2010		
No	5 (2, 7)	
Yes	3 (1, 6)	0.01

NRS: numerical rating scale; Q1: first quartile; Q3: third quartile; [1] Wilcoxon test or Kruskal-Wallis Rank Test. [2]Post-hoc pairwise comparisons; p-values were adjusted using the Benjamini-Hochberg method: [a]group unmarried *vs* married p=0.08; [b]group unmarried *vs* divorced/widowed p=0.01; [c]group married *vs* divorced/widowed p=0.12. [3]Post-hoc pairwise comparisons; p-values were adjusted using the Benjamini-Hochberg method: [a]group surgery *vs* medicine p=0.01; [b]group surgery *vs* rehabilitation p=0.05; [c]group medicine *vs* rehabilitation p=0.64.

Factors associated with moderate-to-severe pain are presented in *Table 3*. No significant associations were found with age, gender, ethnicity, marital status, educational attainment, area of residence, and hospital ward. Male patients were less likely to report moderate-to-severe pain than females, although the association was not significant (adjusted OR 0.62, 95% CI: 0.34-1.12, p=0.12). Similarly, older patients (aged >76 years) had higher odds of reporting moderate-to-severe pain compared with those aged ≤76 years, but this association was also not significant (adjusted OR 1.22, 95% CI: 0.62-2.42, p=0.60). In contrast, experiencing pain in the past 24 hours was significantly associated with moderate-to-severe pain (adjusted OR 2.17, 95% CI: 1.21-3.94, p=0.01). A hospital stay of 4-6 days was also associated with greater odds of moderate-to-severe pain (adjusted OR 2.87, 95% CI: 1.29-6.70, p=0.01), whereas stays of ≥7 days were not (adjusted OR: 0.93, 95% CI: 0.48-1.77, p=0.80). Overall, the association between length of hospital stay and pain severity was significant (p=0.03).

Knowledge of the Law 38/2010

A total of 68% of respondents (155/229) reported being unaware of the law. Patients who were aware of Law 38/2010 had lower odds of experiencing moderate-to-severe pain at the time of the interview (adjusted OR 0.47, 95% CI: 0.25-0.88; p=0.02).

Patient satisfaction and preferences for relief

Most patients reported a high level of satisfaction with pain management: 32% (73/229) stated being satisfied, 45% (104/229) very satisfied, and 14% (32/229) extremely satisfied. A smaller proportion reported being slightly satisfied (7%, 16/229) or not satisfied at all (2%, 4/229). As shown in *Table 4*, patients who were aware of Law 38/2010 had significantly higher odds of reporting high satisfaction with pain control compared to those who were unaware (adjusted OR 2.33, 95% CI: 1.26-4.43; p=0.01). Conversely, experiencing pain in the past 24 hours was associated with lower odds of reporting high satisfaction (adjusted OR 0.52, 95% CI: 0.30-0.90; p=0.02). Older age (>76 years) was associated with reduced odds of high satisfaction (adjusted OR 0.49, 95% CI: 0.26-0.91; p=0.03).

Gender, ethnicity, educational attainment, area of residence, and length of hospitalisation were not associated with patient satisfaction. Patients admitted to rehabilitation had higher odds of reporting high satisfaction compared to those in surgical wards (crude OR 1.59, 95% CI: 0.80-3.24, p=0.20), although this association was not significant.

Figure 1 shows the overall preferences expressed by patients who reported pain, as well as preferences stratified by pain severity. Since patients were allowed to select multiple options, the reported percentages reflect the proportion of respondents endorsing each need. The most commonly reported need was the desire to feel less pain (84.2%), followed by the presence of loved ones (72%) and the need for a comfortable care environment (65.1%). Additionally, 62.6% of patients expressed a need for psychological and emotional support, while 44.4% reported a desire for clear and complete information on pain management. Only 15.9% expressed a desire to receive an opioid. When stratified by pain severity, patients experiencing moderate-to-severe pain expressed a need for pain relief (90% *vs* 67%) and for psychological and emotional support (68% *vs* 48%) compared to those with mild pain. The presence of loved ones was considered important by both groups (70% *vs* 79%). Differences in preferences regarding a comfortable care environment, autonomy in treatment decisions, access to clear information on pain management, and the option to receive an opioid were less marked between the two groups.

DISCUSSION

Pain remains a common challenge among hospitalised patients [34], with prevalence rates in the literature ranging from 38% [35] to 84% [36]. In our study, 84% of patients reported pain at the time of the interview, a figure consistent with findings from a multicentre hospital survey conducted in the Emilia-Romagna region [30]. When compared with that survey, our data

Table 3
Factors associated with pain severity at the interview in hospital. Results from logistic models comparing moderate-to-severe pain (N=142) *versus* mild/no pain (N=87)

	OR_c	95% CI	p-value	OR_a	95% CI	p-value
Age						
≤76 years	Ref.			Ref.		
>76 years	1.61	0.88-3.00	0.13	1.22	0.62-2.42	0.60
Gender						
Women	Ref.			Ref.		
Men	0.67	0.39-1.15	0.20	0.62	0.34-1.12	0.12
Ethnicity						
Non-Caucasian	Ref.			Ref.		
Caucasian	2.56	0.71-10.2	0.20	3.26	0.75-17.1	0.13
Marital status						
Unmarried	Ref.					
Married	1.36	0.69-2.66	0.40			
Divorced/widowed	2.35	1.04-5.44	0.04			
Education[1]						
Higher	Ref.			Ref.		
Lower	1.62	0.95-2.78	0.08	1.46	0.80-2.67	0.20
Residence						
Rural	Ref.					
Urban	0.88	0.49-1.58	0.70			
Wards						
Surgery	Ref.					
Medicine	0.61	0.32-1.16	0.13			
Rehabilitation	0.66	0.34-1.31	0.20			
Days from admission						
1-3 days	Ref.			Ref.		
4-6 days	2.52	1.20-5.53	0.02	2.87	1.29-6.70	0.01
≥7 days	1.04	0.57-1.91	0.90	0.93	0.48-1.77	0.80
Pain in the past 24 hours						
No	Ref.			Ref.		
Yes	2.15	1.25-3.73	0.01	2.17	1.21-3.94	0.01
Knowledge of the Law 38/2010						
No	Ref.			Ref.		
Yes	0.45	0.25-0.79	0.01	0.47	0.25-0.88	0.02

[1]Education was categorised as lower (none, primary, or middle school) and higher (high school or university); OR_c: crude odds ratio; OR_a: adjusted odds ratio; N: number of patients in each pain category; Ref.: reference category; CI: confidence interval.

showed a higher proportion of patients reporting moderate pain (52.4% *vs* 37.1%), and a lower proportion reporting severe pain (9.6% *vs* 15.9%). While not directly measured, this reduction in severe pain may be indicative of improved pain assessment and management in recent years, in line with ministerial and institutional guidelines.

A total of 52% of patients reported experiencing pain in the 24 hours preceding the interview, a proportion lower than found in other studies, including Strohbuecker [37] (63%), Abbot [38] (67%), Quattrin [39] (69%) Costantini [40] (57%) and Das [41] (71%). This difference may reflect contextual variability across healthcare settings or improvements in pain management practices following the introduction of Law 38/2010, although these aspects were not directly assessed. Importantly, our findings showed that experiencing pain in the past 24 hours was significantly associated with a twofold increase in the odds of moderate-to-severe pain (adjusted OR 2.17; 95% CI: 1.21-3.94, p=0.01), suggesting the importance of timely and effective pain control.

This study is one of the few surveys conducted in Italy on the prevalence of pain among hospitalised patients since the introduction of Law 38/2010 [28]. Approved

Table 4
Factors associated with patient satisfaction with pain management. Results from logistic models comparing high/very high satisfaction (N=136) low/no satisfaction (N=93)

	OR_c	95% CI	p-value	OR_a	95% CI	p-value
Age						
≤76 years	Ref.			Ref.		
>76 years	0.53	0.30-0.95	0.03	0.49	0.26-0.91	0.03
Gender						
Women	Ref.			Ref.		
Men	1.00	0.59-1.69	>0.90	1.01	0.57-1.76	>0.90
Ethnicity						
Non-Caucasian	Ref.			Ref.		
Caucasian	1.50	0.40-5.50	0.50	1.93	0.48-7.86	0.30
Marital status						
Unmarried	Ref.					
Married	1.00	0.49-1.99	>0.90			
Divorced/widowed	0.47	0.21-1.04	0.07			
Education[1]						
Higher	Ref.			Ref.		
Lower	1.12	0.66-1.89	0.70	1.54	0.86-2.77	0.20
Residence						
Rural	Ref.					
Urban	0.95	0.53-1.69	0.90			
Wards						
Surgery	Ref.					
Medicine	0.78	0.42-1.46	0.40			
Rehabilitation	1.59	0.80-3.24	0.20			
Days from admission						
1-3 days	Ref.					
4-6 days	0.99	0.49-1.98	>0.90			
≥7 days	1.03	0.56-1.90	>0.90			
Pain in the past 24 hours						
No	Ref.			Ref.		
Yes	0.49	0.28-0.84	0.01	0.52	0.30-0.90	0.02
Knowledge of the Law 38/2010						
No	Ref.			Ref.		
Yes	2.15	1.20-3.96	0.01	2.33	1.26-4.43	0.01

[1]Education was categorised as lower (none, primary, or middle school) and higher (high school or university); OR_c: crude odds ratio; CI: confidence interval; OR_a: adjusted odds ratio; N: number of patients in each pain category; Ref.: reference category; CI: confidence interval.

in March 2010, this law is unique in Europe for guaranteeing citizens the right to adequate pain management, involving not only specialist and general physicians but also nurses and psychologists, and for recognising pain not merely as a symptom, but as a condition in its own right. To date, most European countries have issued guidelines or national strategies on pain, but none has introduced legislation as comprehensive and binding as Italy's Law 38/2010. Notably, no prevalence studies on pain in hospitalised patients have been conducted during the COVID-19 pandemic, as healthcare and research priorities shifted toward emergency response and infection control. These aspects underscore the relevance of our findings both within the Italian context and in the broader European debate on pain management.

A significant association was found between the length of hospital stay and pain intensity in our cohort. This finding is consistent with the results reported by Li-Ying *et al.* [25], who observed that longer hospital stays were associated with higher pain levels at discharge. Persistent, intensive pain can activate complex peripheral and central mechanisms, potentially leading to the transition from acute to chronic pain [42-44]. Ensuring

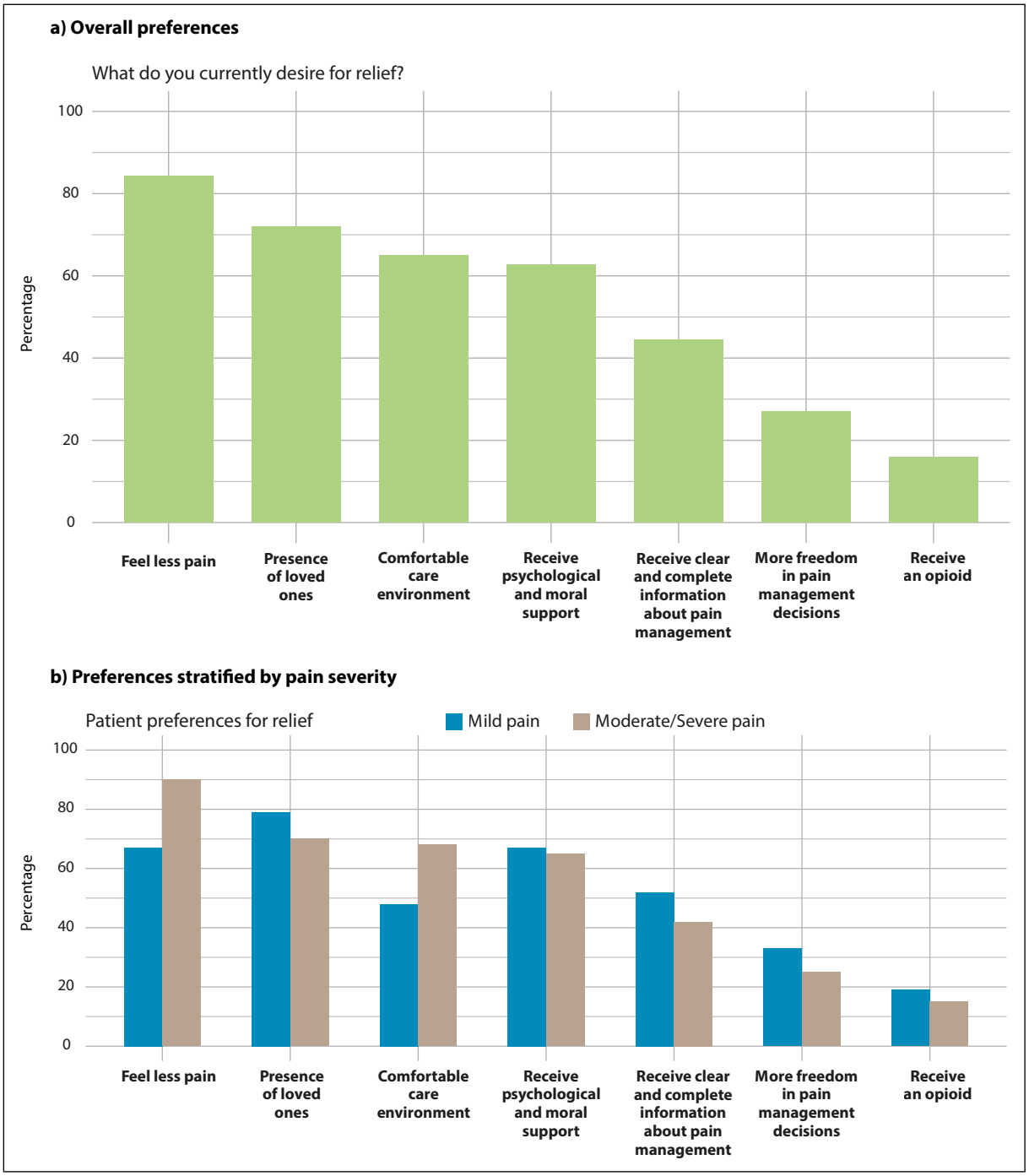


Figure 1
Patient preferences for relief among those reporting pain (N=192)[1].
[1]There are between two to three missing responses for each variable. N: number of patients reporting pain at the time of the interview.

effective pain control during hospitalisation is therefore essential to prevent the development of chronic pain.

Demographic factors, such as gender and age, are well-documented predictors of pain in the literature [29, 35, 40, 45-47]. However, our study did not identify significant gender differences in pain prevalence or intensity ratings. Although women frequently report higher pain intensity across a wide range of clinical and surgical contexts [48], the literature on gender differences in pain severity remains complex and sometimes contradictory. For example, Damico *et al*. [29] found that women were at a higher risk of experiencing pain than men (RR 1.59; 95% CI: 1.29-1.95), with similar findings by Costantini *et al*. [40] (adjusted OR 1.40; 95% CI: 1.23-1.58) and Vallano *et al*. [45] (adjusted OR 1.37; 95% CI: 1.11-1.70). In contrast, Visentin *et al*. [49] reported a lower risk of intense pain in women (adjusted OR 0.80; 95% CI: 0.71-0.92). Biological, psychological,

and socio-cultural factors are thought to contribute to these discrepancies [50]. Social factors, such as child-rearing practices, media portrayals of gender roles, and social learning can shape pain-related behaviours and perceptions [51]. Stereotypes often depict women as more expressive and men as more stoic in their response to pain, which may influence individuals' willingness to report pain and how it is perceived by others [51-53]. In this regard, Falk *et al*. [54] observed that women need to report more intense levels of pain and other distress symptoms for their complaints to be recognised and documented by healthcare professionals.

Regarding age, our analysis showed that patients over 76 years reported significantly higher NRS pain scores compared to those ≤76 years old, based on non-parametric tests. However, logistic regression did not confirm a significant association with moderate-to-severe pain, possibly due to the limited number of older patients. Evidence from the literature remains inconsistent. Li-Ying *et al*. [25] reported a negative correlation between age and pain intensity at discharge. Conversely, Costantini *et al*. [40], found no association between age and pain at the interview, while Vallano *et al*. [45] observed that younger adults (18-39 years) were more likely to report pain than those over 70 years (adjusted OR 1.65; 95% CI: 1.21-2.26). These discrepancies highlight the complexity of age-related differences in pain perception and emphasise the need for further research in more age-balanced samples.

Another important sociodemographic factor considered in our study was educational attainment [55]. Lower educational attainment has been conceptualised as a marker of maladaptive coping strategies that influences the pain experience, and is associated with pain-related beliefs, catastrophizing, and low perceived control [56]. In our study, lower educational attainment was associated with a non-significant increase in the odds of moderate-to-severe pain (adjusted OR 1.46, 95% CI: 0.80-2.67; p=0.20). This finding may reflect limited sample size, which reduces statistical power, as well as the dichotomous classification of educational attainment, which may have obscured more subtle gradients across educational levels.

However, a more specific dimension of knowledge, awareness of Law 38/2010 was significantly associated with a lower risk of moderate-to-severe pain (adjusted OR 0.47, 95% CI: 0.25-0.88; p=0.02), independently of age, gender and educational attainment.

Regarding ward type, our results showed no significant association between ward type and pain intensity. This contrasts with findings from other surveys. For example, Zoega *et al*. [46] observed a higher prevalence of pain in surgical wards compared to medical wards (90% *vs* 80%, p=0.028), while Vallano *et al*. [45] found that patients in orthopaedic and rehabilitation wards were more likely to report pain than those in internal medicine wards (adjusted OR 1.74; 95% CI: 1.21-2.44). Conversely, Damico *et al*. [29] observed no significant differences in pain prevalence across hospital wards (F-statistic 2.088; p=0.081).

Pain management is a recognised indicator of quality of care and an essential criterion for hospital accreditation. Despite the high prevalence of moderate-to-severe pain in our sample, more than 90% of patients expressed satisfaction with the pain management they received, consistent with previous findings [57]. Patient satisfaction may be influenced by psychological and social factors, such as expectations and perceived experiences [58-60]. In our study, satisfaction was assessed using structured questionnaires. However, the absence of a qualitative component is a limitation, as open-ended questions [61] could have offered a deeper understanding of patients' experiences and helped identify additional or unaddressed patient needs.

We observed no significant associations between patient satisfaction and socio-demographic or clinical factors [62, 63]. However, older patients (>76 years) were significantly less likely to report high satisfaction, possibly due to different expectations or a more critical attitude shaped by past healthcare experiences.

Notably, awareness of Law 38/2010 was associated with higher satisfaction. This may indicate that awareness of legal rights is linked to a more positive perception of care. Conversely, patients who experienced pain in the previous 24 hours were significantly less likely to report high satisfaction with pain management, suggesting the importance of timely and effective pain control [57, 63].

Our results revealed that patients' preferences for pain relief included the presence of loved ones, psychological and emotional support, a comfortable care environment, and clear communication about pain management. Although these data were collected through a structured multiple-choice question, they provide a useful overview of patients' perceived needs for relief.

These findings suggest the multidimensional nature of pain experiences and emphasise the importance of a person-centred approach to pain management [64-66].

Limitations

In addition to relying on quantitative self-report questionnaires to assess satisfaction, this study has some limitations. First, it was conducted in a single university hospital (IRCCS Policlinico San Donato), which may limit the generalisability of the findings to other healthcare settings in Italy. However, the single-centre design allowed for a more granular analysis of pain trends within a clearly defined organisational context. Second, specific patient populations, such as those admitted to intensive care or emergency departments, patients with cognitive impairment, and those with language barriers, were excluded, thus limiting the applicability of the results to these groups. Third, the presence of healthcare staff during questionnaire administration may have influenced patient responses, particularly regarding satisfaction, potentially reducing the likelihood of reporting dissatisfaction. Fourth, the questionnaire used in this study was developed pragmatically [23] and was not subjected to formal validation, which may affect the reliability and comparability of the results. However, internal consistency measures such as Cronbach's alpha were not applicable to most of the tool, as it consisted mainly of single-item questions (e.g., pain intensity, satisfaction). Nevertheless, its clinically focused and con-

cise format likely contributed to the very high response rate observed (98%) [22]. Finally, we did not assess pre-existing pain prior to admission or pain specifically related to care procedures. Instead, our data reflect the overall prevalence of pain among hospitalised patients and underscore the importance of conducting repeated pain assessments throughout the hospital stay.

CONCLUSIONS

This cross-sectional study confirms that pain remains a frequent and widespread issue among hospitalised adults, affecting patients across all ward types. Pain experienced in the previous 24 hours was associated with greater pain severity and lower satisfaction with care, suggesting the importance of timely and effective pain control throughout hospitalisation. These findings may inform health policy and service planning aimed at enhancing pain assessment and management in hospital settings.

Funding information

No funding was received for this study.

Data availability statement

The data that support the findings of this study are available on reasonable request from the corresponding Author. The data are not publicly available due to privacy or ethical restrictions.

Declaration on the use of AI

None.

Authors' contributions

The Authors' responsibilities were as follows: MB designed the study, curated the dataset, performed the statistical analysis, and drafted the manuscript. FA and PR checked the code and supervised the analysis. All Authors contributed to data interpretation and revised each draft for important intellectual content. All Authors read and approved the final manuscript.

Ethical approval statement

Ethical approval was obtained from the Ethics Committee of San Raffaele Hospital, Milan, on 15 March 2023 (CE: 34/INT/2023).

Patient consent statement

Participation in the survey was anonymous and voluntary, and informed consent was requested from all patients prior to completing the questionnaire.

Conflict of interest statement

The Authors declare that they have no conflicts of interest.

Received on 10 December 2025.
Accepted on 9 February 2026.

REFERENCES

1. Raja SN, Carr DB, Cohen M, Finnerup NB, Flor H, Gibson S, et al. The revised International Association for the Study of Pain definition of pain: concepts, challenges, and compromises. Pain. 2020;161(9):1976-82.
2. Eliasen MH, Lau CJ, Grønkjær M, Jacobsen MH, Elsborg P, Toxværd CG, Andreasen AH, Benthien KS. Self-reported chronic pain: prevalence and characteristics in the Capital Region of Denmark. Eur J Public Health. 2023;33(Suppl. 2):ckad160.1156. doi: 10.1093/eurpub/ckad160.1156
3. Brattberg G, Thorslund M, Wikman A. The prevalence of pain in a general population. The results of a postal survey in a county of Sweden. Pain. 1989;37(2):215-22.
4. Sjogren P, Ekholm O, Peuckmann V, Grønbaek M. Epidemiology of chronic pain in Denmark: an update. Eur J Pain. 2009;13(3):287-92.
5. Sternbach RA. Pain and "hassles" in the United States: findings of the Nuprin pain report. Pain. 1986;27(1):69-80.
6. Catala E, Reig E, Artes M, Aliaga L, Lopez JS, Segu JL. Prevalence of pain in the Spanish population: telephone survey in 5000 homes. Eur J Pain. 2002;6(2):133-40.
7. Duenas M, Salazar A, Ojeda B, Fernandez-Palacin F, Mico JA, Torres LM, et al. A nationwide study of chronic pain prevalence in the general Spanish population: identifying clinical subgroups through cluster analysis. Pain Med. 2015;16(4):811-22.
8. Duenas M, De Sola H, Salazar A, Esquivia A, Rubio S, Failde I. Prevalence and epidemiological characteristics of chronic pain in the Spanish population. Results from the pain barometer. Eur J Pain. 2025;29(1):e4705.
9. Guido D, Leonardi M, Mellor-Marsa B, Moneta MV, Sanchez-Niubo A, Tyrovolas S, et al. Pain rates in general population for the period 1991-2015 and 10-years prediction: results from a multi-continent age-period-cohort analysis. J Headache Pain. 2020;21(1):52.
10. Zimmer Z, Fraser K, Grol-Prokopczyk H, Zajacova A. A global study of pain prevalence across 52 countries: examining the role of country-level contextual factors. Pain. 2022;163(9):1740-50.
11. Chou KL. Reciprocal relationship between pain and depression in older adults: evidence from the English longitudinal study of ageing. J Affect Disord. 2007;102(1-3):115-23.
12. Rosemann T, Backenstrass M, Joest K, Rosemann A, Szecsenyi J, Laux G. Predictors of depression in a sample of 1,021 primary care patients with osteoarthritis. Arthritis Rheum. 2007;57(3):415-22.
13. Whitlock EL, Diaz-Ramirez LG, Glymour MM, Boscardin WJ, Covinsky KE, Smith AK. Association between persistent pain and memory decline and dementia in a longitudinal cohort of elders. JAMA Intern Med. 2017;177(8):1146-53.
14. Bell T, Franz CE, Kremen WS. Persistence of pain and cognitive impairment in older adults. J Am Geriatr Soc. 2022;70(2):449-58.
15. van der Leeuw G, Eggermont LH, Shi L, Milberg WP, Gross AL, Hausdorff JM, et al. Pain and cognitive function among older adults living in the community. J Gerontol A Biol Sci Med Sci. 2016;71(3):398-405.
16. Macfarlane GJ, Barnish MS, Jones GT. Persons with chronic widespread pain experience excess mortality: longitudinal results from UK biobank and meta-analysis. Ann Rheum Dis. 2017;76(11):1815-22.

17. Duenas M, Ojeda B, Salazar A, Mico JA, Failde I. A review of chronic pain impact on patients, their social environment and the health care system. J Pain Res. 2016;9:457-67.
18. Gregory J, McGowan L. An examination of the prevalence of acute pain for hospitalised adult patients: a systematic review. J Clin Nurs. 2016;25(5-6):583-98.
19. Speak A, Emani S, Vera LA, Sullivan PS, Staley CA, Sharma J, et al. Pain catastrophizing and hospital length of stay in colorectal surgery patients: a prospective cohort study. J Gastrointest Surg. 2022;26(9):1960-2.
20. Regenbogen SE, Mullard AJ, Peters N, Brooks S, Englesbe MJ, Campbell DA Jr, et al. Hospital analgesia practices and patient-reported pain after colorectal resection. Ann Surg. 2016;264(6):1044-50.
21. Levenson JL, Hamer RM, Rossiter LF. Psychopathology and pain in medical in-patients predict resource use during hospitalization but not rehospitalization. J Psychosom Res. 1992;36(6):585-92.
22. Wadensten B, Frojd C, Swenne CL, Gordh T, Gunningberg L. Why is pain still not being assessed adequately? Results of a pain prevalence study in a university hospital in Sweden. J Clin Nurs. 2011;20(5-6):624-34.
23. Becerra-Bolanos A, Armas-Dominguez A, Valencia L, Jimenez-Marrero P, Lopez-Ruiz S, Rodriguez-Perez A. Pain prevalence and satisfaction with pain management in inpatients: a cross-sectional study. Healthcare (Basel). 2023;11(24):3191.
24. Wu CL, Hung YL, Wang YR, Huang HM, Chang CH, Wu CC, et al. Pain prevalence in hospitalized patients at a tertiary academic medical center: exploring severe persistent pain. PLoS One. 2020;15(12):e0243574.
25. Lin LY, Hung TC, Lai YH. Pain control and related factors in hospitalized patients. Medicine (Baltimore). 2021;100(30):e26768.
26. Trentin L, Visentin M, de Marco R, Zandolin E. Prevalence of pain in public hospital: correlation between patients and caregivers. J Headache Pain. 2001;2(2):73-8.
27. Ripamonti C, Zecca E, Brunelli C, Groff L, Boffi R, Caraceni A, et al. Pain experienced by patients hospitalized at the National Cancer Institute of Milan: research project "towards a pain-free hospital". Tumori. 2000;86(5):412-8.
28. Italia. Legge n. 38 del 15 marzo 2010. Disposizioni per garantire l'accesso alle cure palliative e alla terapia del dolore. Gazzetta Ufficiale – Serie Generale n. 65, 19 marzo 2010.
29. Damico V, Murano L, Cazzaniga F, Dal Molin A. Pain prevalence, severity, assessment and management in hospitalized adult patients: a result of a multicenter cross sectional study. Ann Ist Super Sanità. 2018;54(3):194-200.
30. Marri E, Di Ruscio E, Matarazzo T, Gilli G. Indagine sul dolore negli ospedali, negli hospice e in assistenza domiciliare in Emilia-Romagna. Regione Emilia-Romagna: Agenzia sanitaria e sociale regionale; 2012.
31. Downie WW, Leatham PA, Rhind VM, Wright V, Branco JA, Anderson JA. Studies with pain rating scales. Ann Rheum Dis. 1978;37(4):378-81.
32. Williamson A, Hoggart B. Pain: a review of three commonly used pain rating scales. J Clin Nurs. 2005;14(7):798-804.
33. Hjermstad MJ, Fayers PM, Haugen DF, Caraceni A, Hanks GW, Loge JH, et al. Studies comparing numerical rating scales, verbal rating scales, and visual analogue scales for assessment of pain intensity in adults: a systematic literature review. J Pain Symptom Manage. 2011;41(6):1073-93.
34. PAIN OUT Research Group Jena; Chinese PAIN OUT network; Dutch PAIN OUT network; Méxican PAIN OUT network; Serbian PAIN OUT network; Spanish PAIN OUT network, et al. Status quo of pain-related patient-reported outcomes and perioperative pain management in 10,415 patients from 10 countries: analysis of registry data. Eur J Pain. 2022;26(10):2120-40.
35. Melotti RM, Samolsky-Dekel BG, Ricchi E, Chiari P, Di Glacinto I, Carosi F, et al. Pain prevalence and predictors among inpatients in a major Italian teaching hospital. A baseline survey towards a pain free hospital. Eur J Pain. 2005;9(5):485-95.
36. Sawyer J, Haslam L, Daines P, Stilos K. Pain prevalence study in a large Canadian teaching hospital. Round 2: lessons learned? Pain Manag Nurs. 2010;11(1):45-55.
37. Strohbuecker B, Mayer H, Evers GC, Sabatowski R. Pain prevalence in hospitalized patients in a German university teaching hospital. J Pain Symptom Manage. 2005;29(5):498-506.
38. Abbott FV, Gray-Donald K, Sewitch MJ, Johnston CC, Edgar L, Jeans ME. The prevalence of pain in hospitalized patients and resolution over six months. Pain. 1992;50(1):15-28.
39. Quattrin R, Regattin L, Lattuada L, Cerno B, Conzut L, Mesaglio M, et al. Management of the patient's pain in a highly specialized health center. Ig Sanità Pubbl. 2003;59(4):239-52.
40. Costantini M, Viterbori P, Flego G. Prevalence of pain in Italian hospitals: results of a regional cross-sectional survey. J Pain Symptom Manage. 2002;23(3):221-30.
41. Das SK, Dhar SS, Panigrahi A. Prevalence of pain and its characteristics in hospitalized patients in an Indian teaching hospital. Pain Manag Nurs. 2020;21(3):299-303.
42. Voscopoulos C, Lema M. When does acute pain become chronic? Br J Anaesth. 2010;105(Suppl. 1):i69-85.
43. Bell A. The neurobiology of acute pain. Vet J. 2018;237:55-62.
44. Woolf CJ. Central sensitization: implications for the diagnosis and treatment of pain. Pain. 2011;152(Suppl. 3):S2-S15.
45. Vallano A, Malouf J, Payrulet P, Banos JE; Catalan Research Group for studying pain in hospital. Prevalence of pain in adults admitted to Catalonian hospitals: a cross-sectional study. Eur J Pain. 2006;10(8):721-31.
46. Zoega S, Sveinsdottir H, Sigurdsson GH, Aspelund T, Ward SE, Gunnarsdottir S. Quality pain management in the hospital setting from the patient's perspective. Pain Pract. 2015;15(3):236-46.
47. Chen MC, Yeh TF, Wu CC, Wang YR, Wu CL, Chen RL, et al. Three-year hospital-wide pain management system implementation at a tertiary medical center: pain prevalence analysis. PLoS One. 2023;18(4):e0283520.
48. Fillingim RB, King CD, Ribeiro-Dasilva MC, Rahim-Williams B, Riley JL 3rd. Sex, gender, and pain: a review of recent clinical and experimental findings. J Pain. 2009;10(5):447-85.
49. Visentin M, Zanolin E, Trentin L, Sartori S, de Marco R. Prevalence and treatment of pain in adults admitted to Italian hospitals. Eur J Pain. 2005;9(1):61-7.
50. Pieretti S, Di Giannuario A, Di Giovannandrea R, Marzoli F, Piccaro G, Minosi P, et al. Gender differences in pain and its relief. Ann Ist Super Sanità. 2016;52(2):184-9.
51. Robinson ME, Riley JL 3rd, Myers CD, Papas RK, Wise EA, Waxenberg LB, et al. Gender role expectations of pain: relationship to sex differences in pain. J Pain. 2001;2(5):251-7.
52. Osborne NR, Davis KD. Sex and gender differences in pain. Int Rev Neurobiol. 2022;164:277-307.
53. Wise EA, Price DD, Myers CD, Heft MW, Robinson

ME. Gender role expectations of pain: relationship to experimental pain perception. Pain. 2002;96(3):335-42.
54. Falk H, Henoch I, Ozanne A, Ohlen J, Ung EJ, Fridh I, et al. Differences in symptom distress based on gender and palliative care designation among hospitalized patients. J Nurs Scholarsh. 2016;48(6):569-76.
55. Zajacova A, Rogers RG, Grodsky E, Grol-Prokopczyk H. The relationship between education and pain among adults aged 30-49 in the United States. J Pain. 2020;21(11-12):1270-80.
56. Edwards RR, Goble L, Kwan A, Kudel I, McGuire L, Heinberg L, et al. Catastrophizing, pain, and social adjustment in scleroderma: relationships with educational level. Clin J Pain. 2006;22(7):639-46.
57. Comley AL, DeMeyer E. Assessing patient satisfaction with pain management through a continuous quality improvement effort. J Pain Symptom Manage. 2001;21(1):27-40.
58. Linder-Pelz S. Social psychological determinants of patient satisfaction: a test of five hypothesis. Soc Sci Med. 1982;16(5):583-9.
59. Linder-Pelz SU. Toward a theory of patient satisfaction. Soc Sci Med. 1982;16(5):577-82.
60. Avis M, Bond M, Arthur A. Satisfying solutions? A review of some unresolved issues in the measurement of patient satisfaction. J Adv Nurs. 1995;22(2):316-22.
61. Williams B. Patient satisfaction: a valid concept? Soc Sci Med. 1994;38(4):509-16.
62. Alibrandi A, Gitto L, Limosani M, Mustica PF. Patient satisfaction and quality of hospital care. Eval Program Plann. 2023;97:102251.
63. Buli B, Gashaw A, Gebeyehu G, Abrar M, Gerbessa B. Patient satisfaction with post-operative pain management and associated factors among surgical patients at Tikur Anbessa Specialized Hospital: cross-sectional study. Ann Med Surg (Lond). 2022;79:104087.
64. Visentin M. Towards a pain-free hospital. A project and a campaign for the improvement of health care. Recenti Prog Med. 1999;90(6):321-4.
65. Bonakdar RA. Integrative pain management. Med Clin North Am. 2017;101(5):987-1004.
66. Gluyas H. Patient-centred care: improving healthcare outcomes. Nurs Stand. 2015;30(4):50-7; quiz 59. doi: 10.7748/ns.30.4.50.e10186

Ann Ist Super Sanità 2026 | Vol. 62, No. 2: 141-148
DOI: 10.4415/ANN_26_02_06

Optimizing sample preparation for homogeneous mycotoxin distribution in official control analyses

Francesca Debegnach[1], Emanuela Gregori[1], Marianna Rizzo[2], Elena Pannunzi[1], Martina Enza Grieco[1] and Barbara De Santis[1]

[1]*Dipartimento di Sicurezza Alimentare, Nutrizione e Sanità Pubblica Veterinaria, Istituto Superiore di Sanità, Rome, Italy*
[2]*Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria, Rende (CS), Italy*

Abstract

Background. The distribution of mycotoxins within cereal batches is highly heterogeneous. Therefore, to obtain representative samples and accurate analytical results, sample homogenization is a critical preparatory step.

Objective. To provide reliable and standardized instructions for preparing an aggregate grain sample within official control framework, four different homogenization procedures were evaluated. The comparison considered variability in mycotoxin distribution, workload, and time constraints.

Methods. The study was conducted on four aggregate samples, each weighing 10 kg, obtained from a maize grain batch according to regulatory provisions of Regulation (EU) 2023/2782. Analytical variability of aflatoxin (B1 and B2) and fumonisin (B1 and B2) was measured.

Results. The contamination levels in each pooled sample and the degree of homogeneity among the procedures were assessed and compared using the relative standard deviation of repeatability (RSD_r) as acceptability criterion.

Conclusions. The procedures involving fine grinding (particle size <0.5 mm) and aqueous slurry preparation yielded the best results in terms of homogeneity.

Key words
- mycotoxin
- official control
- comminution
- slurry
- homogeneity

INTRODUCTION

Mycotoxins are toxic compounds naturally produced by different types of fungi belonging mainly to the genera *Aspergillus*, *Penicillium* and *Fusarium*. In specific environmental conditions of temperature and humidity, these fungi proliferate on vegetable substrates and produce mycotoxins [1, 2]. Thereby, mycotoxins enter the food chain through contaminated crops intended for food and feed production [3, 4].

Mycotoxins are a significant food safety concern, and as such, they have received considerable attention within the framework of European Union (EU) food legislation. Since 2006, mycotoxins have been included in a set of specialized regulations to ensure consumer protection. With this aim, maximum levels of mycotoxins in food and feed are set and control measures of good farming, storage and processing practices are defined to maintain mycotoxins at levels "as low as reasonably achievable" (ALARA). Maximum levels of contaminants in food, including mycotoxins, are established in Regulation (EU) 2023/915 [5] and subsequent amendments. To verify that the legislation is implemented, the EU food safety system has established a set of rules for official control activities for which sampling, analysis, diagnosis and tests shall be included as appropriate Regulation (EU) 2017/625 being the core reference [6]. The provisions related to sampling and analysis methods for the official control of mycotoxin levels in food products are defined by Regulation (EU) 2023/2782 [7].

Sampling plays a crucial role to obtain representative samples of the lot under control, and this is even more critical for the subject matter of mycotoxins, which are recognized to be heterogeneously distributed in a bulk [8-10]. Every step of the analytical control chain for mycotoxin detection shall include the sampling step and the subsample preparation up to obtain the final laboratory sample for analysis. Each of these steps contributes with diverse sources of variability to the uncertainty associated to the final analytical result. While the laboratory work has succeeded in the definition of standard protocols to measure the source of the analytical un-

Address for correspondence: Barbara De Santis, Dipartimento di Sicurezza Alimentare, Nutrizione e Sanità Pubblica Veterinaria, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy. E-mail: barbara.desantis@iss.it.

certainty, the sampling sources of uncertainty are much higher and difficult to be controlled. It is therefore compulsory to fix criteria which the sampling method should comply with, to have a common approach. The uncertainty that origins because of the implementation of the sampling procedure is out of the scope of this paper. Instead, the aim of the present study is to furnish indications on how to handle the aggregate sample obtained by the implementation of the sampling procedure in official control, to assess the effectiveness of the sample preparation in terms of variability of the mycotoxin content and to guarantee the suitable representativity of the aggregate sample before the arrangement of the official test aliquots, which are used by the laboratory to verify the sample compliance.

According to Regulation (EU) 2023/2782 [7], any food lot shall be sampled following the legislation prescriptions whose guiding criterion is the initial weight of the lot and the weight of the aggregate sample. The incremental samples collected shall be combined to produce an aggregate sample, which, depending on the weight of the lot, can be of a weight from 1 kg to 10 kg. The aggregate sample is considered representative of the lot and the replicate samples (i.e., sample for enforcement, for trade or defense, and for reference or referee), are made available for the lot compliance and for the purposes of the food and feed business operator right of defense, shall be taken from the homogenized aggregate sample. Recent Italian national rule (DL 27/2021) [11] as regards the right of the food business operator (descending from Regulation (EU) 625/2017 [6]) has established the preparation of four (or five in special situations) official aliquots for the accomplishment of the official control. These official aliquots must preserve and guarantee the criterion of representativeness, through an adequate homogenization of the aggregate sample [12, 13].

Assuming the sampling procedure yields a representative aggregate sample of the lot under control, the mycotoxin concentration in the lot is inferred from the analytical result obtained from a small laboratory test portion (typically around 5-50 grams) taken from the official aliquot. Even when sampling provisions are strictly followed according to regulatory procedures to obtain the aggregate sample, special care must be taken to ensure the official aliquots are homogeneous and truly representative. Handling of aggregate sample requires particular attention, especially regarding homogenization. In fact, in the context of official control, decisions on accepting or rejecting a lot are based on the contamination level measured in the laboratory test portion. According to Member Sates rules, the replicate samples for enforcement, defense and reference are envisaged to be taken from the homogenized aggregate sample for the purposes the rights of the food business operator [7]. If the replicate samples are not accurately formed from homogenized aggregate sample, there is a significant risk of misclassification, which can lead to trade disputes as well as economic and health repercussions.

