

ANNALI

DELL'ISTITUTO SUPERIORE DI SANITÀ

Volume **62** No. **3**
2026



A SCIENCE
JOURNAL *for*
PUBLIC
HEALTH



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 2.1

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

Responsibility for the contents and opinions expressed on this journal
rests solely with the Author(s).

ISSN 0021-2571 (print), 2384-8553 (online)

Coden: AISSAW 62 (No. 3)

©Istituto Superiore di Sanità 2026.

Some rights reserved.



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



Printed in September 2026 by Lion Brand srl, Rome

President of the Istituto Superiore di Sanità

Rocco Bellantone

Responsible Director

Antonio Mistretta

Istituto Superiore di Sanità

Editor-in-chief

Enrico Alleva

Istituto Superiore di Sanità

Assistant Editor

Federica Napolitani Cheyne

Istituto Superiore di Sanità

Scientific Committee

Enrico Alleva, Luca Busani, Paola Fattibene, Alessandro Giuliani, Antonio Mistretta,
Maria Teresa Palamara, Carlo Petrini, Emanuele Scafato, Marina Torre

Istituto Superiore di Sanità

Editorial Committee

Annarita Barbaro, Maria Cristina Barbaro, Alessandra Fuglieni, Maria Salvatorina Graziani,
Federica Napolitani Cheyne, Manuela Zazzara

Istituto Superiore di Sanità

OJS Site Administrator

Daniele Cordella

Istituto Superiore di Sanità

Editorial Advisory Board

Chris Beyrer

Johns Hopkins Bloomberg School of Public Health, Baltimore, USA

Jacqueline N. Crawley

Laboratory of Behavioral Neuroscience, NIMH, NIH, Bethesda, USA

Juana Martín de las Mulas González-Albo

Dpto de Anatomía y Anatomía Patológica Comparada, Facultad de Veterinaria, UCO, Córdoba, Spain

Jean-François Delfraissy

Agence Nationale de Recherches sur le sida et les hépatites virales, Paris, France

Tony Fletcher

London School of Hygiene & Tropical Medicine, London, UK

Sui Huang

Institute for Systems Biology, Seattle, USA

Stefania Maggi

CNR Aging Branch Institute of Neuroscience, Padova, Italy

Francesco M. Marincola

Infectious Disease and Immunogenetics Section, NIH, Bethesda, USA

Ana Marušić

Dept of Research in Biomedicine and Health, University of Split School of Medicine, Croatia

Patrick Smeesters

Université Catholique de Louvain, Louvain-La-Neuve, Belgium

Kurt Straif

ISGlobal, Barcelona and Visiting Professor at Boston College

David Vlahov

School of Nursing, University of California, San Francisco, CA, USA

Bernard Zalc (Boris)

Centre de Recherche de l'Institut du Cerveau et de la Moelle épinière, UPMC, Paris, France

The cover image shows the head and the torax of a larva of the species Culex impudicus. The larva is cleared and mounted on a microscope slide to highlight morphological characters for species identification (see the antennal and thoracic setae, the spicules on the antennae and on the thoracic integument). The image is provided by Francesco Severini, Istituto Superiore di Sanità, Rome, Italy.



ANNALI

dell'Istituto Superiore di Sanità

Vol. 62, No. 3 2026

Contents

EDITORIAL

- 207** Refining canine experimental protocols
Laura Ricceri, Federica Pirrone, Stella Falsini and Enrico Alleva

COMMENTARY

- 210** The European Group on Ethics in Science and New Technologies and the opinion on the just ecological transition
Laura Palazzani
- 214** Why do we treat women as if they were men? Rethinking sex and gender in biomedical research and healthcare
Massimo D'Archivio, Anna Ruggieri and Elena Ortona

ORIGINAL ARTICLES AND REVIEWS

- 217** Incidence and factors associated with bystander cardiopulmonary resuscitation in out-of-hospital cardiac arrest in an Italian region
Matteo De Vecchi, Ines Basso, Tommaso Davite, Erika Bassi, Erica Busca, Silvano Andorno, Luca Briacca, Carla Vigliano, Rosanna Vaschetto, Daniele Privitera, Laura Rubino, Salvatore Izzo, Roberto Gioachin and Alberto Dal Molin
- 224** Leptospirosis outbreak in an urban setting: epidemiological and clinical characteristics of the 2025 epidemic in Sarajevo Canton, Bosnia and Herzegovina
Anes Jogunčić, Asaf Salčinović, Agnesa Porović Hodžić, Anisa Bajramović, Zlatan Hamza, Aida Pošković-Bajraktarević, Aysun Tekin and Lal Amos
- 232** Current background human exposure to polybrominated diphenyl ethers: a review
Annalisa Abballe, Isotta Cursi, Valentina Marra, Noemi Milia, Roberto Miniero, Silvia Valentini and Anna Maria Ingelido

- 241** Human-horse interactions as complementary strategies for mental health promotion
Barbara Collacchi, Chiara Ciacchella, Nadia Francia, Stefania Cerino and Francesca Cirulli
- 254** A mixed-methods study of facilitators and barriers of an audit and feedback intervention using key performance indicators for the Emergency Medical System in Northeastern Italy
Laura Deroma, Annarita Tullio, Sabrina Licata and Francesca Valent
- 267** Bisphenol S-induced inflammation: a comparative insight on inflammatory pathway activation in zebrafish and rodent models
Wan Nur Izzati Wan Azhan, Fatin Nadzirah Zakaria, Danial Akmal Fuad Al' Arifin, Nur-Zulaikha Amlie, Evana Kamarudin, Fabeze Paiman, Rio Risandiansyah and Razif Dasiman
- 277** Three-year risk of colorectal cancer by faecal haemoglobin concentration in subjects with a negative FIT
Donella Puliti, Giuseppe Gorini, Adele Caldarella, Teresa Intrieri, Zhanna Tumanova, Gianfranco Manneschi, Carmen Beatriz Visioli, Beatrice Mallardi, Francesca Battisti, Simonetta Bisanzi, Cristina Sani, Giovanna Masala, Marco Zappa and Paola Mantellini
- 285** **Book Reviews, Notes and Comments**
Edited by *Federica Napolitani Cheyne*
- 289** **Publications from International Organizations on Public Health**
Edited by *Anmarita Barbaro*
- 292** **Erratum for:** Legal responses to new psychoactive substances in Europe: countries inside the REITOX network, Norway, and Türkiye
Valeria Aquilina, Francesco Paolo Busardò, Simona Pichini, Giulia Bambagiotti and Silvia Graziano
[Ann Ist Super Sanità 2026 | Vol. 62, No. 2: 100-114 DOI: 10.4415/ANN_26_02_02]

EDITORIAL

Refining canine experimental protocols

Laura Ricceri¹, Federica Pirrone², Stella Falsini¹ and Enrico Alleva¹

¹*Centro di Riferimento per le Scienze Comportamentali e la Salute Mentale, Istituto Superiore di Sanità, Rome, Italy*

²*Dipartimento di Medicina Veterinaria e Scienze Animali, Università degli Studi di Milano, Milan, Italy*

Italy, and in particular the Istituto Superiore di Sanità (ISS), which has long represented a national reference point for animal experimentation, addressed the issue of regulating animal testing by initially implementing the European Directive 86/609/EEC (1986) [1-3]. When this Directive was adopted [4], unfortunately only in 1992, a further restriction was introduced [5], or rather a special legislative pathway was defined, for animal species considered vulnerable in terms of extinction risk (e.g., vulnerable, endangered, rare, near-extinct species, etc.). This pathway incorporated the classification of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES, <https://cites.org/>) into national legislation. In March 2014, four years after the new European Directive on protection of animals used for scientific purposes had been issued [2, 6], new national legislation [7] modified the criteria and evaluation grid for research projects. The ISS remains actively engaged in promoting procedural and methodological ameliorations in 3Rs principles [8-11].