In the case of cereals and nuts, the goal of the homogenization of the aggregate sample, is achievable by two different approaches: the dry milling and the water-slurry mixing. The dry milling involves a milling process on the whole aggregate sample using commercial mills capable of grinding the cereal with appropriate particle size. The slurry mixing involves the simultaneous grinding and blending of the sample with a controlled amount of water through a homogenizer. The preparation of the homogenized aggregate sample shall be carried out following a procedure which guarantees harmonized and standardized operations for the control of particle size distribution and time employed. All the handling of the aggregate sample for the sake of the homogeneity shall be done in an appropriate premise, where technical personnel is available for the implementation of all steps and all the instrumental tools are accessible as well. In case the laboratory takes the burden of the implementation of the homogenization procedure, the whole aggregate sample received shall be submitted to the homogenization procedure.

Several studies investigated the effects of different approaches for preparing the sample and the effects, in terms of accuracy and precision, of different particle size of the testing material on the determination of mycotoxins in foodstuffs. Velasco and Morris [14] evaluated the possibility of extracting aflatoxins from water-slurried products. Copra, maize, cottonseed, cottonseed meal, peanuts, peanut butter and peanut meal were successfully analyzed. The precision of slurry analysis for these products, expressed as coefficient of variation (CV, percent), ranged from 2.6 to 7.8 compared to 5.7 to 20.8 for analysis by standard procedures. The use of water-slurry could successfully reduce the variability associated with aflatoxin analysis because i) the distribution of particles is more uniformly achieved with a slurry than with a dry ground product, and ii) high oil content seeds are readily reduced to a fine particle size whereas, because of clogging, only a coarse grind is possible with conventional mills.

Spanjer *et al.* [15] made a comparison between dry milling and water-slurry mixing as comminuting step preceding analysis of aflatoxin B1 in almonds, pistachios and mixed spices and ochratoxin A in cocoa and green coffee beans. The homogenization process was evaluated in terms of CV, which resulted higher for dry milling than for water-slurry mixing. The authors concluded that sample comminution is best performed by water-slurry mixing which, by producing smaller particles, leads to the lowest possible CVs and reveals the best estimate of the mycotoxin content of a lot, reducing to a minimum subsampling error as well as chances of false-positive or -negative values.

To determine differences in mean aflatoxins contamination and subsample variance from dry and slurry homogenizations, Bircan [16] tested triplicates of 10 kg dried fig samples (naturally contaminated) of six different lots, collected from exporting companies in accordance with the EU legislation. Taking into consideration the waste disposal and storage difficulties associated with slurry homogenization, he considered both dry and slurry homogenization as suitable methods to obtain a homogeneous subsample for such a difficult matrix. Dry mixing had slightly higher CV values

compared with slurry mixing, however he highlighted that to obtain a homogeneous fig dough it is imperative to blend the dried figs sufficiently to break up any large particles, which contribute to the variability.

Lippolis *et al*. [17] compared water-slurry mixing and dry milling procedures investigating the distribution of ochratoxin A (OTA) and deoxynivalenol (DON) in naturally contaminated wheat. Although a normal distribution and a good repeatability of DON measurements was observed for both water-slurry mixing (CV% 4.6) and dry milling (CV% 6.4) procedures, for OTA determination reliable results could be obtained only by slurry mixing (CV% 4.0), whereas dry milling comminution resulted in an inhomogeneous distribution with a high variability (CV% 75.2). This study supports the advantage, in terms of variability of results, derived from the use of the slurry mixing approach to achieve a complete homogenization of the laboratory sample.

Damiani *et al*. [18] evaluated the effect of particle size of comminuted maize on fumonisins recovery during the extraction step. Maize samples were ground, and the resulted flours were separated in different fractions by their particle size (1,000-250 µm). For all samples, the efficiency of the extraction from each fraction increased as particle size decreased. Concentrations in fractions with smaller particle size were 1.3 to 4 times higher than the concentration measured in unfractionated samples. The authors attributed this behavior to an increase of the area available for solvent extraction, considering that the smaller the particle size the higher the surface available for the solvent. This work demonstrated that comminuting the sample into smaller particle size could not only reduce the variance of test results but also affect the accuracy.

All the studies agree on the need of homogenization, but no one has given instruction on how to demonstrate that the protocol adopted ensures complete homogenization. The dry milling or slurry process should obtain particles with appropriate particle size, and the preparation of the homogenized aggregate sample shall be carried out following a procedure which guarantees harmonized and standardized operations for the control of particle size distribution and time employed. A recent audit conducted by the Directorate-General for Health and Food Safety of the European Commission (IT GFA 2023-766) emphasized the importance to provide robust evidence of sample homogeneity for the mycotoxin subject matter. Consequently, the Competent Authority (CA) should ask local CA and official laboratories for comprehensive instructions to demonstrate that achieved homogeneity of the aggregate sample is sufficient. Accordingly, a detailed report on homogeneity testing must be submitted including the number of samples taken and analyzed, the statistical evaluation of the data obtained and, importantly, the acceptance criteria applied for homogeneity assessment.

Slurry preparation is widely recognized as an effective approach for ensuring homogeneity and the European guidance document for competent authorities for the control of compliance with EU legislation on aflatoxins explicitly acknowledges it as an appropriate procedure [19]. However, in the context of official control, not all laboratories may have the capacity or equipment to perform slurry preparation, especially when dealing with large bulk sample quantities. In such cases, grinding the sample is an effective alternative, provided that its effectiveness is thoroughly demonstrated.

In the present study, four different homogenization procedures were evaluated to provide reliable guidance for standardizing the preparation of cereal aggregate samples obtained according to the sampling prescriptions of Regulation (EU) 2023/2782 [7]. The assessment focused on workload, time requirements, and variability in mycotoxin distribution. Homogeneity among the procedures was assessed by measuring the analytical variability of aflatoxins (B1 and B2) and fumonisins (B1 and B2) contamination within each aggregate sample, using the relative standard deviation of repeatability (RSD_r) as acceptability criterion. Additionally, the particle size distribution analysis presented was introduced and considered as a further tool for verifying homogeneity.

MATERIALS AND METHODS

Samples

A 400 kg batch of maize grain was made available by an Italian milling company. After applying the sampling provisions of Regulation (EU) 2023/2782 four times, four 10 kg sacks of maize grain were obtained as aggregate samples representative of the lot. Each aggregate sample was processed using a different procedure: i) hand-mixing of the raw grains (referred to as "grain"); ii) coarse milling, performed using a RAS mill – Romer Analytical Sampling mill (Coring-System Diagnostix GmbH, Gernsheim, Germany) (referred to as "coarse"); iii) fine milling, performed using a Retsch ZM 200 (Retsch GmbH, Haan, Germany) (referred to as "fine"); iv) water-slurry mixing, obtained by the addition of water to the sample in a 1:1 ratio and carried out using a Silverson EX-50 batch mixer (Silverson Machines Ltd, Waterside, Chesham, UK) (referred to as "slurry").

From each homogenized sample, 10 testing aliquots of 25 g maize each were collected. For the dry samples (grain, coarse, and fine), the material was spread evenly on a laboratory bench to form a square with a thickness of approximately two centimeters. This square was then divided into 100 small squares, each containing about 100 g of material. Following a Z-shaped sampling pattern, 10 small squares were selected, from which the material was taken to prepare the 10 testing aliquots. For the slurry samples, aliquots were taken directly from the homogenized slurry.

The test sample were found to be contaminated with aflatoxins B1 and B2, and with fumonisins B1 and B2, therefore the study is focused on these mycotoxins.

Analytical determination

Aflatoxins were analyzed in the samples according to method EN 14123:2007 [20], a high-performance liquid chromatographic method with post-column derivatization and immunoaffinity column cleanup. The application of this method required a matrix extension in order to include maize. For this purpose, the perfor-

mances of the analytical method were verified for the analysis of maize. The values obtained were found to be in compliance with the performance criteria reported in the CEN standard. The analysis of fumonisins were conducted according to method EN 16187:2015 [21]. This analytical procedure employs an immunoaffinity column purification step followed by a high-performance liquid chromatographic detection with a pre-column derivatization using ortho-phthalaldehyde (OPA) and 2-mercaptoethanol. In compliance with quality control procedures, recovery tests were conducted, and QC samples were analyzed to verify the performance of the method during the analytical sessions.

Reagents: chemicals and solvents used for the extraction and clean-up solutions were ACS grade or equivalent (Sigma-Aldrich, St. Louis, USA). Water used was deionized and, for HPLC (high-performance liquid chromatography), purified through a Mill-Q treatment system (Millipore, London, UK). For HPLC analysis, methanol and acetonitrile were HPLC grade (Sigma-Aldrich, St. Louis, USA). Phosphate buffer solution (PBS) was prepared from potassium chloride (0.2 g) (Sigma-Aldrich, St. Louis, USA), potassium dihydrogen phosphate (0.2 g) (Carlo Erba, Milan, Italy), anhydrous disodium hydrogen phosphate (1.2 g) (Panreac Química S.L.U, Castellar del Vallès, Spain), and sodium chloride (8 g) (Sigma-Aldrich, St. Louis, USA) added to distilled water (900 mL). After dissolving, the pH was adjusted to 7.4 and the solution was made to 1 L. Sodium dihydrogen phosphate was from Carlo Erba (Milan, Italy), PBPB (pyridinium hydrobromide perbromide) was from Merck KGaA (Darmstadt, Germany) and OPA was from Sigma-Aldrich (Steineim, Germany). Disodium tetraborate and 2-mercaptoethanol were from Sigma-Aldrich (St. Louis, USA).

Standard solutions: aflatoxins certified standard solution was purchased from Biopure (Tulln, Austria) as a mix in acetonitrile. Fumonisins stock solution was obtained from Biopure (Tulln, Austria) as mix of fumonisins B1 and B2 in acetonitrile:water (50:50, v/v).

Flour sieving

Flour sieving was manually carried out on the coarse and fine flours, by passing them through ISO-certified sieves (Giuliani Tecnologie, Turin, Italy) with meshes of 2.8 mm, 2.0 mm, 1.4 mm, 0.5 mm and 0.212 mm. The percentage of particles passing each sieve was measured by weighing each single fraction and comparing it with the initial amount of material. No sample loss was observed during the sieving.

Laboratory milling equipment

Different milling machines are available on the market with different load capacity of the hopper. In this study the following laboratory milling equipment have been used.

The RAS mill (Romer Analytical Sampling mill) with a 7 kg grain capacity hopper can work with a 2 kg/minutes speed to obtain a coarse milled sample. A Retsch ZM 200, assembled with a 0.5 mm ring sieve and a 3 kg cassette collecting cyclone, able to work with 0.5 kg/minutes speed was employed to obtain a fine milled sample. Finally, a Silverson EX-50 batch mixer was used to prepare the water-slurry maize sample, starting from the maize kernels.

RESULTS AND DISCUSSION

Four 10 kg aggregate maize grain samples were processed to obtain grain, coarse, fine, and slurry testing aliquots. With the aim to verify the grade of homogenization in the four practices, the mycotoxin distribution and variability, and the granulometry distribution were scrutinized.

Aflatoxin and fumonisin contamination distribution

Each of the four 10 kg aggregate samples (grain, coarse, fine and slurry) was sub-sampled to obtain 10 replicates of testing aliquots to be analyzed on the same day. During the analytical sessions, recovery tests were carried out, and quality control (QC) samples were tested to verify method performances. To handle the contamination values, all analytical test values resulted as lower than the limit of quantification were treated with the substitution approach and were replaced by 0 [22].

Mean concentration values (corrected for the recovery and expressed in µg/kg), standard deviations (SD), and RSD_r obtained for grain, coarse, fine and slurry samples for each mycotoxin are reported in *Table 1*.

Table 2 shows data for the sum of aflatoxins, AFs (AFB1+AFB2), and sum of fumonisins, FBs (FB1+FB2). *Figure 1* shows the box plots of single data for AFs and FBs values obtained; to note that box plots for fine and slurry show the lower variability either for AFs or FBs. To note that a 25 g test portion of maize in grain contained approximately 80 maize kernels, whereas 50 g of slurry (water:maize in a 1:1 ratio) contained 25 g of an emulsified, homogenized 10 kg maize kernel sample, without visible intact maize kernels.

The level of variability of aflatoxin and fumonisin content in the aggregate samples was measured by means of RSD_r obtained by 10 independent analyses. Among

Table 1
Mean values, standard deviation (SD) and relative standard deviation of repeatability (RSD_r) for each mycotoxin (replicates n=10)

	Mean±SD (µg/kg)				RSD_r (%)			
	AFB1	AFB2	FB1	FB2	AFB1	AFB2	FB1	FB2
Grain	7.80±18.88	0.99±2.84	12,380±4,654	1,266±1,000	242	285	38	79
Coarse	101.74±140.49	3.74±4.08	14,690±1,195	1,549±493	138	109	8.1	32
Fine	57.92±1.21	2.26±0.31	16,607±919	1,685±366	2.1	14	5.5	22
Slurry	52.78±0.88	2.55±0.08	17,199±865	2,367±402	1.7	3.1	5.0	17

AFB1: aflatoxin B1; AFB2: aflatoxin B2; FB1: fumonisin B1; FB2: fumonisin B2.

Table 2
Mean values, standard deviation (SD) and relative standard deviation of repeatability (RSD_r) for the sum of AFs (AFB1+AFB2) and FBs (FB1+FB2) (replicates n=10)

	Mean (µg/kg)		SD (µg/kg)		RSD_r (%)	
	AFs	FBs	AFs	FBs	AFs	FBs
Grain	8.8	13,647	22	5,119	247	38
Coarse	105	16,239	144	1,374	137	8.5
Fine	60	18,292	1.3	1,198	2.1	6.6
Slurry	55	19,567	0.9	1,123	1.7	5.7

AFs: sum of aflatoxins AFB1 and AFB2; FBs: sum of fumonisins FB1 and FB2.

the four aggregate samples, the RSD_r varied from 1.7% and 3.1% (in slurry) to 242% and 285% (in grain) for AFB1 and AFB2, respectively, and from 5.0% and 17% (in slurry) to 38% and 79% (in grain) for FB1 and FB2, respectively.

RSD_r showed similar trend for each mycotoxin class in the four different procedures, with RSD_r in grain >RSD_r in coarse >RSD_r in fine ≈RSD_r in slurry. In the grain procedure, aflatoxins showed much higher variability than fumonisins (242.2% for AFB1 *vs* 38% for FB1), while in the fine procedure (smaller particle size) variability for aflatoxins was lower than the one for fumonisins (1.7% for AFB1 *vs* 5.7% for FB1).

As regards aflatoxins, when comparing contamination levels across the different procedures, from grain up to water-slurry, the data clearly reveal that aflatoxin contamination tends to be distributed in spots, with highly contaminated individual grains interspersed with uncontaminated ones. In the grain procedure, each testing aliquot represents only few kernels, resulting in a high variability among the 10 analyses. The contamination is detected randomly; therefore, the average contamination is lower compared to other procedures. From grain up to water-slurry, as the degree of comminution increases, the variability decreases, and the average value stabilizes as it is reflected in reduced SD and RSD_r.

As regards fumonisins, the impact of comminution degree on contamination variability is less pronounced. *Fusarium,* a plant pathogen affecting harvested maize, tends to cause widespread contamination throughout the crop. As a result, from grain to water-slurry, a noticeable reduction in variability can be observed (*Table 1* and *2*), along with the stabilization of average contamination levels, reflected by a decreased RSD_r that becomes apparent after the first comminution process (coarse).

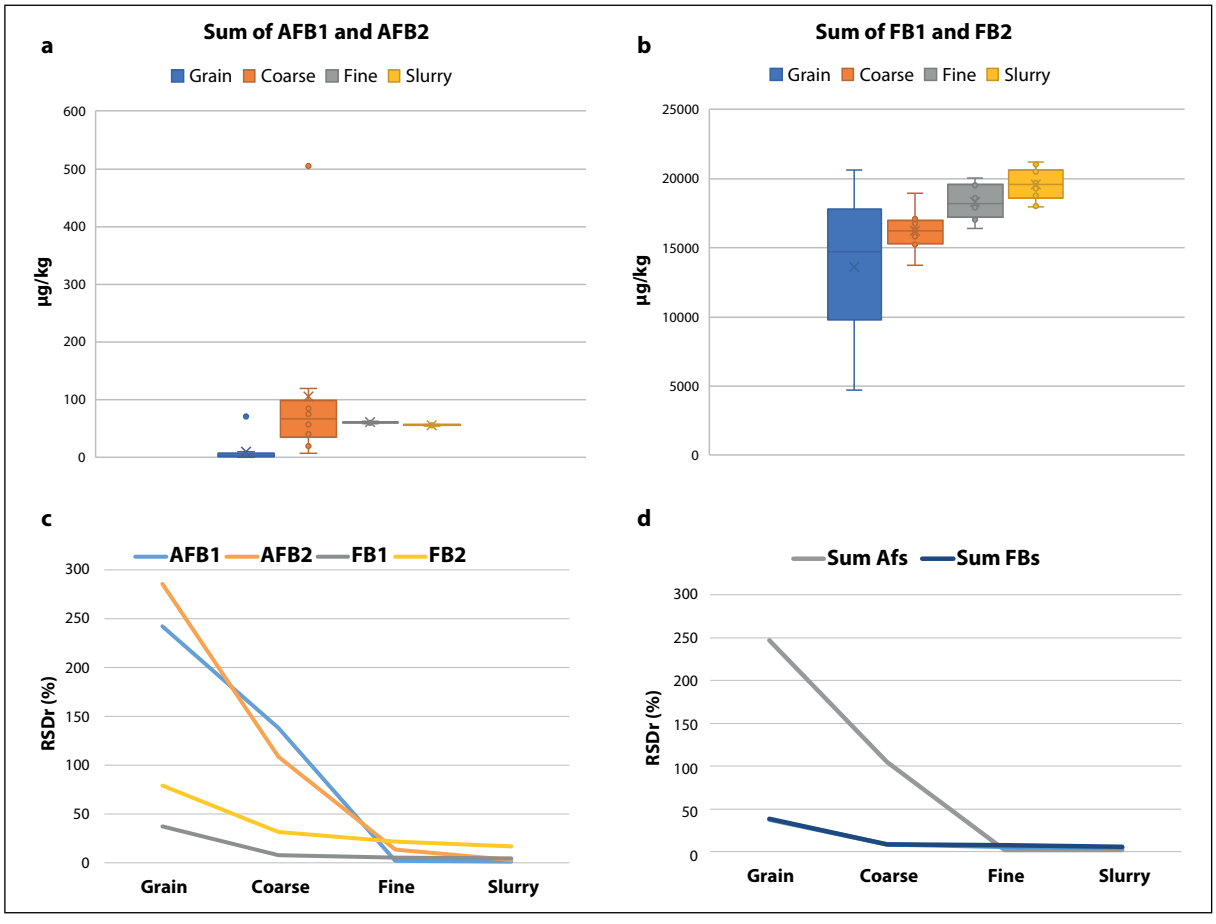


Figure 1
Box plot describing the distribution of the contamination values for the sum of aflatoxins (a) and sum of fumonisins (b), respectively; (c) and (d), trend of RSD_r (relative standard deviation of repeatability) along the four processed samples (grain, coarse, fine and slurry) for the four mycotoxins and for the sum of aflatoxins and fumonisins (d).

Regulation (EU) 2023/2782 [7] (and its amendments) reports prescriptions for analytical requirements of method precision, either in terms of repeatability (the maximum tolerable variability in repeated analyses within one laboratory), and of reproducibility (the maximum tolerable variability among laboratories), and regardless of the mycotoxin analyzed, the RSD_r acceptance criterion is set at 20%. For assessing the homogeneity of samples, the RSD_r can be assumed as a key parameter indicating the effectiveness of the sample preparation.

Taking these RSD_r value as the acceptance criteria of homogeneity, the performances obtained for the sum of AFB1 and AFB2 in grain (247%) and in coarse (137%) are considered unsatisfactory, while the values obtained in fine (2.1%) and in slurry (1.7%) are considered highly satisfactory. On the other hand, for the sum of FBs, while the values obtained for grain (38%) is considered unsatisfactory, the values for coarse (8%), fine (7%) and slurry (6%) are considered all satisfactory. All three procedures are thus considered suitable to homogenize this contaminant and it is confirmed that fumonisins can reach homogeneity with a lower comminution grade. Considered as single, FB1 performed well in coarse, fine and slurry procedure reaching satisfactory and comparable RSD_r values, while FB2 showed insufficient precision for coarse (31.8%), almost sufficient for fine (21.7%) and satisfactory for slurry (17.0%) procedure. The higher variability observed for FB2 highlights its less stable chromatographic signal, which is attributable to limitations in the analytical method. In fact, while FB1 contains a primary amine group that reacts efficiently with OPA forming a stable and highly fluorescent derivative, FB2, lacking this primary amine, produces a weaker derivatization and gives a less stable chromatographic signal.

Fine milling and water-slurry mixing showed comparable variability and average values without statistical difference (t test, 2 tails, same variance, $p>0.05$, accept null hypothesis).

Results obtained in this study are comparable to those reported by other authors [14-18] that investigated and compared procedures of dry milling and water-slurry mixing as successful comminuting step preceding analysis of other matrices and/or mycotoxins. Velasco and Morris [14], for slurried, maize, cottonseed, peanuts, and copra showed aflatoxins CVs below 6%, while for dry milled samples, CVs were in the range between 5% and 21%. Lippolis [17] for slurried wheat reported OTA and DON CVs below 5% and ranges between 6% and 75% for dry milled procedures. This confirms that slurry is the ideal approach for ensuring adequate sample homogeneity and that dry milling procedures needs to be adjusted and standardized to reach comparable performances. It is noteworthy that the variability in dry milling products can be significant among laboratories due to differences in procedures and equipment, making it necessary to establish clear acceptability criteria for the homogeneity assessment.

Granulometry

Comminution is a process that enables reduction of the average particle size of a solid material through crushing, cutting, blending, milling or pulverizing, thereby decreasing its heterogeneity [23]. To evaluate comminution efficiency, the distribution of the particle size of both coarse and fine fractions was studied. A portion of maize flour coarsely milled by using a RAS mill (Romer Analytical Sampling mill), was subject to sieve-shaking fractionation resulting in five particle size fractions (*Figure 2, a-e*): F6, the coarsest fraction (granulometry >2.8 mm) accounted for 3.5%; F5, particles between 2.0 mm and 2.8 mm, made up 26.7%; F4, particles between 1.4 mm and 2.0 mm, were 40.0%; F3, particles ranging from 0.5 mm to 1.4 mm, comprised 20.1%; and F2, the finest fraction (granulometry <0.5 mm), represented 9.7%. Similarly, a portion of maize milled flour underwent to sieve-shaking fractionation, producing three different fractions (*Figure 2, f-h*): F3, the coarsest particles (granulometry >0.5 mm,) with 3.6%, F2, particles sized 0.5-0.212 mm, with 68.7%, and F1, finest particles (granulometry <0.212 mm,), with 27.7%.

Table 3 shows the percentage of particles in each fraction obtained after sieve-shaking fractionation for coarse and fine maize flour.

Taking a threshold fraction of 0.5 mm, the percentage of the particles with granulometry ≤0.5 mm in the coarse milling was 9.7%, while the percentage of par-

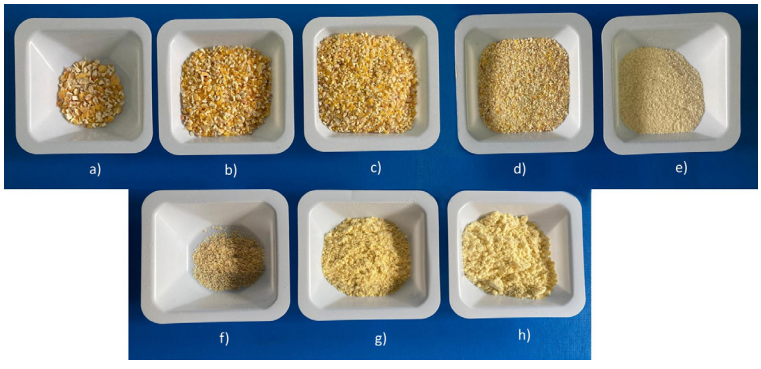


Figure 2
Different fractions obtained after sieve-shaking fractionation of the coarsely milled flour. Particles with granulometry >2.8 mm (a), 2.0-2.8 mm (b), 1.4-2.0 mm (c), 0.5-1.4 mm (d) and <0.5 mm (e). Different fractions obtained after sieve-shaking fractionation of the finely milled flour. Coarsest fraction particles with granulometry >0.5 mm (f), 0.5-0.212 mm (g) and <0.212 mm (h).

Table 3
Percentage of particles of coarse and fine flours in each fraction after sieve-shaking fractionation

Fraction	F1	F2	F3	F4	F5	F6
Sieve (mm)	Ø≤0.212	0.212<Ø≤0.5	0.5<Ø≤1.4	1.4<Ø≤2.0	2.0<Ø≤2.8	Ø>2.8
Particles in coarse flour[1] (%)	-	9.7	20.1	40.0	26.7	3.5
Particles in fine flour[2] (%)	27.7	68.7	3.6	-	-	-

[1]RAS mill-Romer Analytical; [2]Retsch ZM 200-Retsch GmbH.

ticles below the same granulometry in fine flour was 96.4% (68.7+27.7). Following the definition of homogeneity provided in ISO/FDIS 6498:2012 [24], that is, the degree to which a property or a constituent is uniformly distributed throughout a material, a higher proportion of smaller particles indicates greater homogeneity due to increased comminution. The granulometry level and its distribution offer only a rough indication of the potential homogeneity; nonetheless, finer particle size generally corresponds to improved uniformity in the material. Consequently, fine flour exhibits potential homogeneity of the test portions.

Thus, the dry milled sample was considered homogeneous for aflatoxin analysis when finely ground, as demonstrated by an acceptable RSD_r (2.1% for AFs), and when at least 90% of particles exhibited a granulometry below 0.5 mm. For fumonisin analysis, homogeneity was achieved with coarse grinding, where the RSD_r was also acceptable (6.1% for FBs), and at least 60% of the particles with a granulometry below 2 mm.

Therefore, a finely milled dry sample with an RSD_r below 20% and at least 90% of particles smaller than 0.5 mm can be considered suitably homogeneous for aflatoxin analysis. Conversely, for fumonisin analysis, a coarsely milled dry sample with a RSD_r below 20% is considered adequately homogeneous when at least 60% of the particles are smaller than 2 mm.

Working time to carry out the procedures

To handle 10 kg of aggregate maize sample with the RAS mill, 5 minutes were taken, loading the hopper with 2 maize portions, to obtain a coarsely ground sample. Five more minutes were used to disassemble and clean the helical feed worm device and the hopper with a special brush. A grand total of around 20 minutes was taken to obtain the final sample in the coarse procedure.

Using the Retsch ZM 200, to handle 10 kg of maize aggregate sample, 30 minutes were taken. Five more minutes were used to disassemble and properly clean the cassette, the sieve and the rotor. A grand total of around 40 minutes was taken to obtain the final sample in the fine procedure.

Finally, the Silverson EX-50 batch mixer was used to prepare the water-slurry maize sample. For the purpose, 10 kg of maize kernels were placed in a 20 L capacity vessel, and 10 L of water were gradually added to produce a homogeneous paste. Around 15 minutes were taken to obtain a properly slurried and well emulsified sample without visible intact maize kernels. 10 more minutes were taken to clean the rotor by removing the vessel containing the sample and using a 10 L clean vessel to rinse the rotor with water. A grand total of around 30 minutes was taken to obtain the final sample in the slurry procedure.

CONCLUSIONS

The effect caused by the heterogeneous nature of mycotoxin contamination distribution in agricultural products creates major challenges for generating representative samples to be tested during official controls. Sampling regulations specifically addressed to mycotoxins official control establish procedures to be strictly followed to guarantee representativity of the aggregate sample. Due to the high variance in the mycotoxin distribution, the sample preparation to obtain the aggregate sample represents a critical step to be carefully carried out especially in the context of official controls.

In this study, the fine flour procedure (which produces the 96.4% of particles size <0.5 mm) and water-slurry procedure, were the sample preparation options that achieved the highest homogeneity (RSD_r <7%), in 30 and 40 minutes workload respectively.

It was verified that the degree of homogeneity of the 10 kg cereals aggregate sample is closely related to the homogeneization procedure employed. Both the RSD_r and the percentage of particles with size <0.5 mm serve as reliable critera for assessing homogeneity. Furthermore, if a defined percentage of particles (e.g., ≥90%) meet the specific size threshold (e.g., ≤0.5 mm), this particle size criterion can complement the RSD_r and act as a robust proxy for verifying homogeneity, consistent with the reliability of slurry method. Consequently, when a specified percentage of particles falls below the defined size threshold (e.g., from the output of this study, ≥90% <0.5 mm for aflatoxins and 60% <2.0 mm for fumonisins), sample homogeneity can be considered assured (RSD_r <20%). This approach enables a robust and objective assessment of the effectiveness of sample preparation procedures adopted by official laboratories to achieve complete homogenisation of the material.

Authors' contributions

FD, EG, BDS conceived and designed the study; FD, MR, BDS wrote the manuscript; EP, EG, MR did formal analyses; EP, MEG, MR revised and edited the manuscript. All Authors revised the manuscript for important intellectual content and agreed with this article's contents.

Conflict of interest statement

The Authors declare no competing interests.

Received on 19 November 2025.
Accepted on 12 February 2026.

REFERENCES

1. Casu A, Camardo Leggieri M, Toscano P, Battilani P. Changing climate, shifting mycotoxins: a comprehensive review of climate change impact on mycotoxin contamination. Compr Rev Food Sci Food Saf. 2024;23(2);e13323. doi: 10.1111/1541-4337.13323
2. Paterson R, Russell M, Lima N. How will climate change affect mycotoxins in food? Food Res Int. 2010;43(7):1902-14. doi: 10.1016/j.foodres.2009.07.010
3. Marin S, Ramos AJ, Cano-Sancho G, Sanchis V. Mycotoxins: occurrence, toxicology, and exposure assessment. Food Chem Toxicol. 2013;60:218-37. doi: 10.1016/j.fct.2013.07.047
4. Assunção R, Silva MJ, Alvito P. Challenges in risk assessment of multiple mycotoxins in food. World Mycotoxin J. 2016;9(5):791-811. doi: 10.3920/WMJ2016.2039
5. European Union. Commission Regulation (EU) 2023/915 of 25 April 2023 n. 1881/2006 on maximum levels for certain contaminants in food and repealing Regulation (EC). Office Journal of the European Union L 119, 25 April 2023. Available from: http://data.europa.eu/eli/reg/2023/915/oj
6. European Union. Regulation (EU) 2017/625 of the European Parliament and of the Council of 15 March 2017 on official controls and other official activities performed to ensure the application of food and feed law, rules on health and welfare, plant health and plant protection products, amending Regulations (EC) n. 999/2001, (EC) n. 396/2005, (EC) n. 1069/2009, (EC) n. 1107/2009, (EU) n. 1151/2012, (EU) n. 652/2014, (EU) 2016/429 and (EU) 2016/2031 of the European Parliament and of the Council, Council Regulations (EC) n. 1/2005 and (EC) n. 1099/2009 and Council Directives 98/58/EC, 1999/74/EC, 2007/43/EC, 2008/119/EC and 2008/120/EC, and repealing Regulations (EC) n. 854/2004 and (EC) n. 882/2004 of the European Parliament and of the Council, Council Directives 89/608/EEC, 89/662/EEC, 90/425/EEC, 91/496/EEC, 96/23/EC, 96/93/EC and 97/78/EC and Council Decision 92/438/EEC (Official Controls Regulation). Official Journal of the European Union L 95, 15 March 2017. Available from: http://data.europa.eu/eli/reg/2017/625/oj
7. European Union. Commission Implementing Regulation (EU) 2023/2782 of 14 December 2023 No 401/2006 laying down the methods of sampling and analysis for the control of the levels of mycotoxins in food and repealing Regulation (EC). Official Journal of the European Union L, 14 December 2023. Available from: http://data.europa.eu/eli/reg_impl/2023/2782/oj
8. Whitaker TB. Sampling foods for mycotoxins. Food Addit Contam. 2006;23(1):50-61. doi: 10.1080/02652030500241587
9. Miraglia M, De Santis B, Pannunzi E, Debegnach F, Brera C. Mycotoxin concentration data quality: the role of sampling. In: Leslie JF, Bandyopadhyay R, Visconti A (Eds). Mycotoxins: detection methods, management, public health and agricultural trade. CAB International; 2008. p. 185-93. doi: 10.1079/9781845930820.0185
10. Maestroni B, Cannavan A. Sampling strategies to control mycotoxins. In: De Saeger S (Ed). Determining mycotoxins and mycotoxigenic fungi in food and feed. Cambridge, UK: Woodhead Publishing Limited; 2011. p. 3-36. doi: 10.1533/9780857090973.1.3
11. Italia. Decreto Legislativo 2 febbraio 2021, n. 27. Disposizioni per l'adeguamento della normativa nazionale alle disposizioni del Regolamento (UE) 2017/625 ai sensi dell'articolo 12, lettere a), b), c), d) ed e) della legge 4 ottobre 2019, n. 117. Gazzetta Ufficiale – Serie Generale n. 60, 11 marzo 2021.
12. Miraglia M, De Santis B, Minardi V, Debegnach F, Brera C. The role of sampling in mycotoxin contamination: a holistic view. Food Addit Contam. 2005;22(1):31-6. doi: 10.1080/02652030500389055
13. Tittlemier SA, Varga E, Scott PM, Krska R. Sampling of cereals and cereal-based foods for the determination of ochratoxin A: an overview. Food Addit Contam. 2011;28(6):775-85. doi: 10.1080/19440049.2011.559278
14. Velasco J, Morris, SL. Use of water slurries in aflatoxin analysis. J Agric Food Chem. 1976;24(1):86-8. doi: 10.1021/jf60203a003
15. Spanjer MC, Scholten JM, Kastrup S, Jörissen U, Schatzki TF, Toyofuku N. Sample comminution for mycotoxin analysis: dry milling or slurry mixing? Food Addit Contam. 2006;23(1):73-83. doi: 10.1080/02652030500260439
16. Bircan C. Comparison of homogenization techniques and incidence of aflatoxin contamination in dried figs for export. Food Addit Contam. 2009;2(2):171-7. doi: 10.1080/19440040903280521
17. Lippolis V, Pascale M, Valenzano S, Visconti A. Comparison of slurry mixing and dry milling in laboratory sample preparation for determination of ochratoxin A and deoxynivalenol in wheat. J AOAC Int. 2012;95(2):452-8. doi: 10.5740/jaoacint.11-047
18. Damiani T, Righetti L, Suman M, Galaverna G, Dall'Asta C. Analytical issue related to fumonisins: a matter of sample comminution? Food Control. 2019;95:1-5. doi:10.1016/j.foodcont.2018.07.029
19. European Commission. The European guidance document for competent authorities for the control of compliance with EU legislation on aflatoxins. 2010. Available from: https://food.ec.europa.eu/system/files/2021-05/cs_contaminants_catalogue_alfatoxins_guidance-2010_en.pdf
20. Comité Européen de Normalisation. Foodstuffs – Determination of aflatoxin B1 and the sum of aflatoxin B1, B2, G1 and G2 in hazelnuts, peanuts, pistachios, figs, and paprika powder – High performance liquid chromatographic method with post-column derivatisation and immunoaffinity column cleanup. CEN; 2007. (CEN EN 14123:2007).
21. Comité Européen de Normalisation. Foodstuffs – Determination of fumonisin B1 and fumonisin B2 in processed maize containing foods for infants and young children – HPLC method with immunoaffinity column cleanup and fluorescence detection after pre-column derivatisation. CEN; 2015. (CEN EN 16187:2015).
22. European Food Safety Authority. Management of left-censored data in dietary exposure assessment of chemical substances. Scientific report. EFSA J. 2010;8(3):1557. Available from: https://doi.org/10.2903/j.efsa.2010.1557
23. Joint Research Centre Institute for Health and Consumer Protection. Guidelines for sample preparation procedures in GMO analysis. JRC Technical report. Luxembourg: Publications Office of the European Union; 2014. Available from: https://data.europa.eu/doi/10.2788/337005
24. International Organization for Standardization. Animal feeding stuffs – Guidelines for sample preparation, 3rd ed. Geneva: ISO; 2012. (ISO/FDIS 6498:2012).

Ann Ist Super Sanità 2026 | Vol. 62, No. 2: 149-155
DOI: 10.4415/ANN_26_02_07

Evaluating the usefulness of the Mediterranean Diet Quality Index (KIDMED) in assessing eating habits and nutritional status among preschool children

Lutvo Sporišević[1], Anes Jogunčić[2], Damir Šečić[3], Edna Supur[1] and Senka Dinarević-Mesihović[4]

[1]*Public Institution Health Center of Sarajevo Canton, Sarajevo, Bosnia and Herzegovina*
[2]*Department of Epidemiology, Public Health Institute of Sarajevo Canton, Sarajevo, Bosnia and Herzegovina*
[3]*Faculty of Medicine, University of Sarajevo, Sarajevo, Bosnia and Herzegovina*
[4]*Academy of Sciences and Arts of Bosnia and Herzegovina, Sarajevo, Bosnia and Herzegovina*

Abstract

Background. Establishing healthy eating habits during early childhood is essential for long-term health and prevention of non-communicable diseases. The Mediterranean Diet (MD) is recognized as one of the healthiest dietary patterns worldwide. This study aimed to evaluate dietary habits and adherence to the MD among preschool children using the Mediterranean Diet Quality Index (KIDMED) and to explore the influence of parental sociodemographic and anthropometric factors on children's dietary adherence.

Methods. This cross-sectional study included 114 preschool children (24-60 months) from Sarajevo and Orašje, Bosnia and Herzegovina. Data were collected using a modified KIDMED questionnaire, assessing dietary habits and general sociodemographic information. Children's anthropometric parameters were obtained from medical records, and Body Mass Index (BMI) z-scores were calculated using WHO AnthroPlus. Descriptive statistics, group comparisons, and multiple linear regression were performed in RStudio (version 2024.12.0+467), with statistical significance set at $p<0.05$.

Results. The mean KIDMED score was 5.17±2.98 (median 6, interquartile range IQR 3-8), indicating moderate adherence to the MD. Thirty-four percent of children had a poor-quality diet, 36.8% required improvement, and 28.9% showed optimal adherence. Fruit and vegetable consumption was high, but intake of fish, whole grains, nuts, and olive oil was low. Parental education, employment, economic status, and BMI were not significantly associated with children's KIDMED scores (Regression correlation coefficient=0.249, p=0.433).

Conclusions. Preschool children demonstrated moderate adherence to the MD, with clear signs of dietary westernization. Early, family-centered nutritional education is essential to promote and maintain healthy eating habits from early childhood.

Key words

- Mediterranean diet
- KIDMED Index
- preschool children
- dietary habits
- parental influence

INTRODUCTION

Providing optimized nutrition during the initial 1,000 days, spanning from conception through the second birthday, is essential for promoting healthy development [1, 2]. Establishing an appropriate dietary pattern during this early period forms the foundation for a diverse and balanced diet in the future. The overconsumption of high glycemic index foods, excessive intake of red meat and fast food, combined with insufficient consumption of fruits, vegetables, legumes, whole grains, and nuts, as well as low physical activity and prolonged sedentary behavior, plays a crucial role in the onset of obesity and related health conditions [3].

The features of the Mediterranean Diet (MD), widely recognized as one of the healthiest dietary patterns, are essential in preventing obesity, arterial hypertension, cardiovascular diseases, metabolic syndrome, diabetes, dyslipidemia, neurodegenerative diseases, malignan-

Address for correspondence: Anes Jogunčić, Department of Epidemiology, Public Health Institute of Sarajevo Canton, Sprat 3, Dr. Mustafe Pintola 1, 71210 Ilidža, Sarajevo, Bosnia and Herzegovina. E-mail: anes.joguncic@zzjzks.ba.

cies, and other health conditions [4-7]. Adherence to the MD during pregnancy has been shown to positively influence the health of infants and children [4].

The MD is characterized by high consumption of diverse fruits, vegetables, whole grains, legumes, nuts, and seeds; moderate to high intake of fish and seafood; moderate consumption of poultry and dairy products; and low intake of red meat, processed foods, fast food, and sweets. Olive oil serves as the primary source of fat in this dietary pattern [4-6, 8, 9]. The MD has been demonstrated to improve blood lipid profiles, enhance insulin sensitivity, support endothelial and antithrombotic function, and exhibit anti-inflammatory and antioxidant properties. These effects contribute to the prevention of chronic non-communicable diseases, the extension of life expectancy, and the improvement of overall quality of life [5, 10-12].

Pereira-da-Silva and colleagues referenced several studies indicating a negative correlation between adherence to the MD and overweight in children, highlighting both short-term and long-term health implications [13].

The widespread issue of childhood obesity has been acknowledged as a global pandemic [14]. The prevalence of childhood obesity varies depending on the criteria used to define it, socioeconomic factors, dietary habits, levels of physical activity, and other contributing determinants.

According to the World Health Organization (WHO), the global prevalence of obesity in 2016 had tripled compared to 1975. WHO reported that 39 million children under the age of five were overweight or obese in 2020, and 340 million (18%) of children and adolescents aged 5-19 years were overweight or obese in 2016 [15]. Ukraine had the highest prevalence of overweight among European countries for children aged 0 to 6, with 27.8% of boys and 27.3% of girls affected, while Bosnia and Herzegovina also exhibited notable rates, with 17.1% of boys and 17.7% of girls being overweight [16]. This underscores a global trend of continuous growth in overweight and obesity across all age groups and countries. More than 60% of children who are overweight before puberty remain overweight in early adulthood [17].