Over time, behavioural sciences and veterinary ethology at the international level have progressively developed refined methods for assessing psychophysical well-being, particularly in dogs, a species that was widely used in experimental research in the past. For many years now, legislation requires that, in addition to traditional model species (rats, mice, hamsters, quickly joined by zebrafish, etc.), a “non-rodent species” be included at the regulatory level. This requirement originates from the well-known thalidomide accident, in which pregnant women treated with the antiemetic drug experienced severe teratogenic effects in their fetuses. In fact, insufficient toxicological assessment in the rodent species used at the time to detect adverse effects before administration to human beings failed to reveal such outcomes due to absence of proper prenatal treatment evaluations, and to interspecific differences in metabolic processes [12].

At the end of this Editorial, aimed at refining the use of dogs in experimental settings, we provide a selective list of reference articles illustrating the progressive improvement of methodologies related to specific procedures (e.g., urine collection). Finally, we conclude with some helpful suggestions for adhering to the established 3Rs Principle, even at the international level.

Training laboratory animals has been widely recognised as critically important for biological sample collection, as it allows a substantial reduction in the reliance on forced manual restraint, which can rapidly induce aversion and stress responses, as well as on chemical restraint. Indeed, the induction of anesthesia or repeated pharmacological sedation may significantly alter physiological, endocrine, and immunological parameters, thereby introducing confounding variables that can ultimately affect study outcomes. By promoting voluntary cooperation through reward-based training, sample collection procedures can be performed more efficiently, with improved animal welfare and enhanced reliability of the collected data [13, 14].

In this context, it has long been established that, both in cognitive assessment and in routine experimental or veterinary procedures, reward-based training paradigms are significantly more effective than aversion-based approaches in many species, including dogs, cats and non-human primates (who are subject to the most rigorous preventive control).

Reward-based methods are associated with improved learning performance, reduced stress responses, and more stable behavioural and physiological outcomes, whereas aversion-based techniques are linked to increased fear, pessimistic bias, impaired learning ability, and compromised welfare [15]. In support of these principles, the extensive literature on positive reinforcement training (PRT) should be also considered [16-21]. Indeed, European Directive 2010/63/EU [6] and the corresponding Italian Legislative Decree 26/2014 [7], explicitly state in Annex III (General Section): “Establishments shall set up habituation and training programmes suitable for the animals, the procedures, and the length of the project”. This provision is retained in the revised Annex III introduced by Legislative Decree n. 183/2025 [22], which will apply from December 4, 2026.

In many veterinary procedures involving animals in experimental settings (as well as in zoos), PRT has proven extremely useful when the animal is subjected to procedures for which a “standardized state of calmness” is essential for the reliable collection of scientific data. These procedures include blood or urine sampling, swab collection [23] repeated injections oral dosing/volun-

tary ingestion of compounds, monitoring and measurement procedures (e.g., blood pressure measurement, electrocardiogram electrode placement), imaging and diagnostic procedures (e.g., ultrasound examinations, non-restrained radiographic positioning, ophthalmic examinations, and otoscopy) [24], handling management, and welfare-related procedures (e.g., voluntary kennel entry and exit, crate or transport box training, muzzle training, nail and fur toileting, habituation to novel environments or equipment, and target training for positioning, body condition scoring, nail trimming, fur grooming).

An important point concerns the scoring of the degree of suffering (mild, moderate, severe) in dogs. Although not universally validated, dog-specific severity scoring system is currently available, existing regulatory guidance clearly indicates that severity classification must consider not only physical pain, but also suffering and distress, including their emotional and psychological components [6, 25]. In dogs, a highly social and cognitively complex species, distress often manifests primarily as alterations in emotional state rather than as overt signs of physical pain. Indicators of emotional distress in this species include both overt, traditionally recognised behaviours, such as freezing responses and avoidance or withdrawal, and more subtle signs, including lip licking, yawning, head turning, and paw lifting, as well as negative shifts in affective state measurable through cognitive bias paradigms (e.g., pessimistic bias) [18]. These behavioural and cognitive indicators have been consistently associated with a measurable deterioration of welfare, even in the absence of detectable pain. Together, these considerations provide a strong scien-

tific basis for explicitly incorporating emotional distress and its ethological markers into severity assessment for dogs. Such assessments should encompass both overt behaviours and more subtle ethological indicators, in full accordance with Directive 2010/63/EU [6, 7] and with the FELASA/ECLAM/ESLAV guidance [25].

With regard to the use or potential reuse in experiments of the same canine subject, focusing on a particularly critical species, a solid and progressively improving reference protocol has been developed over time [26].

Similarly to cats, dogs do represent a “special case”, in which an enduring dialogue between the scientific community and general public becomes mandatory. The increasing prevalence in single-person households, especially in some Western countries, together with the strong dyadic relationships between human caregivers and family dogs, often fulfilling roles once occupied by broader human social network, has significantly heightened public sensitivity toward canine welfare. This sensitivity exerts powerful influence on how scientific research involving dogs is perceived. Consequently, for such an iconic species, there is a growing necessity to balance public sensitivity for canine quality of life and welfare, with the continued, albeit limited, necessity of experimental activities, conducted within an increasingly morally relevant (3Rs) principle.

Conflict of interest statement

The Authors declare that there are no conflicts of interest.

Received on 15 June 2026.

Accepted on 22 June 2026.

REFERENCES

1. Council of the European Union. Directive 86/609/EEC of 24 November 1986 on the protection of animals used for experimental and other scientific purposes. Official Journal of the European Communities L 358, 18 December 1986.
2. Hartung T. Comparative analysis of the revised Directive 2010/63/EU for the protection of laboratory animals with its predecessor 86/609/EEC: a t4 report. ALTEX. 2010; 27(4):285-303. doi: 10.14573/altex.2010.4.285
3. Manciocco A, Romano E, Zoratto F, Branchi I, Berry A, (Eds). *Sperimentazione animale: aspetti teorici, normativi e applicativi della nuova Direttiva europea 2010/63*. Roma: Istituto Superiore di Sanità; 2011. (Rapporti ISTISAN, 11/39). p.104.
4. Italia. Decreto Legislativo 27 gennaio 1992, n. 116. Attuazione della direttiva n. 86/609/CEE in materia di protezione degli animali utilizzati a fini sperimentali o ad altri fini scientifici. Gazzetta Ufficiale – Serie Generale n. 40, Suppl. n. 33, 18 febbraio 1992.
5. Alleva E. Gli animali servono davvero? La Stampa – TuttoScienze. 23 maggio 1990.
6. Council of the European Union. Directive 2010/63/EU of 22 September 2010 on the protection of animals used for scientific purposes. Official Journal of the European Union L 276, 20 October 2010.
7. Italia. Decreto Legislativo 4 marzo 2014, n. 26. Attuazione della direttiva 2010/63/UE sulla protezione degli animali utilizzati a fini scientifici. Gazzetta Ufficiale – Serie Generale n. 61, 14 marzo 2014.
8. Etna MP, Alleva E, Coccia E. Exploitation of immunological approaches for the quality testing of human vaccines to phase out the use of animals. Ann Ist Super Sanità. 2024;60(1):1-3. doi: 10.4415/ANN_24_01_01
9. De Angelis I, Ricceri L, Vitale A (Eds). *Innovative replacement methods at the Istituto Superiore di Sanità in the spirit of the 3Rs principle*. Roma: Istituto Superiore di Sanità; 2022. (Rapporti ISTISAN 22/18).
10. De Angelis I, Ricceri L, Vitale A. The 3R principle: 60 years taken well. Ann Ist Super Sanità. 2019;55(4):398-9. doi: 10.4415/ANN_19_04_15
11. Vitale A, Ricceri L. The principle of the 3Rs between aspiration and reality. Front Physiol. 2022;13:914939. doi: 10.3389/fphys.2022.914939
12. Botting J. The history of Thalidomide. In: Botting R (Ed). *Animals and medicine: the contribution of animal experiments to the control of disease*. Cambridge: Open Book Publishers; 2015. p. 183-98. doi: 10.11647/OBP.0055
13. Prior H, Blunt H, Crossman L, McGuire A, Stow R, Sewell F. Refining procedures within regulatory toxicology studies: improving animal welfare and data. Animals (Basel). 2021;11(11):3057. doi: 10.3390/ani11113057
14. Layton R, Beggs D, Fisher A, Mansell P, Riddell S, Layton D, et al. A positive-reinforcement training regimen for refined sample collection in laboratory pigs. Animals