Over the years, various indices have been developed to assess diet quality in accordance with dietary guidelines. This study focuses on the Mediterranean Diet Quality Index, designed to evaluate the adherence of children and young people to the MD. The index was originally developed by Serra-Majem *et al*. (2004 version) and later modified by Altavilla *et al*. (2019 version) [18, 19].

Several studies indicate that the Mediterranean Diet Quality Index (KIDMED) is a simple and practical tool for assessing the adequacy of children's dietary habits and their association with nutritional status. In routine medical practice, the assessment of children's eating habits, including the use of the KIDMED questionnaire, often receives insufficient attention. Buja *et al*. (2024) investigated adherence to the MD among Italian children aged 10-11 years using the KIDMED Index and found that higher screen-time and media use were significantly associated with lower diet quality. The study highlighted the early influence of lifestyle habits on nutritional behaviors in childhood. These findings emphasize the importance of early dietary screening and health education to support adherence to the MD [20].

Tambalis *et al*. (2024) analyzed dietary habits in a large national sample of over 177,000 Greek schoolchildren using the KIDMED Index, revealing a clear decline in MD adherence with increasing age. The results indicated that younger children tend to have healthier eating patterns than adolescents. This large-scale study reinforces the value of KIDMED as a tool for monitoring dietary patterns across childhood and adolescence [21].

Bober and Gaszyńska (2025) validated the Polish version of the updated KIDMED 2.0 questionnaire among children and adolescents aged 10-18 years. The study demonstrated satisfactory psychometric properties and confirmed the tool's reliability and applicability for assessing adherence to the MD in youth populations. This supports the broader use of KIDMED 2.0 as a standardized measure for evaluating diet quality in pediatric and adolescent groups [22].

The aim of this study was to assess the dietary habits and nutritional status of the participants and to explore the utility of the KIDMED Index in evaluating their dietary patterns and nutritional status.

METHODS

This cross-sectional study included a total of 152 preschool children and their parents. Of these, 38 parents did not complete the questionnaires and were excluded from further analysis, resulting in a response rate of 78%. All children were in good health and under regular medical supervision at the Sarajevo Health Center and the Orašje Health Center.

The study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki. Ethical approval was obtained from the Ethical Committee of the Health Center of Canton Sarajevo (approval n. 01-6-258/2), and written informed consent was obtained from parents prior to their children's participation.

Parents voluntarily, independently, and anonymously completed a questionnaire adapted for this study, a modified and updated KIDMED questionnaire by Altavilla and Caballero-Pérez (2019), consisting of 16 questions on children's dietary habits. According to the scoring system proposed by the original authors, a positive answer to each item was scored +1, and a negative answer -1.

The total score ranged from -4 to +12, categorized as follows:

- 0-3 points: poor adherence to the MD;
- 4-7 points: average adherence;
- ≥8 points: good adherence to the MD [19].

In addition to dietary questions, the questionnaire included general sociodemographic and anthropometric data: child's age and gender, parental age, parental educational level, family employment and economic status, child's birth weight, current body weight and height, parental Body Mass Index (BMI), duration of

breastfeeding, and age at the introduction of complementary feeding. Children's body weight (kg) and height/length (cm) were extracted from health records collected during routine pediatric checkups as part of vaccination visits, within one month prior to the study. BMI was calculated for each child, and BMI z-scores were derived using the WHO AnthroPlus software [23]. Based on the WHO child growth standards (2006, for ages 0-5 years) [24], nutritional status was categorized as follows: underweight, BMI z-score <-2 SD; normal body weight, BMI z-score from -2 SD to <+1 SD; at risk of overweight, BMI z-score >+1 SD to <+2 SD; overweight, BMI z-score ≥+2 SD to <+3 SD; obese, BMI z-score ≥+3 SD.

Parents self-reported their body weight and height, and BMI was calculated and categorized according to the standard adult classification: underweight (<18.5 kg/m²), normal weight (18.5-24.9 kg/m²), overweight (25-29.9 kg/m²), and obese (≥30 kg/m²). All data were analyzed using RStudio (version 2024.12.0+467). Descriptive statistics were applied to summarize the data: categorical variables were presented as absolute (N) and relative frequencies (%), while continuous variables were expressed as mean ± standard deviation (SD) or median with interquartile range (IQR), depending on data distribution.

Normality of continuous variables was assessed using the Shapiro-Wilk test. Differences between groups (boys *vs* girls) were evaluated using the independent samples t-test for normally distributed variables and the Mann-Whitney U test for non-normally distributed variables. Associations between categorical variables were analyzed using the Chi-square test or Fisher's exact test when appropriate.

To explore the potential influence of parental sociodemographic and anthropometric factors on children's adherence to the MD (KIDMED score), a multiple linear regression analysis was performed. Predictor variables included parental BMI (mother and father), parental age, education level, employment status, family economic status, and parent completing the questionnaire. Statistical significance was set at $p<0.05$ for all analyses.

RESULTS

A total of 114 children were included in the study, of whom 54 (47.4%) were girls and 60 (52.6%) were boys. The mean age of the children was 41.57±12.1 months, ranging from 24 to 60 months. Girls were slightly older than boys (43.6±12.2 *vs* 39.7±11.8 months, p=0.044) (*Table 1*).

The majority of questionnaires were completed by mothers (76.3%), while fathers completed 23.7%. The mean age of mothers was 32.6±5.2 years, and of fathers 34.5±4.7 years, with no statistically significant difference between parents of boys and girls (p=0.372 and p=0.054, respectively). Regarding educational level,

Table 1
General information about participants

		Total	Girls	Boys	p
	Total N (R%)	**114 (100%)**	**54 (47.4%)**	**60 (52.6%)**	
Age of children (months)		41.57±12.1	43.6±12.2	39.7±11.8	0.044
Respondent	Mother	87 (76.3%)	41 (75.9%)	46 (76.7%)	0.926
	Father	27 (23.7%)	13 (24.1%)	14 (23.3%)	
Age of respondent (years)	Mother	32.6±5.2	32.4±5.3	32.8±5.0	0.372
	Father	34.5±4.7	36.0±4.3	33.1±4.8	0.054
Educational level	Elementary school	4 (3.5%)	1 (1.9%)	3 (5.0%)	0.164
	High school	42 (36.8%)	23 (42.6%)	19 (31.7%)	
	College	10 (8.8%)	5 (9.3%)	5 (8.3%)	
	University	52 (45.6%)	20 (37.0%)	32 (53.3%)	
	Master's or PhD	6 (5.3%)	5 (9.3%)	1 (1.7%)	
Employment status of parent which gave information	Unemployed	28 (24.6%)	13 (24.1%)	15 (25.0%)	0.909
	Employed	86 (75.4%)	41 (75.9%)	45 (75.0%)	
Household income	Below average	15 (13.2%)	6 (11.1%)	9 (15.0%)	0.814
	Average	36 (31.6%)	17 (31.5%)	19 (31.7%)	
	Above average	63 (55.3%)	31 (57.4%)	32 (53.3%)	
BMI mother	Normal body weight	78 (68.4%)	42 (77.8%)	36 (60.0%)	0.084
	Overweight	30 (26.3%)	9 (16.7%)	21 (35.0%)	
	Obese	6 (5.3%)	3 (5.6%)	3 (5.0%)	
BMI father	Normal body weight	44 (38.6%)	19 (35.2%)	25 (41.7%)	0.676
	Overweight	50 (43.9%)	24 (44.4%)	26 (43.3%)	
	Obese	20 (17.5%)	11 (20.4%)	9 (15.0%)	

N: frequency; R: row percentage; BMI: Body Mass Index, p: level of statistical significance.

45.6% of respondents had completed university, 36.8% high school, 8.8% college, 5.3% held a Master's or PhD degree, and 3.5% had only elementary education. There were no statistically significant gender differences in parental education (p=0.164). Most responding parents were employed (75.4%), while 24.6% were unemployed (p=0.909). More than half of families (55.3%) reported above-average household income, 31.6% average, and 13.2% below-average income, with no significant differences between parents of boys and girls (p=0.814).

Analysis of maternal BMI showed that 68.4% of mothers had normal body weight, 26.3% were overweight, and 5.3% were obese. Among fathers, 38.6% had normal weight, 43.9% were overweight, and 17.5% were obese. Differences in BMI categories between parents of boys and girls were not statistically significant (p=0.084 for mothers; p=0.676 for fathers).

The mean body weight of all participants was 16.9±3.5 kg, with girls showing slightly higher average weight than boys (17.2±3.6 kg *vs* 16.6±3.4 kg), though this difference was not statistically significant (p=0.088). The mean height was 101.4±10.4 cm, with no significant difference between girls (102.5±10.8 cm) and boys (100.5±10.1 cm, p=0.319) (*Table 2*).

The mean BMI for the entire group was 16.4±2.8 kg/m², and the mean BMI z-score was 0.91±1.50, without significant gender differences (p=0.308 and p=0.946, respectively). The average birth weight of the children was 3,345±587 g, similar between girls (3,377±502 g) and boys (3,316±658 g, p=0.485).

Analysis of breastfeeding showed that the median duration of breastfeeding was 6 months (IQR: 2-12 months). Introduction of complementary feeding after the fourth month of life was reported in 87 children (76.3%). The analysis of food intake based on the KIDMED Index is presented in *Figure 1*.

Analysis of individual KIDMED items revealed that 98.2% of children consumed at least one fruit daily, while 62.3% consumed two or more types of fruit each day. A very high proportion (97.4%) reported eating fresh or cooked vegetables once daily, and 51.8% ate vegetables more than once per day. Regular fish consumption (≥2-3 times per week) was reported by only 17.5% of children. More than one-third of participants (37.7%) visited fast-food restaurants more than once weekly. In contrast, 86.8% reported eating legumes (pulses) more than once per week. Daily consumption of whole-grain pasta or rice was reported by 34.2%, and 32.5% ate whole-grain cereals or bread for breakfast. Nuts were consumed regularly (≥2-3 times per week) by 37.7%, while olive oil was used at home by 38.6% of families. Breakfast skipping was reported by 32.5% of children. The majority (86.0%) consumed a dairy product (milk or yogurt) for breakfast, but 63.2% also reported eating commercially baked goods or pastries for breakfast. Slightly more than half (51.8%) consumed two yogurts and/or 40 g of cheese daily. Frequent consumption of sweets and candies (several times per day) was observed in 43.9% of children. Overall, the findings indicate moderate adherence to the Mediterranean dietary pattern among the surveyed preschool children.

The mean KIDMED Index score was 5.17±2.98, while the median value was 6 (IQR: 3-8) (*Figure 2*). Based on the KIDMED classification, 39 children (34.2%) had a very low-quality diet, 42 (36.8%) required improvement of their dietary pattern to align with the Mediterranean model, and 33 (28.9%) demonstrated an optimal MD. While fruit and vegetable consumption was high, suggesting awareness of healthy food choices, the low frequency of fish intake and limited consumption of whole-grain foods and olive oil point to deviations from traditional Mediterranean habits. The high proportion of children consuming fast food, pastries for breakfast, and sweets several times per day reflects the growing influence of Western dietary patterns. These results underline the need for early nutritional education and family-based interventions to reinforce healthy eating habits and improve adherence to the MD from early childhood.

A multiple linear regression model was constructed to examine the influence of parental sociodemographic and anthropometric characteristics on children's adherence to the MD, expressed through the KIDMED Index. The independent variables included parental BMI (mother and father), parental age, level of education, employment status, family material status, and which parent completed the questionnaire (mother or father).

The overall model did not reach statistical significance ($F(7,106)=1.004$, p=0.433), with a correlation coefficient of R=0.249 and an explained variance of 6.2% ($R^2=0.062$; adjusted $R^2=0.000$). This indicates a weak and statistically non-significant relationship between the examined parental factors and the children's KIDMED Index. None of the predictors demonstrated a significant individual effect on children's adherence to the MD, suggesting that within this cohort, parental

Table 2
Anthropometric characteristics of the children

	Total		Girls		Boys		p
	Mean	SD	Mean	SD	Mean	SD	
Weight (kg)	16.9	3.5	17.2	3.6	16.6	3.4	0.088
Height (cm)	101.4	10.4	102.5	10.8	100.5	10.1	0.319
BMI (kg/m²)	16.4	2.8	16.5	2.9	16.4	2.7	0.308
BMI z-score	0.91	1.50	1.01	1.58	0.82	1.43	0.946
Mass at birth (g)	3,345	587	3,377	502	3,316	658	0.485

SD: standard deviation; BMI: Body Mass Index. BMI z score – Body Mass Index z-scores were calculated based on World Health Organization growth standards.

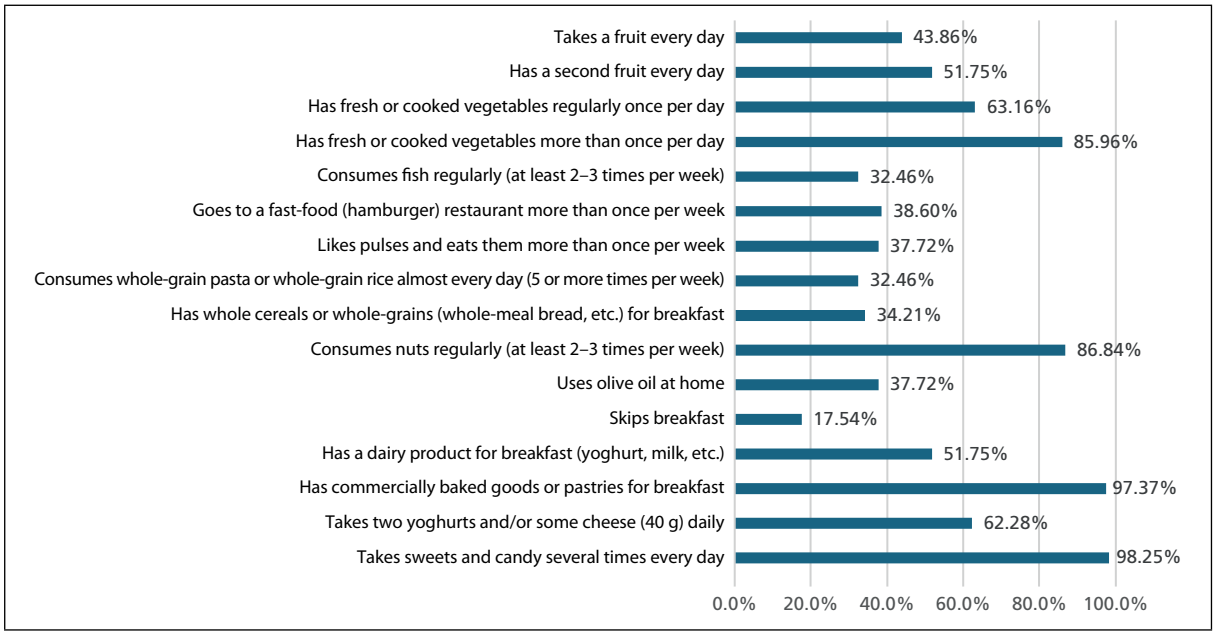


Figure 1
Food intake according to the KIDMED questionnaire.
KIDMED: Mediterranean Diet Quality Index.

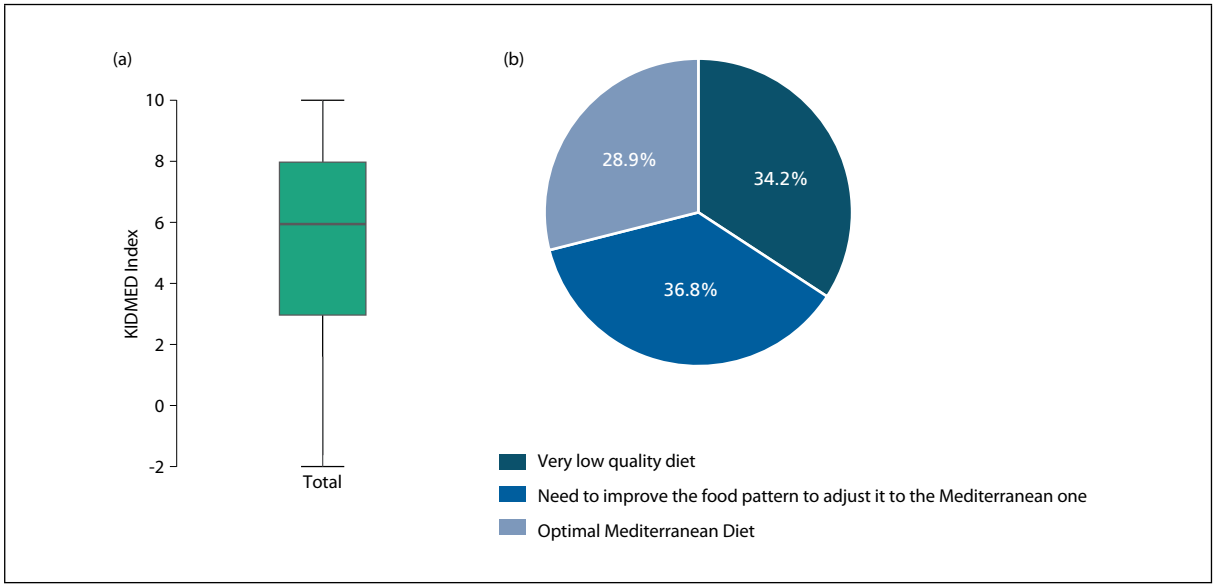


Figure 2
KIDMED Index value (a) and classification (b).

demographic, educational, economic, or BMI characteristics did not substantially influence dietary quality.

DISCUSSION

The mean KIDMED score observed in this study (5.17±2.98) indicates a moderate adherence to the MD among preschool children. These findings are comparable to those reported in Greek, Spanish, and Italian preschool populations, where mean scores typically range from 4.5 to 6.5 points [13, 22, 23, 25-27]. Similar to previous studies, the present research highlights a high intake of fruits and vegetables but insufficient consumption of fish, whole grains, nuts, and olive oil – core components of the traditional Mediterranean pattern [6, 12, 18, 19]. This imbalance suggests a gradual shift from traditional dietary habits toward more Westernized eating behaviors, characterized by greater consumption of fast food, sweets, and processed bakery products [3, 28].

Parental influence is considered a key determinant of children's dietary behavior, yet in this study, no significant association was found between parental education, employment, economic status, or BMI and the KIDMED Index. These findings align with those of

Bober and Gaszyńska [22] and Pereira-da-Silva *et al*. [13], who also observed limited predictive power of sociodemographic variables in explaining MD adherence in preschool children. However, other studies have reported mixed evidence – some suggesting that higher parental education and healthier parental BMI are associated with better KIDMED scores [8, 26, 28], while others attribute dietary differences primarily to environmental and cultural factors such as food availability and family mealtime practices [26, 30]. The weak correlation found in the current regression model ($R=0.249$, $R^2=0.062$) supports the notion that structural and environmental determinants may outweigh individual parental characteristics in shaping dietary habits during early childhood.

When compared with recent large-scale studies, the proportion of children demonstrating optimal adherence (28.9%) is relatively consistent. Tambalis *et al*. [21] reported similar results in over 170,000 Greek schoolchildren, while Buja *et al*. [20] found comparable rates of moderate to high adherence among Italian children. Conversely, lower adherence levels have been observed in non-Mediterranean European populations, suggesting that geographical and cultural proximity to the Mediterranean basin still offers a modest protective influence [23, 29].

The observed trends underscore the urgent need for early nutrition education that promotes the principles of the MD – particularly regular fish consumption, the use of olive oil, and the inclusion of whole grains and nuts in daily meals. Interventions targeting both parents and preschool institutions may be especially effective, as the early years represent a critical period for establishing long-term dietary preferences [1, 2, 31]. Consistent with global recommendations [15, 17], the findings reinforce that public-health strategies should focus not only on nutrient adequacy but also on dietary patterns that support lifelong metabolic and cardiovascular health.

Despite the valuable insights provided, this study has certain limitations, including its cross-sectional design and reliance on parent-reported dietary data, which may introduce recall bias. Nevertheless, the findings contribute to the limited body of evidence on MD adherence among preschool children in Southeast Europe. The observed patterns highlight both strengths – such as regular fruit and vegetable intake – and weaknesses, notably low fish and whole-grain consumption. Future research should explore longitudinal relationships between early dietary patterns, parental behaviors, and later health outcomes, as well as evaluate the effectiveness of preschool- and family-based nutrition interventions.

CONCLUSIONS

The study showed moderate adherence to the MD among preschool children, with high fruit and vegetable intake but low consumption of fish, whole grains, nuts, and olive oil. One-third of participants had poor dietary quality, while only about one-quarter achieved optimal adherence. Parental sociodemographic and anthropometric factors did not significantly influence children's KIDMED scores. The KIDMED Index proved to be a simple and effective screening tool for assessing dietary habits and identifying deviations from the Mediterranean pattern in preschool children of the Bosnian population. These findings emphasize the importance of early nutritional education to promote healthy eating habits from early childhood.

Conflict of interest statement

None.

Authors' contributions

Conceptualization: AJ; data collection: LS and ES; data analysis: AJ; writing-original draft preparation (Introduction): DŠ and ES; writing-discussion and interpretation: AJ and LS; review and final editing: SDM. All Authors have read and approved the final manuscript.

Received on 29 October 2025.
Accepted on 12 February 2026.

REFERENCES

1. Beluska-Turkan K, Korczak R, Hartell B, Moskal K, Maukonen J, Alexander DE, et al. Nutritional gaps and supplementation in the first 1000 days. Nutrients. 2019;11(12):2891. doi: 10.3390/nu11122891
2. Schwarzenberg SJ, Georgieff MK; Committee on nutrition. Advocacy for improving nutrition in the first 1000 days to support childhood development and adult health. Pediatrics. 2018;141(2):e20173716. doi: 10.1542/peds.2017-3716
3. Clemente-Suárez VJ, Beltrán-Velasco AI, Redondo-Flórez L, Martín-Rodríguez A, Tornero-Aguilera JF. Global impacts of Western diet and its effects on metabolism and health: a narrative review. Nutrients. 2023;15(12):2749. doi: 10.3390/nu15122749
4. Mascarenhas MR. Pediatric anti-inflammatory diet. Pediatr Ann. 2019;48(6):e220-e5. doi: 10.3928/19382359-20190515-02
5. Schwingshackl L, Morze J, Hoffmann G. Mediterranean diet and health status: active ingredients and pharmacological mechanisms. Br J Pharmacol. 2020;177(6):1241-57. doi: 10.1111/bph.14778
6. Gantenbein KV, Kanaka-Gantenbein C. Mediterranean diet as an antioxidant: the impact on metabolic health and overall wellbeing. Nutrients. 2021;13(6):1951. doi: 10.3390/nu13061951
7. Davis C, Bryan J, Hodgson J, Murphy K. Definition of the Mediterranean diet: a literature review. Nutrients. 2015;7(11):9139-53. doi: 10.3390/nu7115459
8. Kanellopoulou A, Giannakopoulou SP, Notara V, Antonogeorgos G, Rojas-Gil AP, Kornilaki EN, et al. The association between adherence to the Mediterranean diet and childhood obesity: the role of family structure. Nutr Health. 2021;27(1):39-47. doi: 10.1177/0260106020952600
9. Naureen Z, Bonetti G, Medori MC, Aquilanti B, Velluti V, Matera G, et al. Foods of the Mediterranean diet:

lacto-fermented food, the food pyramid and food combinations. J Prev Med Hyg. 2022;63(2 Suppl. 3):E28-E35. doi: 10.15167/2421-4248/jpmh2022.63.2S3.2744
10. Stromsnes K, Mas-Bargues C, Gambini J, Gimeno-Mallench L. Protective effects of polyphenols present in Mediterranean diet on endothelial dysfunction. Oxid Med Cell Longev. 2020;2097096. doi: 10.1155/2020/2097096
11. Sofi F, Macchi C, Abbate R, Gensini GF, Casini A. Mediterranean diet and health status: an updated meta-analysis and a proposal for a literature-based adherence score. Public Health Nutr. 2014;17(12):2769-82. doi: 10.1017/S1368980013003169
12. Dominguez LJ, Di Bella G, Veronese N, Barbagallo M. Impact of Mediterranean diet on chronic non-communicable diseases and longevity. Nutrients. 2021;13(6):2028. doi: 10.3390/nu13062028
13. Pereira-da-Silva L, Rêgo C, Pietrobelli A. The diet of preschool children in the Mediterranean countries of the European Union: a systematic review. Int J Environ Res Public Health. 2016;13(6):572. doi: 10.3390/ijerph13060572
14. Dabas A, Seth A. Prevention and management of childhood obesity. Indian J Pediatr. 2018;85(7):546-53. doi: 10.1007/s12098-018-2636-x
15. World Health Organization. Obesity and overweight. Geneva: WHO; 2021. Available from: https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight
16. Branca F, Nikogosian H, Lobstein T. The challenge of obesity in the WHO European region and the strategies for response. Copenhagen: WHO Regional Office for Europe; 2007.
17. Nittari G, Scuri S, Petrelli F, Pirillo I, Di Luca NM, Grappasonni I. Fighting obesity in children from European WHO member states: epidemiological data, medical-social aspects, and prevention programs. Clin Ter. 2019;170(3):e223-e30. doi: 10.7417/CT.2019.2137
18. Serra-Majem L, Ribas L, Ngo J, Ortega RM, García A, Pérez-Rodrigo C, Aranceta J. Food, youth and the Mediterranean diet in Spain: development of KIDMED, Mediterranean Diet Quality Index in children and adolescents. Public Health Nutr. 2004;7(7):931-5. doi: 10.1079/phn2004556
19. Altavilla C, Caballero-Pérez P. An update of the KIDMED questionnaire, a Mediterranean Diet Quality Index in children and adolescents. Public Health Nutr. 2019;22(14):2543-7. doi: 10.1017/S1368980019001058
20. Buja A, Grotto G, De Battisti E, Baldovin T, Chiamenti G, Baldo V. Media usage and adherence to the Mediterranean diet in children. Acta Biomed. 2024;95(1):e2024116.
21. Tambalis KD, Panagiotakos DB, Psarra G, Sidossis LS. Dietary habits among 177,091 Greek schoolchildren by age: the EYZHN study. Public Health Nutr. 2024;27(4):870-9.
22. Bober E, Gaszyńska E. Validation of KIDMED 2.0 PL – Mediterranean Diet Quality Index for Polish children and adolescents. Nutrients. 2025;17(2):318. doi: 10.3390/nu17162636
23. World Health Organization. Application tools: WHO AnthroPlus software. Geneva: WHO; 2009.
24. Bloem M. The 2006 WHO child growth standards. BMJ. 2007;334(7596):705-6. doi: 10.1136/bmj.39155.658843.BE
25. Vidal-Peracho C, Lafuente-Ibáñez de Mendoza I, et al. Association between parental education, BMI, and adherence to the Mediterranean diet in Spanish preschoolers. Nutrients. 2023;15(5):1124. doi: 10.3390/nu15051124
26. Caruso R, Toffolutti V, et al. Adherence to the Mediterranean diet and quality of life in early childhood: results from a multicenter European study. Public Health Nutr. 2022;25(11):3074-83.
27. Fernández-García JC, Martínez R, et al. Socioeconomic disparities and dietary patterns in preschool children: analysis using KIDMED Index. Int J Environ Res Public Health. 2023;20(9):5682. doi: 10.3390/ijerph20095682
28. Giampieri F, Rosi A, Frias-Toral E, et al. Unhealthy ultra-processed food, diet quality and adherence to the Mediterranean diet in children and adolescents: The delicious Project. Foods. 2025;14:2648. doi: 10.3390/foods14152648
29. Zaragoza-Martí A, Giner-Morales R, et al. Factors associated with low KIDMED scores in Mediterranean children aged 3-6 years. Nutrients. 2023;15(8):1876. doi: 10.3390/nu15081876
30. Pérez-Rodrigo C, Gil Á, et al. Promoting healthy dietary patterns in early childhood: evidence from Mediterranean countries. Nutrients. 2022;14(9):1841. doi: 10.3390/nu14091841
31. Colapinto CK, Graham J, et al. Early childhood nutrition and dietary quality indicators: global lessons for Mediterranean adherence. Nutrients. 2024;16(5):1159. doi: 10.3390/nu16051159

Ann Ist Super Sanità 2026 | Vol. 62, No. 2: 156-162
DOI: 10.4415/ANN_26_02_08

Women's experiences of membrane sweeping for labour induction: a phenomenological qualitative study

Jessica Preziosi[1,2], Silvia Salvi[1,2], Michelangela Danza[2], Elena Romano[1], Erika Colagiovanni[1], Francesca Rizzi[1], Silvia Buongiorno[1], Stefano Fruci[1], Sascia Moresi[1] and Gloria Anderson[1,2]

[1]*Fondazione Policlinico Agostino Gemelli IRCCS, Rome, Italy*
[2]*Dipartimento di Scienze della Vita e Sanità Pubblica, Università Cattolica del Sacro Cuore, Rome, Italy*

Abstract

Background. Membrane sweeping is a common but still invasive procedure offered to hasten labour in prolonged pregnancy. Rising labour induction rates have increased the use of interventions like membrane sweeping, though its impact on women's experiences remains unclear.

Aim. To explore women lived experiences of membrane sweeping in a tertiary Italian maternity unit and identify their informational needs.

Methods. Twenty women ≥40+0 to 41+6 weeks' gestation who underwent membrane sweeping were interviewed in-hospital 24-48 h postpartum. Semi-structured interviews (mean 18 min) were analysed with combined inductive-deductive content analysis.

Findings. Three themes emerged i) clarity and timing of information ii) baseline knowledge and information-seeking behaviour iii) physical and emotional experience of the procedure in relation to the onset of labour. In addition, some women reported the procedure as painful or required further pharmacological induction of labour, adding a layer of dissatisfaction in their narrations. However, most women stated that they would undergo membrane sweeping again in a future pregnancy.

Conclusions. When embedded in shared decision-making, membrane sweeping can be a well-accepted initial induction option. Moreover, structured antenatal education could be recommended.

Key words

- induction of labour
- informed consent
- membrane sweeping
- women's experiences

INTRODUCTION

Labour induction is the iatrogenic stimulation of uterine contractions before the onset of spontaneous labor, when maternal or fetal well-being is threatened by medical or obstetric complications [1]. Over the past decade, global induction rates have risen to about 25 percent, affecting both low- and high-risk pregnancies [1]. Available techniques span from pharmacological methods – such as oxytocin or prostaglandins – to mechanical or procedural approaches, including artificial rupture of membranes, double-balloon catheterisation, and membrane sweeping [2]. Several guidelines [3, 4] recommend membrane sweeping to reduce the need for formal pharmacological induction in post-term pregnancies.

Membrane sweeping is a procedure in which a clinician inserts a gloved finger through the cervix and gently detaches the lower pole of the membranes from the uterine wall [5]. This manoeuvre is intended to stimulate local prostaglandin release, promoting cervical ripening. Thus, this simple, low-cost intervention that can be done in the outpatient setting, increases the likelihood of spontaneous labour within 48 hours by roughly 20 percent and therefore diminishes reliance on pharmacological agents [6]. A Cochrane systematic review and other studies confirm that membrane sweeping does not significantly heighten maternal or perinatal risk compared with drug-based induction [6, 7], explaining its widespread adoption in everyday obstetric practice. If serious complication are rare, common side effects are mild vaginal bleeding, irregular uterine contractions and discomfort during procedure. In fact, membrane sweeping can be uncomfortable and may feel intrusive to some women [6].

Understanding women's subjective experiences is essential regardless of broader debates about obstetric violence [8, 9], yet most research focuses on timing and efficacy [10, 11], largely overlooking women's informational needs, psychological responses, and personal narratives. A recent qualitative systematic review

Address for correspondence: Michelangela Danza, Università Cattolica del Sacro Cuore, Largo Francesco Vito 1, 00168 Rome, Italy. E-mail: michelangela.danza@unicatt.it.

identified only one study that explored the procedure from women's perspectives [12]. This knowledge gap is concerning considering the rising global induction rates [6, 13] and the potential role of membrane sweeping in reducing pharmacological inductions. Because experiences are likely to differ across organisational and cultural settings, additional qualitative research is needed to refine clinical practice in line with women's preferences and expectations.

Thus, we aim to explore the experiences of women undergoing membrane sweeping at the Obstetric and Obstetric Pathology Unit of the Agostino Gemelli University Hospital-IRCCS, aiming to inform clinical practice and advance a more woman-centred model of care.

MATERIALS AND METHODS

We conducted a descriptive phenomenological study, reported following COREQ (Consolidated criteria for reporting qualitative research) [14]. A phenomenological lens is appropriate when the goal is to illuminate participants lived experiences, particularly in fields with limited prior evidence [15]. The study was approved by our local Ethical Committee (Protocol ID 7350 of February 10, 2025).

Participants

Eligible women were recruited between 10 February and 25 March 2025 through the post-term pregnancy outpatient clinic of the Obstetric and Obstetric Pathology Unit at Agostino Gemelli University Hospital IRCCS. Inclusion criteria were age >18, BMI 18-30 kg/m^2, singleton uncomplicated pregnancy, fluent Italian, no previous attempt at membrane sweeping in the current pregnancy. Gestational age was determined according to crown-rump length in the first trimester. Participation was voluntary, and all women were assured of data confidentiality and of their right to withdraw at any time without affecting their care. Exclusion criteria were maternal age <18; inadequate proficiency in Italian; or no clinical indication for membrane sweeping.

In accordance with our institutional protocol, all women with an otherwise uncomplicated singleton pregnancy reaching 40 weeks' gestation undergo ultrasonographic assessment of amniotic fluid volume and a non-stress fetal test. Immediate induction of labour is typically indicated if anhydramnios is detected on ultrasound (single deepest vertical pocket <2 cm) [16] or if cardiotocography criteria for normality are not met. Otherwise, routine induction of labour is scheduled no later than 41+0/3 weeks' gestation. When clinically indicated, membrane sweeping may be offered during these visits as part of the standard care pathway, but never portraited as mandatory. When membrane sweeping was clinical indicated, the study procedures were explained in detail by an obstetrician and a research midwife. Women who expressed interest were provided with additional information regarding the study's aims and methodology and subsequently gave written informed consent.

Data collection and analysis

Women who gave written consent were interviewed 24-48 hours after birth, while still hospitalised in the Obstetrics Unit. Interviews took place in the women's single rooms, in accordance with the current clinical practice, and always in the presence of the newborn. In most cases, a support person (typically the partner) was also present during the interview.

A PhD research midwife and young research midwife with no clinical role in their care carried out face-to-face, semi-structured interviews of roughly half an hour, guided by a flexible topic schedule (8 domains; *File 1S available online as Supplementary Material*) designed to explore women's views on membrane sweeping.

After the interview, each participant completed an anonymous questionnaire capturing socio-demographic and obstetric details. All conversations were audio-recorded, then the verbatim were transcribed and anonymised. Immediately afterwards, the interviewer added contextual observations and reflexive notes to a field diary to enrich subsequent analysis. All data were stored and managed in compliance with the General Data Protection Regulation (GDPR), ensuring confidentiality, anonymity, and secure handling of participants' information.

Data analysis

We analysed the transcripts with qualitative content analysis that blended deductive and inductive coding [17, 18]. Two researchers independently (ER, research midwife and JP, PhD midwife) read each transcript line by line, identified meaning units – words or passages that reflected the women's experiences – and assigned initial codes. Deductive codes stemmed from the study questions, whereas inductive codes emerged directly from the data, capturing unexpected insights. The coders compared their work and resolved differences through discussion; when consensus was elusive, a third researcher (GA, PhD midwife) was consulted to reach and agreement [19]. Codes were analysed with the support of dedicated sheets in Microsoft Excel. Data collection and analysis proceeded concurrently, allowing iterative refinement of the coding frame and progressively deeper interpretation [20]. Recruitment ceased when theoretical saturation was reached and no new codes or categories appeared [21].

RESULTS

Between February and March 2025, 20 women were interviewed. Their principal sociodemographic and obstetric characteristics are summarised in *Table 1*. The median age of the participants was 33 years (IQR: 29.5;36). All first sweeps occurred at 40+1 (IQR: 40+0 - 40+2) weeks of gestation. Four women received a second sweep at 40+3 weeks of gestation. Five (25%) subsequently required pharmacological induction; the remaining 15 (75%) entered spontaneous labour within 48 h.

The analysis categorized women's narratives into three overarching themes, each with its own layers of meaning (*Figure 1*): i) clarity and timing of information, encompassing two sub-themes that captured how and when details were provided; ii) baseline knowledge and information-seeking behaviour, articulated through three sub-themes reflecting women's prior understand-

Table 1
Sociodemographic and obstetric characteristics

Participants	Value
Women, n (%)	20 (100%)
Age, years-median (IQR)	33 (29.5-36)
Italian citizenship, n (%)	19 (95%)
Nulliparous, n (%)	10 (50%)
Repeated membrane sweep, n (%)	4 (20%)
Gestational age at first membrane sweeping, weeks-median (IQR)	40+1 (40+0 - 40+2)
Pharmacological induction required, n (%)	8 (40%)

IQR: Interquartile range; %: percentage; n: number of women.

ing, misconceptions, and sources of learning; and iii) physical and emotional experience of the procedure, described across four sub-themes that ranged from physical sensations to emotional responses. Together, these themes sketch a comprehensive picture of how women perceived, understood, and lived through membrane sweeping.

Clarity and timing of information

The first theme that emerged from the data, relates to the information provided to women regarding the membrane sweeping procedure and the process through which verbal informed consent was obtained. This theme captures women's experiences of being informed – both in terms of clarity and timing – and how this influenced their sense of autonomy and involvement in the decision-making process.

Information received before and during the procedure

A recurrent, cross-cutting theme was the need to feel properly informed – not through technical minutiae, but via clear, straightforward explanations of the manoeuvre – because such communication anchored women's sense of agency and genuine informed consent. Most women described receiving simple, step-by-step guidance that they found reassuring: *"Before the procedure I was told exactly how it would be done… The gynaecologist said it might be a bit uncomfortable, but I could ask her to stop at any time"*. One woman, who had actively requested the sweep, echoed this sentiment: *"They explained every step very clearly, and that made me feel part of the decision"*. This

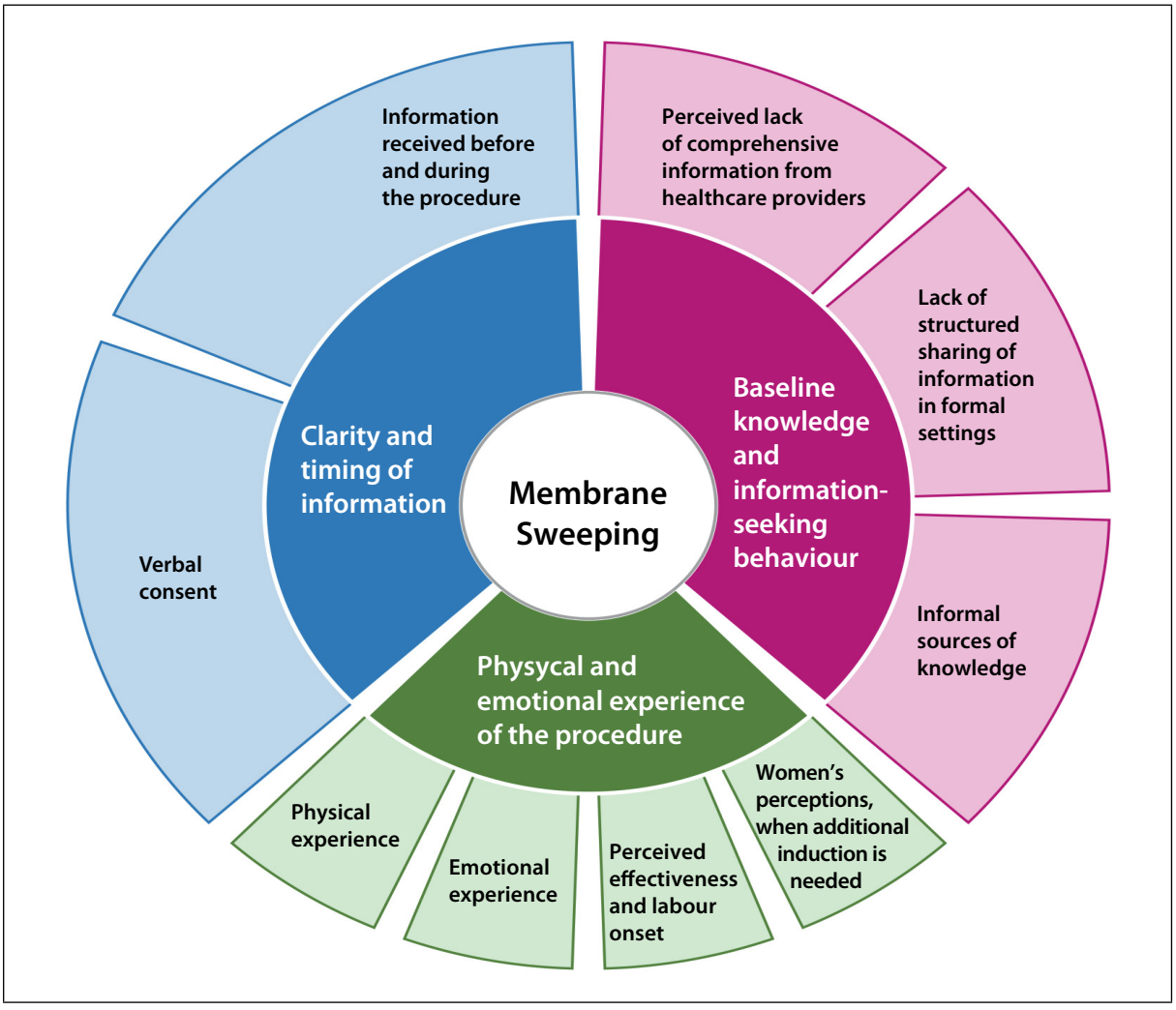


Figure 1
Emerging themes from women's perceptions of membrane sweeping.

transparency fostered respect and inclusion: *"I didn't feel pressured; I felt at ease"*, another noted. Yet not all accounts were positive. One participant suspected the sweep had been performed without her fully grasping it: *"The gynaecologist told me it just 'a little help, not a membrane sweep"*. Her experience highlights how even subtle lapses in communication can erode trust and leave women uncertain about the care they had received.

Verbal consent

Nearly all women recalled that clinicians explicitly sought their verbal consent before proceeding with the manoeuvre, a gesture they consistently interpreted as both respectful and collaborative. This practice reinforced their sense of emotional safety and personal autonomy: *"The doctor told me that if the conditions were right, they could proceed, but only with my consent"*. Another echoed, *"(…) and they asked for my consent"*, while a third reflected, *"I felt included in the decision to perform the procedure proposed by the doctor"*.

Baseline knowledge and information-seeking behaviour

The second theme dealt with women's grasp of the procedure itself, meaning the extent to which they possessed a conscious, accurate understanding of what membrane sweeping entails and why it is offered.

Perceived lack of comprehensive comprehension of the procedure

Although every woman recalled receiving a brief explanation of membrane sweeping, many acknowledged that they still did not grasp its full meaning or implications. One woman noted, *"They told me it was a manoeuvre to help release hormones and start labour, but I couldn't picture what would actually happen."* Another remarked, *"I understood it might speed things up, yet I had no idea about possible downsides or alternatives"*. Those who were familiar with the topic reported having done further research on it, stating: *"I was aware of the various induction methods, both mechanical and otherwise, but before consenting, I made sure to look deeper online, even with images, into the membrane sweeping procedure"*. Several reported the medical explanation of the procedure as *"quick"* or *"lacking depth"*, leaving them feeling under-briefed: *"When the doctor suggested it, I said yes – but only afterwards did I realise I hadn't really understood what I'd agreed to"*. A few had learned of the technique for the first time in that very moment: *"Before they mentioned it, I didn't even know membrane sweeping existed"*. Consequently, although the consent was formally requested, many women felt they lacked genuine comprehension of the procedure and its broader consequences.

Lack of structured sharing of knowledge in formal settings

Several women noted that membrane sweeping had never been addressed in their antenatal classes or other structured education sessions, so they first encountered the concept only when it was offered late in pregnancy. One woman reflected, *"I was poorly informed at the time it was proposed. Going forward, I would have liked to know more during pre-birth classes"*.

Informal sources of knowledge

For the women who approached the outpatient visit already aware of membrane sweeping, that knowledge almost always came from informal sources rather than from healthcare professionals. They mentioned browsing websites and forums: *"I was already somewhat informed from reading on the internet"* or consulting popular pregnancy books: *"During my first pregnancy, I read a book about it"*. Personal networks were equally influential: *"I knew about the manoeuvre because my cousin had it two months ago"*. Social media stood out as a particularly powerful channel, shaping both awareness and expectations: *"I got information from social media and friends, mainly people online who had already given birth"*.

Physical and emotional experience of the procedure

This theme encompasses women's physical sensations and emotional reactions to membrane sweeping, along with their views on how it influenced labour. Most women recalled the procedure favourably, highlighting both its tolerability and its perceived effectiveness: *"Overall, I would repeat the manoeuvre; it was a positive and effective experience"*.

Physical experience

Most women depicted membrane sweeping as *"uncomfortable but bearable*," underscoring its brief duration and the clinicians' considerate technique. One participant noted, *"They let me feel with my fingers the maximum pressure they would apply, so I understood exactly what was happening"*. Only one woman described the manoeuvre as distinctly painful, yet even they emphasised that the short time it took helped to offset the discomfort: *"It was painful at times. Painful, especially when they touch your cervix. You know you have to do it, and that it doesn't l last long, but it is painful"*.

Emotional experience

Women's emotional reactions spanned a spectrum from relief, particularly among those eager to avoid pharmacological induction, to anxiety about *"interfering"* with a natural childbirth process. One woman admitted, *"I was scared at the idea of intervening in something that might have happened naturally on its own"*. Another described the procedure as *"annoying but tolerable"*, adding that she *"hoped it would work, especially because I wanted to avoid pharmacological induction"*. Women also reported the possibility of undergoing membrane sweeping again in subsequent pregnancies. *"If I were to have another pregnancy, I would undergo membrane sweeping again because it's not painful and it increases the chances that labour will start naturally, without needing other induction methods"*. The clinical team was often tempered as *"someone to trusts a lot"*, so women *"felt relieved doing it since completely relied on the doctors' clinical experience"*.

Perceived effectiveness and labour onset

Many women attributed the onset of labour to the sweep itself, citing early physical signs, such as contractions, loss of the mucus plug, or spontaneous rupture of membranes, within 24-48 hours from the procedure: *"Right after I went home, I began feeling the uterine con-*

tractions; the next morning I lost the mucus plug, and that evening my waters broke". For these women, the procedure offered a welcome sense of momentum: "*I think it worked, because labor started within 48 hours*". Others valued the sweep as a middle ground between passive waiting and pharmacological induction, giving them a measure of control: "*I felt ready for the manoeuvre and saw it as a more thorough check-up*", and "*It's good that it's done and then you can go home. Being at home longer helps me cope with contractions*". This perceived labour progress was emotionally reassuring: "*I had a good experience and believe it triggered labour*". Yet a few women were ambivalent, feeling uneasy about initiating labor artificially even while recognising its potential benefits: "*I felt weird, really anxious, even if I knew this manoeuvre could help me*". Together, these accounts show how membrane sweeping can simultaneously foster empowerment, relief, and lingering uncertainty, depending on individual women expectations and values.

Women's perceptions, when additional induction is needed

Despite largely favourable reports, a subset of women still required pharmacological induction or caesarean section, underscoring the wide variation in clinical trajectories. One woman reflected, *"The sweep wasn't effective for me. I needed a pharmacological induction for my vaginal birth after caesarean section, and ultimately, I had a caesarean section"*. Another woman recalled, *"They performed an additional sweep while I was already on misoprostol; a few hours later my waters finally broke"*. Such experiences highlight the need for flexible, individualised care plans and acknowledge that membrane sweeping may not work for everyone.

DISCUSSION

This qualitative study is one of the few published articles to explore women's perceptions of membrane sweeping, focusing on their informational needs, decision-making processes and lived experience of the procedure. Women valued transparent, step-by-step explanations and the opportunity to provide their consent; however, the depth of counselling received, and women's baseline knowledge of the procedure varied widely. Physical discomfort was generally described as tolerable, and many participants framed membrane sweeping as an acceptable "*middle ground*" between passive waiting and pharmacological induction. A minority of women experienced information on membrane sweeping as cursory and remained uncertain about possible risks and alternatives, underscoring persistent gaps in antenatal education.

All women reported receiving verbal explanations and feeling genuinely involved in the decision-making process. This a finding contrasts with previous studies in which many women reported to be inadequately informed and felt excluded from the clinical decisions process [22, 23]. Previous work linked this lack of involvement to heightened anxiety and a loss of control, whereas respectful, well-informed, shared care was shown to enhance the childbirth experience [23]. Although most women in our sample considered the information received as "*adequate*", several still would have received a deeper explanation of the procedure's purpose, risks, and benefits. This echoes prior researches [23, 24], that underscored the need for comprehensive, anticipatory information as a foundation for truly shared decision-making.

Baseline understanding of membrane sweeping differed widely across women. Some arrived at the outpatient clinic with prior knowledge gleaned from the internet or their personal networks, while others first encountered the concept of "membrane sweeping" at the moment of the visit. These accounts underline how digital media and peer communities now shape maternity-related health literacy. Some women said that they would have welcomed structured, anticipatory information on membrane sweeping in their antenatal classes, suggesting a broader unmet educational need. This finding echoes calls for more comprehensive childbirth preparation to support truly informed choices [24]. Rather than postponing discussion until an induction is clinically indicated, introducing the risks and benefits of induction, membrane sweeping included, during routine antenatal classes may be beneficial, given evidence that course attendees feel significantly better prepared than non-attendees [25].

Most women described membrane sweeping as "*uncomfortable but bearable*"; only a few found it distinctly painful, while emphasising that the short duration of the manoeuvre and their trust in the clinical team kept the pain manageable. Emotionally, reactions ranged from relief, especially among women keen to avoid pharmacological induction, to anxiety about intervening in what they perceived as a natural process. Nevertheless, many viewed the sweep favourably because it either appeared to hasten labour or allowed them to remain at home longer before admission. Nearly all women reported feeling "*relieved*" once the procedure was over, and they were convinced that it might help initiate labour. Although the knowledge of the study could have heightened clinicians' attentiveness, a potential source of positive-response bias, our data suggest that thorough counselling and clear information women's understanding and overall experience are substantially improved. No negative experiences were reported, and several women said they would readily choose membrane sweeping in a future pregnancy because they did not find it traumatic. Taken together, these findings reinforce the perception of membrane sweeping as a generally tolerable first-line option for induction. By offering a low-intervention alternative and potentially averting more invasive, and often less satisfactory, methods, membrane sweeping may enhance both clinical outcomes and maternal satisfaction [22].

Overall, our findings highlight the pivotal role of trust between women and their care providers in shaping childbirth experiences. One woman valued being able to return home after the sweep, noting that the familiar environment made the onset of labour feel more manageable. This aspect echoed in studies reporting women's higher maternal satisfaction with outpatient, rather than in-hospital, induction [26]. This evidence aligns with others reporting that high-quality support, from clinicians and personal companions alike, helps

women cope with labor and birth pain [27, 28]. Clear, timely communication and shared decision-making not only enhance women's experience of membrane sweeping but also promote a more positive childbirth journey as a whole. To that end, healthcare professionals should remain attentive to women's preferences, offer personalised counselling, and provide psychologically supportive care throughout the induction process.

Study limitations

First, all membrane sweeps were performed exclusively by obstetricians, so the study does not capture potential differences in technique or counselling that midwives might bring; this limits the transferability of our findings across different professional groups. Moreover, since our research was conducted in a single maternity unit in Italy, its applicability to settings with different resources, protocols, or populations may be limited.

CONCLUSIONS

To our knowledge, this is the first qualitative study to examine women's subjective experiences of membrane sweeping rather than its clinical success alone in Italy. Given that most women described the procedure positively, membrane sweeping merits consideration as a first-line induction option before escalating to pharmacological methods. Mixed-methods studies could be particularly valuable for exploring the phenomenon in greater depth, offering a more comprehensive understanding of both clinical outcomes and personal narratives.

Authors' contributions

JP, GA, SS: contributed to the study design, data interpretation, literature search, and manuscript writing; SB, ER, JP, SF, SM: were involved in data collection; ER, JP, GA: contributed to data analysis and figure generation; SS, SF, SB, MD, EC, FR: contributed to the revision of the manuscript. All Authors read and approved the final version of the paper.

Conflict of interest statement

The Authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Received on 2 January 2026.
Accepted on 17 March 2026.

REFERENCES

1. World Health Organization. WHO recommendations for induction of labour. Geneva: WHO; 2011.
2. Alfirevic Z, Keeney E, Dowswell T, Welton N, Medley N, Dias S, et al. Which method is best for the induction of labour? A systematic review, network meta-analysis and cost-effectiveness analysis. Health Technol Assess. 2016;20(65):1-584.
3. National Institute for Health and Care Excellence. Inducing labour (NG207). London: NICE; 2021.
4. Società Italiana di Ginecologia e Ostetricia. Linee guida per l'induzione del travaglio di parto. Rome: SIGO; 2022. Available from: https://www.sigo.it/wp-content/uploads/2022/02/LG15_Induzione_Travaglio_Parto.pdf
5. Boulvain M, Stan C, Irion O. Membrane sweeping for induction of labour. Cochrane Database Syst Rev. 2005;2005(1):CD000451. doi: 10.1002/14651858.CD000451.pub2. Update in: Cochrane Database Syst Rev. 2020;2:CD000451. doi: 10.1002/14651858.CD000451.pub3
6. Finucane EM, Murphy DJ, Biesty LM, et al. Membrane sweeping for induction of labour. Cochrane Database Syst Rev. 2020;2(2):CD000451. doi:10.1002/14651858.CD000451.pub3
7. Ugezu CH, Corcoran P, Dunn EA, Burke C. Does membrane sweep work? Assessing obstetric outcomes and patient perception of cervical membrane sweeping at term in an Irish obstetric population: a prospective multi-centre cohort study. Ir J Med Sci. 2020;189(3):969-77. doi: 10.1007/s11845-020-02191-w
8. Lundh CØ, Øvrum AK, Dahl B. Women's experiences with unexpected induction of labor: a qualitative study. Eur J Midwifery. 2023;7:7. doi: 10.18332/ejm/161481
9. World Health Organization. The prevention and elimination of disrespect and abuse during facility-based childbirth: WHO statement. Geneva: WHO; 2014. Available from: https://iris.who.int/handle/10665/134588
10. Hassan AM. Membrane sweeping to induce labor in post-term pregnant women: success rate and outcomes. Cureus. 2023;15(3):e36942. doi: 10.7759/cureus.36942
11. Coates R, Cupples G, Scamell A, McCourt C. Women's experiences of induction of labour: qualitative systematic review and thematic synthesis. Midwifery. 2019;69:17-28. doi: 10.1016/j.midw.2018.10.013
12. Roberts J, Evans K, Spiby H, Evans C, Pallotti P, Eldridge J. Women's information needs, decision-making and experiences of membrane sweeping to promote spontaneous labour. Midwifery. 2020;83:102626. doi: 10.1016/j.midw.2019.102626
13. Vogel JP, Souza JP, Gülmezoglu AM. Patterns and outcomes of induction of labour in Africa and Asia: a secondary analysis of the WHO Global Survey on Maternal and Neonatal Health. PLoS One. 2013;8(6):e65612. doi: 10.1371/journal.pone.0065612
14. Tong A, Sainsbury P, Craig J. Consolidated criteria for reporting qualitative research (COREQ): a 32-item checklist for interviews and focus groups. Int J Qual Health Care. 2007;19(6):349-57. doi: 10.1093/intqhc/mzm042
15. Creswell JW, Poth CN. Qualitative inquiry and research design: choosing among five approaches (4th ed.). Thousand Oaks, CA: SAGE Publications; 2018.
16. Wax JR, Pinette MG. The amniotic fluid index and oligohydramnios: a deeper dive into the shallow end. Am J Obstet Gynecol. 2022;227(3):462-70. doi:10.1016/j.ajog.2022.04.016
17. Graneheim UH, Lundman B. Qualitative content analysis in nursing research: concepts, procedures and measures to achieve trustworthiness. Nurse Educ Today. 2004;24(2):105-12.
18. Elo S, Kyngäs H. The qualitative content analysis process. J Adv Nurs. 2008;62(1):107-15. doi: 10.1111/j.1365-2648.2007.04569.x
19. Nowell LS, Norris JM, White DE, Moules NJ. The-

matic analysis: striving to meet the trustworthiness criteria. Int J Qual Methods. 2017;16(1):1-13. doi: 10.1177/1609406917733847

20. Braun V, Clarke V. Using thematic analysis in psychology. Qual Res Psychol. 2006;3(2):77-101. doi: 10.1191/1478088706qp063oa
21. Fusch PI, Ness LR. Are we there yet? Data saturation in qualitative research. Qual Rep. 2015;20(9):1408-16. doi: 10.46743/2160-3715/2015.2281
22. Blanc-Petitjean P, Dupont C, Carbonne B, Salomé M, Goffinet F, Ray CL; MEDIP Study Group. Methods of induction of labor and women's experience: a population-based cohort study with mediation analyses. BMC Pregnancy Childbirth. 2021;21(1):621. doi: 10.1186/s12884-021-04076-x
23. Coates D, Donnolley N, Foureur M, Henry A. Women's experiences of decision-making and attitudes in relation to induction of labour: a survey study. Women Birth. 2021;34(2):e170-e177. doi: 10.1016/j.wombi.2020.02.020
24. Simpson KR, Newman G, Chirino OR. Patients' perspectives on the role of prepared childbirth education in decision making regarding elective labor induction. J Perinat Educ. 2010;19(3):21-32. doi: 10.1624/105812410X514396
25. Coates D, Goodfellow A, Sinclair L. Induction of labour: experiences of care and decision-making of women and clinicians. Women Birth. 2020;33(1):e1-e14. doi: 10.1016/j.wombi.2019.06.002
26. Sangskär H, Berglin L, Sengpiel V, Svanberg T, Svensson M, Berter B, Elden H, Sjögren P, Strandell A, Carlsson Y. Safety, effectiveness, women's experience, and economic costs of outpatient induction in women with uncomplicated pregnancies: a systematic review and meta-analyses. Int J Gynaecol Obstet. 2023;161(2):343-55. doi: 10.1002/ijgo.14625
27. Bohren MA, Hofmeyr GJ, Sakala C, Fukuzawa RK, Cuthbert A. Continuous support for women during childbirth. Cochrane Database Syst Rev. 2017;7(7):CD003766. doi: 10.1002/14651858.CD003766.pub6
28. World Health Organization. WHO recommendations: intrapartum care for a positive childbirth experience. Geneva: WHO; 2018.

Ann Ist Super Sanità 2026 | Vol. 62, No. 2: 163-178
DOI: 10.4415/ANN_26_02_09

Addressing food and nutrition insecurity in European countries: a scoping review of strategies and policies

Maria Lucia Specchia[1*], Drieda Zace[1*], Maria Gabriella Cacciuttolo[1], Emanuele La Gatta[1], Luigi Petrella[1], Rosaria Messina[1], Flavia Beccia[1], Alberto Capriolo[1], Gilberto Turati[2] and Maria Luisa Di Pietro[1]

[1]Sezione di Igiene, Dipartimento di Scienze della Vita e Sanità Pubblica, Università Cattolica del Sacro Cuore, Rome, Italy
[2]Dipartimento di Economia e Finanza, Università Cattolica del Sacro Cuore, Rome, Italy
**These Authors contributed equally to this manuscript as co-first Authors*

ABSTRACT

Background. Food and nutrition insecurity (FNI) is a major public health concern, due to its association with a variety of adverse health outcomes.

Objective. The aim of this article is to provide a description of the strategies and policies to mitigate FNI in European countries, with a particular focus on economic, agriculture and social macro-areas.

Methods. A scoping review, following the Preferred Reporting Items for Systematic reviews and Meta-Analyses extension for Scoping Reviews (PRISMA) guidelines, was performed to map any study design, reporting policies and strategies that tackle FNI through food and nutrition security (FNS), access, utilization, or stability.

Results. The review identified 13 documents from institutional websites and 6 scientific articles, published from 2005 to 2022. The included papers highlight a multifaceted approach to addressing food insecurity, with policies covering several dimensions. The policies identified can be categorized into several key themes: agriculture and fisheries, nutrition, environment, sustainability, economy, stakeholder's views, COVID-19 pandemic and other crisis, vulnerable populations (women, children, migrants) and information campaigns. The review describes in detail several policies, examining their effectiveness and the challenges faced in ensuring that these initiatives result in tangible improvements in food access, affordability, and nutritional quality, particularly for vulnerable populations.

Conclusions. The results show a current lack of a specific and coherent policy framework on FNI at a European level. Policy fragmentation is mainly due to the multidimensional nature of FNI, which has interconnections with many different areas of the food system. The findings suggest a comprehensive approach, incorporating several policy measures and involving multiple stakeholders to ensure sustainable and equitable access to nutritious food.

Key words
- food insecurity
- nutrition insecurity
- policy strategies
- sustainability

BACKGROUND

Food and nutrition insecurity (FNI) can be defined as the limited or uncertain availability of nutritionally adequate and safe foods or limited or uncertain ability to acquire foods in socially acceptable ways [1]. Conversely, food security (FS) exists when all people, at all times, have physical and economic access to sufficient, safe and nutritious food to meet their dietary needs and food preferences for an active and healthy life [2]. Nutrition security has long been defined by the Food and Agriculture Organization (FAO) as "*a situation that exists when secure access to an appropriately nutritious diet is coupled with a sanitary environment, adequate health services and care, in order to ensure a healthy and active life for all household members*".

The traditional four pillars of food security – availability, access, utilization, and stability – as well as two additional dimensions – agency and sustainability – are included in the six-dimensions framework for food security [3, 4].

Address for correspondence: Maria Gabriella Cacciuttolo, Dipartimento di Scienze della Vita e Sanità Pubblica, Università Cattolica del Sacro Cuore, Largo Francesco Vito 1, 00168 Rome, Italy. E-mail: maria.cacciuttolo01@icatt.it.

Food security can be considered at different levels: individual, household, national, regional (continental) and global [5].

Definitions of food security and nutrition security reflect the contexts to which they are applied. If we consider the Sustainable Development Goal 2 (SDG 2: Zero Hunger) that aims to end all forms of hunger and malnutrition by 2030, it refers primarily to the availability of food in the world's poorest countries. As we move up the rankings of countries' wealth (or average income), the meaning of food and nutrition security shifts to the accessibility and quality of food for the most vulnerable segments of the population. In addition to the lower class, middle-income earners are similarly struck by impoverishment, particularly throughout times of crisis. This puts them in a vulnerable economic situation, which affects their access to food and places them at risk for a decline in their general health.

Food security is a major social determinant of health. Food security significantly impacts individual well-being and places a heavy burden on society, posing a serious public health challenge and straining healthcare systems. According to the FAO, food insecurity has been steadily rising since 2014. In 2019, around 2 billion people globally lacked regular access to safe and nutritious food, and by 2022, this number grew by 400 million (SDG Indicator 2.1.2) [2, 6]. The COVID-19 pandemic worsened global hunger, with 9.2% of the world population affected in 2022, up from 7.9% before the pandemic. The FAO also projects that nearly 600 million people will face hunger by 2030 – 119 million more than if the pandemic and the war in Ukraine had not occurred [7].

We have concentrated our research on the European region's countries since the topic of food security is not as well investigated in middle- and high-income countries as it is in low-income countries. Furthermore, the international crises, including the pandemic and the conflicts, have led to segments of the population sliding into poverty. In Europe, those most vulnerable to food insecurity include people with low incomes, women, the elderly, renters, single-person and lone-parent households, individuals with lower educational levels, people with disabilities, and those outside the labor market.

Food insecurity is linked to a range of health issues, including a higher risk of infectious diseases, poor oral health, injuries, and chronic conditions like diabetes, obesity, heart disease, mental health disorders, and other long-term illnesses [8, 9].

Addressing food insecurity requires comprehensive strategies that cover food production, distribution, affordability, nutrition education, social protection, and healthcare interventions. A holistic approach ensures that everyone has access to safe and nutritious food, as well as the information and resources needed to make healthy choices [10].

Within the European Union, a macro-regional strategy allows neighboring countries to collaborate on shared challenges and better utilize regional potential [11].

In public health, a strategy refers to an organized plan for addressing health issues at local, regional, or national levels, while a policy is a broader action plan, often less formalized than a strategy [12]. To tackle food and nutrition insecurity and improve public health, it is essential to design and implement policies and strategies that focus on vulnerable groups. This study aims to identify the strategies and policies European countries have adopted to address food and nutritional insecurity, with an emphasis on economic, agricultural, and social sectors.

METHODS

This scoping review was carried out and reported following the "Preferred Reporting Items for Systematic reviews and Meta-Analyses extension for Scoping Reviews" (PRISMA-ScR) Checklist [13]. The review protocol was submitted and registered on the Open Science Framework (OSF) portal [14] and is available at https://doi.org/10.17605/OSF.IO/3254P.

Aim and research question

The research question for this scoping review was formulated following the PICOS framework as follows:

Population: all demographic groups residing in countries within the World Health Organization (WHO) European region;

- Intervention/exposure: food and nutrition insecurity;
- Comparison: not applicable;
- Outcome: policies and strategies implemented to address food and nutrition insecurity;
- Study design: any study design, report, or document providing insights into the review question.

Scientific literature search

Search strings

Applying the "Humans", "English", filters, the search string employed for the PubMed database was:

(("Food Supply"[Mesh] OR "food security" OR "food insecurity" OR "food access" OR "food availability" OR "household food insecurity" OR "household food security" OR "nutrition security" OR "nutrition insecurity") AND (policy OR mitigation OR strategy OR measures OR management OR guideline OR regulation OR law OR legislation OR framework) AND Europe).

The search string employed for the Scopus database was:

(({food security} OR {food insecurity} OR {food access} OR {food availability} OR {nutrition security} OR {nutrition insecurity}) AND ({policy} OR {strategy} OR {regulation} OR {legislation} OR {law} OR {guideline} OR {framework}) AND Europe)

The search string employed for the Web of Science (WoS) database was:

ALL=("Food Supply" OR "food security" OR "food insecurity" OR "food access" OR "food availability" OR "household food insecurity" OR "household food security" OR "nutrition security" OR "nutrition insecurity") AND ALL=(policy OR mitigation OR strategy OR measures OR management OR guideline OR regulation OR law OR legislation OR framework) AND ALL=(Europe)

Eligibility criteria

All articles retrieved from the search strategy were imported to the Rayyan software [15] and duplicates were removed. The initial screening, based on title and abstract, was conducted independently by two research-

ers. At each stage, researchers worked in a double-blind manner, and any discrepancies were resolved through re-examination of the study or document followed by discussion. If necessary, another member of the review team was consulted to reach a consensus.

Inclusion and exclusion criteria

Studies of any design that reported on policies or strategies aimed at addressing food and nutrition security, implemented in countries within the WHO European region, were included. Articles that did not meet these inclusion criteria were excluded.

Desk research

Desk research was carried out on a curated selection of websites from international agencies and government or non-government organizations. These entities were chosen for their prominence in addressing food and nutrition insecurity, making them highly likely to publish relevant material. The organizations were identified as key sources of information pertinent to the topics under review. The documents published by these entities ranged from guidelines and consultative reports to mandatory laws and regulations. For example, documents from the FAO and WHO, as United Nations bodies, primarily serve policy-related functions, while those from the European Commission typically represent legally binding laws and regulations.

The agencies and organizations included in the search were Community Research and Development Information Service (CORDIS), European Union (EU) Publications, United Nations iLibrary, FAO, European Commission, WHO, WHO Regional Office for Europe, United Nations Children's Fund (UNICEF), World Food Programme (WFP), and the US Department of Agriculture. A complete list of the websites used for the desk research is provided in the *Supplementary Material available online*. When institutional websites referenced secondary sources, those sources were also reviewed.

The search was conducted using keywords such as food security, nutrition security, food insecurity, nutrition insecurity, strategy, policy, and Europe. No temporal limitations were applied, ensuring a thorough exploration of available resources.

Data extraction

Data coming from both the literature search and desk research were reported into a dedicated Excel data extraction form.

The following information was retrieved for each document: first author/official organizations/agency/institution, title, publication year, country, study design, target population, sample size, policy/strategy characteristics (type, duration, setting), measure of effectiveness/impact of the policy/strategy.

A qualitative synthesis of the identified data was performed; the results were summarized through an analysis of the included studies and documents.

RESULTS

Characteristics of included studies and documents

The scientific literature search and desk research yielded a total of 2,921 articles and 29 documents, respectively. Among these, 6 articles [16-21] and 13 [22-34] documents met the inclusion criteria and were ultimately included in the review (see *Figure 1*, *Table 1* and *Table 2*).

Scientific literature search

The six included papers from the scientific literature spanned a timeframe from 2016 to 2022 and encompassed a variety of study types, including one review, case study, dynamic panel analysis, econometric analysis, qualitative study, and quasi-experimental study, respectively. Regarding the primary macro areas addressed, two papers focused on "*agriculture initiatives*", another two papers examined "*school meals*", one paper explored "*social, environmental, economic, and ethical dimensions*" of food insecurity, and one paper delved into "*legislation*" implications. In terms of food insecurity dimensions, three papers addressed the issue of "*food availability*", while the remaining three papers discussed issues related to "*access to food*". Additionally, one paper also discussed the "*utilization of resources*" in addressing food insecurity (*Table 1*).

Desk research

The thirteen included documents were published in a timeframe from 2005 to 2022 (*Table 2*). The origin/source of the documents included FAO, International Fund for Agricultural Development (IFAD), UNICEF, WFP, WHO; European Commission; United States Mission to the European Union (USEU); Department for Environment, Food and Rural Affairs of the UK; Russian Federation. Eight documents focused on availability of food, six addressed issues related to agency in food access, four documents also discussed the utilization of resources, three addressed concerns about food sustainability, and two addressed access to food. The primary settings of policy application were as follows: for five documents, "*agriculture initiatives*"; for four documents, "*legislation*"; for four documents, "*education*"; for three documents, the topic was "*trade*"; for two documents, the topic was "*marketing law*"; for two documents, "*health promotion*"; for one document, "*school meals*"; for one document, "*crisis response*"; and for one document, "*innovation*."

The policies' themes

Considering the vast types of documents that were included in the review and the different themes that the policies discussed, we report the results based on these categories: agriculture and fisheries, nutrition, environment, sustainability, economy, stakeholder's views, COVID-19 pandemic and other crisis, vulnerable populations (women, children, migrants), information campaigns. However, it is important to highlight that many documents concerned more than one theme, since the various aspects and dimensions of food and nutrition insecurity are correlated between them.

Agriculture and fisheries policies

The analyses of the effects of agricultural production, land use, farms, and trade on Food and Nutrition Se-

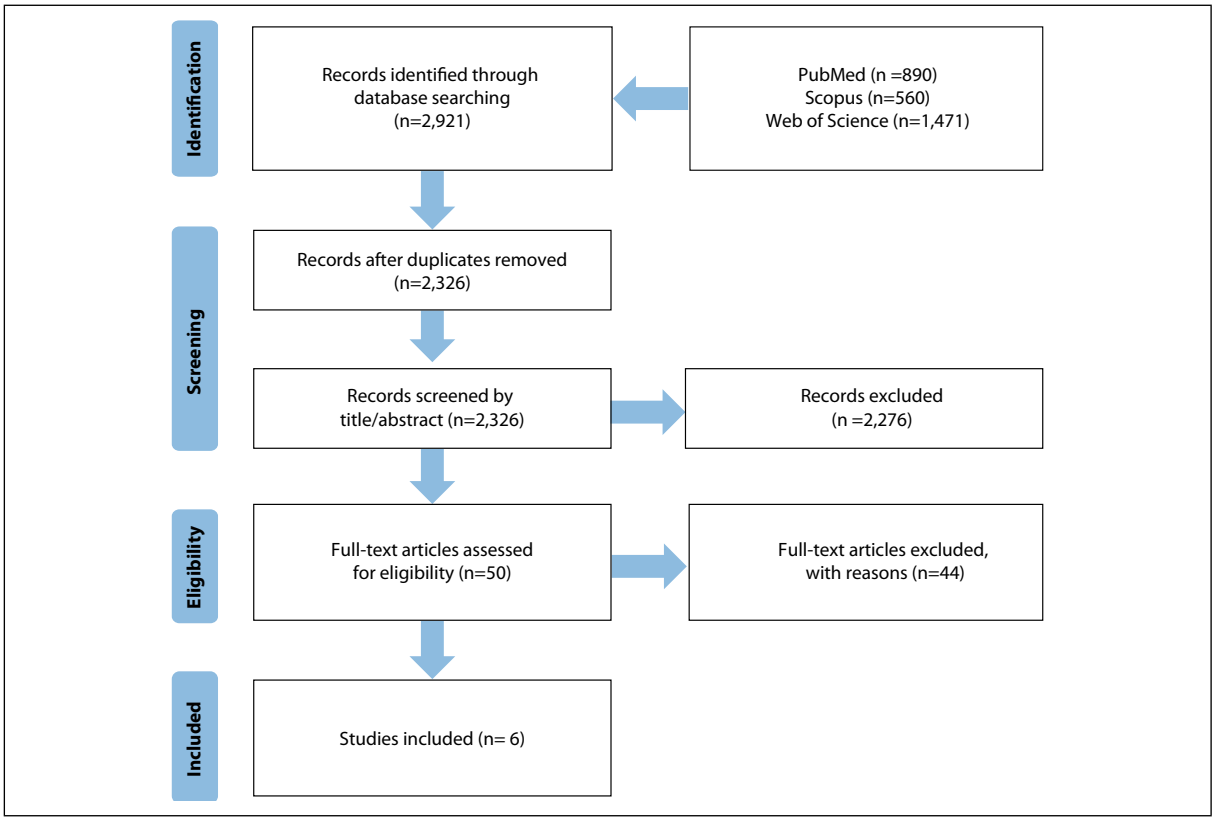


Figure 1
Flowchart of the selection process based on the scientific literature research.

Table 1
Characteristics of studies identified by the scientific literature search

Authors [reference]	Country	Target population	Study design	Strategy or policy description (intervention)	Macro-area (economic, agriculture, nutrition…)	Food security dimensions	Results (strategy or policy outcome or impact)
Rutten *et al.*, 2018 [16]	Several European countries	European consumer	Review	This paper presents the vision of the *Sustainable EU Food and Nutrition Security* (SUSFANS) project on how to advance research in support of policy and practice on Sustainable *Food and Nutrition Security* (FNS) in the European Union, as developed by its partners from academia, public and private sectors.	Social, environmental, economic, ethical	Availability	The conceptual framework and modelling of sustainable FNS in the European Union can be used to provide foresight on future pathways for food production and consumption.
Toma *et al.*, 2021 [17]	Poland, Romania, Latvia	Small farms in Latvia, Poland, and Romania	Case study (SWAT based data synthesis and interpretation)	*Common Agricultural Policy* (CAP)	Agriculture initiatives, legislation	Availability	Issues for small farms to contribute to food and nutrition security: (1) market integration through short supply chains, hygiene regulation and training, and quality regulations (2) agricultural knowledge and innovation systems for small farms, including the farm advisory systems, (3) rural infrastructure and services, innovative digital services, synergies and alignment with cohesion funds and (4) simplification of agri-environment and climate measures.

Continues

Table 1
Continued

Authors [reference]	Country	Target population	Study design	Strategy or policy description (intervention)	Macro-area (economic, agriculture, nutrition…)	Food security dimensions	Results (strategy or policy outcome or impact)
Fusco *et al.*, 2020 [18]	Italy	European countries	Dynamic panel analysis	The *Common Agricultural Policy* (CAP, 2014-2020) provides economic aid to farmers to improve agricultural productivity and ensure stable and inexpensive food supply. In order to achieve adequate levels of food security, many countries have recognized the importance of trade policies (trade openness), developing reforms to reduce taxes on incoming goods and contributing to the growth of the international market aimed at eradicating poverty and improving the availability of food.	Agriculture initiatives	Availability	From the results of the dynamic panel analysis emerged that commercial opening has, on average, a statistically significant net positive impact on the food security of European countries, both from an energy and nutritional point of view. Based on the results obtained, in order to ensure quantity and quality of food supplies, it would be desirable for the European Union to adopt a liberal trade policy, which should represent a complement and not a substitute for domestic development policies.
Jensen *et al.*, 2016 [19]	Denmark	Citizens	Econometric analysis	In Denmark, a tax on saturated fat in food products was introduced on the 1st of October 2011, as a supplement to existing taxation on sugar, chocolate, candy, ice cream and soft drinks.	Nutrition	Access	Minimising access to non-nutritional food could play a crucial role in nutrition and food security.
Long *et al.*, 2017 [20]	United Kingdom	Parents of children who attend holiday clubs	Qualitative study	Holiday clubs, which provide free meals (usually breakfasts, lunches, and snacks) for children, and sometimes parents, when children are not in school. Food may be provided in a variety of settings, including schools and community groups. These programmes also provide a space for parents and children to socialise, learn and participate in a variety of healthy activities. Many holiday clubs offer time for exercise, play and nutrition skills training for children and their families, as well as opportunities for other members of the child's household (e.g., parents and siblings) to visit at least once per week.	Nutrition	Access	The holiday clubs are largely focused on households that face food insecurity: a large percentage of children attending holiday clubs (42%, 16 out of 38 respondents) came from households that could be defined as suffering from food insecurity, and a significant number of children in these programmes (24%, 9 out of 38 respondents) came from households that were not only food insecure, but also faced frequent episodes of hunger. The results of this study also suggested that children who came from food insecure households disproportionately benefited from the holidays clubs, when compared with children who came from food secure households.
Crilley *et al.*, 2022 [21]	United Kingdom	Children aged 7-16	Quasi-experimental study	Holiday clubs have the intention of providing children with nutritious food to replace their free school meal, alongside activities, during the school holidays. In government-funded schools in England, the *School Food Standards* define minimal nutritional standards to ensure children are provided with nutritious meals. To meet these standards, school caterers are provided with advice on the types of foods to serve and how much to serve. These standards aim to help children to develop healthy eating habits.	School meals	Access, utilization	The results of the current study show that children have a better diet quality score on an attending club day compared to a non-attending club day, highlighting that children are more likely to adhere to the United Kingdom *Eatwell Guidelines* when they attend the club compared to days they do not attend. Greater adherence would be expected to bring numerous health benefits at a population level, including reduced prevalence to type 2 diabetes, lower rates of cardiovascular disease, and colorectal cancer and increased life expectancy.

SUSFANS: Sustainable EU Food and Nutrition Security; FNS: Food and Nutrition Security; CAP: Common Agricultural Policy; SWAT: Soil and Water Assessment Tool.

Table 2
Characteristics of documents retrieved from the desk research

Title [reference]	Source (institution)	Publication year	Country	Food security dimensions	Setting	Strategy or policy description (intervention)	Target population	Results (strategy or policy outcome or impact)
World Health Organization (WHO) European Action Plan for Food and Nutrition Policy 2007-2012 [22]	WHO Regional Office for Europe	2008	European countries	Availability, access, utilization, agency	Health promotion, school meals, legislation	The document encompasses 6 action areas including one targeted on mothers and children with the following aims: promoting optimal foetal nutrition by ensuring maternal nutrition from preconception, providing advice on diet and food safety to pregnant women, providing micronutrient supplementation as required; promoting and supporting breastfeeding; promoting appropriate and safe complementary feeding of infants and young children; taking community-based initiatives to ensure adequate provision of complementary foods, sufficient micronutrient intake and proper nutritional care of infants and young children; promoting development of pre-school and school nutrition and food safety policies and programs, guidelines for healthy school meals, healthy food provision in canteens. Other aims from other areas include policy actions on trading, economy and legislation.	Mothers, children, general population	The sixth action area includes the creations of surveillance systems for nutritional status, food availability and consumption, physical activity, and food safety. The *Health Impact Assessment* method is used to evaluate the impact of programs and policies.
Protecting children from the harmful impact of food marketing: policy brief [23]	WHO	2022	Global	Agency	Marketing law	Governments are urged to implement comprehensive policy approaches to limit the marketing of foods that contribute to an unhealthy diet. In Turkey and Ireland restrictions are applied for unhealthy food marketing to children aged under 18 years. In Ireland, commercial communications for unhealthy food products and/or services are not permitted in children's programs. In Turkey, the *Regulations on Principles and Procedures of Broadcasting Services* restricts advertising of unhealthy food and beverages before, during or after children's television programs: if such food is advertised during non-children's programs, health promotion messages must be displayed.	Children	Reduce children's exposure to such marketing in order to provide the best protection for all children.

Continues

curity (FNS) were thoroughly considered by the papers included in the review. The *Sustainable EU Food and Nutrition Security* (SUSFANS) project provided an illustrative example through the EU-focused agricultural sector model called *Common Agricultural Policy Regionalised Impact* (CAPRI). CAPRI could provide detailed agricultural input and responses to climate change, water availability and demand, and also offered insights into agricultural prices and the environmental impacts of agriculture [16].

The policy measures integrated within the *Common Agricultural Policy* (CAP) of the EU held potential for fostering the contribution of small farms to FNS in Central and Eastern Europe (CEE). In a review of European rural and agricultural policies in CEE, the authors proposed eight "*themes*" that should be incorporated into these policies: people and communities; better infrastructure and connectivity; access to land; access to affordable credit; agricultural knowledge and innovation systems; availability and quality of farm labor; natural resources and climate; products, markets and marketing [17].