- (Basel). 2025;15(4):471. doi: 10.3390/ani15040471
15. Casey RA, Naj-Oleari MCP, Campbell S, Mendl MT, Blackwell EJ. Dogs are more pessimistic if their owners use two or more aversive training methods. *Sci Rep*. 2021;11(1):19023. doi: 10.1038/s41598-021-97743-0
 16. Schapiro SJ, Magden ER, Reamer LA, et al. Behavioral training as part of the health care program. In: Weichbrod RH, Thompson GAH, Norton JN (Eds). *Management of animal care and use programs in research, education, and testing*. 2nd ed. Boca Raton, FL: CRC Press/Taylor & Francis; 2018. Chapter 32. doi: 10.1201/9781315152189-32
 17. Kurien BT, Everds NE, Scofield RH. Experimental animal urine collection: a review. *Lab Anim*. 2004;38(4):333-61. doi: 10.1258/0023677041958945
 18. De Castro ACV, Fuchs D, Morello GM, Pastur S, De Sousa L, Olsson IAS. Does training method matter? Evidence for the negative impact of aversive-based methods on companion dog welfare. *PLoS One*. 2020;15(12):e0225023. doi: 10.1371/journal.pone.0225023
 19. Kendrick J, Stow R, Ibbotson N, Adjin-Tettey G, Murphy B, Bailey G, et al. A novel welfare and scientific approach to conducting dog metabolism studies allowing dogs to be pair housed. *Lab Anim*. 2020;54(6):588-98. doi: 10.1177/0023677220905330
 20. Desforges E. Challenges and solutions surrounding environmental enrichment for dogs and cats in a scientific environment. *Animals (Basel)*. 2021;11(10):2980
 21. Bastos APM, Warren E, Krupenye C. What evidence can validate a dog training method? *Learn Behav*. 2025;53(3):227-8. doi: 10.3758/s13420-024-00658-9
 22. Italia. Decreto Legislativo 2 dicembre 2025, n. 183. Modifiche al decreto legislativo 4 marzo 2014, n. 26, in attuazione della direttiva (UE) 2024/1262, che modifica la direttiva 2010/63/UE per quanto riguarda i requisiti per gli stabilimenti e per la cura e la sistemazione degli animali e per quanto riguarda i metodi di soppressione degli animali. *Gazzetta Ufficiale – Serie Generale* n. 282, 4 dicembre 2025.
 23. Ballav M, Borah T, Xie B, Henneman ZJ, Siudzinski TR, Harris S, Elliott M, Nancollas GH. Kinetics of canine dental calculus crystallization: an in vitro study on the influence of inorganic components of canine saliva. *J Colloid Interface Sci*. 2014;425:20-6. doi: 10.1016/j.jcis.2014.03.029
 24. Berns GS, Brooks AM, Spivak M. Functional MRI in awake unrestrained dogs. *PLoS One*. 2012;7(5):e38027. doi: 10.1371/journal.pone.0038027
 25. Smith D, Anderson D, Degryse AD, et al. Classification and reporting of severity experienced by animals used in scientific procedures: FELASA/ECLAM/ESLAV Working Group report. *Lab Anim*. 2018;52(1 Suppl.):5-57. doi: 10.1177/0023677217744587
 26. Ecuer E, Boxall J, Louwerse AL, Mikkelsen LF, Moons CP, Roth M, Spiri AM. FELASA recommendations for the rehoming of animals used for scientific and educational purposes. *Lab Anim*. 2023;57(5):572-82. doi: 10.1177/00236772231158863

COMMENTARY

The European Group on Ethics in Science and New Technologies and the opinion on the just ecological transition

Laura Palazzani

Dipartimento di Giurisprudenza, Economia, Politica e Lingue Moderne, Libera Università Maria Santissima Assunta (LUMSA), Rome, Italy

Abstract

This article analyses the opinion of the European Group on Ethics in Science and New Technologies (EGE) *Towards a just green transition: addressing structural inequities*. The article first presents the origins, role and advisory function of the EGE within the European Union, then examines the ethical framework proposed for the green transition. The opinion develops a multidimensional understanding of justice, including distributional, corrective, transitional, procedural, recognition, global, intergenerational and planetary dimensions. Particular attention is devoted to the capability approach, which allows justice to be assessed in relation to human flourishing, health, social inclusion and ecological sustainability. The article argues that the green transition must be governed through transparent, participatory and accountable institutions, capable of protecting vulnerable groups, reducing structural “inequities” and aligning technological progress with human rights. From a public health perspective, the just green transition is not only an environmental necessity, but also a democratic and ethical imperative. The article concludes that the ecological transition can be sustainable only if it is guided by justice, solidarity and respect for human dignity.

Key words

- green transition
- justice
- equity

THE EUROPEAN GROUP ON ETHICS IN SCIENCE AND NEW TECHNOLOGIES: ORIGINS, DEVELOPMENTS AND ROLE

The European Group on Ethics in Science and New Technologies (EGE) was established in 1991 (its original name was the Group of Advisers on the Ethical Implications of Biotechnology) with the institutional role of advising the President of the European Commission on ethical guidelines for policies and regulation. It is a body composed of experts; it is independent, pluralistic and interdisciplinary (generally covering the disciplines of natural and social sciences, new technologies and the philosophy of new technologies, ethics and law).

The aim of the documents produced is to provide a comprehensive and concise analysis of a specific topic, generally at the direct request of the Presidency of the European Commission. The EGE's task is to draw up a document which, on the basis of an analysis of the arguments for and against the design and/or use of a tech-

nology, outlines ways of acquiring a critical awareness of the problems arising from technologies, providing a valuable guide for policies and regulation, rooted in scientific and ethical reasons. The growing importance of the EGE over the years is due to the quality of its output, and to the growing awareness of the importance of European governance with a scientific and ethical framework. Ethics is not called upon to place limits on scientific and technological progress, but rather to identify ethical requirements for the design and application of technologies that are compatible with fundamental human rights, in defence of human integrity, freedom as autonomy and responsibility, and justice.

Regulating new technologies is difficult: regulations often require continuous revision and reformulation in light of new scientific discoveries and societal transformations. The EGE's function therefore has the delicate role of ethical mediation between science/technology on the one hand and society/governments on the other.

The task of mediation is divided into several levels: descriptive, dialogical-dialectical and orientational/consultative. At the analytical level, the committee describes emerging problems: the description is generally complex and requires specific, even specialist, interdisciplinary skills. Within the committee, there is constant study, research, in-depth analysis and updating, with internal expertise and external hearings. At the dialogical-dialectical level, the committee is a forum for discussion, which is essential given the pluralism that characterises the current debate at the theoretical level. This pluralism manifests itself at the scientific, ethical and legal levels: even in the scientific field, there is often no single interpretation of phenomena; in ethics, we live in a postmodern society with fragmentation of values; in law, there are different ways of understanding the law and the relationship between law, ethics and science. The most difficult and delicate moment in the work of a committee is the effort, through discussion, to seek minimum or maximum points of agreement, where possible, expressed in the recommendations. It is not a question of reaching a compromise, but rather a mediation, a sharing or a consensus through overlap or intersection.

The opinions and documents of the EGE aim to provide conceptual tools to those who govern and to society in general to understand often complex, dynamic and changing problems and their relevance and urgency, outlining possible scenarios and identifying the paths to be taken in terms of public policies. These paths should seek a balance between the needs of scientific and technological progress and the protection of human beings, recognising the primacy of the human being over science.

The EGE has addressed a wide range of topics. These include issues related to the beginning of human life (prenatal diagnosis, cloning, research on human embryos), the end of human life, research on humans (genetic engineering, biobanks, international trials), new health technologies and emerging technologies (brain implants, doping, nanomedicine, synthetic biology, information and communication technologies, digitalisation, robots, artificial intelligence – AI, biotechnology and patents, genetically modified organisms, animal testing, food safety, energy, security and surveillance, digital democracy, and the pandemic).