At EU level, the *Farm to Fork Strategy*, published by the European Commission on May 2020, delineated 27 actions aimed at transforming the way EU food was produced, processed, transported, presented, and sold, comprehensively addressing the challenges of sustainable food systems. Specifically, the strategy sought to reward and strengthen farmers, fishers, and other operators in the food chain who had already transitioned to sustainable practices, while also facilitating the transition for others and creating additional opportunities

Table 2
Continued

Title [reference]	Source (institution)	Publication year	Country	Food security dimensions	Setting	Strategy or policy description (intervention)	Target population	Results (strategy or policy outcome or impact)
European strategy for child and adolescent health and development – Action tool [24]	WHO Regional Office for Europe	2005	European countries	Access, utilization, agency	Education, work legislation, marketing law	The tool is composed of 8 tables. The first regards maternal and newborn health and the second is about nutrition. Each table is divided in several priorities, and for each priority are presented three levels of action: cross sector, health system and health services actions. Some of those actions are aimed to fight malnutrition of pregnant women and mothers, promote quality improvement, continuous education and equitable access to antenatal care staff and services; promotion and support to exclusive breastfeeding, through the legislation improvement to protect working mothers, the stimulation of media to represent it as the norm, the health professionals training; malnutrition and micronutrient deficiencies prevention in at risk infants, also in kindergartens, schools and day care centers; regulations to avoid distribution of unhealthy snacks and soft drinks in school cafeterias, to incorporate nutritional education in school curricula and to regulate food advertising for children and adolescents in the media.	Mothers and neonates, children, and adolescents	Monitoring and evaluation of maternal and newborn health services and of nutritional status.
European Food and Nutrition Action Plan 2015-2020 [25]	WHO Regional Office for Europe	2015	European countries	Agency, utilization	Health promotion, education	Promote policies aimed at influencing food production, marketing, availability, affordability, public awareness, healthcare professionals' knowledge. Specific aims are: addressing food consumption habits; promoting correct nutrients intake; reducing food and nutrition insecurity for certain vulnerable populations; investing in nutrition at the earliest possible stage, before and during pregnancy; improving the citizens ability to make healthy choices; encouraging the use of social media and new techniques to promote healthier food choices and lifestyles.	Mothers and neonates, children, and general population	Create healthy food and drink environments. Promote the gains of a healthy diet throughout life, especially for the most vulnerable groups. Reinforce health systems to promote healthy diets. Support surveillance, monitoring, evaluation, and research. Strengthen governance, alliances, and networks to ensure a health-in-all-policies approach.

Continues

for their businesses. Moreover, it underscored the importance of establishing a conducive food environment to promote the adoption of healthy and sustainable diets, which could enhance consumers' health and quality of life while reducing society's health-related costs [28, 29, 31].

The European Commission monitored the implementation of the *Unfair Trading Practices Directive* by member states, collaborating with co-legislators to enhance agricultural rules that strengthened the position of farmers (e.g., producers of products with geographical indications), their cooperatives, and producer organizations in the food supply chain. The marketing standards were revised to "*support the adoption and distribution of sustainable products in agriculture, fisheries, and aquaculture, while strengthening the integration of sustainability criteria and considering their potential impact on reducing food loss and waste*" [29].

Nutrition policies

Based on individual-level data from five member states (Denmark, Netherlands, Czech Republic, Italy, France), the SUSFANS project modelled the nutritional adequacy of diets using EU dietary guidelines and nutrient reference values developed by the European Food Safety Authority. A range of sustainability metrics was added to these individual food intake patterns to model current and future diets that were environmentally "*Sustainable, Healthy, Affordable, Reliable, and Palatable*" (SHARP) [16].

As for nutrition quality, the Commission highlighted that harmonized mandatory front-of-pack nutrition

Table 2
Continued

Title [reference]	Source (institution)	Publication year	Country	Food security dimensions	Setting	Strategy or policy description (intervention)	Target population	Results (strategy or policy outcome or impact)
Better food and nutrition in Europe: a progress report monitoring policy implementation in the WHO European Region [26]	WHO	2018	European countries	Agency, utilization	Legislation, education	Promoting healthy diets in school settings, through the limitation of *High in saturated Fat Salt and Sugar* (HFSS) products and the inclusion of free or subsidized fruits and vegetables, of food or nutrient based standards for the foods and meals available in schools, of nutrition education and skills to increase nutrition literacy; acting on taxes and subsidies to influence purchasing behaviors; promoting and supporting breastfeeding with several measures, including full implementation of the International *Code of Marketing of Breast-milk Substitutes*, effective training of health workers and peer counsellors to provide support to all mothers.	General population	Questionnaire results: WHO European region countries have adopted measures to promote healthy diets in schools (96%). 58% of countries have a school fruit and vegetable scheme, 23% countries reported a ban on vending machines on school premises, 25% reported salt covered under the mandatory standards in schools and 58% of countries reported standards for foods and beverages served for lunch in school canteens and cafeterias. A growing number of countries in the European Region have introduced health-related taxes on specific foods or nutrients with the objective of influencing what people buy and eat. 98% of countries reported that they provided counselling on breastfeeding, mainly in hospitals (65%) and care clinics (49%); 73% of countries were implementing the *Baby-friendly Hospital Initiative*.
The state of food security and nutrition in the world 2022 [27]	International Fund for Agricultural Development (IFAD), United Nations Children's Fund (UNICEF), Food and Agriculture Organization (FAO), and WHO	2022	European countries	Availability, sustainability	Agriculture initiatives	The European cities of Copenhagen and Vienna implemented procurement policies requiring a given percentage of food to be organic. Recent discussions of agricultural policy reform in the European Union (EU) (*Farm to Fork Strategy*) and the United Kingdom (UK) (*New Agriculture Bill*) have stressed the importance of considering the health and environmental sustainability of food production as desirable public goods that are to be supported. A "*public money for public goods*" approach could render subsidies to nutritious foods that are important for public health and environmental sustainability politically more feasible than past production-centred approaches. For example, the *European Union's Common Agricultural Policy* took up around 35% of the European Union's budget in 2020. In Tajikistan, the *Livestock and Pasture Development Project II* aimed at enhancing livestock productivity and rural livelihoods while reducing the ecological footprint of livestock herds on pastures. The project established rotational pasture plans, water points, veterinary services, breeding techniques and fodder production, alongside capacity building and strengthening of social capital implemented through *Pasture Users' Unions*.	General population	The policies adopted by Copenhagen and Vienna stimulated an increased supply of organic fruits, vegetables, and other products. *The Livestock and Pasture Development Project II* in Tajikistan increased livestock weight by 30%, milk production by 99%, and generated higher income from livestock by 110%.

Continues

Table 2
Continued

Title [reference]	Source (institution)	Publication year	Country	Food security dimensions	Setting	Strategy or policy description (intervention)	Target population	Results (strategy or policy outcome or impact)
EU food supply and food security – contingency plan [28]	European Commission	2021	European countries	Availability	Agriculture initiatives	The *Farm to Fork Strategy* includes the development of a contingency plan to ensure the EU's food supply and food security in the event of future crises. Building on the lessons learned from the COVID-19 pandemic and other recent events, the Commission plans to develop a set of procedures to be followed in times of crisis. This includes establishing an EU crisis response mechanism to effectively prepare and respond to critical events that could threaten the EU's food security.	Vulnerable to food security population	Enhance the knowledge of and mitigate to the extent possible the vulnerabilities and risks and to create and maintain the procedural capability to respond in a swift, coordinated, and cooperative way relying on a mix of EU policies that support the resilience of the system and provide crisis management tools.
A Farm to Fork Strategy for a fair, healthy and environmentally-friendly food system [29]	European Commission	2020	European countries	Availability, sustainability	Agriculture initiatives, crisis response	The Commission will step up its coordination of a common European response to crises affecting food systems in order to ensure food security and safety, reinforce public health and mitigate their socio-economic impact in the EU. The plan will set up a food crisis response mechanism coordinated by the Commission and involving Member States. It will be comprised of various sectors (agriculture, fisheries, food safety, workforce, health and transport issues) depending on the nature of the crisis.	General population	The Commission invites all citizens and stakeholders to engage in a broad debate to formulate a sustainable food policy including in national, regional, and local assemblies.
Food Security Doctrine of the Russian Federation [30]	President of the Russian Federation	2020	Russian Federation	Availability	Trade	The new *Doctrine* lists achievement of a positive balance of trade in agricultural products and raw materials and food, as well as fulfilment of the export potential taking into account the priority of self-sufficiency in such goods domestically and within the Eurasian Economic Union among the key tasks for ensuring food security.	General population	The strategic goal of ensuring food security is to provide the country's population with safe, quality, and affordable farm products, raw materials and food in the quantities that satisfy the balanced food consumption rates.
EU Green Deal [31]	United States Department of Agriculture – Foreign Agricultural Service	2021	European countries	Availability, sustainability	Agriculture initiatives	This quarterly report covers January through March 2021 and provides details on the status of the *EU Green Deal* objectives and strategies including the *Farm to Fork Strategy, Biodiversity Strategy, the Climate Target Plan, the EU Methane Strategy, and the Circular Economy Action Plan.*	General population	Not disclosed

Continues

labelling at the EU-level could inform consumer decisions and should be integrated on FNS policies. Additionally, the EU *Action Plan for Organic Production* suggests that national organic action plans – focused on boosting consumption, increasing production, and enhancing sustainability – could promote organic farming and consumption [31].

The WHO (2018) report "*Better food and nutrition in Europe: a progress report monitoring policy implementation in the WHO European Region*" emphasized that promoting healthier food availability and limiting "*High Fat, Salt, and Sugar*" (HFSS) products could be achieved through strategies like subsidizing fruits and vegetables, modifying food presentation at purchase points, enhancing nutrition education, and implementing food standards in schools. Schools played a vital role in fostering healthy eating habits, with evidence showing that nutrition education is most effective when it includes practical skills like cooking and food literacy rather than just providing information. In this report particular attention is paid to malnutrition, considering its double burden referring to the coexistence of undernutrition with overweight and obesity or diet-related Non-Communicable Diseases (NCDs). Pregnant and

Table 2
Continued

Title [reference]	Source (institution)	Publication year	Country	Food security dimensions	Setting	Strategy or policy description (intervention)	Target population	Results (strategy or policy outcome or impact)
Food Security Strategy of the Republic of Moldova for the period of 2023-2030 [32]	FAO	2022	Republic of Moldova	Availability	Agriculture initiatives, trade	Strategic vision and priorities of the Government regarding ensuring the country's food security, the development of sustainable food and management systems of risks in the context of international crisis situations.	General population	The specific objectives of the *Strategy* are: improving the governance of the field of food security in order to prevent and more effectively manage potential food insecurity crises; facilitation of international trade with agro-food products and means of production necessary for the agricultural sector; development of an efficient and safe local market; increasing production and productivity in the agrifood sector; development of mechanisms to reduce security risks food for vulnerable groups.
Seasonal migration in Europe and Central Asia in the context of the COVID-19 pandemic [33]	FAO	2021	Europe and European Central Asia	Availability	Social legislation	In the Republic of Moldova, was set a minimum unemployment benefit per month mostly to the benefit of returned migrant workers. The National Fund for the Development of Agriculture and Rural Environment has included returning migrant workers in the list of applicants for advance start-up grants. United Nations agencies, jointly with state agencies, have developed a socio-economic roadmap to respond to the consequences of COVID-19.	Migrant workers engaged in seasonal employment	Immediately formulate measures to support the socio-economic reintegration of returnees and improve the living conditions of their families. Develop policies and programs to help the socio-economic impact of COVID-19 on returnees interested in engaging in agricultural work. Assess how to make better use of remittance flows to generate new employment opportunities in the rural and agricultural sectors. Engage in short-term recovery actions to address the food insecurity problems of repatriates and remittance-receiving households.
A plan for public procurement: enabling a healthy future for our people, farmers and food producers [34]	Department for environment, food, and rural affairs of UK	2014	UK	Agency	Trade, innovation, education	An online portal or 'marketplace' has been established to allow supply chains, caterers, and customers to trade. It is particularly focused on providing a place where small and medium enterprises are better able to supply the public sector. Established a new *Action Group* which brings together the farming and food supply industries with *Research Technology Organizations* and government funders to identify and advance the innovation required to improve competitiveness. A *Food Procurement Information Service* will provide a helpline service, including a website with information, and details of workshops to support catering managers and food procurers in the public sector, and food producers and processors wishing to supply it.	Farmers, providers, chain supply in UK	A balanced scorecard and toolkit for procurers: an enabling approach that is consistently applied for catering and food procurement across government. A strong partnership: across the public, private sectors and beyond. Enduring action: for the longer term.

lactating women, along with young children, were particularly vulnerable to micronutrient deficiencies. Efforts to combat acute malnutrition included maternal and antenatal care improvements, support for exclusive breastfeeding, appropriate complementary feeding, and nutrient-rich dietary interventions [26].

The European Commission prioritized reversing rising overweight and obesity rates in the EU by 2030. The aim of the *Farm to Fork Strategy* was to transition to a more plant-based diet with less red and processed meat and more fruits and vegetables, reducing health risks and environmental impact. This strategy aimed

to reduce food system's climate footprint, strengthen its resilience, and ensure food security, nutrition, and public health [26].

Environmental policies

Toma *et al.* illustrated the importance of incorporating environmental considerations into policies concerning FNS through a case study of Romania. The Romanian authorities had introduced a pilot agri-environment-climate measure in 2014-2020 designed to *"promote farm-level adaptation to water scarcity in areas facing the most extreme effects due to climate change advocating for the simplification of agri-environment and climate measures"* [17].

Climate changes are likely to have impacted agriculture and food; hence, FNS policies should have encompassed climate aspects such as the EU *Climate Law*, which aimed for climate neutrality by 2050 while achieving net-zero greenhouse gas emissions and reducing net greenhouse gas emissions by at least 55% compared to 1990 levels by 2030 [31].

Furthermore, the *European Green Deal* is a policy that aims to make Europe the first climate-neutral continent by 2050, greening and transforming the EU economy to become carbon neutral. It maps a new, sustainable, and inclusive growth strategy to *"boost the economy, improve people's health and quality of life, care for nature, and leave no one behind"* [29, 31].

Economic policies

Regarding food demand and supply, the SUSFANS report on the AgriPrice4Cast project, which provided seasonal prices based on short-term yield forecasts, was noteworthy. The seasonal price forecasts for the EU allowed for the planning of emergency measures in cases of harvest outages in the rest of the world and/or the design of storage and other stabilization measures [16].

Trade openness should be a key element in policy-making. This is supported by a study conducted by Fusco *et al.*, which found that increasing trade had a positive effect on food security in European countries – improving both the reliability of food supply and the quality of nutrition [18].

The WHO (2018) report highlighted the significant role of price in food choices, with taxes and subsidies influencing purchasing behavior and encouraging healthier eating. This approach has gained interest as a tool to improve diets and prevent NCDs. Common strategies include taxing sugar-sweetened beverages and trans-fat-rich foods. Denmark led the way in 2003 by setting a national limit on industrial trans fats, and by 2018, 15 countries had similar regulations [26].

The analysis of the *Food Security Strategy* of the Republic of Moldova showed that low-income levels among the population, inadequate pension and social benefits, and the continual unchecked rise in food prices did not ensure food security in its most critical dimension - the affordability of food. This underscored the imperative to bolster the country's economy [32].

In 2020, the President of the Russian Federation signed the *Food Security Doctrine* for Russia, replacing the *Doctrine* adopted in 2010. The document served as a strategic plan outlining goals, objectives, and key directions of the government's socioeconomic policy to guarantee the food security of the Russian Federation. It provided a framework for developing regulatory legal acts concerning food security, agricultural, and fishery sectors, taking into account the recommendations of the FAO regarding the marginal propensity of imports and food stocks [30].

Sustainability of food and nutrition security

Rutten *et al.* focused on the sustainability of FNS, dissecting it into three dimensions: economic/business sustainability, social/cultural/health sustainability, and environmental/climate sustainability. Factors influencing FNS sustainability encompassed primary producers, food chain actors, and consumers. Metrics for evaluating the contribution of the European agricultural and fisheries sectors to FNS included production quantities, prices, and nutrient availability and supply, as well as the income of entrepreneurs (economic sustainability), resource use, environmental externalities (environmental sustainability), and trade in food and nutrients. Notably, food loss and waste at various stages of the food chain significantly contributed to the environmental pressure exerted by the food system [16].

Assessing the status and sustainability of FNS in the EU, requires models that project food and nutrition supply and demand while accounting for market dynamics, policy impacts, and sustainability factors. The SUSFANS toolbox addresses these requirements by integrating advanced micro-level models of consumer nutrition behaviour with macro-level models of food demand and supply [16].

The British Department for Environment, Food, and Rural Affairs emphasized the importance of supply chains in food security by launching an online marketplace to facilitate trade among suppliers, caterers, and customers. They also established a new action group to drive innovation by connecting the farming and food supply industries with research organizations and government funders to identify. Additionally, a *Food Procurement Information Service* was launched providing a helpline service, a resource website, and workshops to support public sector catering managers, food procurers, producers, and processors [34].

The WHO Regional Office for Europe launched the *First Action Plan for Food and Nutrition Policy* (2000-2005) to help EU member states reduce food-related illnesses and foster a sustainable environment [35]. This was followed by two additional action plans: the *2007-2012 Action Plan* which outlined six priority areas to tackle primary public health challenges concerning nutrition, and the *2015-2020 Action Plan*, a comprehensive strategy aimed at reducing food-related diseases, strengthening food system governance, and improving population nutrition [25, 35].

Stakeholders' views

Policy guidance concerning sustainable FNS in the EU was recommended to incorporate stakeholders' perspectives across its diverse dimensions in defining objectives and indicators, as well as in determining the relative importance weights for each indicator [16].

Furthermore, fostering cooperation and partnerships among various stakeholders was considered crucial. This included collaboration between central and local public administration authorities, non-governmental organizations, and international development partners. Such partnerships facilitated holistic and coordinated efforts towards achieving sustainable FNS goals [32].

Food security and COVID-19 pandemic and other crises

During the early stages of the COVID-19 pandemic, several challenges arose that disrupted the normal functioning of the food supply chain and posed risks to food security These challenges included border controls, which restricted the free movement of people and goods, hampering the operation of the single market and affecting consumers. Additionally, market disruptions occurred as demand for certain products significantly declined.

In response to these challenges, the European Commission recognized the need for a coordinated approach to ensure food security, safety, and public health while mitigating the socio-economic impacts within the EU. To address this, the *Farm to Fork Strategy* incorporated a *Contingency Plan* to safeguard food supply and security across the EU during crises. The plan established an EU-wide food crisis response mechanism coordinated by the European Commission and involving member states. It aimed to address sectoral vulnerabilities in agriculture, fisheries, aquaculture, food safety, labor, health, and transport. Functioning as a permanent forum, this mechanism would facilitate coordination at the EU level, with participation from member states and potentially stakeholders from the food supply chain. In a crisis, the forum would serve as the primary operational body to coordinate the response [28, 29].

Focus on particularly vulnerable categories

Children

Food-related policies prioritized addressing children's needs, recognizing them as a vulnerable group affected by food insecurity. In 2005, the WHO Regional Committee for Europe adopted the *European Strategy for Child and Adolescent Health and Development*, which identified seven priority areas, including maternal and newborn health and nutrition, and recommended actions across three levels: cross-sectoral policies, health systems, and health services. A toolkit accompanied the strategy, offering guidance for policy development. In terms of nutrition, the strategy outlined six key objectives: ensuring adequate nutrition for pregnant women and mothers, promoting and supporting exclusive breastfeeding, preventing malnutrition and micronutrient deficiencies, addressing overweight and obesity, promoting oral and dental health, and ensuring appropriate monitoring and evaluation of nutritional status [24].

Additionally, research by Long *et al.* emphasized the potential of holiday clubs in England to reduce food insecurity, highlighting their importance for food-insecure households and advocating for equal access to such services [20].

The 2018 WHO report *Better food and nutrition in Europe* highlighted the critical importance of early-life nutrition for establishing growth patterns, feeding practices, taste preferences, and dietary habits. It emphasized that children who had been breastfed were less likely to become overweight or obese or to develop type-2 diabetes in adulthood. The report recommended exclusive breastfeeding for the first six months and continued breastfeeding up to two years. However, the European Region had the lowest breastfeeding rate among all WHO regions. To promote breastfeeding the report called for the full implementation of the *International Code of Marketing of Breast-milk Substitutes*, alongside legislation, monitoring, and health workers training. The *Baby-Friendly Hospital Initiative* also played a key role in supporting breastfeeding [26]. Another key focus of food policies was protecting children from the harmful effects of marketing unhealthy foods high in fat, sugar, or salt. In 2022, WHO, in collaboration with UNICEF, published *Protecting children from the harmful impact of food marketing: policy brief*. This document aimed to guide policymakers on how to protect children from unhealthy food marketing, particularly by enforcing specific regulations on food advertising [23].

Women

The *Better food and nutrition in Europe* (WHO, 2018) report, highlighted the growing focus on maternal nutrition before and during pregnancy, particularly its impact on the later risk of NCDs and obesity in children. It emphasized that both undernourishment and overnutrition during pregnancy required urgent attention.

The report also noted the coexistence of overnutrition (due to excessive energy intake) and undernutrition (stemming from micronutrient deficiencies) in pregnant women, particularly in Eastern Europe. Pregnancy was considered an opportune time to monitor nutritional status and promote healthy behaviours. Vitamin and supplementation programs were highlighted as an essential component of care for pregnant women and women of reproductive age across many countries in the region [26].

Migrants

Migration status is closely linked to poverty, which can limit social networks and impact access to food security programs. These programs often benefit those with stronger social connections rather than those from truly food-insecure households [20]. Also, periods of crisis often disproportionately affect migrant populations. In response to the socio-economic challenges posed by COVID-19, several countries in Europe and Central Asia implemented measures to mitigate the pandemic's impact on migrants, recognizing migration as a critical factor influencing food security. For instance, the Republic of Moldova, set the minimum unemployment benefit at MDL 2,775 per month (USD 157), and developed a socio-economic response plan in collaboration with UN agencies. Similarly, Tajikistan introduced a strategy to support migrants, women, and youth, while digital platforms were created to connect these groups for advocacy and learning. In Kyrgyzstan, the government provided food and shelter to those in extreme need and establishing a migrant support fund [33].

Information campaigns

The *Better food and nutrition in Europe* report (WHO, 2018) emphasized the importance of dietary guidelines from reliable sources which provide accessible advice on healthy eating, portion sizes, and commonly consumed foods [26].

Front-of-package labelling was highlighted as a key tool for conveying nutritional information, particularly for complex processed foods, and influencing consumer choices. The European Commission proposed harmonizing mandatory front-of-pack nutrition labelling and extending mandatory origin labels to certain products [26, 29]. The *French Nutri-Score* labelling system has emerged as a frontrunner, with several countries, including France, Belgium, Spain, the Netherlands, and Germany, already adopting it domestically. As a means to reduce food waste and loss in the EU, the Commission also announced that it would propose revisions to EU rules relating to date marking ("*use by*" and "*best before*" dates) [31].

DISCUSSION

This scoping review aimed to identify the strategies and policies European countries have adopted to address food and nutritional insecurity, with an emphasis on economic, agricultural, and social sectors. The results highlight the complexity of policy approaches addressing food insecurity in the European region. The integration of policies across agriculture, nutrition, environment, economy, and sustainability underscores the multidimensional nature of FNS. However, challenges remain in ensuring that these policies translate into tangible improvements in food access, affordability, and nutritional quality, particularly for vulnerable populations.

The European Union's CAP and initiatives such as the *Farm to Fork Strategy* have demonstrated potential in shaping sustainable food production and supporting small farms, particularly in CEE. However, the sectoral nature of agricultural interventions suggests that while these policies contribute to food availability, they do not fully address socioeconomic disparities and income inequalities that influence food access [16, 17, 28, 29, 31].

Moreover, food availability and stability can be severely disrupted by animal health crises, as the control of infectious livestock diseases frequently involves large-scale culling measures with direct consequences for food supply, prices, and market stability. Outbreaks such as bovine spongiform encephalopathy, highly pathogenic avian influenza, and African swine fever have demonstrated how disease-control policies, while essential for public and animal health, can generate unintended food security risks by reducing the availability of key animal-source foods.
These impacts are amplified in highly concentrated production systems and can disproportionately affect populations that rely on animal products as primary protein sources, a dynamic widely recognized in international assessments of food system shocks, including FAO analyses of crises affecting agriculture and food security [36].

Nutrition-focused policies have made strides in promoting healthier food environments, with front-of-pack labelling and school-based interventions proving effective influencing dietary choices [31]. However, the persistent issue of malnutrition – both undernutrition and overnutrition – indicates the need for stronger regulatory frameworks and enforcement mechanisms. The review underscores the role of targeted fiscal policies, such as taxation of unhealthy foods and subsidies for healthier options, in modifying consumer behaviour. The challenge remains in ensuring that these policies are implemented equitably across member states to address disparities in diet-related health outcomes [26].

Climate change is an increasingly critical factor affecting food security, and policies such as the EU *Climate Law* and the *European Green Deal* aim to mitigate its impact by promoting sustainable agricultural practices [29, 31]. The case study from Romania highlights how national-level environmental policies can integrate water scarcity adaptation measures, yet broader EU-wide strategies are necessary to ensure consistency and effectiveness across diverse geographic and climatic conditions [17]. These policies should concern activities that substantially contribute to climate change mitigation or climate change adaptation, including agriculture and bioenergy.

The role of economic policies in food security is evident in the use of price forecasting tools and trade policies that influence food affordability [16]. Trade openness, as highlighted in the reviewed studies, has generally had a positive impact on food security in European countries. However, issues such as rising food prices, low-income levels, and inadequate social benefits in countries like Moldova underscore the need for more comprehensive economic strategies to improve food affordability [32].

Fiscal policies, including taxes on unhealthy foods and subsidies for nutritious options, have shown promise, but their effectiveness depends on their design and enforcement at the national level [26].

Sustainability remains a central theme in food security policies, with research emphasizing economic, social, and environmental dimensions. The SUSFANS toolbox provides valuable modelling capabilities to predict the sustainability of food systems, but its practical application in policymaking requires further refinement. Reducing food loss and waste is a key strategy in improving food system sustainability, yet implementation across the food supply chain varies significantly among EU member states. Strengthening collaboration between policymakers, industry stakeholders, and researchers is crucial in advancing a more sustainable food system. Partnerships between government agencies, non-governmental organizations, and international bodies have facilitated more holistic approaches to food security, yet disparities in stakeholder influence may lead to imbalances in policy outcomes. Future efforts should focus on inclusive governance structures that promote equitable participation [16].

The COVID-19 pandemic served as a stress test for European FNS policies, revealing both strengths and vulnerabilities in the food supply chain. While the EU's

contingency planning helped maintain food availability, initial disruptions in logistics and market dynamics highlighted areas requiring improvement. The establishment of a coordinated EU food crisis response mechanism represents a step forward in crisis preparedness, yet further refinements are necessary to enhance resilience against future shocks, including economic downturns and climate-related crises [28, 29].

The findings underscore the significance of targeted food policies and interventions addressing the needs of particularly vulnerable groups, including children, women, and migrants. Children are among the most susceptible to food insecurity due to their developmental needs and dependence on caregivers. Policies such as the *European Strategy for Child and Adolescent Health and Development* (WHO, 2005) have provided comprehensive frameworks for addressing nutritional deficiencies, promoting breastfeeding, and reducing obesity risks [24]. However, despite these efforts, challenges such as low breastfeeding rates in the WHO European Region persist, necessitating more aggressive implementation of supportive measures, including the *Baby-Friendly Hospital Initiative* and stricter regulation of breast-milk substitute marketing [26]. Additionally, the role of holiday clubs in England as an intervention for food-insecure households demonstrates a promising approach to mitigating childhood food insecurity, yet broader access and sustainability remain key concerns [20]. Maternal nutrition plays a crucial role in both maternal and child health outcomes, influencing the long-term risk of noncommunicable diseases and obesity in offspring. The dual burden of malnutrition – both undernutrition and overnutrition – among pregnant women highlights the need for a nuanced approach that includes both dietary interventions and micronutrient supplementation [24]. The prevalence of malnutrition in Eastern Europe underscores persistent inequalities in maternal healthcare access and nutritional resources, necessitating more robust and equitable food policy measures. Ensuring that maternal nutrition programs reach all pregnant women, particularly those in disadvantaged socio-economic conditions, remains an ongoing challenge that must be addressed through strengthened healthcare services and social support networks [26].

The food security challenges faced by migrants are further complicated by economic instability, social exclusion, and limited access to support services. The COVID-19 pandemic exacerbated these vulnerabilities, prompting various European and Central Asian governments to implement emergency measures, including financial assistance and food provision programs. However, the effectiveness of these interventions varies, and structural inequalities persist in many regions. Programs targeting migrants often benefit those with better social networks, rather than reaching the most vulnerable individuals. Thus, future policies should prioritize inclusive strategies that ensure equitable food distribution, targeted financial support, and increased access to nutritional education [20, 26].

CONCLUSIONS

The analysis of diverse scholarly papers and institutional documents highlights the complex and multifaceted nature of food and nutrition security, encompassing key dimensions such as food availability, access, utilization, and sustainability. These aspects can be effectively addressed through a broad range of policy measures and interventions across various sectors, including agriculture, nutrition, environment, economics, and stakeholder engagement. Special attention must be given to vulnerable populations, such as children, women, and migrants, recognizing their unique needs and challenges in accessing nutritious and affordable food. The COVID-19 pandemic has further exposed vulnerabilities within the food supply chain, underscoring the urgent need for coordinated responses to crises affecting food systems. In response, there is an increasing focus on sustainability in food policies, with efforts to incorporate environmental considerations, reduce food waste, and promote sustainable agricultural practices. In summary, the findings call for a comprehensive approach to food and nutrition security in Europe, involving a wide array of policy measures, stakeholder engagement, and international collaboration. However, significant challenges remain, particularly in addressing the root causes of food insecurity, ensuring equitable access to nutritious food, and building resilience against future crises. Continuous research, monitoring, and evaluation of policy effectiveness are essential for advancing sustainable and inclusive food systems in Europe.

Conflicts of interest statement

The Authors declare no conflict of interest.

Ethical approval

Ethical approval for this type of study is not required by our institute.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Authors' contributions

The study was conceived and designed by MLdP and MLS, with input from all the Authors. Data collection was conducted by ELG, LP, MGC, MRM. Data analysis was conducted by ELG, LP, MGC, MRM, AC and interpretation was conducted by DZ, ELG. The draft was written in all the parts with the contribution of DZ, MGC, ELG, LP, MRM, FB, AC. Critical revision of the article was made by MLS, MLdP and GT.

The approval of the final version to be submitted was approved by all the Authors the paper prior to submission.

Received on 27 October 2025.
Accepted on 8 April 2026.

REFERENCES

1. United States Department of Agriculture. Food security in the US – Measurement. USDA; 2026. Available from: https://www.ers.usda.gov/topics/food-nutrition-assistance/food-security-in-the-us/measurement#security
2. Food and Agriculture Organization, International Fund for Agricultural Development, United Nations Children's Fund, World Food Programme, World Health Organization. The state of food security and nutrition in the world 2019. Safeguarding against economic slowdowns and downturns. Rome: FAO; 2019. (SOFI series, 2019). doi: 10.4060/CA5162EN
3. Clapp J, Moseley WG, Burlingame B, Termine P. Viewpoint: the case for a six-dimensional food security framework. Food Policy. 2022;106:102164. doi: 10.1016/j.foodpol.2021.102164
4. High Level Panel of Experts on food security and nutrition of the Committee on World Food Security. Food security and nutrition: building a global narrative towards 2030. A report by the HLPE. Rome: HLPE; 2020. (HLPE Report n. 15). Available from: https://openknowledge.fao.org/handle/20.500.14283/ca9731en
5. Sumsion RM, June HM, Cope MR. Measuring food insecurity: the problem with semantics. Foods. 2023;12(9):1816. doi: 10.3390/foods12091816
6. Food and Agriculture Organization, International Fund for Agricultural Development, United Nations Children's Fund, World Food Programme, World Health Organization. The state of food security and nutrition in the world. Rome: FAO; 2020. (SOFI series, 2020). doi: 10.4060/ca9692en
7. Food and Agriculture Organization, International Fund for Agricultural Development, United Nations Children's Fund, World Food Programme, World Health Organization. In brief to the state of food security and nutrition in the world 2023. Rome: FAO; 2023. (SOFI series, 2023). doi: 10.4060/cc6550en
8. National Institute on Minority Health and Health Disparities. Food accessibility, insecurity and health outcomes. Bethesda, MD: NIMHD; 2023. Available from: https://www.nimhd.nih.gov/resources/understanding-health-disparities/food-accessibility-insecurity-and-health-outcomes
9. Policy Research to Improve Food Security. What are the implications of food insecurity for health and health care? Toronto: Proof; 2023. Available from: https://proof.utoronto.ca/food-insecurity/what-are-the-implications-of-food-insecurity-for-health-and-health-care/
10. Food and Agriculture Organization. Report of the World Food Summit. Rome: FAO; 1996. (WFS 96/REP). Available from: https://www.fao.org/4/w3548e/w3548e00.htm
11. European Commission. What the European Commission does in strategy and policy. Brussels: EC. Available from: https://commission.europa.eu/about-european-commission/role-european-commission/strategy-and-policy_en
12. Guest C, Ricciardi W, Kawachi I, Lang I (Eds). Oxford handbook of public health practice. Oxford: Oxford University Press; 2013. doi: 10.1093/med/9780199586301.001.0001
13. Tricco AC, Lillie E, Zarin W, O'Brien KK, Colquhoun H, Levac D, Moher D, Peters MDJ, Horsley T, Weeks L, Hempel S, Akl EA, Chang C, McGowan J, Stewart L, Hartling L, Aldcroft A, Wilson MG, Garritty C, Lewin S, Godfrey CM, Macdonald MT, Langlois EV, Soares-Weiser K, Moriarty J, Clifford T, Tunçalp Ö, Straus SE. PRISMA extension for scoping reviews (PRISMA-ScR): checklist and explanation. Ann Intern Med. 2018;169(7):467-73. doi: 10.7326/M18-0850
14. Cacciuttolo MG. PROTOCOL: addressing food and nutrition insecurity in European countries: A scoping review of strategies and policies. OSF; 2023. doi: 10.17605/OSF.IO/3254P
15. Ouzzani M, Hammady H, Fedorowicz Z, Elmagarmid A. Rayyan – a web and mobile app for systematic reviews. Syst Rev. 2016;5(1):210. doi: 10.1186/s13643-016-0384-4
16. Rutten M, Achterbosch TJ, Boer IJM de, Cuaresma JC, Geleijnse JM, Havlík P, Heckelei T, Ingram J, Leip A, Marette S, Meijl H van, Soler L-G, Swinnen J, Veer P van't, Vervoort J, Zimmermann A, Zimmermann KL, Zurek M. Metrics, models and foresight for European sustainable food and nutrition security: the vision of the SUSFANS project. Agric Syst. 2018;163:45-57. doi: 10.1016/j.agsy.2016.10.014
17. Toma I, Redman M, Czekaj M, Tyran E, Grivins M, Sumane S. Small-scale farming and food security – policy perspectives from Central and Eastern Europe. Glob Food Secur. 2021;29:100504. doi: 10.1016/j.gfs.2021.100504
18. Fusco G, Coluccia B, Leo FD. Effect of trade openness on food security in the EU: a dynamic panel analysis. Int J Environ Res Public Health. 2020;17(12):4311. doi: 10.3390/ijerph17124311
19. Jensen JD, Smed S, Aarup L, Nielsen E. Effects of the Danish saturated fat tax on the demand for meat and dairy products. Public Health Nutrition. 2016;19(17):3085-94. doi: 10.1017/S1368980015002360
20. Long MA, Stretesky PB, Graham PL, Palmer KJ, Steinbock E, Defeyter MA. The impact of holiday clubs on household food insecurity – pilot study. Health Soc Care Community. 2018;26(2):e261-9. doi: 10.1111/hsc.12507
21. Crilley E, Brownlee I, Defeyter MA. The diet of children attending a holiday programme in the UK: Adherence to UK food-based dietary guidelines and school food standards. Int J Environ Res Public Health. 2021;19(1):55. doi: 10.3390/ijerph19010055
22. World Health Organization. European Action Plan for food and Nutrition Policy 2007-2012. Geneva: WHO; 2008. Available from: https://iris.who.int/handle/10665/349767
23. United Nations Children's Fund, World Health Organization. Protecting children from the harmful impact of food marketing: policy brief. New York, NY: UNICEF; 2022. Available from: https://www.who.int/publications/i/item/9789240051348
24. World Health Organization Regional Office for Europe. European strategy for child and adolescent health and development. Action tool. Copenhagen: WHO; 2005. Available from: https://iris.who.int/handle/10665/349955
25. World Health Organization Regional Office for Europe. European food and nutrition Action Plan 2015-2020. Copenhagen: WHO; 2015. Available from: https://www.who.int/europe/publications/i/item/9789289051231
26. World Health Organization Regional Office for Europe. Better food and nutrition in Europe: a progress report monitoring policy implementation in the WHO European Region. Copenhagen: WHO; 2018. Available from: https://iris.who.int/handle/10665/345370
27. International Fund for Agricultural Development, United Nations Children's Fund, Food and Agriculture Organization, World Health Organization. The state of food security and nutrition in the world 2022. Rome: FAO; 2022. (SOFI series, 2022). doi: 10.4060/cc0639en

28. European Commission. EU food supply and food security – contingency plan. Brussels: EC; 2020. Available from: https://ec.europa.eu/info/law/better-regulation/have-your-say/initiatives/12770-EU-food-supply-and-food-security-contingency-plan_en
29. European Commission. Communication from the Commission to the European Parliament, the Council, the European Economic and Social Committee and the Committee of the Regions. A Farm to Fork Strategy for a fair, healthy and environmentally-friendly food system. Brussels: EC; 2020. (COM(2020) 381 final). Available from: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:52020DC0381
30. United States Department of Agriculture Foreign Agricultural Service. Presidential Decree n. 20 validating Food Security Doctrine of the Russian Federation. Brussels: USDA; 2020. Available from: https://leap.unep.org/en/countries/ru/national-legislation/presidential-decree-no-20-validating-food-security-doctrine
31. United States Department of Agriculture Foreign Agricultural Service. EU Green Deal – April 2021 Update. Brussels: USDA; 2021. Available from: https://www.fas.usda.gov/data/gain/2021/04/european-union-eu-green-deal-april-2021-update
32. Food and Agriculture Organization. Food Security Strategy of the Republic of Moldova for the period of 2023-2030. Rome: FAO; 2022. Available from: https://www.fao.org/faolex/results/details/en/c/LEX-FAOC215849/
33. Food and Agriculture Organization. Seasonal migration in Europe and Central Asia in the context of the COVID-19 pandemic. Rome: FAO; 2021. doi: 10.4060/cb5718en
34. Department for Environment, Food and Rural Affairs. A plan for public procurement: enabling a healthy future for our people, farmers and food producers. London: DEFRA; 2014. Available from: https://www.gov.uk/government/publications/a-plan-for-public-procurement-food-and-catering
35. World Health Organization Regional Office for Europe. The First Action Plan for Food and Nutrition Policy: WHO European Region 2000-2005. Copenhagen: WHO; 2001. Available from: https://iris.who.int/handle/10665/108379
36. Food and Agriculture Organization. The impact of disasters on agriculture and food security 2025. Rome: FAO; 2025. doi: 10.4060/cd7185en

Ann Ist Super Sanità 2026 | Vol. 62, No. 2: 179-189
DOI: 10.4415/ANN_26_02_10

Spontaneous orienting of untrained companion dogs naïve to human epilepsy toward odor samples from an unfamiliar human in a controlled non-social paradigm: a proof-of-concept

Federica Pirrone[1], Gianfranco Cancelli[2] and Mariangela Albertini[1]

[1]*Dipartimento di Medicina Veterinaria e Scienze Animali, Università degli Studi di Milano, Lodi, Italy*
[2]*Il Collare d'Oro, Cologno Monzese (MI), Italy*

Abstract

Background. Evidence for spontaneous seizure detection by dogs remains limited. This study examined whether ictal odor cues elicit behavioral responses in untrained companion dogs.

Objective. To test whether dogs naïve to human epilepsy show spontaneous discrimination of ictal versus interictal odor.

Methods. Thirty dogs, without prior seizure exposure, freely investigated three odor stations (ictal, interictal, blank) using sweat samples from a single unfamiliar donor in controlled, non-social conditions.

Results. Dogs were more likely to investigate the ictal odor first than expected by chance (Monte Carlo p=0.029), indicating an early orienting bias. No differences emerged in sustained engagement. Structured exploratory patterns occurred only in odor conditions (ictal p=0.001; interictal p=0.002), not in the control (p=0.715).

Conclusions. Ictal odor may carry salience sufficient to influence initial attention in naïve dogs. However, findings are based on a single donor and require replication with multiple individuals to assess generalizability.

Key words

- canine
- seizure alert dogs
- human epilepsy
- volatile organic compounds
- olfaction

INTRODUCTION

Epilepsy is a chronic neurological disorder characterized by recurrent epileptic seizures and affects approximately 1% of the human population worldwide. According to data from the Istituto di Ricerche Farmacologiche Mario Negri, Milan, Italy, nearly 500,000 individuals in Italy are diagnosed with epilepsy, with approximately 30,000 new cases reported annually. Patients with epilepsy face a significantly increased risk of mortality, psychiatric and somatic comorbidities, and adverse effects related to antiseizure medications [1].