THE DOCUMENT ON THE JUST GREEN TRANSITION

The document *Towards a just green transition: addressing structural inequities* (11 February 2026) [1] starts from a recognition of the urgency of the topic due to the context of the triple crisis of climate change, biodiversity and ecological degradation. The document discusses the concept of justice, and its normative basis (what it means to ensure “equality” and “leave no one behind”) in the context of the relationship between humans and the environment, recognising its growing and progressive complexity. The aim is not only to analyse the issue theoretically, but also to offer practical suggestions for including justice in policies.

The main innovative points may be underlined as described below.

The analysis of the multilevel concept of justice and the capability approach

Of particular interest is the analysis of the concept of justice to be applied from the design stage of policies for ecological transition: justice not only in the sense of recognising equality and not exacerbating existing inequalities, but also in the proactive sense of understanding the reasons for structural inequalities in order to overcome them, building more resilient societies. In the face of “disruptive” changes in the fast ecological transition, characterized by uncertainty and unpredictability, it is essential to change traditional approaches, with an innovative conceptualization of justice in a multilevel framework. The EGE emphasises that scientific evidence “remains crucial” in informing policy, but that policies should also integrate the multidimensional concept of justice, considering different levels:

- *Distributional justice* requires an equitable allocation of both burdens and benefits. Climate policies may disproportionately affect low-income households, rural populations, or workers in carbon-intensive sectors. The ethical commitment to “leave no one behind” should go beyond preventing additional harm and actively reduce existing structural injustices through targeted compensation mechanisms. From a public health perspective, this aligns with the principle of proportionate universalism: policies should be universal, but always tailored to needs;
- *Corrective justice* underscores historical responsibility: communities that have suffered from pollution, extractive industries, or economic marginalisation deserve recognition and remediation. This is particularly important in regions historically dependent on coal or heavy industry, where environmental degradation and health disparities often coexist;
- *Transitional justice* recognises that even necessary transitions can generate loss of employment, identity, or community cohesion. Ethical governance demands anticipatory measures to keep justice in ecological transition, with retraining programmes, social protection, green collective bargaining, and community participation in shaping new economic pathways. Without such measures, social protest movements may undermine both democratic legitimacy and climate objectives;
- *Procedural justice* means fair access to decision-making, through trust, transparency, and inclusive communication, which is considered essential for compliance with collective measures. Climate policies, often more disruptive and long-term, require even deeper democratic engagement. Citizens’ assemblies, participatory and inclusive consultation mechanisms represent important initiatives that should actively include marginalised groups, as migrants, elderly populations, rural communities, and people with disabilities;
- *Recognitional justice* demands cultural sensitivity. For communities whose identities are tied to specific ways of life, transition policies may be experienced as existential threats. Ethical governance requires acknowledging these attachments, rather than dismissing them as irrational resistance;
- *Global justice and solidarity* require the awareness that

green transition cannot be confined within European borders and the recognition that high-income regions avoid displacing environmental burdens onto lower-income countries. Trade agreements, due diligence directives, and carbon border mechanisms should be aligned with human rights principles, given the global interdependence;

- *Intergenerational and intragenerational justice* mean considering the rights and interests of future persons, and present persons, in both social and ecological sustainability. Importantly, intergenerational justice also intersects with intra-generational equity. Policies that impose austerity-like sufficiency measures on disadvantaged populations, while leaving affluent consumption patterns intact, would violate principles of fairness;
- *Planetary justice* further broadens the moral community to include non-human life. While not fully elaborated in the text, the recognition of biodiversity and ecosystem integrity as morally relevant entities challenges anthropocentric policy frameworks. Public health, increasingly aligned with “one health” and “planetary health” paradigms, must integrate this expanded ethical horizon.

In the context of the multilevel dimensions of justice, the M. Nussbaum’s capabilities approach is invoked in the document to help make justice operational beyond the economic and financial dimension. It is considered the impact of policies on the dimension of “human flourishing”, and therefore on life, health, bodily integrity, the senses, imagination, thought, emotions, practical reason, and the relationship with other species and the environment. A transition that protects ecosystems while undermining basic capabilities, through unemployment, energy poverty, or social exclusion, would fail on justice grounds; conversely, failure to respect planetary limits compromises the health and capabilities of both present and future generations.

It is the capabilities approach that allows justice to be established in a flexible way, promoting justice for individuals, communities and nations at an intercontinental and global level, while also considering non-human beings and future generations. This approach, proposed by the EGE, combines several dimensions: “intersectionality”, taking into account vulnerabilities related to age, gender and socio-economic status; “spatiality”, taking into account the many levels of policy and regulation, with the need for policies that are applicable in different geographical contexts; and “temporality”, recognising the intergenerational dimension and reflecting on urgency and ecological integrity.

Ethical governance of green transition

The document highlights the risks of delays or decisions not to prioritise the green transition.

The EGE insists on the need for “ex ante” and “ex post” assessment of risks and benefits in decisions regarding the green transition, with particular reference to social and cultural impact, with the provision of compensation for the most vulnerable groups through transparent and inclusive policies. The aim is to achieve a fair and proportionate distribution of burdens and benefits,

strengthening social protection (with particular reference to employment and the minimum wage).

The EGE is aware that the green transition can cause tensions, which require democratic deliberation. It is important to clearly develop “indicators” (such as well-being, health, sustainability, equity, environmental costs) that are useful as a coordinated, strengthened and implemented monitoring mechanism, through inclusive interoperable platforms. Each European country should clearly publish its environmental policies and measure the results of their social impact in terms of equity.

In the EGE’s view, the just transition should be legally binding at European level, tailored to socio-economic realities, with compensatory measures in national plans. To this end, detailed reports on the involvement of local populations in the evaluation of green policies are necessary. Europe should promote “green bargaining” for the protection of workers, giving them the tools, including educational tools, to cope with a green economy. It is also a question of clarifying and addressing market contradictions in order to link well-being and sustainability. Governments at all levels must address the contradictions inherent in the market in the green transition, seeking to reconcile “economic objectives” and “ethical imperatives”, competitiveness, sustainability and equity as interdependent objectives.

Governance should be “adaptive” and “flexible”, capable of governing systemic and interconnected phenomena, such as the relationship between humans and non-human species and the environment in a global and planetary sense, and the long-term impact on the relationship between present and future generations.

The document proposes a holistic approach of resilience, inclusiveness, equity, solidarity, and sustainability, seeking to bridge the gap between the economy and society, developing robust sustainability criteria and transparent reporting methods to prevent the so-called “greenwashing”. Compensation measures such as financial assistance, tax exemptions, and incentives should be available to those who are disadvantaged (e.g., in terms of property ownership or job opportunities).

Technological progress should be aligned with ecological and social justice, with respectful technical solutions aimed at strengthening justice beyond the borders of the European Union, taking into account fundamental human rights, in a perspective of global solidarity. Social labour policies should be closely linked and adapted to the green transition, and ecological policies should always comply with human rights. Circular economy policies should be promoted in Europe, with a reduction in waste and the encouragement of sustainable consumption and production.

Sustainability in Europe should always have a global perspective, with particular reference to middle- and low-income countries. Transparency in administrative policies should be improved, simplified and made understandable to all. The risks of global inequality should be mitigated with awareness.

Forms of participation

Governance must be transparent, participatory, and enforceable.

Communication, participation and inclusiveness should be strengthened. The success of the green transition will depend on participation, such as citizen active involvement, which is a prerequisite for building trust. Communication should be clear, transparent and reflective of European diversity, involving, in an inclusive way, the most vulnerable. Public participation should be systematically and procedurally guaranteed, and Member States should be “obliged” to establish permanent forums for participation and organize the fight against disinformation campaigns.

Europe should promote civic education on environmental justice, integrate “ecological and climate literacy”, and “ecological critical awareness” and encourage ethical and critical reflection, including through the role of artists, educators and journalists who can promote initiatives that strengthen the community’s bonds and ties on these issues. Education should promote resilience.