Epileptic seizures are typically short-lasting (generally lasting less than 2 minutes) and early diagnosis and appropriate treatment can achieve effective seizure control in a large proportion of cases. However, approximately 30% of patients are drug-resistant, representing a substantial therapeutic challenge [2]. Moreover, epileptic seizures typically occur unpredictably [2] and may remain unrecognized or go unnoticed by patients, either because they occur without overt behavioral manifestations [3, 4] as in the case of focal nonconvulsive seizures, or because postictal impairment of awareness and memory prevents accurate recall [5]. This results in unreliable seizure reporting [6, 7], with studies showing that patients may fail to document more than 50% of their seizures [8]. Such underreporting poses major challenges for diagnosis, prognosis, and the evaluation of treatment efficacy. In clinical practice and research settings, patients or caregivers are in fact commonly asked to maintain seizure diaries, which serve as outcome measures, to inform risk assessment and support seizure forecasting. Accurate seizure documentation is therefore essential for sound clinical and scientific practice [2].

In this context, there is a growing need for complementary systems capable of enhancing patients' and caregivers' awareness of seizure episodes, particularly in cases characterized by impaired recognition. Cur-

Address for correspondence: Federica Pirrone, Dipartimento di Medicina Veterinaria e Scienze Animali, Università degli Studi di Milano, Via dell'Università 6, 26900 Lodi, Italy. E-mail federica.pirrone@unimi.it.

rently available technologies are largely based on implanted electroencephalographic (EEG) recording devices combined with predictive algorithms [9]. Although promising, these approaches are invasive, carry non-negligible risks, and their accuracy and feasibility for routine clinical use remain under debate. Moreover, some patients may be reluctant to undergo implantation procedures [10]. Other commercially available systems, such as accelerometers, motion sensors, or multimodal detectors, are primarily designed to identify convulsive movements and alert caregivers; however, they do not provide advance warning to the individual experiencing the seizure [11]. Animal-assisted detection strategies, including trained or spontaneously responsive dogs, have attracted increasing scientific interest as a potential non-invasive complementary approach. This interest stems from the well-documented effectiveness of dogs' exceptional olfactory abilities in a wide range of applied contexts, including tracking, drug and explosive detection, finding human victims of disasters and searching for human remains [12-15]. Within the medical field, macrosmatic species – particularly dogs, but also rodents – have been investigated as diagnostic or screening tools for a range of human conditions, most notably in cancer detection (e.g., lung, breast, prostate, and skin cancers), as well as in infectious diseases such as COVID-19 and tuberculosis [16-20].

Since 1999, dogs have been trained either to respond once a seizure has begun (seizure response dogs, SRDs), or to display premonitory behaviors before the individual becomes aware of an impending seizure (seizure alert dogs, SADs) [21]. However, with the exception of one prospective study [22], which monitored seizure frequency over a 48-week period in ten patients referred to a seizure alert dog service and reported a significant reduction in seizure frequency during and after the dog training period, this acquired ability of dogs has so far been evaluated scientifically primarily through guardian- or trainer-reported questionnaires or retrospective analyses [9, 21-24]. While these studies suggest potential clinical benefits associated with trained seizure alert dogs, they did not experimentally test dogs' performance accuracy and reliability. Importantly, seizure-related responding/alerting behaviors have also been reported in untrained pet dogs [24-26]. However, these reports remain largely anecdotal or, again, based on guardian- and trainer-reported surveys conducted in domestic environments, which do not provide experimental evidence of spontaneous seizure-detection abilities under controlled conditions.

Another aspect that remains poorly understood is the sensory modality underlying seizure detection in dogs. Dogs may rely on visual cues, such as subtle behavioral or postural changes, olfactory cues, or a combination of both, emitted by patients prior to seizure onset or during the ictal phase. Dogs' ability to detect human diseases through olfaction is well established [18-20], owing to their olfactory sensitivity, which is orders of magnitude greater than that of humans, and their capacity to detect volatile organic compounds (VOCs) associated with condition-specific metabolic alterations.

Maa *et al.* [27] conducted a larger and more rigorous prospective laboratory study to investigate the olfactory detection of seizure-associated VOCs in humans by professionally trained service dogs under blinded conditions, reporting high discrimination accuracy between ictal and interictal odors (approximately 94% sensitivity and over 96% specificity). In a previous work, Catala *et al.* [28] trained dogs to perform a predefined behavior in response to seizure-associated odors from a small cohort (five patients), demonstrating high discrimination accuracy (a sensitivity of 87% and a specificity of 98%). These findings support the validity of seizure-related VOCs as a potential biomarker detectable by trained dogs. However, when dogs are deliberately trained to detect and respond to these odor cues, as in these studies, their responses reflect a learned process rather than a spontaneous sensory reaction, making it difficult to disentangle intrinsic olfactory sensitivity from the effects of reinforcement, attentional focus, and repeated exposure. Essentially, these studies did not clarify whether olfaction represents a primary sensory modality spontaneously used by dogs, or whether it becomes dominant as a result of training and odor-specific learning. In an attempt to clarify this aspect, Powell *et al.* [29] examined the behavioral responses of naïve pet dogs exposed to seizure-associated sweat samples collected from unknown individuals with epilepsy. The odors were delivered via a remote system positioned directly beneath the seated guardian's thighs, thereby creating the impression that the scent originated from the dog's own guardian. Dogs displayed increased attention and proximity-seeking behaviors in response to seizure samples. However, the odor stimuli were presented within an established dog–owner relationship, meaning that the observed responses occurred in a strongly relational context; therefore, the potential influence of familiarity, attachment, prior experience, or contextual learning on spontaneous olfactory sensitivity cannot be excluded. Consequently, it remains unclear whether seizure-associated odors elicit a spontaneous behavioral response in untrained dogs, independent of learning, social context, or prior experience. More broadly, the precise contribution of canine olfaction to seizure detection remains undercharacterized.

The present study reports results from the first experimental phase of a broader research project aimed at addressing this question, focusing on spontaneous exploratory behavior in untrained dogs with no prior exposure to epilepsy and no experience with medical detection tasks. Sweat odor samples were collected from an unfamiliar person and presented to dogs within a socially neutral experimental paradigm. By removing potential confounds related to training, experience, familiarity, and the dog-guardian relationship, this approach was designed to directly assess whether odors present during the ictal state may be intrinsically salient to dogs and elicit spontaneous orienting responses prior to any form of learning. Given the exploratory nature of this proof-of-concept design, the study aimed to provide initial evidence to inform subsequent steps toward the identification of potentially generalizable seizure-related odor signatures. A deeper understanding of the contribution of olfactory strategies engaged by dogs in seizure

detection will refine current knowledge of the underlying physio-ethological mechanisms involved. Such knowledge could be leveraged to better support episode recognition and reporting through these valuable tools, particularly in vulnerable individuals whose seizure onset or ictal phase goes unrecognized or unnoticed, either because episodes lack overt behavioral manifestations or because conscious awareness is impaired.

MATERIALS AND METHODS

This study was part of a larger research project on seizure alert dogs that received approval from the Ethics Committee (Ref. no. CE_116/21, 23.11.21) and the Animal Welfare Committee (OPBA_67_2024) of the University of Milan and was conducted in compliance with national and EU legislation and institutional guidelines. Written informed consent was obtained from all human participants after they received detailed information about the procedures; however, dog guardians were not informed about the specific aim of the study. In the case of minor participants, written informed consent was provided by a legal guardian. Participation was entirely voluntary, both with respect to providing sweat samples and to allowing dogs to take part in the activities.

Participants (patients)

Eligible patients for the general project were individuals of either gender, older than 6 years, with a diagnosis of focal epilepsy. They could be either hospitalized, admitted to neurological units for prolonged presurgical video-electroencephalographic (video-EEG) monitoring aimed at characterizing the electroclinical features of seizures in view of potential surgical treatment, or non-hospitalized patients. In the present study, we selected sweat samples from a non-hospitalized 6-year-old female volunteer receiving care at Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Italy. Consistent with the exploratory proof-of-concept design of the present study, samples from a single patient were used to provide a standardized odor stimulus across all dogs. This approach minimized inter-individual variability in odor profile, thereby enabling a more controlled comparison of ictal versus interictal conditions across dogs.

Sample collection and handling

Sweat was selected as the biological matrix, in line with previous epilepsy-related studies [27-29], because VOCs released during epileptic seizures readily diffuse into sweat and because its collection is entirely non-invasive. Sweat samples were collected by a familiar caregiver wearing nitrile gloves. Sterile gauze pads (10×10 cm) were used and applied with a double pass to the skin of the neck and/or forehead.

Sweat samples were collected at two different time points, with three samples obtained from the patient at each sampling occasion: 1) an interictal baseline condition, during seizure-free intervals i.e., at least 6 hours before or after a seizure to exclude potential pre-ictal or post-ictal influences, in line with Catala *et al.* [28] and Elger *et al.* [2] an ictal condition, during focal seizures or during the convulsive phase of secondarily generalized focal seizures. After collection, each gauze pad was subsequently cut into four sections, yielding 12 subsamples per patient for each sampling phase, which were placed into hermetically sealed polymeric tubes (Securitainer tube 29×63 mm, Nolato, Netherlands), bearing the subject's ID. Samples were transferred within 45 minutes to a monitored refrigerator at 4 °C, and subsequently transported under refrigerated conditions (+4 to +8 °C) to the Animal Physioethology Laboratory of the dog testing center, Department of Veterinary Medicine (DIVAS), University of Milan, Lodi (Italy). Samples were accompanied by detailed documentation specifying sampling conditions and timing and were stored at -18 °C until testing. Previous research has shown that freezing preserves human body odor samples without altering how they are perceived [30].

Dogs

All dogs involved in the study were privately owned companion dogs aging at least 1 year old, recruited from friends and acquaintances, with no reported prior experience in scent detection, olfactory discrimination tasks, or formal training involving odor cues. Importantly, all dogs were naïve to the odor of the seizure, meaning that they had no known prior exposure to epileptic seizures, either in humans or other dogs. This criterion was adopted to ensure that any observed behavioral responses would reflect spontaneous reactions to the presented odor stimuli rather than prior learning or experience. Dogs had to be free from overt signs or existing diagnoses of distress and/or pain during the experimental session. Additionally, dogs needed to show interest in new situations and objects, as novel objects were involved in the sniffing task they would undergo if they were enrolled. Thirty-four companion dogs aged 1-14 years (median age=5.5 years), including 11 males (one neutered) and 23 females (19 spayed) were recruited for this study through word of mouth at the National Association Assistance Dogs Il Collare d'Oro and at the Department of Veterinary Medicine and Animal Sciences, University of Milan. Overall, the sample comprised 10 mixed-breed dogs and 24 purebred dogs representing 14 breeds, including Labrador Retriever, Australian Cattle Dog, Belgian Shepherd (Tervuren), Border Collie, Jack Russell Terrier, Wirehaired Pointing Griffon (Korthals), English Setter, Wirehaired Dachshund, Maremma Hound, Czechoslovakian Wolfdog, Chihuahua, Lagotto Romagnolo, Dachshund, and Airedale Terrier. Dogs were accompanied by their guardians throughout all experimental phases (see the following section on Experimental setup and testing), and their behavior during the sniffing phase was video-recorded for subsequent analysis. The final sample size met and exceeded the minimum required for the study, as determined by an a priori power analysis conducted in R (pwr package) and WebPower. The analysis assumed a medium-to-large effect size (f=0.40), a significance level of α=0.05, and a desired statistical power of 0.80, yielding a minimum required sample of 25 dogs.

Experimental setup and testing

Behavioral testing of dogs was conducted at the Animal Physioethology Laboratory of the Department of

Veterinary Medicine, University of Milan, Lodi, Italy. Three odor stations were arranged on the floor in a semicircular configuration, equidistant (130 cm apart) and positioned 300 cm from a central starting point, where the guardian was seated and released the dog at the beginning of each trial. Each station consisted of a plastic pot mounted on a fixed base (49x35 cm) containing the samples and covered with a perforated metal plate (15x15 cm), allowing diffusion of volatile compounds while preventing direct contact with the biological sample. After testing the first ten dogs, we observed that 40% of them (4/10) did not approach the experimental setup during the sniffing phase and remained in proximity to their guardian, despite having freely explored the room during the preceding acclimatization phase (see below for details). The only procedural difference between the acclimatization and sniffing phases was the presence of the sniffing stations on the floor. To facilitate spontaneous approach to the stations, an identical empty food bowl (16 cm diameter) was placed next to each pot at a fixed distance of approximately 2 cm *(Figure 1a, b)*. Following this adjustment, failure to approach the experimental setup was no longer observed in subsequent dogs.

The three stations contained: A) a polymeric tube filled with a sweat sample collected during an ictal phase, B) a polymeric tube filled with a sweat sample collected during an interictal baseline phase from the same individual, and C) a polymeric tube filled with a clean gauze pad serving as a control. Each dog was tested once and constituted a single independent observational unit in the experimental design. Each trial comprised two sequentially structured phases separated by a 5-minute break: an acclimatization phase and a sniffing phase.

Acclimatization phase (10 min): before the sniffing phase, the guardian and the dog entered the laboratory room and were welcomed by two researchers (MA and FP), who provided instructions and then left the room. In particular, the guardian was instructed to sit on a chair at the starting point, release the dog to explore freely, and remain seated facing away from the interior of the room, including the area where the odor stations would later be placed. During this time, the guardian completed documentation provided by the researchers, to prevent any visual or verbal interaction with the dog. This procedure closely mirrored the sniffing phase, with the sole difference being the absence of the odor stations positioned on the floor in the center of the room.

Break (5 min): at the end of the acclimatization phase, only one researcher (MA) re-entered the room. The guardian was then asked to leash the dog and temporarily leave the laboratory to allow the experimental setup to be arranged. To minimize position-related interference, the left-center-right position of each odor station was pseudorandomized across trials using a true random number generator (http://www.random.org), such that all possible presentation sequences (e.g., ABC, BCA, CAB) were counterbalanced across dogs. At the end of each session, the slots were changed, and the sample stations were thoroughly cleaned with a vapor machine (Vaporetto PRO 90 Turbo, Polti, Italy).

Sniffing phase (2 minutes): the guardian and the dog re-entered the room, while the researchers exited and remained in an adjacent room for the duration of the phase. The guardian sat at the starting point, unleashed the dog, and turned away from the experimental area while completing additional documentation. The dog was thus free to explore the room and the experimental setup without guidance, commands, or reinforcement

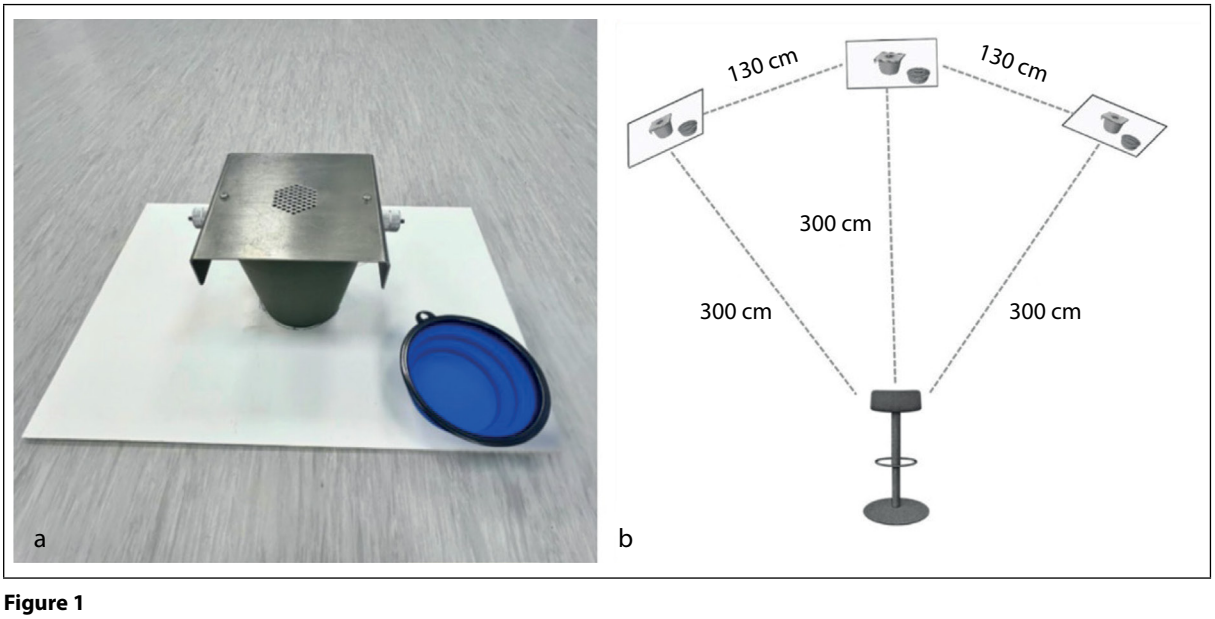


Figure 1
a) Detail of the experimental odor station; b) Experimental testing room and spatial arrangement of the odor stations. Three odor stations were arranged on the floor in a semicircular configuration. Stations were spaced 130 cm apart from each other and positioned 300 cm from a central frontal starting point, marked by the guardian's chair. From this position, the dog was released and allowed to explore the experimental setup freely during the sniffing phase.

(Figure 2). Dog behavior was monitored remotely by a single researcher (FP), who had not arranged the setup, using two wall-mounted digital video cameras (GoPro HERO7, Italy), operated from the adjacent room. The combined recordings provided complete visual coverage of the dogs, ensuring continuous monitoring of exploratory behavior and enabling the detection of any relevant signs of severe distress or anxiety that would have led to immediate interruption of the trial, while also allowing accurate coding of the predefined behavioral parameters during subsequent video analysis. In addition, guardians were explicitly informed that they could stop the test at any time if they had concerns regarding their dog's well-being or safety. After 2 minutes, both researchers re-entered the room and formally terminated the trial. The experiment was conducted under double-blind conditions as the spatial position of ictal, interictal, and control samples was unknown to the guardian-dog dyads and to the monitoring researcher (FP), while the researcher responsible for arranging the odor stations was neither present in the testing room nor had any visual access to the room during the sniffing phase, including remote access via the video monitoring system.

Video coding

The video-recorded sniffing phase was analyzed using PotPlayer (v. 1.7). To minimize the risk of unconscious confirmation bias, the analysis was conducted without access to information regarding the sequence of odor samples presented [31]. Behavioral responses were quantified using the following variables, which captured different temporal and functional aspects of olfactory exploration:

1. sniffing duration, defined as the total time (in seconds) spent sniffing each station during the 2-minute sniffing phase and treated as a continuous variable in subsequent analyses. Longer sniffing was considered an index of sustained attention to the corresponding olfactory stimulus, reflecting the extent to which an odor maintained the dog's engagement over time. Evidence of prolonged engagement with the ictal odor would be indicated by longer sniffing duration at that station compared to the interictal and control stations (A_duration > B_duration and C_duration);
2. sniffing frequency, defined as the number of distinct sniffing episodes directed toward each station during the 2-minute sniffing phase and treated as a continuous variable in subsequent analyses. This measure was used as an index of repeated engagement with the odor source, reflecting the dog's tendency to return to and re-explore a given stimulus. A higher level of repeated engagement with the ictal odor would be suggested by a higher sniffing frequency at the ictal station relative to the other conditions (A_freq >B_freq and C_freq);
3. initial orienting behavior, assessed by recording the first station investigated after release. This measure was used as an index of immediate attentional bias toward the olfactory stimuli. For inferential purposes, a dichotomous variable (A_first) was derived to indicate whether the ictal station was approached first (yes/no). In addition, to analyze how dogs distributed their attention across stimuli over time, the order of olfactory investigation was recorded for each station (A, B, and C) as the position in the exploration sequence (first, second, or third), reflecting the order in which stimuli were selected during spontaneous exploration. Stations that were not investigated during the trial were coded as "not sniffed";
4. approach-related measures, which included a) non-investigation and b) latency to first sniff. Non-investigation was defined as the absence of sniffing at a given station during the sniffing phase and quantified as the proportion of dogs that did not investigate each station. Latency to first sniff was defined as the time elapsed (in seconds) from the moment the dog was released to the first sniffing interaction with the first station approached. This parameter was analyzed as a continuous variable to assess quantitative differences in exploratory onset across odor conditions. In addition, to facilitate the characterization of distinct behavioral profiles of initiation, latency to first sniff was dichotomized using the sample median (short *vs* long latency) for specific analyses. This categorization allowed the identification of qualitatively different patterns of initial engagement, potentially reflecting differences in stimulus salience or approach-related processes, that could not be captured solely by comparisons of central tendency.

In addition to exploration-related measures, potential indicators of distress were quantified. Specifically, the frequency of scratching, yawning, and panting was recorded for each dog during the experimental session.

Finally, to further characterize dogs' exploratory strategies, the subsample of dogs (n=24) tested under conditions in which both the pot and the bowl were consistently present at all three stations was selected for additional analyses. Specifically, an Object variable was introduced to characterize orienting strategies within each approached station. The Object variable was coded categorically to indicate whether dogs investigated the pot only (P), the bowl only (B), both objects with the pot contacted first (PB), and both objects with

Figure 2
Dog freely exploring one of the odor stations during the sniffing phase.

Table 1
Distribution of sniffing order across stations in all dogs (n=30)

	First n (%)	Second n (%)	Third n (%)	Not sniffed n (%)
Station A	14 (46.7)	4 (13.3)	4 (13.3)	8 (26.7)
Station B	9 (30.0)	12 (40.0)	2 (6.7)	7 (23.3)
Station C	7 (23.3)	8 (26.7)	7 (23.3)	8 (26.7)

Station A: ictal; station B: interictal; station C: empty (control). Percentages are calculated within station (row percentages) for each station.

the bowl contacted first (BP). To verify that these dogs were representative of the full sample, all previously defined behavioral measures were re-evaluated in this subsample.

Statistical analyses

Of the 34 dogs initially involved, four failed to interact with the odor stations during the sniffing phase, remaining at the starting point near the guardian, and were therefore excluded from the analyses. Unless otherwise specified, these were conducted on both the full sample (n=30) and on the subsample of dogs (n=24) tested under conditions in which both the pot and the bowl were consistently present at all three stations.

To exclude potential procedural biases, the distribution of presentation sequences (ABC, ACB, BAC, BCA, CAB, CBA) was examined using chi-square goodness-of-fit tests. In this analysis, as well as in all subsequent applications of chi-square tests throughout the study, Monte Carlo significance estimates were applied when expected cell counts were small (≤5) to ensure accurate p-value estimation. In addition, adjusted standardized residuals were inspected to identify specific categories contributing to significant deviations from expected frequencies. To control for spatial biases, the effect of the ictal station's spatial position (left, center, right) on dogs' initial orienting behavior was examined based on its location within the presentation sequence for each trial. The association between the spatial position of station A and the likelihood of being approached first was evaluated using chi-square tests of independence. Preliminary inspection of the data revealed that latency variables were positively skewed across stations. Given the non-normal distribution of the data and the limited sample size, non-parametric statistical tests were used for all inferential analyses.

Sniffing duration and sniffing frequency were treated as continuous variables and compared across odor conditions (ictal, interictal, control) using Friedman tests. The relationship between sniffing duration and sniffing frequency was examined using Spearman's rank correlation coefficient, computed separately for each odor condition in the full sample. The strength of correlation was classified as absent (0.00-0.09), weak (0.10-0.29), moderate (0.30-0.49), or strong (0.50.1.00) [32]. The correlation analyses were exploratory and aimed at characterizing the structure of olfactory exploration rather than testing multiple independent hypotheses. Therefore, no correction for multiple comparisons was applied.

Initial orienting behavior (first station investigated) was analyzed using chi-square goodness-of-fit tests to evaluate deviations from a uniform distribution across stations. Sniffing order (first, second, third) was analyzed for each station using chi-square goodness-of-fit tests against a uniform distribution. Differences in non-investigation across stations were analyzed using Cochran's Q test. Latency to first sniff was analyzed as a continuous variable using Kruskal–Wallis tests according to the identity of the first station approached. To specifically examine latency patterns associated with the ictal odor, additional analyses were restricted to trials in which the ictal station was chosen first. In addition, the variable was dichotomized using the sample median as a conservative cut-off, defining short (≤median) and long (>median) latencies. Differences in the distribution of short and long latencies across first-choice stations were assessed using chi-square tests with Monte Carlo estimation of exact p-values. The Pot-Bowl (PB) variable was examined in the 24-dog subsample using chi-square goodness-of-fit tests. All statistical analyses were performed using IBM SPSS Statistics (version 30), with statistical significance set at p≤0.05.

RESULTS

In the total sample (n=30), sniffing order, reported as frequencies and percentages in *Table 1*, differed significantly across stations. Specifically, the distribution of sniffing order for station A significantly deviated from a random distribution (χ^2=8.93; Monte Carlo p=0.029, *Figure 3*). Inspection of residuals indicated that this effect was primarily driven by an overrepresentation of first investigations at the ictal station (14 observed *vs* 7.5 expected; residual =+6.5), accompanied by underrepresentation in the second (4 *vs* 7.5; residual =–3.5) and third positions (4 *vs* 7.5; residual =–3.5), while

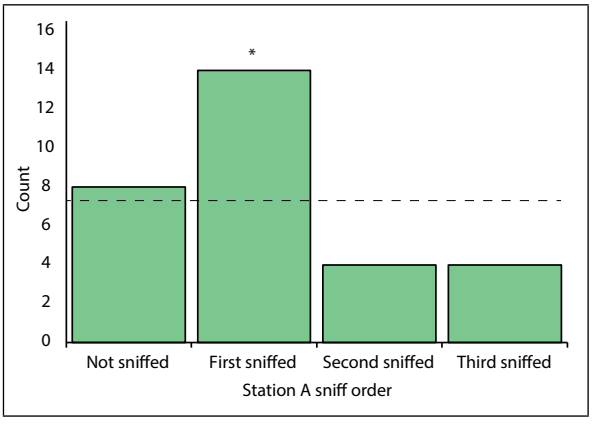


Figure 3
Distribution of sniffing order for station A. The dashed line represents the expected frequency under a uniform distribution (n=7.5 per category). *p≤0.05 based on adjusted standardized residuals.

non-investigation showed only a minimal deviation (8 *vs* 7.5; residual =+0.5). For station B, the distribution of sniffing order did not reach statistical significance (χ^2=7.07; Monte Carlo p=0.070). However, residual inspection suggested a tendency toward second-position investigations (12 *vs* 7.5; residual =+4.5). For station C, sniffing order was fully consistent with a random distribution (χ^2=0.13; Monte Carlo *p*=1.000), with small residuals across categories (range −0.5 to +0.5), indicating no positional bias. No significant differences were observed in the proportion of dogs that failed to investigate the three stations (Cochran's Q test: Q=0.12; Monte Carlo p=1.000). The number of dogs that did not investigate each station was comparable (ictal: 8/30; interictal: 7/30; empty: 8/30), indicating that the samples obtained during the ictal condition were not selectively avoided.

The chi-square goodness-of-fit test showed no significant deviation from a uniform distribution of presentation sequences (χ^2=2.40, p=0.791), thus excluding potential sequence biases. The likelihood of the ictal station being approached first was not influenced by its spatial position (left, center, or right). Specifically, no significant association was observed between the position of station A and first investigation (χ^2=1.69; Monte Carlo p=0.468).

In the total sample of dogs (n=30), no significant differences were observed for any other behavioral variables between the ictal and interictal or control conditions. Descriptive and inferential statistics for continuous variables (sniffing duration, sniffing frequency, and latency to first sniff) are reported in *Table 2* as medians and interquartile ranges (IQR; 25th-75th percentiles), with distributional properties further summarized by skewness and kurtosis. Sniffing duration and sniffing frequency were strongly and positively correlated within each odor condition (ictal A: Spearman's ρ=0.83, p=0.001; interictal B: ρ=0.81, p=0.001; control C: ρ=0.79, p=0.001), indicating a consistent association between sustained and repeated engagement across odor conditions. Latency to first sniff was positively skewed across all stations, particularly for the interictal (B) and control (C) stations, with higher skewness and kurtosis values indicating the presence of extreme latency values.

When latency to first sniff was examined dichotomized using the sample median (10.5 sec), the overall association between first choice and latency category did not reach statistical significance (Pearson's χ^2=4.35; Monte Carlo p=0.165). Descriptively, however, distinct exploratory profiles emerged across conditions *(Table 3)*.

Across the full sample (n=30), no behavioral signs of distress, fear, or anxiety were observed. Stations that were not investigated were simply ignored and were not associated with any observable signs of discomfort or withdrawal.

In the subsample of dogs that were presented with both the pot and the bowl at all three stations during the sniffing task (n=24), the PB variable revealed a non-random distribution of sniffing choices across container types *(Table S1 available online as Supplementary material)*. For both the ictal (A) and interictal (B) stations, the distribution of PB categories significantly deviated from a uniform distribution (A: p=0.001; B: p=0.010), indicating the adoption of a structured sniffing strategy within odor-containing stations.

Inspection of residuals indicated that, for station A, the deviation from uniformity was primarily driven by a marked overrepresentation of the BP category (12 observed *vs* 4.5 expected; residual =+7.5), accompanied by underrepresentation of the B category (1 *vs* 4.5; residual =−3.5) and the PB category (2 *vs* 4.5; residual = −2.5). A comparable pattern was observed for station B, where the BP category was again overrepresented (11 *vs* 4.8; residual =+6.3), while both the P (2 *vs* 4.8; residual =−2.8) and B (2 *vs* 4.8; residual =−2.8) categories were underrepresented. In contrast, no significant deviation from uniformity was observed for the control station (p=0.715). Residuals for station C remained small across categories (range −1.7 to +1.3), indicating the absence of a structured exploratory pattern, with the pot never being sniffed.

Table 2
Descriptive statistics of interest-related variables in all dogs (n=30)

Variable	Median	IQR (25-75)	Skewness	Kurtosis	P
Duration (sec)					Friedman test
Station A	2	0.0-2.0	0.633	−0.175	
Station B	2	0.5-3.0	0.239	−0.341	0.811
Station C	1	0.0-3.0	1.295	2.121	
Frequency (n)					Friedman test
Station A	1	0.0-1.0	-0.422	0.042	
Station B	1	1.0-1.0	-0.192	0.459	0.713
Station C	1	0.0-1.0	-0.298	−0.295	
Latency to first sniff (sec)					Kruskal-Wallis test
Station A	12	4.0-21.0	1.158	0.628	
Station B	5	3.0-10.0	2.892	8.516	0.16
Station C	37	8.5-59.0	1.161	1.300	

Station A: ictal; station B: interictal; station C: empty (control); IQR: interquartile range.

Table 3
Latency to first sniff relative to the sample median (10.5 sec)

Station	n	Short latency n (%)	Long latency n (%)	Latency pattern
A	14	8 (57.1)	6 (42.9)	Mixed (both rapid and delayed initiation)
B	9	7 (77.8)	2 (22.2)	Predominantly short (rapid initiation)
C	7	2 (28.6)	5 (71.4)	Predominantly long (delayed initiation)

Station A: ictal; station B: interictal; station C: empty (control); Total sample size (n=30).

Results for all other behavioral variables in this subsample of dogs (n=24) were consistent with those observed in the full sample (*see Supplementary materials available online for details on statistical analyses)*. Importantly, also in this subset, the distribution of presentation sequences did not significantly deviate from a uniform distribution (χ^2=1.00; Monte Carlo p=0.984), indicating the absence of systematic biases in the order of presentation of the odor stations under standardized testing conditions. The probability of the ictal station being sniffed first did not differ as a function of its spatial position (χ^2=0.59; Monte Carlo p=0.878). It is worth noting that, for the distribution of sniffing order, differences in statistical significance emerged for stations A and B, with station A significant in the full sample but not in the subsample (χ^2=5.67; Monte Carlo p=0.144), and the reverse pattern observed for station B (subsample: χ^2=8.33; Monte Carlo p=0.044). These differences likely reflect sampling-related variability associated with the reduced sample size, which can affect the stability of p-values in discrete data, without altering the underlying pattern of behavior, which remained consistent with that observed in the full sample. Indeed, inspection of residuals indicated a tendency for station A to be sniffed first more often than expected (11 *vs* 6; residual =+5.0). For station B, the sniffing-order distribution significantly deviated from chance, primarily driven by an overrepresentation of second-position investigations (10 *vs* 6; residual =+4.0) and an underrepresentation of third-position investigations (2 *vs* 6; residual =−4.0). For station C, similarly to the full sample, sniffing order was consistent with a random distribution (χ^2=1.33; Monte Carlo *p*=0.753), with residuals remaining small across categories (range −2.0 to +2.0), confirming the absence of a structured positional bias.

DISCUSSION

In the present study, we sought to determine whether untrained companion dogs, naïve to epileptic seizures, show spontaneous differential responses to odor samples collected during the ictal state compared with interictal samples and blank controls under controlled experimental conditions, within a socially neutral laboratory paradigm. The aim was to isolate the olfactory component from seizure-related learning, familiarity, and socially mediated reinforcement. In this initial experimental phase, all dogs were presented with odor samples originating from a single individual in order to provide a standardized stimulus across trials.

Across behavioral measures, odor associated with seizure samples selectively influenced initial orienting behavior. Dogs were significantly more likely to investigate the ictal station first, whereas no differences emerged in sniffing duration, sniffing frequency, non-investigation, or latency to first sniff. Thus, this odor did not increase sustained exploration, repeated investigation, or approach speed. Instead, it selectively biased the prioritization of the first behavioral choice during the free-exploration phase of the task. This profile is consistent with stimulus salience expressed at the level of early behavioral prioritization, rather than prolonged or repeated exploratory engagement, suggesting that dogs prioritize the ictal station without increasing exploratory processing relative to the other conditions. In other words, the ictal samples appear to influence initial station selection at the onset of exploration, without modulating sustained exploratory investment. Some explanations may account for the observed patterns, which likely reflect different facets of stimulus salience and may coexist. First, the observed effect may reflect perceptual salience. If odor cues present in samples collected during the ictal state contain distinctive VOCs, they may be rapidly detectable and sufficiently discriminable upon initial sampling. In this case, extended investigation would not be necessary for perceptual differentiation, and exploration would not increase beyond the initial approach. Under this account, prioritization reflects efficient sensory discrimination rather than enhanced motivational engagement. Second, the effect may reflect biological salience, whereby odor cues present in samples collected during the ictal state signal a physiologically altered state that is behaviorally relevant even in the absence of prior learning. Stations containing ictal samples may thus be preferentially selected because it conveys information about an atypical biological condition. Importantly, biological salience does not necessarily imply strong motivational valence, a distinction widely discussed in incentive-salience models of reward processing [33, 34]. Within this framework, a stimulus may act as a potent attentional attractor despite lacking, or even before acquiring, clear appetitive or aversive value. If differential appetitive or aversive value were driving behavior, one would expect corresponding modulation of sniffing duration, frequency, or repeated returns, which was not observed. Thus, the present data do not support motivational valence as the primary driver of the observed dynamics. It is worth noting that no behavioral indicators of distress, anxiety, or withdrawal were observed while dogs were exploring the stations or in their immediate proximity, suggesting that the stimuli, including odor cues present in the ictal samples were not aversive within the stan-

dardized experimental context. As described in the Materials and Methods section, in initial trials conducted prior to the introduction of the bowl, a small number of dogs remained at a distance from the apparatus and did not approach the stations. This pattern likely reflected limited spontaneous engagement with the setup rather than a specific response to the odor stimuli. The inclusion of the bowl functioned as an approach facilitator and resolved this issue.

The heterogeneity in latency among dogs that first approached the ictal station further refines the interpretation of biological salience. Although inferential analyses of latency did not reveal statistically significant differences, descriptive patterns showed that the ictal condition was selectively prioritized while being associated with both rapid and delayed initiation, whereas the interictal odor was approached rapidly but typically in second position, suggesting uniform detection without selective prioritization, and the control condition showed delayed initiation and random sampling order, consistent with reduced behavioral relevance. Such a configuration suggests that the stimulus present in ictal samples may be both perceptually distinctive and biologically relevant yet variably interpreted at the individual level. Its prioritization at the selection stage might be consistent with heightened perceptual discriminability and possible activation of novelty-driven orienting mechanisms. At the same time, the coexistence of rapid and delayed latencies indicates that the stimulus may be less predictable or less readily categorizable than baseline (interictal) human odor, thereby engaging differential appraisal processes across individuals.

Importantly, this variability did not translate into increased sniffing duration or frequency, arguing against strong motivational valence as the driver of the observed dynamics. Taken together, these findings suggest that odor cues present in samples collected during the ictal state operate primarily at the level of stimulus selection and early orienting, potentially driven by perceptual distinctiveness, reduced predictability, and biological rather than motivational relevance. The absence of differential motivational engagement further supports the interpretation that the observed response reflects a spontaneous, pre-associative detection mechanism rather than an experience-dependent, value-driven process. However, because the present study employed odor samples from a single donor, the observed orienting bias cannot be assumed to reflect a seizure-specific olfactory signature shared across individuals. An alternative explanation that cannot be excluded is that dogs responded to stimulus-specific olfactory features present in the samples obtained during the ictal condition from this individual.

Analysis of exploratory strategy within stations (Object variable) revealed structured sampling behavior for both ictal and interictal odors, but not for the blank control. In particular, in odor-containing stations, the distribution differed significantly from chance, with dogs predominantly exhibiting the BP pattern (both objects, bowl first) and showing fewer single-object inspections, especially bowl-only interactions. Because the pattern was comparable across ictal and interictal conditions, it is unlikely to reflect a seizure-specific effect. Instead, the presence of human odor per se seems to organize local investigation once a station has been selected. Because the bowl was identical across conditions, contained no food, and provided no differential reinforcement, its motivational value was constant and unrelated to seizure state or human trace. The concurrent marked underrepresentation of bowl-only (B) inspections suggests that dogs did not treat the bowl as a preferred object per se. Rather, the bowl likely functioned as an initial point of contact that facilitated engagement with the station, from which dogs subsequently proceeded to sample the pot. Thus, seizure-related salience appears to operate at the level of between-station prioritization, whereas within-station sampling structure reflects general exploratory dynamics, with dogs initially engaging the more familiar and positively associated entry point before proceeding to the pot. In contrast, the control station showed no structured distribution, and pot-only inspections were absent, indicating that in the absence of relevant odor the micro-sequence was not consistently expressed. In this respect, the inclusion of the bowl appears methodologically justified: it promoted approach to the apparatus without biasing odor discrimination, allowing between-station prioritization effects to emerge without increasing failure to approach or avoidance of the experimental setup. From a practical standpoint, these findings suggest that incorporating a neutral but positively valenced engagement cue may enhance participation in laboratory detection paradigms without confounding stimulus discrimination. This principle may be important for the design of standardized olfactory testing protocols and for early phases of training in applied detection contexts, where reliable approach to the odor source is required before shaping learned detection behaviors.

To our knowledge, this is the first study specifically designed to characterize the spontaneous olfactory strategy of untrained dogs responding to seizure-associated human odor under controlled, socially neutral conditions. Prior research with untrained dogs has largely identified socially directed behaviors, such as staring, proximity seeking, pawing, or nudging, as indicators of seizure-related responsiveness [26, 35-37]. Although informative, these responses occur within relational contexts and cannot isolate the underlying structure of olfactory exploration. By contrast, the present paradigm quantified multiple temporal dimensions of spontaneous odor investigation (initial orienting, latency, duration, frequency, and within-station sampling strategy) in the absence of dyadic cues, reinforcement histories, or social referencing, enabling early sensory-attentional processes to be examined independently of social interaction. Despite its strengths, this study has some limitations that should be acknowledged. Particularly, the study does not determine the chemical identity or specificity of the volatile compounds involved, nor does it address discrimination between epileptic and non-epileptic seizure events. Moreover, the use of samples from a single individual, while allowing strict control of inter-individual odor variability in this exploratory phase, limits the generalizability of the findings.

Future studies including multiple donors are required to determine whether the observed behavioral prioritization reflects seizure-related physiological cues that are shared across individuals or stimulus-specific odor features.

CONCLUSION

Odor cues present in samples collected during the ictal state may have selectively biased initial orienting in untrained, naïve dogs within a controlled, nonsocial paradigm, without increasing the duration or frequency of exploration. This dissociation between first choice and sustained investigation suggests that odor cues present in samples collected during the ictal state act primarily as early salience-driven signals rather than cues influencing ongoing exploratory processing, indicating intrinsic perceptual and/or biological salience sufficient to bias spontaneous selection in the absence of learning or reinforcement, without evidence of strong motivational valence. By isolating olfactory cues from social interaction and controlling for stable individual odor signatures, primary sensory processing was distinguished from socially mediated alerting. From a mechanistic perspective, the results are consistent with the possibility that ictal-related VOCs engage canine olfaction at an early sensory-attentional level. From a mechanistic perspective, the results are consistent with the possibility that VOCs present in samples collected during the ictal state may contribute to the observed behavioral prioritization by engaging canine olfaction at an early sensory-attentional level. Ongoing research within the same project is extending this approach to a multi-donor design to determine whether such effects truly reflect generalizable seizure-related olfactory cues.

Authors' contributions

FP: Writing – original draft, Writing – review & editing, Validation, Supervision, Methodology, Investigation, Formal analysis, Conceptualization. GC: Writing – review & editing, Methodology, Investigation, Conceptualization. MA: Writing – review & editing, Supervision, Methodology, Data curation, Conceptualization.