CONCLUSIONS

The opinion analysed, represents an ethically ambitious and normatively coherent framework for socio-ecological transformation. Its strength lies in its multi-dimensional conception of justice, integration of ethical governance with human rights, and recognition of democratic participation as foundational.

From a public health standpoint, the transition is not merely an environmental strategy but a comprehensive approach to safeguarding the determinants of health,

reducing inequities, and promoting human flourishing within ecological limits. The EGE asks that ethical aspirations should translate into enforceable standards, measurable equity outcomes, and institutional accountability. Without robust implementation, justice risks remaining rhetorical.

The green transition, according to the EGE, is both a moral imperative and a test of democratic maturity. If guided by justice, solidarity, and respect for human dignity, it offers an opportunity to build resilient societies capable of thriving within planetary boundaries. If pursued without ethical vigilance, it may deepen divides and erode trust.

The choice is not between green and just: the ecological transition should be just, or it will not be sustainable.

Conflict of interest statement

The Author declares no conflict of interest.

Received on 16 March 2026.

Accepted on 28 April 2026.

REFERENCES

1. European Group on Ethics in Science and New Technologies. Towards a just green transition: addressing structural inequities (Opinion n. 35, 11 February 2026). Luxembourg: Publications Office of the European Union; 2026. doi: 10.2777/5343947

COMMENTARY

Why do we treat women as if they were men? Rethinking sex and gender in biomedical research and healthcare

Massimo D'Archivio, Anna Ruggieri and Elena Ortona

Centro di Riferimento per la Medicina di Genere, Istituto Superiore di Sanità, Rome, Italy

Abstract

Modern medicine largely relies on a male-default model, incorrectly assuming that findings from male bodies can be universally generalized. This paradigm results in structural biases that lead to diagnostic delays, inadequate treatments, and inequitable health outcomes for women. Although sex involves biological attributes and gender reflects socio-cultural roles, both dimensions are frequently overlooked in research design and data interpretation. Significant differences exist at cellular levels and in disease manifestations, yet studies often fail to provide sex-stratified analyses, obscuring unique symptoms and mechanisms. Furthermore, women face higher risks of adverse drug reactions because pharmacological guidelines often ignore sex-specific metabolic profiles. Moving toward precision healthcare requires structural changes, such as mandatory sex-disaggregated reporting and the integration of gender-responsive variables. Ultimately, acknowledging that women are not simply smaller versions of men is a scientific necessity for ensuring an accurate and inclusive healthcare system.

Key words

- sex
- gender
- gender-sensitive medicine
- precision medicine
- health equity
- biomedical research

INTRODUCTION

Despite decades of scientific progress and growing awareness of biological diversity, modern medicine still largely relies on a model historically derived from male bodies. From preclinical research to clinical trials and therapeutic guidelines, the assumption that findings obtained in male organisms can be generalized to women continues to shape biomedical science and healthcare delivery. This paradigm has important consequences: it contributes to diagnostic delays, inadequate treatments, differential adverse drug reactions, and ultimately inequitable health outcomes.

The question "Why do we treat women as if they were men?" is therefore not rhetorical, but reflects a structural limitation embedded in research design, data interpretation, and clinical practice. While *sex* refers to biological attributes such as chromosomes, reproductive organs, and hormonal profiles, *gender* encompasses sociocultural roles and exposures that influence health. These dimensions interact across the life course and should be systematically integrated into research and care [1]. Yet, historically, women have been underrepresented or insufficiently analyzed, resulting in a knowledge base that does not fully capture sex- and gender-specific determinants of health.

This commentary examines the origins of this bias, its consequences across the research continuum, and the need to integrate sex and gender considerations into preclinical, clinical, and pharmacological studies, highlighting opportunities for more equitable and effective healthcare.

HISTORICAL ROOTS OF THE MALE DEFAULT MODEL

The male body has long been considered the default in biomedical research. Early physiological, pharmacokinetic, and toxicological studies were predominantly conducted in male animals and participants. This imbalance was driven by concerns about hormonal variability, potential pregnancy risks, and the perceived complexity of female biology, leading to the systematic exclusion of women from many trials until the late twentieth century [2].

The effects of this bias persist. Many clinical guidelines still rely on evidence derived mainly from male populations. Even when women are included, results are often not stratified by sex, obscuring important differences in disease mechanisms, symptom presentation, and treatment responses. This lack of disaggregated analysis reinforces the assumption of uniform applicability.

In addition, gender-related factors – such as occupational exposure, caregiving roles, healthcare access, and sociocultural norms – are rarely incorporated into study design. As a result, the interaction between biological and social determinants remains insufficiently explored.

SEX DIFFERENCES IN PRECLINICAL RESEARCH

Sex differences emerge at cellular and molecular levels and influence fundamental biological processes. Cells from female and male organisms differ in chromosomal composition, gene expression, epigenetic regulation, and metabolism. These differences persist even *in vitro*, independent of circulating hormones.

For example, female cells often show greater resilience to stressors such as oxidative stress, metabolic challenges, and inflammation, supported by stronger antioxidant responses, more efficient mitochondrial function, and activation of cytoprotective mechanisms like autophagy [3]. In contrast, male cells may be more prone to apoptosis under similar conditions, with differences reported in mitochondrial dynamics, reactive oxygen species production, and DNA repair capacity [3].

Despite this evidence, preclinical studies frequently fail to report the sex of cells or animal models, limiting reproducibility and potentially leading to incomplete conclusions [4]. Considering sex as a biological variable is essential to improve translational relevance.

SEX AND GENDER IN CLINICAL RESEARCH

Clinical research also reflects the legacy of the male default model. Women remain underrepresented in many trials, particularly in early phases and in areas such as cardiovascular, neurological, and pharmacological research. Even when participation is balanced, sex-disaggregated analyses are not consistently performed [2].

This gap is especially relevant in conditions that present differently in women. Cardiovascular disease, for instance, often manifests with atypical symptoms, contributing to underdiagnosis and delayed treatment [5]. Autoimmune diseases – more common in women – have historically received less attention despite their significant burden [6]. Differences in pain perception, immune responses, and metabolism further influence disease progression and treatment outcomes.

Gender-related factors add a further layer of complexity. Differences in environmental exposures, access to care, and social determinants – such as caregiving roles and socioeconomic status – affect disease risk, adherence, and recovery. Ignoring these aspects limits the effectiveness of clinical interventions and contributes to persistent disparities.

LIFESTYLE AND NUTRITION

Lifestyle and nutritional recommendations are often perceived as gender-neutral, yet they are frequently based on evidence that does not adequately account for sex-specific biological differences or gender-related determinants. As in other areas of medicine, a “one-size-fits-all” approach prevails, potentially limiting the

effectiveness of preventive strategies. Women and men exhibit distinct metabolic profiles, nutrient requirements, and physiological responses to dietary interventions [7]. For instance, hormonal fluctuations across the female life course, from menstrual cycles to postmenopause, significantly modulate energy metabolism, nutrient absorption, and needs for essential micronutrients like iron and calcium [8]. Furthermore, gendered social roles often dictate food choices (e.g., women tending toward vegetable-rich diets) [9], which in turn affect the risk of chronic disease such as type 2 diabetes and cardiovascular disease.

Failure to integrate both sex and gender perspectives into lifestyle medicine reflects the same structural bias observed in biomedical research more broadly. Incorporating sex-disaggregated data and gender-sensitive variables into nutritional science and prevention strategies is therefore essential to move beyond generalized recommendations and toward more precise and equitable approaches to health promotion.

PHARMACOLOGICAL IMPLICATIONS

Sex differences in pharmacokinetics and pharmacodynamics are well established [10]. Variations in body composition, gastric motility, enzyme activity, renal clearance, and hormonal regulation influence drug absorption, distribution, metabolism, and elimination. As a result, women may experience different efficacy profiles and a higher risk of adverse reactions. These concepts are important also when considering vaccine efficacy and safety. It is generally known that women develop stronger immune responses to vaccines and undergo more frequent and more severe adverse reactions [11, 12]. Consistent with drugs, also vaccines pharmacokinetics and pharmacodynamics differ by sex; females often experience higher serum concentrations of vaccine components due to lower body weight and differences in body composition; differences in body fat (higher in women) and muscle mass (higher in men) can impact the distribution of vaccines from the injection sites.