Acknowledgement

We wish to thank our colleague Patrizia Piotti for her initial contribution to the conceptual development of this study, along with Valeria Giulia Traversi, Daniele Griffini, and Giorgia Gobbi for their invaluable support with dog recruitment and logistical arrangements. We are also grateful to all the caregivers and dogs who participated in the study.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the Authors used ChatGPT-5.2 to improve the readability of the manuscript during its preparation. The Authors have reviewed and edited the content and take full responsibility for the final publication.

Conflict of interest statement

The Authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Received on 6 March 2026.
Accepted on 24 March 2026.

REFERENCES

1. Schmidt D, Schachter SC. Drug treatment of epilepsy in adults. BMJ. 2014;348:g254. doi: 10.1136/bmj.g254
2. Elger CE, Hoppe C. Diagnostic challenges in epilepsy: seizure under-reporting and seizure detection. Lancet Neurol. 2018;17(3):279-88. doi: 10.1016/S1474-4422(18)30038-3
3. Viana PF, Duun-Henriksen J, Glasstëter M, Dümpelmann M, Nurse ES, Martins IP, et al. 230 days of ultra long-term subcutaneous EEG: seizure cycle analysis and comparison to patient diary. Ann Clin Transl Neurol. 2021;8(1):288-93. doi: 10.1002/acn3.51261
4. Mielke H, Meissner S, Wagner K, Joos A, Schulze-Bonhage A. Which seizure elements do patients memorize? A comparison of history and seizure documentation. Epilepsia. 2020;61(7):1365-75. doi: 10.1111/epi.16550
5. Langston ME, Tatum WO. Focal seizures without awareness. Epilepsy Res. 2015;109:163-8. doi: 10.1016/j.eplepsyres.2014.10.018
6. Fattouch J, Di Bonaventura C, Lapenta L, Casciato S, Fanella M, Morano A, et al. Epilepsy, unawareness of seizures and driving license: The potential role of 24-hour ambulatory EEG in defining seizure freedom. Epilepsy Behav. 2012;25(1):32-5. doi: 10.1016/j.yebeh.2012.07.001
7. Inoue Y, Mihara T. Awareness and responsiveness during partial seizures. Epilepsia. 1998;39(S5):7-10. doi: 10.1111/j.1528-1157.1998.tb05142.x
8. Schulze-Bonhage A, Richardson MP, Brandt A, Zabler N, Dümpelmann M, San Antonio-Arce V. Cyclical underreporting of seizures in patient-based seizure documentation. Ann Clin Transl Neurol. 2023;10(10):1863-72. doi: 10.1002/acn3.51880
9. Elger CE, Mormann F. Seizure prediction and documentation–two important problems. Lancet Neurol. 2013;12(6):531-2. doi: 10.1016/S1474-4422(13)70092-9
10. Arthurs S, Zaveri HP, Frei MG, Osorio I. Patient and caregiver perspectives on seizure prediction. Epilepsy Behav. 2010;19(3):474-7. doi: 10.1016/j.yebeh.2010.08.010
11. Jory C, Shankar R, Coker D, McLean B, Hanna J, Newman C. Safe and sound? A systematic literature review of seizure detection methods for personal use. Seizure. 2016;36:4-15. doi: 10.1016/j.seizure.2016.01.013
12. Lit L, Crawford CA. Effects of training paradigms on search dog performance. Appl Anim Behav Sci. 2006;98(3-4):277-92. doi: 10.1016/j.applanim.2005.08.022
13. Hebard C. Use of search and rescue dogs. J Am Vet Med Assoc. 1993;203(7):999-1001.
14. Maejima M, Inoue-Murayama M, Tonosaki K, Matsuura N, Kato S, Saito Y, et al. Traits and genotypes may predict the successful training of drug detection dogs. Appl Anim Behav Sci. 2007;107(3-4):287-98. doi: 10.1016/j.applanim.2006.10.005

15. Gazit I, Terkel J. Domination of olfaction over vision in explosives detection by dogs. Appl Anim Behav Sci. 2003;82(1):65-73. doi: 10.1016/S0168-1591(03)00051-0
16. Yılmaz BN, Hayta Y, Küçükmutaf H, Süzer N, Seyidoğlu N, Aydin C. Developing an Effective Training Protocol for Biodetector Rats: A Preliminary Study. J Anatol Environ Anim Sci. 2025;10(3):230-6. doi: 10.35229/jaes.1639518
17. Beyene N, Sitotaw AL, Telila EG, Gebre HA, Alemu NB, Burny R, et al. The use of rats to detect drug-resistant TB. Public Health Action. 2023;13(1):1-3. doi: 10.5588/pha.22.0059
18. Pirrone F, Albertini M. Olfactory detection of cancer by trained sniffer dogs: A systematic review of the literature. J Vet Behav. 2017;19:105-17. doi: 10.1016/j.jveb.2017.03.004
19. Pirrone F, Piotti P, Galli M, Gasparri R, La Spina A, Spaggiari L, et al. Sniffer dogs performance is stable over time in detecting COVID-19 positive samples and agrees with the rapid antigen test in the field. Sci Rep. 2023;13(1):3679. doi: 10.1038/s41598-023-30897-1
20. Mazzola SM, Pirrone F, Sedda G, Gasparri R, Romano R, Spaggiari L, et al. Two-step investigation of lung cancer detection by sniffer dogs. J Breath Res. 2020;14(2):026011. doi: 10.1088/1752-7163/ab716e
21. Kirton A, Winter A, Wirrell E, Snead OC. Seizure response dogs: Evaluation of a formal training program. Epilepsy Behav. 2008;13(3):499-504. doi: 10.1016/j.yebeh.2008.05.011
22. Strong V, Brown S, Huyton M, Coyle H. Effect of trained Seizure Alert Dogs on frequency of tonic-clonic seizures. Seizure. 2002;11(6):402-5. doi: 10.1053/seiz.2001.0656
23. Flegg PJ. Seizure-alerting and -response behaviors in dogs living with epileptic children. Neurology. 2005;64(3):581. doi: 10.1212/WNL.64.3.581
24. Martos Martinez-Caja A, De Herdt V, Boon P, Brandl U, Cock H, Parra J, et al. Seizure-alerting behavior in dogs owned by people experiencing seizures. Epilepsy Behav. 2019;94:104-11. doi: 10.1016/j.yebeh.2019.02.001
25. Edney A. Dogs and human epilepsy. Vet Rec. 1993;132(14):337-8. doi: 10.1136/vr.132.14.337
26. Strong V, Brown SW. Should people with epilepsy have untrained dogs as pets? Seizure. 2000;9(6):427-30. doi: 10.1053/seiz.2000.0429
27. Maa E, Arnold J, Ninedorf K, Olsen H. Canine detection of volatile organic compounds unique to human epileptic seizure. Epilepsy Behav. 2021;115:107690. doi: 10.1016/j.yebeh.2020.107690
28. Catala A, Grandgeorge M, Schaff JL, Cousillas H, Hausberger M, Cattet J. Dogs demonstrate the existence of an epileptic seizure odour in humans. Sci Rep. 2019;9(1):4103. doi: 10.1038/s41598-019-40721-4
29. Powell NA, Ruffell A, Arnott G. The untrained response of pet dogs to human epileptic seizures. Animals. 2021;11(8):2267. doi: 10.3390/ani11082267
30. Lenochova P, Roberts SC, Havlicek J. Methods of Human body odor sampling: the effect of freezing. Chem Senses. 2008;34(2):127-38. doi: 10.1093/chemse/bjn067
31. Haidet KK, Tate J, Divirgilio-Thomas D, Kolanowski A, Happ MB. Methods to improve reliability of video-recorded behavioral data. Res Nurs Health. 2009;32(4):465-74. doi:10.1002/nur.20334
32. Schober P, Boer C, Schwarte LA. Correlation coefficients: appropriate use and interpretation. Anesth Analg. 2018;126(5):1763-8. doi: 10.1213/ANE.0000000000002864
33. Berridge KC, Robinson TE. What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? Brain Res Rev. 1998;28(3):309-69. doi: 10.1016/S0165-0173(98)00019-8
34. Robinson TE, Berridge KC. Incentive-sensitization and addiction. Addiction. 2001;96(1):103-14. doi: 10.1046/j.1360-0443.2001.9611038.x
35. Dalziel DJ, Uthman BM, Mcgorray SP, Reep RL. Seizure-alert dogs: a review and preliminary study. Seizure. 2003;12(2):115-20. doi: 10.1016/S105913110200225X
36. Kirton A, Wirrell E, Zhang J, Hamiwka L. Seizure-alerting and -response behaviors in dogs living with epileptic children. Neurology. 2004;62(12):2303-5. doi: 10.1212/WNL.62.12.2303
37. Strong V, Brown SW, Walker R. Seizure-alert dogs - fact or fiction? Seizure. 1999;8(1):62-5. doi: 10.1053/seiz.1998.0250

Ann Ist Super Sanità 2026 | Vol. 62, No. 2: 190-196
DOI: 10.4415/ANN_26_02_11

External quality assessment programs in cytogenomics in the Lombardy Region, Italy (2018-2024): performance and trends

Fabio Pasotti[1], Valentina Achille[2], Simona Da Molin[1], Giovanna Azzarà[1], Giuseppa Liga[1], Giuseppe Moriello[1], Barbara Zaccaria[1], Lorenza Romitti[2], Silvia Russo[3], Maria Iascone[4], Sabine Stioui[5] and Sabrina Buoro[1]

[1]*Centro Regionale di Coordinamento della Medicina di Laboratorio, Regione Lombardia, Milan, Italy*
[2]*Laboratorio di Genetica Medica, ASST Grande Ospedale Metropolitano Niguarda, Milan, Italy*
[3]*Laboratorio di Genetica, IRCCS Istituto Auxologico Italiano, Milan, Italy*
[4]*Laboratorio di Genetica, ASST Papa Giovanni XXIII, Bergamo, Italy*
[5]*Laboratorio di Genetica Medica, Centro Diagnostico Italiano SpA, Milan, Italy*

Abstract

Background. This study summarizes seven years (2018-2024) of external quality assessment (EQA) activities in cytogenomics conducted in the Lombardy Region, Italy, across four diagnostic sectors: chromosomal microarray (CGH/SNP-array), prenatal cytogenetics, postnatal cytogenetics, and oncohematology.

Methods. A descriptive analysis was performed of the regional EQA cycles, focusing on analytical concordance and on the type and evolution of non-conformities over time.

Results. Analytical concordance remained consistently high across all EQA cycles, with correct identification of the primary findings in CGH-array and constitutional cytogenetics. Early rounds were mainly characterized by incomplete reporting, International system for human cytogenomic nomenclature (ISCN) inaccuracies, limited interpretive content, and insufficient communication of methodological limitations, whereas more recent cycles showed progressive convergence toward national (SIGU) and international (ACMG/ClinGen, European) standards. In microarray (CGH/SNP-array) exercises, inter-laboratory variability shifted from basic interpretive and reporting deficiencies to more advanced issues of standardization in 2023-2024. Prenatal and postnatal cytogenetics showed progressive improvement in report structure, ISCN compliance, and methodological completeness. Oncohematology remained the most challenging area, with persistent variability in complex karyotype reconstruction despite improved metaphase adequacy and more complete ISCN reporting.

Conclusions. These findings support the role of regionally coordinated EQA schemes in promoting harmonization of cytogenomic practice, continuous quality improvement, and public health governance.

Key words

- cytogenomics
- external quality assessment
- cytogenetics
- chromosomal microarray
- oncohematology

INTRODUCTION

Cytogenomic diagnostics, including chromosomal microarray analysis, classical cytogenetics and cytogenetic investigation of hematologic malignancies, are essential components of constitutional and cancer genetics. Ensuring accuracy, reproducibility and harmonization across laboratories requires the implementation of external quality assessment (EQA) systems. International initiatives such as ACMG/ClinGen recommendations for constitutional copy number variation (CNV) interpretation [1], European guidelines for cytogenomic analysis [2], and long-standing UK NEQAS/GenQA and EMQN programs (see the GenQA/UK NEQAS and EMQN websites) have progressively shaped quality standards. In Italy, quality assurance in cytogenetics has a solid tradition. The pioneering national project on standardization and quality assurance led by the Istituto Superiore di Sanità laid the groundwork for coordinated external assessment in the early 2000s [3]. This effort subsequently evolved into the

Address for correspondence: Fabio Pasotti, Centro Regionale di Coordinamento della Medicina di Laboratorio, Regione Lombardia, Piazza dell'Ospedale Maggiore 3, 20162 Milan, Italy. E-mail: fabio.pasotti@ospedaleniguarda.it.

Italian EQA Programme, including the cytogenetics scheme, and documented substantial improvements in laboratory performance following the introduction of explicit poor-performance criteria [4]. These national findings align with international experience, including more recent EQA models for karyotype analysis such as that proposed by Weng *et al*. [5], and reinforce the role of EQA as a driver of laboratory harmonization. In this context, the Regional Coordination Center for Laboratory Medicine (CRCMedLab) established, in 2018, a structured EQA scheme dedicated to cytogenomic diagnostics, covering chromosomal microarray (CGH/SNP-array), prenatal cytogenetics, postnatal cytogenetics and oncohematology. The objective of this manuscript is to present a consolidated longitudinal analysis of seven years of activity (2018-2024), comparing early and recent performance and evaluating the evolution of laboratory compliance, analytical concordance for the primary findings and reporting quality in relation to national and international EQA schemes.

MATERIALS AND METHODS

Diagnostic areas and EQA design

The cytogenomics EQA program of the Lombardy Region covered four diagnostic areas: prenatal cytogenetics, postnatal cytogenetics, hemato-oncological cytogenetics (oncohematology), and CGH/SNP-array analysis. Between 2018 and 2024, the program was implemented continuously and included both prospective and retrospective exercises across the four diagnostic sectors. The number of participating laboratories by year and diagnostic area is summarized in *Table 1*. Across the study period, the mean number of participating laboratories was 16 for CGH/SNP-array, 21 for postnatal cytogenetics, 17 for prenatal cytogenetics, and 19 for oncohematology. The overall distribution of EQA cycles is reported in *Table 2*. Two main types of exercises were organized:

- prospective assessments: DNA samples were distributed to participating laboratories, which performed routine analyses according to their standard protocols and submitted complete reports;
- retrospective assessments: laboratories were asked to send a report of pathological cases previously diagnosed in their routine activity during the preceding semester, using anonymized clinical and laboratory data.

In the prospective approach, all laboratories received the same control material and were requested to perform array-based analyses exactly as in routine practice. In the retrospective approach, laboratories re-submitted reports and interpretations for real cases they had previously managed, allowing evaluation of reporting quality, adherence to guidelines and interpretive consistency. The cytogenomics EQA scheme was coordinated by CRCMedLab and involved all diagnostic laboratories in the Lombardy Region performing cytogenetic and cytogenomic analyses.

Data submission and evaluation

For both the prospective and retrospective approaches, laboratories submitted results through the CRCMedLab website (www.qualitalaboratorilombardia.it). For each exercise, the following elements were evaluated, where applicable:

- analytical performance (detection of the main chromosomal abnormality or CNV);
- karyotype reconstruction;
- ISCN nomenclature accuracy and completeness;
- diagnostic interpretation and genotype-phenotype correlation;
- structure and completeness of the written report, including clerical accuracy and methodological limitations.

After the first three experimental years of the regional EQA program, a structured, penalty-based scoring

Table 1
Number of participating laboratories by year and diagnostic area in the Lombardy (Italy), cytogenomics EQA program (2018-2024)

Diagnostic Area	2018	2019	2020	2021	2022	2023	2024
CGH/SNP-array	19	-	-	18	15	17	16
Postnatal cytogenetics	-	25	22	-	22	19	19
Prenatal cytogenetics	-	17	-	16	16	19	19
Hemato-oncological cytogenetics	-	-	20	20	21	17	15

EQA: external quality assessment; CGH/SNP-array: chromosomal microarray.

Table 2
Cytogenomics EQA cycles conducted in Lombardy, Italy (2018-2024)

Diagnostic area	2018	2019	2020	2021	2022	2023	2024	Total number of exercises
CGH/SNP-array	X(p)			X(p)	X(r)	X(p)	X(p)	5
Postnatal cytogenetics		X(r)	X(r)		X(r)	X(r)	X(r)	5
Prenatal cytogenetics		X(r)		X(r)	X(r)	X(r)	X(r)	5
Hemato-oncological cytogenetics			X(r)	X(r)	X(r)	X(r)	X(r)	5
Total	1	2	2	3	4	4	4	20

EQA: external quality assessment; p: prospective; r: retrospective.

system with a maximum score of 30 was introduced for exercises requiring formal report-based evaluation. Three domains were assessed, each with a maximum score of 10 points: analysis (genotyping/nomenclature), interpretation, and report structure/content. Scoring was penalty-based: each domain was initially assigned a score of 10, from which predefined penalties were subtracted according to the evaluation criteria. Severe errors were classified as unsatisfactory performance, whereas predefined minor and major deficiencies led to specific score deductions. The full scoring framework is summarized in *Table 3*.

Data analysis was descriptive in nature and aimed at summarizing laboratory performance across EQA cycles. Error frequencies, proportions, and score distributions were compared over time to identify performance trends across diagnostic sectors and across early versus recent exercises. No formal inferential statistical testing was performed, as the primary aim of the EQA program was quality monitoring and harmonization rather than hypothesis testing. Evaluation criteria were aligned with SIGU recommendations for cytogenetic and cytogenomic diagnosis (available from the SIGU website), ISCN nomenclature, and international recommendations

Table 3
Penalty-based scoring system used for formal report evaluation in the Lombardy (Italy) cytogenomics EQA program. Each category was assigned a maximum score of 10 points. Scoring was penalty-based, with predefined deductions applied according to the evaluation criteria. Severe errors were classified as unsatisfactory performance

Category	Evaluation criterion	Applicable scheme(s)	Penalty/ outcome
Analysis (genotyping/ nomenclature)	Severe error: expected result incorrectly identified, including missed or incorrect identification of the abnormality	All schemes	Unsatisfactory performance
	ISCN formula: minor error (e.g., spacing, incorrect use of symbols)	All schemes	-0.5
	ISCN formula: incomplete	All schemes	-0.5
	ISCN formula: incorrect (misleading with respect to the analytical result, causing interpretive problems)	All schemes	-2
	ISCN formula: absent	All schemes	-2
	Explanation of the ISCN formula understandable to non-experts: incomplete (e.g., missing description of genotypic sex, observed abnormality, type of imbalance/rearrangement, chromosomes or chromosome arms involved, or missing indication of normal/abnormal karyotype)	All schemes	-0.5
	Explanation of the ISCN formula understandable to non-experts: incorrect or absent	All schemes	-1
	Karyotype reconstruction: incorrect in one of the evaluated karyotypes	Prenatal, postnatal, oncohematology	-0.5
	Karyotype reconstruction: incorrect in more than one of the evaluated karyotypes	Prenatal, postnatal, oncohematology	-1
	Number of metaphases analyzed: inadequate but reported in the report	Prenatal, postnatal, oncohematology	-0.5
	Number of metaphases analyzed: inadequate and not reported in the report	Prenatal, postnatal, oncohematology	-1
	Banding resolution: inadequate but reported in the report	Prenatal, postnatal, oncohematology	-0.5
	Banding resolution: inadequate and not reported in the report	Prenatal, postnatal, oncohematology	-1
	Same item previously penalized in earlier EQA exercises	All schemes	-5
Interpretation	Severe error: interpretation incorrect (e.g., misleading interpretation) or absent	All schemes	Unsatisfactory performance
	Missing clinical indication/indication not reported	All schemes	-1
	Incorrect indication of reproductive risk for the patient and/or the couple	All schemes	-1
	Failure to recommend extension of testing to family members, where appropriate	All schemes	-1
	Suggestion to perform inappropriate genetic analyses	All schemes	-2
	Incomplete: lack of correlation with the diagnostic question	All schemes	-1
	Incomplete: lack of prognostic risk information (when applicable) or lack of recommendation for multidisciplinary consultation/oncohematology consultation	Oncohematology	-1
	Incomplete: lack of recommendation for further diagnostic work-up/no reference to additional ongoing investigations	Oncohematology	-1

Continues

Table 3
Continued

Category	Evaluation criterion	Applicable scheme(s)	Penalty/ outcome
	Incomplete: lack of indication of recurrent abnormality or lack of recommendation for multidisciplinary consultation/oncohematology consultation	Oncohematology	-1
	Failure to comply with applicable recommendations/guidelines	All schemes	-1
	Same item previously penalized in earlier EQA exercises	All schemes	-5
Report structure and content	Language/terminology inadequate and potentially misleading	All schemes	-0.25
	Confusing report template or lack of clear structure	All schemes	-0.25
	Missing date of sample collection	All schemes	-0.25
	Missing date of sample receipt	All schemes	-0.25
	Missing report date	All schemes	-0.25
	Missing identification of the requesting physician or institution	All schemes	-0.25
	Incomplete patient identification (missing name, surname, or date of birth, as provided by the referring center/accompanying form in prospective exercises)	All schemes	-0.25
	Incorrect patient identification (incorrect name, surname, or date of birth, as provided by the referring center/accompanying form in prospective exercises)	All schemes	-1
	Missing indication of sex (as provided by the referring center/accompanying form in prospective exercises)	All schemes	-0.5
	Missing sample identification code	All schemes	-0.25
	Missing specimen/material examined	All schemes	-0.25
	Missing identification of the analyst and/or signature of the laboratory director or delegated staff	All schemes	-0.25
	Missing page numbering (e.g., 1 of 1)	All schemes	-0.25
	Missing indication for testing/diagnostic suspicion/clinical information	All schemes	-1.5
	Turnaround time not respected	Prenatal, postnatal, oncohematology	-1
	Absence of method description	All schemes	-0.25
	Missing information on test limitations	All schemes	-0.25
	Information on test technique insufficient or incorrect (e.g., sequenced region and/or mutation investigated and/or kit used)	All schemes	-0.5
	Analytical sensitivity and specificity absent or insufficient	All schemes	-0.5
	Same item previously penalized in earlier EQA exercises	All schemes	-5

EQA: external quality assessment; ISCN: International system for human cytogenomic nomenclature.

for CNV interpretation [1]. Final reports of each EQA exercise were made available on the CRCMedLab website (www.qualitalaboratorilombardia.it) within twenty working days after the program deadline.

RESULTS

CGH/SNP-array analysis

As shown in *Table 1*, participation in the CGH/SNP-array scheme ranged from 15 to 19 laboratories across active cycles. One case was distributed in each CGH/SNP-array EQA cycle, for a total of five cases across the study period. All distributed cases involved clinically relevant CNVs; no borderline CNVs were included. Across the five CGH/SNP-array EQA cycles, analytical concordance remained consistently high, with correct identification of the primary pathogenic copy-number variant in all exercises. However, the earliest array-based rounds showed substantial heterogeneity in interpretive and reporting quality. In 2018, although no laboratory failed to identify the clinically relevant abnormality, interpretation was fully correct in only 2/19 laboratories (10.5%), whereas 14/19 (73.7%) were judged correct with observations and 3/19 (15.8%) were classified as incorrect. Report content showed observations in all submissions (19/19, 100%), and omission of methodological limitations was also recorded in all reports. The most frequent deficiencies concerned omission of key analytical limitations, incomplete syndrome/OMIM reporting, and insufficient integration between genomic findings and clinical interpretation. By 2023, the profile had become more structured, with 17 laboratories evaluated and a mean score of 27.632/30. At this stage, evaluator comments were focused less on basic interpretive omissions and more on standardization issues, including use of the hg38 genome build, explicit indication of OMIM references, parental testing recommendations in terminal deletions, and clearer definition of CNV classification

criteria and methodological limitations. A further improvement was observed in 2024, when 16 laboratories were evaluated with a mean score of 28.945/30. In this round, residual comments were mainly limited to refinement of reporting practice, such as more explicit citation of interpretive guidelines, clearer indication of the number of genes involved in large CNVs, and improved consolidation of methodological characteristics and analytical limitations. Overall, these findings indicate that inter-laboratory variability progressively shifted from substantial interpretive and reporting deficiencies toward more advanced issues of report harmonization and standardization.

Hemato-oncological diagnostics (oncohematology)

As shown in *Table 1*, participation in the oncohematology scheme ranged from 15 to 21 laboratories across active cycles. Oncohematology showed the highest variability among the diagnostic sectors. The earliest evaluations identified recurring issues including inadequate metaphase numbers, incomplete description of clonal evolution, and errors in karyotype reconstruction. Quantitative comparison between 2020 and 2023 is reported in *Table 4*. Karyotype reconstruction errors increased from 2/20 (10%) in 2020 to 6/17 (35%) in 2023. Over the same period, inadequate metaphase number decreased from 6/20 (30%) to 2/17 (11.8%). ISCN syntax errors remained substantial, accounting for 7/20 cases (35%) in 2020 and 6/17 (35.3%) in 2023, whereas missing ISCN reports decreased from 2/20 (10%) to 0/17 (0%). Overall, these findings indicate improved adequacy of metaphase analysis and more complete ISCN reporting, together with persistent variability in the handling of complex karyotypes.

Table 4
Comparison of oncohematology EQA performance (2020 *vs* 2023)

Parameter	2020	2023
Participating laboratories	20	17
Karyotype reconstruction errors	2/20 (10%)	6/17 (35%)
Inadequate metaphase number	6/20 (30%)	2/17 (11.8%)
ISCN syntax errors	7/20 (35%)	6/17 (35.3%)
Missing ISCN reports	2/20 (10%)	0/17 (0%)
Mean total score	n/a	25.42/30

EQA: external quality assessment; ISCN: International system for human cytogenomic nomenclature; n/a: not applicable.

Table 5
Comparison of prenatal EQA performance (2019 *vs* 2024)

Parameter	2019	2024
Participating laboratories	17	19
Correct overall evaluation	16/17 (94%)	19/19 (100%)
Incorrect ISCN	1/17 (6%)	0/19 (0%)
Mean total score	n/a	29.61/30
Completeness of key elements	Variable	Near-complete

EQA: external quality assessment; ISCN: International system for human cytogenomic nomenclature; n/a: not applicable.

Prenatal diagnostics

As shown in *Table 1*, participation in the prenatal diagnostics scheme ranged from 16 to 19 laboratories across active cycles. The first prenatal EQA exercise, conducted in 2019, showed a generally good level of analytical performance, with 94% of centers providing a correct overall evaluation. However, a detailed review of the submitted reports highlighted several recurrent deficiencies, particularly in sections describing the analytical process. Missing or inconsistently reported elements included the culture method employed, the number of metaphases analyzed, the recommendation for genetic counseling and the unexpanded acronyms. These gaps reflected the heterogeneity of reporting practices before the introduction of updated national guidelines. A marked improvement emerged in the 2024 cycle, which included 19 participating laboratories. In this round, all laboratories achieved a correct analytical result (100%), and the mean total score reached 29.61/30, indicating a high level of adherence to expected standards. Reporting was generally complete and aligned with the updated SIGU recommendations, with almost all laboratories providing thorough methodological descriptions, complete ISCN notation and clearly structured reports. This evolution is summarized in *Table 5*, which illustrates the progression from heterogeneous and partially incomplete reporting in 2019 to a more standardized and comprehensive approach in 2024.

Postnatal diagnostics

As shown in *Table 1*, participation in the postnatal diagnostics scheme ranged from 19 to 25 laboratories across active cycles. Postnatal cytogenetics EQA exercises conducted between 2019 and 2024 demonstrated consistently high analytical concordance, with all participating laboratories correctly identifying the main chromosomal abnormality in each evaluation. However, qualitative review of submitted reports revealed recurrent deficiencies, particularly in the earlier cycles (2019-2021). The most frequent issues concerned administrative inaccuracies, unexpanded acronyms, heterogeneous methodological descriptions, and outdated ISCN terminology. In some reports, the postnatal origin of the analyzed karyotype was not explicitly stated, potentially generating ambiguity in clinical interpretation.

Because postnatal EQA assessments were primarily based on qualitative review of full written reports, numerical frequencies for each specific reporting deficiency were not available for all cycles. Nevertheless, assessor reports consistently documented progressive improvement over time. In the most recent cycles (2023-2024), report structure became more standardized, documentation of analytical methods improved, and interpretive comments were more complete and clinically relevant.

DISCUSSION

The Lombardy Regional EQA program demonstrates consistently strong analytical performance across all cytogenomic fields, a trend that mirrors both Italian national experiences and international comparison initiatives. In CGH/SNP-array analysis, analytical concor-

dance remained uniformly high throughout all cycles, consistent with findings from major international EQA schemes such as EMQN and GenQA (see the corresponding program websites) as well as Australasian microarray programs [6], where technical sensitivity is excellent while interpretive variability is the predominant issue.

The CGH/SNP-array scheme showed a clear temporal shift in the nature of non-conformities. In the earliest round, variability was driven mainly by incomplete interpretive content and omission of methodological limitations, despite correct identification of the primary genomic imbalance. By contrast, the later rounds showed a more mature reporting profile, in which the main residual issues concerned harmonization of terminology, use of updated genome builds, explicit reference to interpretive guidelines, and more structured integration of genomic and clinical information. In this context, the 2024 round can be regarded as the most standardized among the evaluated array cycles, not because a formal statistical ranking was applied across all historical rounds, but because evaluator comments were largely restricted to refinement-level issues and the mean score was higher than in 2023. The improvements observed in Lombardy – particularly in ISCN accuracy, structured reporting, and adherence to ACMG/ClinGen and European cytogenomic guidelines [1, 2], as well as updated SIGU recommendations (available from the SIGU website) – reflect broader trends toward harmonization in cytogenomic practice.

The Italian experience provides an important historical framework for interpreting these results. Early national quality-assurance initiatives, such as the Italian Project on Standardization and Quality Assurance led by the Istituto Superiore di Sanità [3], demonstrated the impact of structured evaluation in reducing inter-laboratory variability. Subsequent national EQA activities, including the Italian External Quality Assessment scheme in classical cytogenetics and the National Cytogenetics EQA Program (2013-2016), confirmed that explicit performance criteria and standardized reporting frameworks were effective in improving banding documentation, metaphase selection and ISCN accuracy [4, 7]. The Lombardy dataset reproduces these observations locally: analytical concordance remains high in all sectors, whereas the most frequent non-conformities involve ISCN nomenclature, incomplete documentation of methodological limitations and variability in interpretive content. The regional scheme also adds value by systematically evaluating CGH/SNP-array performance – an aspect only partially addressed in national cytogenetics programs.

In prenatal and postnatal cytogenetics, the progressive standardization observed in Lombardy paralleled both Italian and broader European experience. Early cycles were characterized by variability in administrative completeness, ISCN formatting, and documentation of analytical methods, whereas later evaluations, particularly after publication of the updated SIGU recommendations in 2023, showed clearer report structure, improved methodological documentation, and more consistent interpretive comments. These findings support the view that sustained EQA participation contributes not only to maintenance of analytical concordance, but also to harmonization of report quality and adherence to evolving professional standards. These trends mirror the European shift toward harmonized reporting advocated by recent cytogenomic guidelines [2] and updated SIGU recommendations (available from the SIGU website).

Oncohematology remained the most challenging diagnostic field. The increase in karyotype reconstruction errors observed in the most recent retrospective evaluation cycles should be interpreted cautiously and may reflect multiple contributing factors, including the complexity of cases derived from routine diagnostic activity and differences in laboratory workflows. At the same time, the reduction in inadequate metaphase number and the disappearance of missing ISCN reports indicate improved technical adequacy and more complete reporting. Overall, these findings suggest that the main residual source of variability lies in the interpretation and formal description of complex clonal architectures. This finding should be interpreted cautiously studies focusing on cytogenetic analysis of hematologic malignancies have shown that even experienced laboratories may struggle with accurate reconstruction and ISCN description of complex or chimeric karyotypes [5, 8, 9]. This finding should be interpreted cautiously and may reflect multiple contributing factors, including the complexity of cases derived from routine diagnostic activity. At the same time, the progressive improvement in ISCN completeness and the reduction in inadequate metaphase counts suggest improved technical adequacy despite persistent interpretive challenges.

Taken together, the Lombardy EQA data indicate consistently high analytical concordance across diagnostic sectors, accompanied by recurrent but progressively reduced variability in interpretation and reporting. This distribution of strengths and weaknesses mirrors national and international EQA experiences in cytogenetics and molecular genetics [1-9] and reinforces the principle that EQA programs are most effective when assessing the entire diagnostic process, not only the technical laboratory component but also reporting structure, clinical interpretation and communication of analytical limitations. Several strategic priorities emerge for future development of the regional program:

- further harmonization of structured reports, including mandatory sections and consistent documentation of key analytical elements;
- ongoing ISCN training, with emphasis on complex oncologic karyotypes and array-derived notations;
- progressive integration of NGS-based methods applied to cytogenomic analysis into the EQA portfolio, reflecting current shifts in diagnostic workflows;
- strengthened collaboration with national and international EQA providers, ensuring dynamic alignment with evolving best-practice standards and improved cross-laboratory comparison.

Within this broader context, the Lombardy cytogenomics EQA program represents a mature and integrated regional implementation of principles developed over two decades of Italian and European efforts in cy-

togenetics quality assurance. Its longitudinal structure, comprehensive inclusion of cytogenomic technologies and strong linkage to regional governance represent important strengths supporting ongoing improvement and harmonization across laboratories.

CONCLUSIONS

The results of the Lombardy cytogenomics EQA program confirm the effectiveness of a regionally coordinated initiative in strengthening the quality and reliability of cytogenomic diagnostics. Between 2018 and 2024, laboratories consistently achieved high analytical concordance across all sectors, accompanied by progressive improvements in reporting completeness, ISCN compliance and adherence to national and international guidelines.

The program proved particularly valuable in identifying persistent critical areas, notably the interpretation and reporting of complex oncologic karyotypes and the standardized communication of methodological limitations. These findings support the implementation of targeted training initiatives and continued harmonization of reporting practices.

Future integration of emerging technologies, including NGS-based cytogenomic approaches, into the EQA framework will be essential to reflect evolving diagnostic workflows. Overall, this experience underscores the value of sustained EQA activities as a public health tool for promoting continuous quality improvement, harmonized laboratory practices and alignment with evolving best practices in cytogenomics.

Authors' contributions

FP: conceived and coordinated the study, supervised data interpretation and drafted the manuscript; GL, GM, BZ, SB: contributed to supervision and manuscript revision; GA, VA, SDM, SR, MI, SS: contributed to data extraction, evaluation and manuscript review. All Authors approved the final manuscript.

Conflict of interest statement

The Authors declare no conflicts of interest.

Use of artificial intelligence

Artificial intelligence tools (ChatGPT, OpenAI) were used exclusively for language editing and stylistic refinement. Scientific content, data analysis and interpretations were entirely performed, reviewed and validated by the Authors.

Received on 20 January 2026.
Accepted on 26 March 2026.

REFERENCES

1. Riggs ER, Andersen EF, Cherry AM, et al. Technical standards for the interpretation and reporting of constitutional copy-number variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics (ACMG) and the clinical genome resource (ClinGen). Genet Med. 2020;22(2):245-57. doi: 10.1038/s41436-019-0686-8
2. Silva M, de Leeuw N, Mann K, et al. European guidelines for constitutional cytogenomic analysis. Eur J Hum Genet. 2019;27(1):1-16. doi: 10.1038/s41431-018-0244-x
3. Taruscio D, Falbo V, Floridia G, et al. Quality assessment in cytogenetic and molecular genetic testing: the experience of the Italian Project on Standardisation and Quality Assurance. Clin Chem Lab Med. 2004;42(8):915-21. doi: 10.1515/CCLM.2004.148
4. de Stefano MC, Floridia G, Censi F, et al. The Italian National External Quality Assessment Program in Cytogenetics: 4 years of activity (2013-2016) following the introduction of poor performance criteria. Ann Ist Super Sanità. 2018;54(2):109-16. doi: 10.4415/ANN_18_02_06
5. Weng BH, Shen XY, Xu YL, et al. A novel external quality assessment of chromosomal karyotype analysis based on chimera image assembly. Ann Clin Lab Sci. 2020;50(5):674-80.
6. Wright DC, Adayapalam N, Bain N, et al. Chromosome microarray proficiency testing and analysis of quality metric data trends through an external quality assessment program for Australasian laboratories. Pathology. 2016;48(6):586-96. doi: 10.1016/j.pathol.2016.05.009
7. Floridia G, Falbo V, Censi F, et al. The Italian external quality assessment scheme in classical cytogenetics: four years of activity. Community Genet. 2008;11(5):295-303. doi: 10.1159/000121401
8. Rack KA, van den Berg E, Haferlach C, et al. European recommendations and quality assurance for cytogenomic analysis of haematological neoplasms. Leukemia. 2019;33(8):1851-67. doi: 10.1038/s41375-019-0378-z
9. Hastings RJ, Cavani S, Dagna Bricarelli F, et al. Cytogenetic guidelines and quality assurance: a common European framework for quality assessment for constitutional and acquired cytogenetic investigations. Eur J Hum Genet. 2007;15(5):525-7. doi: 10.1038/sj.ejhg.5201809

Ann Ist Super Sanità 2026 | Vol. 62, No. 2: 197-202
DOI: 10.4415/ANN_26_02_12

Book Reviews, Notes and Comments

Edited by
Federica Napolitani Cheyne

MERAVIGLIE DI UN MARE FERITO
Viaggio di un ecologo intorno al Mediterraneo
Giuseppe Notarbartolo di Sciara
Milano: Enrico Damiani Editore; 2025
416 p.
ISBN 9791254560716
29,90 €

[Wonders of a wounded sea: An ecologist's journey around the Mediterranean]

Giuseppe Notarbartolo di Sciara is a marine ecologist, biologist, and conservationist who has long been able to skillfully convey through these pages – starting from his childhood memories – all his love and, above all, his insatiable curiosity for the sea. In a "One Health" vision, his passionate writing may serve as a punctual avenue of convergence between classical biomedicine and biological oceanography

Born in Venice, a splendid city surrounded and permeated by water (but also, as the writer points out, already at that time poor in marine animal biodiversity compared to today), the author, still a child, begins discovering a magical world during a tedious Sunday. Lying on his stomach, idly resting on a *mascareta* (a typical Venetian boat), he observes the life unfolding in the crystal-clear water.

The "micro-theater" that appears before his eyes – animated by slow hermit crabs, colorful sea anemones, graceful brittle stars (ophiuroids), and small shore crabs – fascinates him so deeply that this universe becomes the solid and lifelong passion of his existence. It is a striking example of how an engaging childhood passion can transform into the enduring and mature professionalism of a marine biologist.

This volume is therefore capable of powerfully igniting the reader's curiosity toward the blue labyrinths of an incredibly fascinating planet – one that has always inspired poets and philosophers – rich in creatures with bizarre shapes, microscopic organisms or gigantic beings, algae and plants that are colorful and multifaceted. At the same time, it provides greater awareness of the serious risks shared by these forms of life, many of which are in grave danger of extinction or severe decline. An ecological disruption that may involve the health not only of our aquatic ecosystems, but impact the one of human population.

In the mid-1980s, after nearly a decade spent in the United States working closely with the oceanographer Walter Munk, his mentor, and after earning a PhD in Marine Biology from the University of California, Notarbartolo returned to Italy determined to raise awareness among a population still largely inattentive to the conservation of ecosystems. From this desire emerged, in the early years of the new millennium, the idea of undertaking a (imaginary) journey – actually the sum of many journeys he had chosen – of 4,600 miles to recount the wonders of the Mediterranean and to instill a greater ecological awareness in human beings and of their fundamental role in protecting the environment. The "fantastical" voyage aboard the boat *Pantoporia* is also an inner journey in which the author experiences a deep fusion with the aquatic environment. It represents an inner growth and a strengthening of his determination to fight for the good of a nature that is perceived as external but is in reality part of ourselves.

Reading the book allows us to discover the strengths and vulnerabilities of the Mediterranean, the importance of winds and ocean currents, and their current fragile balance due to global warming and the looming danger caused by the socio-economic interests of the human species.

A first step could be for each of us to understand the damage humanity has caused to the aquatic environment and the creatures inhabiting it. For this reason, the book is recommended both to the vast audience of nature lovers and environmentalists and, above all, to the many professionals in the biomedical field who are sensitive to the rapidly emerging "One Health" issues among the potential readers.

The main message coming from the text is in fact a warning about the reckless and catastrophic actions of human activities which, over the centuries, have caused the impoverishment and destruction of many areas of our beloved sea. *Homo sapiens* is compared to "a nine-headed Hydra unable to avoid polluting the very cradle of the civilization it has created, and which in its destructive impulse will end up wiping itself out along with everything else".

Within the pages of this volume – sometimes playful but deeply scientific, given the author's credentials (formerly President of ICRAM, the Central Institute for Applied Scientific and Technological Research on the Sea, later incorporated into ISPRA, the Italian Institute for Environmental Protection and Research) – one can read stories of encounters with a varied fauna. Among them are the swift pods of orcas that amaze with their Machiavellian and highly effective strategy for hunting tuna – the same "mischievous" ones that often amuse themselves by striking the hulls of ships they encounter, sometimes causing significant damage without ever causing sinkings.