Historically, drug dosing recommendations have been based largely on male populations, contributing to inappropriate dosing and increased side effects in women. Differences in QT interval prolongation, sedation, and metabolism have been observed across multiple drug classes. Hormonal fluctuations during the menstrual cycle, pregnancy, and menopause can further modify responses, yet are rarely accounted for in trial design.

Achieving precision medicine requires integrating sex-specific pharmacological data, ensuring adequate female representation, and considering hormonal status.

THE ROLE OF RARE AND UNDERSTUDIED CONDITIONS

Bias also affects rare and understudied conditions. Many diseases with female predominance, particularly autoimmune and inflammatory disorders, remain insufficiently characterized. Limited data hinder diagnosis and delay therapeutic innovation. Women with rare dis-

eases often experience longer diagnostic pathways due to both biological complexity and systemic bias.

Studying these conditions offers valuable insight into sex-specific mechanisms, including immune regulation and hormonal signaling, with broader implications for common diseases.

FROM SEX-BLIND RESEARCH TO GENDER-RESPONSIVE MEDICINE

Moving beyond a sex-blind approach requires structural changes. Preclinical studies should include both female and male models and report sex clearly [2]. Clinical trials must ensure balanced recruitment and perform sex-disaggregated analyses, while pharmacological research should evaluate sex-specific responses.

Equally important is the integration of gender-related variables, including social determinants, occupational exposures, and healthcare access. Mixed-method approaches can better capture these interactions.

Regulatory agencies, funding bodies, and journals play a key role by requiring sex-disaggregated reporting and transparent methodologies. Education and training are also essential to improve awareness among researchers and clinicians.

IMPLICATIONS FOR CLINICAL PRACTICE

Integrating sex and gender into clinical practice improves diagnosis and treatment. Clinicians should consider sex-specific symptoms, risk factors, and responses. Laboratory reference ranges, imaging interpretation, and scoring systems may require adjustment [2].

Gender-sensitive approaches also enhance patient-centered care by accounting for social context, caregiving roles, and health behaviors, supporting more effective decision-making.

TOWARD EQUITABLE AND PRECISION HEALTHCARE

Incorporating sex and gender into research aligns with the goals of precision medicine. Recognizing biological and social diversity improves reproducibility, strengthens translational relevance, and supports equitable care.

Understanding both differences and similarities between women and men enables more accurate risk assessment, better prevention, and optimized treatments – benefiting all individuals.

CONCLUSIONS

The persistence of the male default model reflects historical practices, methodological limitations, and insufficient integration of sex and gender variables. Yet evidence clearly shows that these factors influence disease mechanisms, treatment responses, and outcomes.

Achieving equitable healthcare requires systematically incorporating sex and gender across all stages of research. This effort involves researchers, clinicians, institutions, and policymakers. Embracing biological diversity and social complexity will improve scientific rigor and patient care.

Recognizing that women are not simply smaller versions of men is both a matter of fairness and a scientific necessity. Integrating sex and gender into medicine is essential for a more accurate, inclusive, and effective healthcare system.

Conflict of interest statement

The Authors declare no conflicts of interest.

Received on 6 May 2026.

Accepted on 18 May 2026.

REFERENCES

- Ortona E, Delunardo F, Baggio G, Malorni W. A sex and gender perspective in medicine: a new mandatory challenge for human health. Preface. *Ann Ist Super Sanità*. 2016;52(2):146-8. doi: 10.4415/ANN_16_02_02
- Cattaneo A, Bellenghi M, Ferroni E, et al. Recommendations for the application of sex and gender medicine in preclinical, epidemiological and clinical research. *J Pers Med*. 2024;14(9):908. doi: 10.3390/jpm14090908
- Maselli A, Matarrese P, Straface E, Canu S, Franconi F, Malorni W. Cell sex: a new look at cell fate studies. *FASEB J*. 2009;23(4):978-84. doi: 10.1096/fj.08-114348
- Buoncervello M, Marconi M, Carè A, Piscopo P, Malorni W, Matarrese P. Preclinical models in the study of sex differences. *Clin Sci (Lond)*. 2017;131(6):449-69. doi: 10.1042/CS20160847
- Antoncecchi E, Lillo A, Moscucci F, et al. Cardiovascular health in women: a consensus document of the Italian Cardiovascular Societies. *J Cardiovasc Med (Hagerstown)*. 2026;27(1):58-75. doi: 10.2459/JCM.0000000000001829
- Ortona E, Pierdominici M, Maselli A, Veroni C, Aloisi F, Shoenfeld Y. Sex-based differences in autoimmune diseases. *Ann Ist Super Sanità*. 2016;52(2):205-12. doi: 10.4415/ANN_16_02_12
- Mauvais-Jarvis F, Bairey Merz N, Barnes PJ, et al. Sex and gender: modifiers of health, disease, and medicine. *Lancet*. 2020;396(10250):565-82. doi: 10.1016/S0140-6736(20)31561-0
- Feskens EJM, Bailey R, Bhutta Z, Biesalski HK, Eicher-Miller H, Krämer K, Pan WH, Griffiths JC. Women's health: optimal nutrition throughout the lifecycle. *Eur J Nutr*. 2022;61(Suppl 1):1-23. doi: 10.1007/s00394-022-02915-x
- Silenzi A, Giona L, Zanchi G, et al. Gender differences in eating habits and lifestyles of young students: observational evaluation from MaestraNatura program. *Front Nutr*. 2025;12:1653154. doi: 10.3389/fnut.2025.1653154
- Younes S. The relationship between gender and pharmacology. *Curr Res Pharmacol Drug Discov*. 2024;7:100192. doi: 10.1016/j.crphar.2024.100192
- Ferroni E, Mateo-Urdiales A, Bietta C, et al. Sex differences in response to COVID-19 mRNA vaccines in Italian population. *Epidemiol Infect*. 2024;152:e139. doi: 10.1017/S0950268824001079
- Anticoli S, Capanna S, Volpin A, et al. Sex differences in response to HBV vaccination in a cohort of health care workers. *Vaccine X*. 2024;22:100605. doi: 10.1016/j.jvacx.2024.100605

Incidence and factors associated with bystander cardiopulmonary resuscitation in out-of-hospital cardiac arrest in an Italian region

Matteo De Vecchi¹, Ines Basso², Tommaso Davite³, Erika Bassi², Erica Busca², Silvano Andorno², Luca Briacca², Carla Vigliano⁴, Rosanna Vaschetto², Daniele Privitera⁵, Laura Rubino⁶, Salvatore Izzo⁶, Roberto Gioachin^{7*} and Alberto Dal Molin^{2*}

¹Dipartimento Emergenza Urgenza, Azienda Socio-Sanitaria Territoriale Grande Ospedale Metropolitano Niguarda, Milan, Italy

²Dipartimento di Medicina Traslazionale, Università degli Studi del Piemonte Orientale "Amedeo Avogadro", Novara, Italy

³Dipartimento di Emergenza Urgenza, Azienda Socio-Sanitaria Territoriale Ovest Milanese, Magenta, Italy

⁴Dipartimento di Emergenza-Urgenza e Accettazione, Azienda Ospedaliero-Universitaria SS. Antonio e Biagio e Cesare Arrigo, Alessandria, Italy

⁵Department of Life Sciences and Medicine, Faculty of Science, Technology and Medicine, University of Luxembourg, Esch-sur-Alzette, Luxembourg

⁶Dipartimento di Emergenza Urgenza, Azienda Ospedaliero-Universitaria Maggiore della Carità, Novara, Italy

⁷Dipartimento Management e Gestione Reti di Emergenza, Azienda Sanitaria Zero, Torino e Novara, Italy

*These Authors should be considered co-last Authors

Abstract

Introduction. Out-of-hospital cardiac arrest (OHCA) is a major cause of mortality, and early bystander cardiopulmonary resuscitation (CPR) is critical for survival. This study assessed OHCA incidence and factors associated with bystander-initiated CPR in the Piedmont region of Northern Italy.

Methods. A retrospective cross-sectional analysis was conducted using 2022 administrative data from an Emergency Medical Dispatch Centre serving 851,538 residents. All confirmed OHCA cases were included. Demographic, clinical, and contextual variables were extracted. Descriptive statistics summarized case characteristics, and comparisons between events with and without bystander CPR were performed using chi-square or Fisher's exact tests.

Results. Among 905 confirmed cases, the incidence was 106 per 100,000 inhabitants. Most patients were male (57%), with a mean age of 76 years, and arrests occurred predominantly at home (87%). Bystander CPR was documented in 644 cases, occurring in 39.9%, mainly independently. CPR was more frequent in rural areas ($p=0.002$) and public settings ($p<0.001$). Return of spontaneous circulation occurred in 6.4% and was strongly associated with bystander intervention ($p<0.001$).

Conclusions. Fewer than half of eligible patients received bystander CPR, highlighting gaps in community readiness. Strengthening public training and dispatcher-assisted CPR may improve outcomes.

Key words

- out-of-hospital cardiac arrest
- emergency medical services
- cardiopulmonary resuscitation
- prehospital care
- retrospective studies
- CPR in Italian OHCA

INTRODUCTION

Out-of-hospital cardiac arrest (OHCA) is a major global health challenge and represents the third leading cause of death in industrialized countries [1, 2].

Its global incidence ranges from 30 to 170 cases per 100,000 inhabitants annually [3-5], with marked variation across countries and regions, largely attributable to differences in data collection, population characteris-

tics, and emergency care systems. This heterogeneity is particularly evident in Italy, where the absence of a national cardiac arrest registry has resulted in fragmented epidemiological information [6]. Despite advances in resuscitation and post-arrest care, survival remains low, with an overall rate of approximately 8% and favourable neurological outcomes in fewer than 3% of patients at hospital discharge [7].

It is estimated that about half of OHCA are witnessed by bystanders [8]. When promptly initiated, cardiopulmonary resuscitation (CPR) and the use of semi-automatic external defibrillators (AEDs) by lay responders represent the most critical link in the chain of survival [9]. Bystander CPR significantly improves outcomes, with immediate initiation after collapse, even before AED deployment, associated with a two- to threefold increase in survival probability [10, 11].

Several community-based projects worldwide have aimed to improve awareness among both adults and children about recognising OHCA and promptly initiating resuscitation [12-14]. Nevertheless, despite these initiatives, the proportion of people in Europe who provide resuscitation while awaiting professional assistance varies widely, ranging from 17% to 78% [15].

Multiple factors influencing the likelihood of bystanders initiating resuscitative manoeuvres have been identified in the literature [16]. These include sociodemographic variables (age, gender, and level of education), contextual circumstances (location of the arrest, presence of other witnesses, relationship to the victim), psychological aspects (confidence, fear of harming the victim, or fear of legal consequences), and practical factors (prior CPR training, availability of AEDs, and dispatcher-assisted CPR) [17-21]. Importantly, many of these determinants are modifiable. For example, public training campaigns, integration of CPR teaching into school curricula, and dispatcher-assisted instructions have been shown to improve laypeople's willingness and ability to intervene, whereas broader availability and accessibility of AEDs address infrastructural barriers [2]. Conversely, factors such as advanced age of the bystander or a familial relationship to the victim, which may be associated with higher emotional distress, are more difficult to modify [17]. Despite this knowledge, existing evidence remains incomplete and strongly shaped by contextual factors such as healthcare organization, cultural attitudes, and local infrastructure [22]. Such variability suggests that a deeper understanding of context-specific dynamics could provide valuable insights for improving bystander intervention.

In Italy, the pre-hospital emergency service is publicly funded and organized on a regional basis within the framework of the National Health Service. To improve efficiency and resource management, since 2021, in the Piedmont region, the system has been coordinated by Azienda Zero. Emergency calls are managed by four medical dispatch centres, which assess case severity, coordinate the most appropriate response, and, in the event of OHCA, provide CPR instructions to laypeople [23].

To the best of our knowledge, no recent studies have investigated the occurrence of OHCA and the rate of bystanders-initiated CPR in the Piedmont region [6].

In the absence of a national registry, recommended by international guidelines [24], collecting local data is essential to gain insights into this event, thereby informing emergency service organization and guiding targeted public awareness initiatives [6]. Accordingly, this study aimed to: i) estimate the incidence and outcomes of OHCA cases managed by an Emergency Medical Dispatch Centre in Piedmont; ii) determine the proportion of events in which bystanders initiated CPR prior to Emergency Medical Services (EMS) arrival; and iii) compare the characteristics and outcomes of cases with and without bystander-initiated CPR.

METHODS

Study design

We conducted a retrospective cross-sectional study based on administrative data from an Emergency Medical Dispatch Centre in the Piedmont region. Reporting followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines [25].

Setting

We collected data between January 1 and December 31, 2022, from an Emergency Medical Dispatch Centre in Piedmont, serving a catchment population of 851,538 residents across five provinces [12]. The centre manages an average of 250-300 emergency calls per day. Prehospital coverage is provided by approximately 60 basic life support (BLS) ambulances, staffed by emergency medical technicians with regional rescue qualifications, and 18 advanced life support (ALS) ambulances, staffed by physicians and/or critical care nurses. Additional support is ensured by a helicopter emergency medical service, staffed by an anesthesiologist-intensivist, a critical care nurse, and a technician from the Alpine and Speleological Rescue Service.

Data sources and measurement

We identified all suspected cases of OHCA managed by the Piedmont Emergency Medical Dispatch Centre, regardless of patient age, through the telephone dispatch code. Each case was included if subsequently confirmed as OHCA by the on-scene crew assessment. Inter-hospital transfers and cancelled dispatches (e.g., involvement of the continuing care service or caller withdrawal) were excluded.

From each medical record, we extracted the following variables: i) demographics: patient age and sex; ii) event characteristics: EMS response time of the first emergency unit, type of vehicle dispatched (BLS or ALS ambulance), location of the event (home, public place, workplace, street, sport facility, other), province, and urbanisation level (rural or urban); iii) clinical information: presumed cause of cardiac arrest (e.g., cardiovascular, respiratory, metabolic, traumatic, neurological, drug overdose, gastrointestinal, other, or unknown), as recorded by the responding unit; iv) bystander response: whether CPR was initiated (yes/no), and if so, whether it was started independently or dispatcher-assisted; v) outcomes: death or return of spontaneous circulation (ROSC) in the out-of-hospital setting.

The study was approved by the local Ethics Committee (CE187/2024). Data were extracted by authorized clinical personnel, anonymized with progressive numerical codes, and stored in an encrypted database developed using Microsoft Excel.

Statistical analysis

Categorical variables were summarized as absolute counts and percentages, while continuous variables were reported as means with standard deviations or medians with interquartile ranges, as appropriate. Group comparisons for categorical variables were performed using the chi-square test; when expected cell counts were <10, Fisher's exact test was applied. A p-value <0.05 was considered statistically significant. Statistical analyses were performed using Stata version 19 (Stata-Corp, College Station, TX, USA).

RESULTS

A total of 1,646 suspected OHCA cases were identified. Of these, 721 were excluded because on-scene crews did not confirm cardiac arrest, 18 due to cancelled dispatches, and 2 because they involved urgent inter-hospital transfers for time-dependent conditions. In total, 905 cases of cardiac arrest were confirmed during the study period (Table 1).

In 2022, the annual incidence of OHCA was 106 per 100,000 inhabitants (95% CI: 99-113). Most patients were male (57%), with a mean age of 76.4 years (± 16). On average, men were younger than women (73.4 vs 80.5 years). The likely cause was mainly related to cardiovascular issues. Most events occurred at home (87%) and were managed by an ALS ambulance staffed with both a physician and a critical care nurse (80.3%), with a median response time of 8 minutes (IQR 5-13). In particular, the EMS response time was ≤ 5 minutes in 31.7% of cases (n=287) and ≤ 10 minutes in 64.3%.