There is also the sensational encounter with Risso's dolphins, large delphinids with round heads that produce vocalizations "which sometimes resemble irreverent raspberries," while performing fascinating undulating movements of their tails emerging from the water. The author recounts with enthusiasm the captivating choreographic movements of mobula rays (described as "miniature mantas"), the close encounter with a pilot whale that surfaces with its "imposing big head" and, for a suspended instant, meets the gaze of *Homo sapiens*, filled with wonder before it. His universe is profoundly human in the sense of a scholar immersing himself in an ecosystem and intuits and characterizes its inner functioning.

Above all, the volume allows us to understand the long and often troubled path that led to the creation of the first officially protected marine area on Earth – a celebrated, well-known, and paradigmatic case of Italian scientific excellence that became an operational reality and a reference point for the international community of environmental, evolutionary, and biomedical scholars.

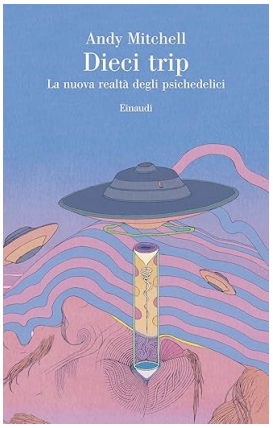
This internationally significant achievement is only the first major step, or rather "the starting point", toward the creation of a healthier world, a reality full of awareness thanks to the work of women and men of good will. This is precisely one of the goals of the so-called Third Mission that universities and research institutions pursue in order to promote scientific literacy among ordinary citizens.

The successes achieved by scientific groups such as the one described here are encouraging, but we also know that we must never lower our guard against alarming problems such as pollution, overfishing, illegal fishing, and navigation activities that disregard environmental damage. These "Horsemen of a Marine Apocalypse" will always represent risk factors for the Earth's ecosystem, yet they will increasingly be countered by the determination and perseverance of those who understand that we are all part of the same planet, without separation.

A change of approach is absolutely necessary, even if it will take time to be fully conveyed to the general public. According to the author, a new awareness of respect for living beings may already be seen in the next generations. This will heal the distance between humanity and nature, transforming the dangerous idea of wanting to dominate something that, if destroyed, could cause great catastrophes even for us, *sapiens* yet "reckless" beings.

"Who, if not us, can defeat the monster, if the monster is ourselves?".

Stella Falsini and Enrico Alleva
Istituto Superiore di Sanità, Rome, Italy
stella.falsini@iss.it
enrico.alleva@iss.it



DIECI TRIP
La nuova realtà degli psichedelici
Andy Mitchell
Translation by Chiara Veltri
Torino: Einaudi; 2025
370 p.
26,00 €
ISBN 9788806267360

[Ten trips: a psychedelic adventure]

Ten trips by Andy Mitchell is situated within a unique historical moment for psychedelics, arguably the only one truly comparable to the 1960s: the so-called psychedelic renaissance. Unlike that earlier period, however, this new resurgence does not emerge from countercultural circles, but from universities, hospitals, biotech start-ups, and healthcare institutions. It marks a renewed centrality in public discourse, achieved through scientific legitimation, clinical practice, and the language of evidence; indeed, this transformation constitutes the book's central focus. Mitchell, a British clinical neuropsychologist, draws on ten experiences with different substances in markedly diverse settings not as a purely narrative device, but as a means of examining the internal tensions of the present historical moment. *Ten Trips* is neither an uncritical celebration of psychedelics nor a straightforward experiential memoir; rather, it offers a thoughtful and at times uneasy reflection on what may be at risk of being lost as these substances regain social and clinical acceptability.

The book stages a fracture that has become increasingly visible today. On the one hand are the traditionalists, often more idealistic in orientation, for whom the psychedelic experience is inseparable from ritual and symbolic context, including the forest setting, the presence of a shaman, and a dimension that might be described as spiritual or initiatory. On the other hand are the pragmatists, or realists, who regard the "trip" as a side effect, if not an outright obstacle: something to be reduced, controlled, or even eliminated in order to render psychedelics compatible with clinical protocols and with integration into public healthcare systems. Within this framework, therapeutic value would not lie in the subjective experience itself, but rather in the underlying neurobiological mechanisms, which could in principle be isolated without necessarily involving visions, ego dissolution, or intense altered states of consciousness.

One of the principal merits of *Ten Trips* is that Mitchell refuses to resolve this tension in ideological terms. He offers neither a reconciled synthesis nor a definitive answer; indeed, he insists that, at present, such an answer simply does not exist. His position is deliberately suspended, though not neutral: the book clearly highlights the risk that the psychedelic renaissance, precisely in its attempt to become mainstream, may end up amputating what makes these substances culturally, his-

torically, spiritually, and politically significant. If the trip is entirely removed, attenuated, or treated as a mere inconvenience, psychedelics risk being confined to a purely technical, clinical use, stripped of imaginative and symbolic depth, becoming, in effect, a new SSRI. In such a scenario, the psychedelic renaissance might be remembered not as a genuine paradigm shift, but as a brief historical moment: a period in which psychedelics re-emerged only to be normalized and reframed within Western biomedical paradigms, to the point of losing the "trip" itself.

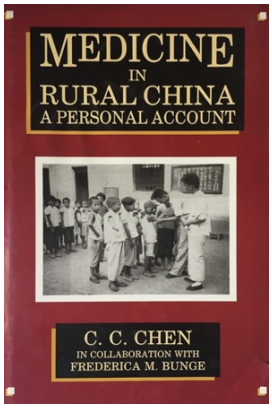
And yet, from a clinical and institutional perspective, this "loss" may not necessarily be a loss. The possibility of separating antidepressant efficacy from the psychedelic experience is not merely a theoretical or neuroscientific question; it represents a pivotal issue for the organization of healthcare services. Indeed, it may determine whether we are dealing with a treatment destined to remain confined to a small number of highly specialized centres, requiring complex settings, complex staffing requirements and associated costs, or with an intervention that could be integrated into routine clinical practice. If it were possible to demonstrate that the therapeutic effect is at least partly independent of altered states, psychedelics could become more manageable, more readily standardizable, and, above all, more accessible to patients, potentially also within the national healthcare systems (e.g., Italian National Health Service). From this standpoint, a reduction of the trip would not necessarily represent an impoverishment; rather, it could be a precondition for broader equity of access and for systemic sustainability.

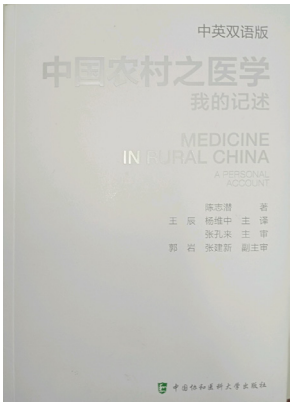
It is worth recalling the role that the Istituto Superiore di Sanità (ISS, Italian National Institute of Health) played in the early discussion on the potential psychopharmacological value of psychoactive agents. In 1973, Marcello Baraghini organized a workshop on this topic. In preparation, he initially contacted Nobel Laureate Daniele Bovet, founder and long-time head of the Laboratorio di Chimica Terapeutica at ISS and, at that time, Director of the Laboratorio di Psicobiologia e Psicofarmacologia at Consiglio Nazionale delle Ricerche (CNR) in Rome. Bovet referred him to Giorgio Bignami, who was then leading the Reparto di Psicofarmacologia within the Laboratorio di Farmacologia at ISS, directed by Vincenzo Longo (Giambruno Legnaioli, *in verbis*). In the years that followed, however, research in this field was largely overshadowed by growing social and political concerns surrounding psychoactive substances, which came to be viewed primarily as drugs of abuse rather than as potential therapeutic agents.

From a stylistic perspective, the book privileges reflection over immersive narrative. Readers expecting vivid and extended descriptions of the experiences may find themselves disoriented: Mitchell frequently interrupts the narrative to broaden the discussion to philosophy, the history of psychiatry and psychedelics, research policy, and the political economy of mental health. This choice is consistent with the book's broader ambitions, although at times it makes the reading more demanding. Yet it is precisely this density that makes *Ten Trips* relevant for a readership seeking not entertainment, but critical tools to understand what is currently unfolding in the field of psychedelics. More broadly, it will appeal to readers interested in the current and future role of psychedelics and in the assumptions that shape their contemporary resurgence.

In sum, *Ten Trips* places the narratives of individual journeys within a broader reflection on the historical moment in which these experiences become possible and legitimate, yet at the same time under threat. Mitchell neither endorses a romantic vision nor a technocratic one; rather, he compels the reader to confront an uncomfortable question: what are we willing to sacrifice – experience, mystery, subjective transformation, even the idea of "multiverses" – so that psychedelics may finally enter the realm of "normality"? It is a question that remains open, rendering the book not a conclusion but a critical exploration of a transition still unfolding.

Francesca Zoratto and Antonio Inserra
Istituto Superiore di Sanità, Rome, Italy
francesca.zoratto@iss.it; antonio.inserra@iss.it





MEDICINE IN RURAL CHINA
A personal account
C.C. Chen
in collaboration with Frederica M. Bunge
Berkeley (CA): University of California Press; 1989
237 p.
ISBN-10: 0520062981
82,00 €
On the right: front cover of the original edition in Chinese

This substantial and dense volume opens with an elegant photographic sequence providing a visual prelude to the narrative. Images of traditional social organization, together with a final photograph depicting a group of physicians in 1981, frame a text that unfolds with remarkable clarity and narrative fluency, at times recalling the most engaging works of contemporary historical writing.

As suggested by its title, the book offers a personal perspective – therefore a "personal account" – by a

health professional who combines medical expertise with a strong historical sensibility and an ability to adopt broad, even visionary perspectives. Through this lens, the author reconstructs a crucial historical trajectory in the development of medical sciences and rural sociology, spanning from the early decades of the twentieth century to the threshold of the present day.

As the editor emphasizes in the introduction, the sixty years covered by these memoirs in the field of community medicine have witnessed more progress in human health than the preceding two millennia. Globally, infant mortality rates have been reduced by more than half, while life expectancy has nearly doubled.

The pivotal figure, Dr. Chen, started his medical studies in the 1920s. Only decades later – by the mid-1980s – did international institutions such as the World Health Organization and the United Nations Children's Fund fully commit themselves to the ambitious goal of universal child immunization by 1990. Within this broader historical process, Chen emerges not merely as an observer but as an active protagonist.

Indeed, he can be regarded as a conductor orchestrating many of the principles that would later be codified under the concept of primary health care. Widely recognized as a pioneer in this field, Chen's work is here commemorated through a narrative that illustrates the evolution of healthcare in China, including the gradual integration of traditional Chinese medicine with "modernized" (by exploiting a likely Western-centric terminology) organizational and institutional models. The book thus also offers an implicit comparison with Western approaches to healthcare planning and strategic organization.

The volume is structured into three main sections. The first, *Pre-Liberation China*, introduces the late imperial period up to 1911, focusing primarily on the role of traditional medicine. It then examines the profound social transformations affecting medical education during the period between 1912 and 1928. The narrative follows the intellectual and personal journey of a medical student travelling from Chengdu to Beijing, a trajectory that reflects broader transformations in Chinese society. The section concludes with a discussion of pioneering initiatives in rural health and early models of community medicine, as well as the international attention attracted by these experiences. Particularly noteworthy are the passages devoted to healthcare under wartime conditions between 1937 and 1949.

The second part, *Post-Liberation China*, covers the period from 1949 to 1976 and concludes with a chapter dedicated to the decade 1976-1987, entitled *A New Era in Health Development*. This section arguably represents the conceptual core of the book and merits careful reading, as it traces the institutional consolidation and expansion of China's public health system.

The third and final section, *Reflections on the Health Experience*, may prove the most challenging for international readers. Here the author reflects on the structural difficulties faced by a vast developing country whose social progress has been closely intertwined with the development and organization of its healthcare system. The concluding chapters – *The Process of Rural Development: Lessons from China* and *Issues for the Future* – are particularly stimulating. They invite reflection not only on the Chinese experience but also on broader managerial and policy questions at a time when healthcare systems worldwide are undergoing continuous processes of reform and reorganization.

Ultimately, the book offers valuable insights into how a nation confronted profound public health challenges and gradually addressed them through social innovation, institutional reform, and technological progress. For readers interested in the historical development of community medicine and health policy, this work represents both a historical testimony and a thoughtful reflection on the long-term dynamics of healthcare transformation.

Enrico Alleva, Benedetta Pieri and Andrea Piccioli
Istituto Superiore di Sanità, Rome, Italy
enrico.alleva@iss.it

IL SÉ DIGITALE
Dai neuroni specchio alla mediazione tecnologica
Vittorio Gallese
Milano: Raffaello Cortina Editore; 2026
272 p.
ISBN 978-88-3285-844-0
€ 16.00

[The digital self: from mirror neurons to technological mediation]

For some time now, we have been asking ourselves what kinds of anthropological transformations are being brought about by the new technological dimension in which we are becoming increasingly interconnected. At times, the tone of this reflection becomes openly alarming. Some scholars point to possible modifications at the neurobiological level and – perhaps in the not-too-distant future – even on a genetic, or rather epigenetic, basis. Another crucial issue concerns how our subjectivity and our relationship with the world and with the Other (another individual of our species, whether familiar or unfamiliar) are being transformed in light of new technological devices.

This is the field of inquiry of Vittorio Gallese's latest book, which begins with a simple question: *"What happens to empathy and reciprocity when the bodily presence of the other is digitally mediated or replaced by an avatar?"* (p. 60). The premise from which this well-known neuroscientist – and neurophilosopher, a rather original attribute – develops his argument is the inadequacy of a form of reductionism that has become very influential, namely the view that considers the brain an autonomous organ detached from the body.

The scientifically significant discovery and characterization of the now paradigmatic *mirror neurons* – to which Gallese himself contributed more than twenty-five years ago – has shown that these neural mechanisms enable us not only to emulate the movements and feelings of the Other, but also to understand them by internalizing and physically experiencing them. This major step in neuroscience has led to the key concepts of aesthetic intercorporeality and embodied simulation. The latter does not simply activate interactivity; rather, it leads to the "internalization, at a subpersonal level, of neural circuits" (p. 54), producing a pre-reflective, automatic, and non-intentional form of understanding.

In his fluid and pleasantly readable book, Gallese essentially reflects on the new ontophenomenology of the mind, a topic of great relevance both for scientists belonging to the ever-expanding biomedical and neuroscientific community and for clinicians dealing with mental suffering. For this reason, the book is likely to attract psychiatrists and clinical psychologists, particularly those concerned with the emerging, post-COVID increase in psychological difficulties among children and adolescents.

Gallese's book first and foremost reaffirms the centrality of the physical dimension in relation to the digital one. It does so by offering a concise reflection on the bodily self – conceived not as a closed entity but as a relational one – and also on the very notion of art, which is no longer seen as a secondary domain of knowledge but rather as a central locus of experience and understanding. The act of representation thus becomes the manifestation of an extended mind that contributes to creating the world and the surrounding reality. New technologies – including those applied to art – act even more powerfully in this regard.

In the second part, the scholar turns instead to examining the digital self, which "is not a distant avatar, but an embodied projection that returns to the body, constrains it, and shapes it" (p. 101) and, much later – using an even more fitting term – *reformats* it.

In this new scenario, the distinction between subject and object becomes increasingly blurred, since, as Gallese reiterates, "intersubjectivity is no longer understood as a representational and inferential enterprise, but as an embodied, dynamic, and situated process, in which the Other is experienced even before being conceptualized" (p. 58). All this applies to immersive practices such as those offered by virtual reality, the metaverse, and, more broadly, by media environments. These had already been theorized more than sixty years ago by Marshall McLuhan, the first scholar to realize that mass media function as bodily extensions, anticipating the touchscreen by several decades. As the author emphasizes, it constitutes "a radical perceptual shift that reformulates the sensory conditions of our being in the world" (p. 127).

Technology is no longer merely a means, within the ontophenomenological perspective formulated by Gallese (following Simondon), but rather a genuine ontological operator, capable of modifying the body–world relationship. This inevitably recalls Benjamin and Debord, particularly when discussing the widespread ontological aestheticization that permeates every sphere of contemporary society, leading to the observation that "digital capitalism is an aesthetic machine". Yet once technologies have exhausted their generative function, they tend to slide into spectacularization and into forms of potentially very risky surveillance. Indeed, Gallese overturns Debord's famous assumption by arguing that it is no longer capital/spectacle – having reached such a degree of accumulation – that becomes image; rather, it is the accumulation of images that becomes capital (p. 109).

One of the chapters of the book focuses on screenology (a distinct area within media studies). The screen is conceived as an "environment" – here once again recalling McLuhan. "Is it possible to inhabit the image without being subjected to it?" Gallese asks. "Who has the right to appear?" These are ethical and political questions within the current visual regime, in which everything must be shown and displayed, yet where an excess of image production may paradoxically lead to its disappearance (as suggested by Baudrillard and Virilio).

When examining the digitalization of the self, several issues arise, including mediated and post-relational empathy, enacted through the relationships we establish not only with the Other via digital interfaces but also directly with non-human entities (AI, chatbots, robots). Here we reach perhaps the most delicate point of Gallese's essay. While reaffirming the primacy of the human mind, the only one that is truly embodied, he reverses the terms of the debate by sidestepping the classic question: *Can machines think?* For the author, the real issue is not whether AI can be conscious – since it cannot become embodied in a biological substrate – but rather: "what happens to us when we begin to think and act as if it were?" (p. 192).

The risk, therefore, is not so much that AI will replace the human population that uses it, but that it will shape it, or worse, orient it, suggesting what we should think and desire. On platforms such as Character.AI or Replika, for instance, seemingly concrete affective dynamics can develop between humans and machines. According to Gallese, another danger accompanies this process: a form of disturbing reflexivity, whereby human beings themselves begin to reason like machines, conceiving of themselves as simulacra, as disembodied interfaces.

The Digital Self, in conclusion, systematizes a series of theories that are by now widely established across aesthetics, neuroscience, and media theory, helping us to gain a deeper understanding of the new relational phenomenology and the renewed status of the mind in the age of the algorithm. It does so through a clear and fluid style, culminating in a final, constructive note: the proposal of a radical and political aesthetics, "not as decoration but as resistance" (p. 211), which might enable us to inhabit new technologies critically. The author's dynamic way of weaving together the various aspects of such a delicate contemporary issue is one of the book's most significant strengths, especially for younger readers or students.

Gallese extends this argument to the field of art, which, he argues, must have the courage to oppose the "perceptual domestication imposed by algorithms".

This, moreover, already occurs in the works of artist-activists who employ AI, datism, and other new media while subjecting them to what might be described as a continuous détournement (once again a Debordian notion), aimed at demystifying their use in the service of power, whether governmental or technocratic. Gallese speaks of "interruption" or of a counter-aesthetics, but the underlying idea remains the same.

And yet, this seems to us the somewhat more problematic part of his overall argument. Although he cites as examples mainly installation works – perhaps not coincidentally by artists such as Molly Soda Goliath, Zach Blas Lewis, Hito Steyerl, Mette Ingvartsen, Grayson Perry, and Anicka Yi – such an aesthetics remains somewhat too general. One thing is certain: within this new epistemic horizon, the concept of the operativity of images replaces the now anachronistic notion of mere representation.

However, when the scholar attempts to reformulate his initial question "what does it mean to be human in the age of technological mediation?" into the more uncertain "what can we still become?" (p. 244), the resulting outlook does not convey a particularly convincing optimism. The danger of an ethically unsustainable attempt to artificially amplify human capacities for economic gain – something often discussed within the biomedical community – may well be lurking in the background.

For biomedical readers, such a volume does represent a possibly useful and challenging cultural experience. Author Gallese in in fact among the leaders of the group gloriously led by Giacomo Rizzolatti, MD, globally recognized for his innovative hypothesis about both the physiological and the patho-physiological role of "mirror neurons", neural cells they characterized in monkeys and humans. From such an innovative finding, long-range speculations are fruitfully explored and expanded, particularly in a progressively digitalized world.

One solution, perhaps, would lie in making autonomous choices, without allowing the algorithm to decide for us. Yet is this option still truly possible? Or are we already moving toward a condition of collective standardization, an existential and experiential horizon in which it is not so much machines that develop feelings, but human beings who gradually lose their capacity for empathy?

Stella Falsini[1], Enrico Alleva[1] and Bruno Di Marino[2]
[1]*Istituto Superiore di Sanità, Rome, Italy*
[2]*Accademia Nazionale di Belle Arti, Rome, Italy*
enrico.alleva@iss.it

Ann Ist Super Sanità 2026 | Vol. 62, No. 2: 203-205
DOI: 10.4415/ANN_26_02_13

Publications from International Organizations on Public Health

Edited by
Annarita Barbaro

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS (FAO)

Rub A. **Fiscal policy repurposing as a tool to enable healthy diets. A review of available evidence**. Rome: FAO 2026; 44 p. ISBN 978-92-5-140561-1. This technical report reviews how fiscal policy repurposing – through taxes and subsidies – can be used as a lever to promote healthier diets and advance global nutrition and non-communicable diseases targets. It first maps the international policy landscape using a documentary scan of multilateral frameworks, grey literature, and global databases, tracing how fiscal measures have been progressively endorsed in nutrition and food systems agendas. It then systematically assesses effectiveness based on 29 systematic, umbrella and scoping reviews (2015-2025), covering around 900 primary evaluations and simulations of sugar-sweetened beverage (SSB) taxes, taxes on foods hights: SSB taxes reduce consumption, HFSS taxes deliver modest gains when rates are high, and subsidies increase intake of nutritious foods. Yet most evidence comes from high-income settings, and long-term health effects remain underexamined. Current fiscal systems still favour taxes over positive incentives and prioritize staples over nutrient-dense foods. Coherent fiscal repurposing therefore represents a promising but underused strategy to improve diet quality and equity.

Evaluation of certain contaminants in food: One-hundred-and-first report of the Joint FAO/WHO Expert Committee on food additives. WHO Technical Report Series, No. 1061. Rome: FAO and WHO 2026; 82 p. ISBN 978-92-5-140589-5. This report represents the conclusions of the One-hundred-and-first meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), which met at WHO headquarters in Geneva, Switzerland, in October 2025 to evaluate the contaminant of arsenic in food. The Committee assessed biochemical, toxicological and dietary exposure data on inorganic arsenic, small organoarsenic species (including dimethylarsinate [DMAV] and methylarsonate [MMAV]) and complex organoarsenic species (arsenobetaine, arsenocholine, arsenosugars and arsenolipids). The Committee also considered whether the risks associated with exposure to a combination of arsenic species present in food and water could be assessed. Analytical methods, transfer from feed to food, sampling protocols, effects of processing, prevention and control, and levels of contamination are also described. This report will be useful to those involved in controlling contaminants in foods, government and food regulatory officers, and those employed in industrial testing laboratories and toxicological research facilities.

2026 Global Report on Food Crises. Joint analysis for better decisions. Rome: FAO, WFP and GNAFC 2026; 80 p. ISBN 978-92-5-140659-5. The Global Report on Food Crises (GRFC) 2026 reveals that acute food insecurity and malnutrition remain at alarmingly high and deeply entrenched levels, with crises increasingly concentrated in a core group of countries. In its tenth edition, the report shows that acute hunger has doubled over the past decade, with two famines declared last year for the first time in its history. As a flagship publication of the Global Network Against Food Crises (GNAFC), the GRFC serves as the key reference for understanding acute food insecurity at global, regional and country levels. Produced through a collaborative effort among 18 partners, it provides a consensus-based assessment of acute food insecurity and malnutrition in countries affected by food crises, with the aim of informing and guiding both humanitarian and development responses.

INTERNATIONAL SCIENCE COUNCIL (ISC)

Preparing national research ecosystems for AI – third edition (2026). Paris: International Science Council 2026; 167 p. Recognizing the urgent need to develop appropriate regulatory and institutional frameworks – for the development and use of AI technologies in general, and for science and research specifically – the ISC's Centre for Science Futures has mapped the integration of AI into research and science ecosystems across select countries around the world, with a particular focus on the Global South. Following the approach of past editions, the aim was to deliberately include case studies from countries of small to medium size. The aim is to offer insight into approaches being adopted by countries of similar size and capacity, to inform those tasked with spearheading the preparation of the research environment for AI. With eight new case studies, this edition now presents perspectives from 26 countries. These outlooks highlight how AI is transforming scientific research faster than national systems, policies and governance frameworks can respond. As the global AI landscape continues to evolve rapidly, each case study should be

understood in the context of the period in which it was written. The report addresses both the advancements made and the challenges faced in this field, making it a valuable read for science leaders, policymakers, AI professionals, and academics.

Towards gender equality in scientific organizations: assessment and recommendations. Paris: International Science Council 2026; 127 p. This report presents the most comprehensive global assessment to date of gender equality in scientific organizations. It reports the findings of a 2025 global study conducted jointly by the International Science Council (ISC), the Inter Academy Partnership (IAP), and the Standing Committee for Gender Equality in Science (SCGES). The analysis draws on institutional data from 136 organizations, survey responses from nearly 600 scientists, and a dozen interviews with representatives of scientific organizations. Together, these sources support a multilevel assessment of women's representation, participation, leadership, and recognition, combining structural analysis with lived experience. Building on global online surveys carried out in 2015 and 2020, the study provides a ten-year perspective on progress and persistent gaps. It identifies structural barriers to gender equality and highlights areas where institutional policies and practices have contributed to measurable change.

UNITED NATIONS ENVIRONMENT PROGRAMME (UNEP)

Our planet. Our purpose: UNEP annual report 2025. Copenhagen: United Nations Environment Programme 2026; 52 p. In 2025, UNEP worked in 151 countries, delivering science to support policymaking, providing advice to governments, supporting businesses to become more sustainable, and leading community-level projects that improved lives and livelihoods. The 2025 Annual Report explores UNEP's results and impacts across climate action, nature and land action, pollution action and more. In a time when multilateralism is more important than ever, it looks at how UNEP uniquely brings the world together to tackle global challenges, while underpinning decision-making processes with science, data and policy support.

EUROPEAN FOOD SAFETY AUTHORITY (EFSA)

EFSA (European Food Safety Authority), Dujardin B, Gómez Ruiz J Á, Ioannidou S, Salvatore S, Smeraldi C, Tard A, Gutiérrez Linares A. **The 2024 European Union monitoring report on food additives and flavourings (1st pilot)**. EFSA Journal 2026, 24(4), e10070. This EFSA report presents the results of the 2024 European Union (EU) pilot monitoring programme on food additives and flavourings, in response to a mandate from the European Commission. This pilot focused on three food additives, green S (E 142), ponceau 4R (E 124) and tartrazine (E 102), and two flavourings, caffeine (FL No 16.016) and pulegone. The aim of the monitoring programme was to evaluate the occurrence and use of these substances, estimate chronic dietary exposure across population groups and compare the outcomes with previous EFSA assessments. Monitoring data were collected from 22 EU Member States and five food business operators, comprising 18,296 analytical results from 8943 food samples and 663 use levels. Dietary exposure was assessed using three refined exposure scenarios: non-brand-loyal, brand-loyal and food-supplement 'consumers only' scenarios. Different challenges and misreporting were identified when preparing the monitoring report that might have biased, to different extents, the dietary exposure estimations for the substances assessed. Therefore, conclusions derived in the framework of this first pilot are not yet considered to be sufficiently robust for decision-making, and further actions will be taken to strengthen the collection and analysis of the monitoring data in the next report. Overall, the first pilot represents an essential preparatory step towards a sustainable EU monitoring system for food additives and flavourings. The lessons learned will be used to strengthen data quality, improve exposure assessment methodologies and ensure more reliable and accurate outcomes in subsequent annual monitoring reports.

EFSA (European Food Safety Authority), Bearth A, Jansen T, Mazzocchi M, Verbeke W, Alaveras G, Kanellakopoulou A, et al. **Frequency of consumption of different fish, crustacean and mollusc species contributing to methylmercury exposure and consumer awareness of national advice on their consumption**. EFSA Journal 2026, 24(2), e9865. Following a request of the European Commission, EFSA assessed fish and other seafood consumption patterns and consumer awareness of related health risks and benefits across the 27 Member States, Iceland and Norway. Awareness of existence of consumption national advice and to which extent this advice influence consumers consumption behaviour was also examined. To address these objectives, two surveys were conducted in 2023 and 2024 among adolescents, adults and pregnant women. Data were collected through computer-assisted telephone interviews by means of a combined Food Propensity and Awareness Questionnaire. The surveys covered 38 fish species grouped by their maximum levels of mercury (1.0, 0.5 and 0.3 mg/kg). Respondents were asked about consumption frequency, awareness of contaminants and knowledge of national dietary advice. The analysis showed that fish and other seafood consumption increased between the two surveys across all countries and species categories, regardless of whether updated advice was issued. Awareness of chemical contaminants was generally low, with mercury being the most recognised contaminant. Awareness of national advice was moderate and slightly higher among pregnant women but reported changes in consumption behaviour linked to this advice were lim-

ited. Information sources also played a role in shaping consumer behaviour, and these varied per country and population group.

WORLD HEALTH ORGANIZATION (WHO)

Global hepatitis report 2026. Geneva: World Health Organization 2026; 94 p. ISBN 978-92-4-012238-3 (electronic version) ISBN 978-92-4-012239-0 (print version). The Global hepatitis report 2026 provides the most comprehensive and up-to-date assessment of the global burden of hepatitis B (HBV) and hepatitis C (HCV), which together account for more than 95% of deaths related to viral hepatitis. The report highlights the progress in response efforts at global, regional and country levels, in the context of global commitments, strategies and targets. The report is organized around 4 major topics: an overview of global commitments, strategies and targets related to viral hepatitis; the status of the HBV and HCV epidemics and trends in the period 2015–2024; progress in coverage of interventions for prevention, diagnosis and treatment; and priorities for action at global and regional levels to accelerate progress towards the 2030 elimination targets, and associated country examples. This report is primarily based on data compiled from Member States in periodic rounds of global hepatitis data collection managed by WHO and model-based estimates of incidence, prevalence and mortality. In the 2025 round, 140 WHO Member States reported data, an increase from 113 in the previous round in 2023. Additional data sources include the WHO Global Health Observatory and databases managed by other UN agencies.

Indicators for human exposures to zoonotic pathogens. Geneva: World Health Organization 2026; 29 p. This report presents a WHO-PREZODE collaboration to develop and validate standardized indicators that assess the risk of zoonotic disease emergence by modelling pathogen circulation in animals and the risk of animal to human zoonotic spillover. The proposed indicators are intended to be actionable, i.e., to reflect the impact of the implementation of a prevention strategy along the process of zoonotic pathogen emergence and over time. Using expert-driven data from various contributors, the indicators were shown to correlate with documented Avian Influenza Virus and MERS-CoV events and to be actionable in simulating and guiding effective zoonotic disease prevention strategies. The indicators were also tested through a simulation of prevention strategies targeting specific aspects of zoonotic disease transmission. These simulations showed the actionability of the proposed indicators in guiding effective prevention strategies. This initiative provides actionable tools to measure, monitor, and mitigate the emergence of zoonotic diseases.

SHAKE the salt habit, 2nd ed. Geneva: World Health Organization 2026; 117 p. ISBN 978-92-4-012034-1. The document outlines a structured approach to developing a comprehensive sodium reduction strategy, including preparatory steps such as establishing governance mechanisms, engaging stakeholders and investing in data systems. It presents a set of evidence-based policies and interventions, with an emphasis on mandatory approaches, including food reformulation, nutrition labelling, public procurement standards, marketing restrictions, fiscal measures and behaviour change communication. Supported by a logic model linking actions to health and economic outcomes, the guidance also covers implementation considerations, capacity-building, monitoring, enforcement and evaluation. Intended primarily for policymakers and programme managers, it supports the development of effective, context-adapted interventions to reduce sodium intake, strengthen health systems and contribute to the prevention and control of noncommunicable diseases.

Instructions to Authors

Annali dell'Istituto Superiore di Sanità is a peer reviewed quarterly science journal which publishes research articles in biomedicine, translational research and in many other disciplines of the health sciences. The journal includes the following material: original articles, reviews, commentaries, editorials, brief and technical notes, book reviews. The publication of Monographic Sections on *Annali ISS* has been discontinued. In case you wish to present a limited number of coordinated contributions on specific themes concerning priorities in public health, please contact the Editorial office. If only regional or Italian data are presented in the manuscript, these should be compared with similar data available at European or international level. *Annali* follows the Recommendations for the Conduct, Reporting, Editing, and Publications of Scholarly Work in Medical Journals, issued by the International Committee of Medical Journal Editors (ICMJE) recently updated with a specific section II.A.4. on Artificial Intelligence (AI)–Assisted Technology. www.icmje.org.

MANUSCRIPT SUBMISSION

Manuscripts should be submitted online to https://annali.iss.it/.
The submission should include:
- cover letter where the authors declare that the manuscript has not been published or submitted for publication elsewhere;
- manuscript;
- tables and figures;
- authors' contributions statement (individual contribution to the manuscript);
- conflict of interest statement (a conflict of interest exists when authors or their institutions have financial or personal relationship with other people or organizations that could inappropriately bias conduct and findings of the study);
- permission to reproduce figures, if appropriate.

Receipt of author's paper will be acknowledged by an e-mail containing an identification number which should be used in future correspondence.
If Artificial intelligence (AI)-assisted technologies were used in the production of the submitted work, both the cover letter and the submitted work clearly state this and provide detail on their use.

NOTE FOR ISS AUTHORS

Authors whose affiliation is Istituto Superiore di Sanità can submit their paper only after completing the usual ISS authorization procedure (Infoweb).

REVIEW PROCEDURE

Each paper submitted to *Annali* is subjected to the following procedures:
- it is reviewed by the Editor-in-Chief for general suitability;
- if it is judged suitable, qualified referees are selected and a peer review process takes place;
- based on the recommendations of the referees and replies of the authors, the Editor-in-Chief decides whether the article should be accepted, modified or rejected;
- once the paper has been accepted, authors will receive proofs from the editorial office, which should be corrected and returned (usually within three working days).

MANUSCRIPT PRESENTATION

Please ensure that your manuscript follows these guidelines.
Manuscripts should be written in good English, as concisely as possible to allow a clear understanding of the text. The title should be followed by the complete name of the authors, their affiliations – only one per author and in the original language – town and country. The name of the Working Group should appear at the end of the by-line; its composition should be reported before the References, names and affiliations of each member are required. The name and address, telephone and e-mail of the corresponding author should also be indicated. On the same page a running head of no more than 40 characters (including spaces) should be included.
Original articles should normally be organized into different sections (*i.e.*: Introduction, Materials and methods, Results, Discussion, Conclusions). In the Methods section a specific paragraph on the adopted statistical analysis should necessarily be included.
Each article should be accompanied by:
- an abstract of about 150 words; the abstract should be structured when required (such as in original articles);
- key words up to a maximum number of five (MeSH headings, whenever possible. Refer to: www.nlm.nih.gov/mesh/meshhome.html).

Tables and figures should be kept to a minimum and be presented only if necessary.
Authors should deal responsibly and effectively with security issues that might be raised by their papers (see: Statement on Scientific Publication and Security *Science* 2003;299:1149).
This journal has adopted the SAGER reporting Guidelines for Sex and Gender Equity in Research.
These guidelines apply to original research articles and review papers. Authors should use the terms sex and gender carefully in order to avoid confusing both terms. Where subjects can also be differentiated by gender (shaped by social and cultural circumstances), the research should be conducted similarly at this additional level of distinction. Where the subjects of research comprise organisms capable of differentiation by sex, the research should be designed and conducted in a way that can reveal sex-related differences in the results, even if these were not initially expected.
Please consult the guidelines (https://researchintegrityjournal.biomedcentral.com/articles/10.1186/s41073-016-0007-6).

Authors are also encouraged to use fair, accurate and respectful language, but preferences can change and vary across groups and individuals and can also evolve overtime. The following guidelines may help in use of a correct terminology in the area of HIV: https://www.cdc.gov/stophivtogether/library/stop-hiv-stigma/fact-sheets/cdc-lsht-stigma-factsheet-language-guide.pdf
https://www.hptn.org/resources/HIVLanguageGuide
https://unesdoc.unesco.org/ark:/48223/pf0000144725
The name of the bioresource (and identifier, if available) which provided samples/data useful for the conduct of the study should be reported in extense, either in the Material and methods section or in the Acknowledgements.

LENGTH OF THE TEXT

To provide a text that meets the requirements of our publication:
• the *letter* to the Editor should be about 450 words; it does not need an abstract. Please contact the editorial office in advance if you wish to submit a Letter;
• the *editorial* should be no longer than 1,000 words; editorials are submitted on invitation. Please contact the editorial office in advance if you wish to submit an editorial;
• the *commentary,* 2,000 words; the commentary is an opinion piece or reflection on recent papers previously published on *Annali ISS* or elsewhere; an abstract is required; please contact in advance the editorial office;
• the *brief note*, 3,000 words, including about 15 references, one table and one figure;
• the *article,* 6,000 words, including about 40 references, three tables and two figures;
• the *review* should be no longer than 10,000 words, including no more than 100 references up to a maximum of four tables and three figures.
• *Supplementary materials* should be no longer than two printed pages. If necessary, authors could invite readers to contact them.

FORMATTING GUIDELINES

Text

• Use Times New Roman font, 10 point, single spaced;
• do not use the automated features of your application (endnotes, headers, footers, especially for references);
• avoid using bold characters to emphasise words or sentences within the text;
• indicate clearly titles of chapters and subchapters avoiding numbering.

Tables and figures

They should be understandable also without reference to the text and should be numbered in Arabic numerals in a consecutive and independent way according to their citation within the paper.
Tables should be presented on a separate sheet and preceeded by a title. Each column within the table should have a heading. Abbreviations should be reported in full in the legend.
Figures should be loaded as separate files. The following file formats are acceptable: JPEG, TIFF or EPS. Vectorial images (graphs, flow charts, schemes, and other non bitmap material) should be in Excel, Adobe Illustrator, Microsoft Power Point so as to allow the editorial formatting of the material.
Figures are redrawn into the *Annali* style by our in-house illustrators.
Photographs must have a minimum resolution of 300 dpi.
Captions should be presented on a separate sheet and contain a sufficient explanation of their object. They should be concise but comprehensive.

REFERENCES

All references in the text must be numbered in square brackets, *i.e.,* [1, 2, 3-6], and mentioned at the end of the article in the order in which they are quoted. They should conform to the "Recommendations for the Conduct, Reporting, Editing, and Publications of Scholarly Work in Medical Journals" (www.icmje.org), according to the following examples.
Titles of periodicals should be abbreviated in accordance with the Medline abbreviation of the US National Library of Medicine (www.nlm.nih.gov/bsd/aim.html).
Online journal articles can be cited using, in addition to the complete citation, the DOI number. Do not insert websites among the References but directly in the text in parentheses, where necessary.

Articles in journal

Bozzuto G, Ruggieri P, Molinari A. Molecular aspects of tumor cell migration and invasion. Ann Ist Super Sanità. 2010;46(1):66-80. doi: 10.4415/ANN_10_01_09

Books and chapters in a book

Godlee F, Jefferson T. Peer review in health sciences. London: BMJ Books; 1999.
Van Weely S, Leufkens HGM. Background paper: orphan diseases. In: Kaplan W, Laing R (Eds). Priority medicines for Europe and the world – a public health approach to innovation. Geneva: World Health Organization; 2004.

Proceedings

Fadda A, Giacomozzi C, Macellari V. Comparative measurements to validate a new telemetric pressure insoles system. In: 2. International Symposium on measurement, analysis and modelling of human functions. 1. Mediterranean Conference on measurement. Workshop on evaluation check of traceability. Proceedings. Genova: June 14-16, 2004. p. 425-7.

Technical reports

Della Seta M, Di Benedetto C, Leone L, Pizzarelli S, Siegmund U. ETHICSWEB technical guides. Manual for the creation of standards and guidelines for sharing information about knowledge organization systems on ethics and science. Roma: Istituto Superiore di Sanità; 2011. (Rapporti ISTISAN, 11/32).

Legislation

Italia. Decreto legislativo 29 ottobre, n. 419. Riordinamento del sistema degli enti pubblici nazionali, a norma degli articoli 11 e 14 della legge 15 marzo 1997, n. 59. Gazzetta Ufficiale – Serie Generale n. 268, 15 ottobre 1999.
US Social Security Administration. Evidentiary require-

ments for making findings about medical equivalence. Final rules. Fed Reg. 2006 Mar 1;71(40):10419-33.
The authors should check that each reference cited in the text appears in the reference list and viceversa. References should not include works submitted for publication but not yet accepted or unpublished results, etc. These can be mentioned in the text in parentheses.

CONVENTIONS

All Latin or foreign words should be in italics. The authors should use internationally accepted abbreviations. All abbreviations should be spelled out in full the first time they occur in the text, followed by the shortened term in parentheses; afterwards use the abbreviation only. Avoid abbreviations in the title of the manuscript.
For writing symbols, quantities and units of measurements refer to the International Systems of Units (SI) and the ISO standards.

LICENSES AND AGREEMENTS

Please, contact the Editorial Office for any other information. Annali Editorial Office - Scientific Communication Service
Istituto Superiore di Sanità
Viale Regina Elena, 299 00161 Rome, Italy
Tel.: +39 06 49902945 Fax: +39 06 49902253
E-mail: annali@iss.it
www.iss.it/annali - https://annali.iss.it/