In 28.8% of cases, medical records did not specify whether bystander CPR was performed. Among the 644 cases with available data, bystander CPR was reported in 39.9% (n=257), mostly initiated independently (90.7%).

ROSC in the out-of-hospital setting was achieved in 6.4% of patients. For this group of patients, the median EMS response time was 7 minutes (IQR 4-10).

Comparisons between cases with and without bystander-initiated CPR are reported in Table 2. Bystander CPR was significantly less frequent in OHCA cases occurring at home compared with other locations ($p < 0.001$). Similarly, the likelihood of bystander intervention was lower in urban areas ($p = 0.002$), where median EMS arrival time was 6 minutes compared with 11 minutes in rural settings ($p < 0.001$).

Overall, bystander CPR was associated with a higher probability of achieving ROSC in the out-of-hospital setting ($p < 0.001$). Specifically, ROSC was more frequent in the home setting compared with other locations ($p < 0.001$), while no significant difference was observed between rural and urban areas ($p = 0.415$).

DISCUSSION

The findings of this study, based on data from an Emergency Medical Dispatch Centre in Piedmont

Table 1

Demographic and clinical characteristics of the 905 confirmed OHCA cases

	N=905	%
Sex, male*	518	57.24
Age, in years*		
<65	168	18.58
65-74	160	17.70
75-84	239	26.44
≥ 85	337	37.28
Presumed cause		
Cardiovascular	875	96.69
Neurological	19	2.10
Respiratory	4	0.44
Other	4	0.44
Trauma	2	0.22
Unknown causes	1	0.11
Province*		
n. 1	359	39.71
n. 2	187	20.69
n. 3	180	19.91
n. 4	172	19.03
Other	6	0.66
Level of urbanization*		
Rural	453	50.11
Urban	451	49.89
Location of the event		
Home	788	87.07
Street	45	4.97
Public place	12	1.33
Workplace	3	0.33
Sport facility	3	0.33
Other	54	5.97
Type of vehicle dispatched*		
ALS with physician and nurse	726	80.31
BLS	152	16.81
Helicopter	18	1.99
ALS with nurse	8	0.88
Arrival time		
Median time (Q1-Q3), in minutes	8 (5-13)	
Bystander CPR		
No	387	42.76
Yes	257	28.39
Not reported	261	28.83
CPR initiation by bystanders		
Independently	233	90.7
Dispatcher-assisted	24	9.3
Outcome*		
Death	843	93.56
ROSC in the out-of-hospital setting	58	6.44

*Missing data: sex (n=2), age (n=1), province (n=1), level of urbanization (n=1), type of vehicle dispatched (n=1), outcome (n=4); OHCA: out-of-hospital cardiac arrest; ALS: advanced life support; BLS: basic life support; CPR: cardiopulmonary resuscitation; ROSC: return of spontaneous circulation; Q1-Q3: first-third quartile.

during 2022, offer new insights into the epidemiology of OHCA in this region. More than 40% of cases occurred in patients younger than 75 years, confirming that OHCA also affects a considerable proportion of relatively younger individuals. Bystander CPR was reported in 39.9% of cases with available data. CPR was most frequently initiated independently, and its occurrence was strongly associated with a higher probability of achieving ROSC in the out-of-hospital setting. Moreover, both home location and urban setting were

Table 2

Comparison of OHCA cases with and without bystander-initiated cardiopulmonary resuscitation

	Bystander CPR initiated N=257	Bystander CPR not initiated N=387	P-value
Sex, male*	154 (59.92)	219 (56.59)	0.444
Age, in years*			
<65	55 (21.40)	72 (18.65)	0.255
65-74	59 (22.96)	72 (18.65)	
75-84	70 (27.24)	107 (27.73)	
≥85	73 (28.40)	135 (34.88)	
Presumed cause			
Cardiovascular	246 (95.72)	376 (97.16)	0.325
Other cause	11 (4.28)	11 (2.84)	
Province**			
n. 1	114 (44.53)	141 (36.81)	0.280
n. 2	49 (19.14)	83 (21.67)	
n. 3	47 (18.36)	82 (21.41)	
n. 4	46 (17.97)	77 (20.11)	
Level of urbanization*			
Rural	149 (57.98)	176 (45.60)	0.002
Urban	108 (42.02)	210 (54.40)	
Location of the event			
Home	183 (71.21)	358 (92.51)	<0.001
Street	24 (9.34)	14 (3.62)	
Public setting (public place, workplace, sport facility)	16 (6.23)	1 (0.25)	
Other	34 (13.22)	14 (3.62)	
Outcome			
Death	211 (83.07)	376 (97.41)	<0.001
ROSC in the out-of-hospital setting	43 (16.93)	10 (2.59)	

*Missing data: sex (n=2), age (n=1), level of urbanization (n=1); **for the province variable, four cases classified as "Other" were excluded; therefore, the denominator in the "Bystander CPR not initiated" group was 383.

OHCA: out-of-hospital cardiac arrest; CPR: cardiopulmonary resuscitation; ROSC: return of spontaneous circulation.

Bold values indicate statistically significant p-values ($p < 0.05$).

linked to a lower likelihood of bystander intervention.

We identified an annual incidence of 106 OHCA per 100,000 inhabitants. This figure is consistent with European data, which report a mean incidence of 82.3 per 100,000 (95% CI: 31-243) [15] and aligns with the pooled estimate of a recent systematic review of 42 Italian studies, which found an incidence of approximately 86 per 100,000 (95% CI: 10-190) [6]. Although within the expected range, our estimate is slightly higher than both the Italian and European averages. Several factors may account for this difference, including the older age structure of the Piedmont population compared with the national average [26, 27] differences in EMS organization and case ascertainment [28] and potential regional variations in cardiovascular risk profiles, comorbidity burden, and lifestyle factors [29].

Notably, bystander CPR was initiated in almost 40% of cases. Although this estimate may be underestimated due to missing documentation in over one third of the medical records, the observed proportion falls below the European average (61%) [15], while exceeding pooled national estimates (26%) [6]. Importantly, with the observed higher likelihood of ROSC, our findings confirm the crucial role of bystander-initiated CPR in improving clinical outcomes [30], while carrying minimal risk of injury for patients [31]. To further increase CPR initiation, some system-level strategies have been proposed. For example, smartphone applications that alert trained volunteer responders in the vicinity of a

cardiac arrest can reduce response times and improve the quality of resuscitation [14, 32]. This approach may be particularly valuable in settings where EMS arrival is delayed or where the presence of potential bystanders is limited, such as private homes or sparsely populated regions. In such contexts, the systematic involvement of first responders such as police officers or firefighters may prove especially effective, as they are often able to reach patients before medical teams, thereby contributing to higher survival rates [33, 34].

Most bystanders initiated CPR independently, without guidance from the Emergency Centre. This pattern may reflect the positive impact of regional public training initiatives and community awareness campaigns, which likely increased laypersons' confidence in responding to cardiac arrest. At the same time, it highlights a potential underutilization of dispatcher-assisted CPR, a key system-level intervention known to increase CPR rates and improve patient outcomes, particularly when bystanders are untrained or hesitant [35]. In Italy, the provision of standardized pre-arrival instructions by emergency dispatchers, covering basic CPR and the use of AEDs while awaiting emergency vehicles, is mandated by national legislation [36]. Nonetheless, notable regional differences persist in the adoption of protocols, availability of technological resources, and continuing education programs for dispatchers [37]. Optimizing dispatcher-assisted CPR depends not only on the technical content of the instructions but also on how they

are delivered. Innovations such as video support during emergency calls, simplified and standardized language, and the use of metronome cues or motivational prompts can enhance clarity, sustain compression quality, and increase bystander confidence [38]. Equally important are dispatcher-related factors, including dedicated training and the adoption of structured protocols or centralized systems, which together may strengthen the consistency and effectiveness of guidance provided to callers [38].

In our cohort, ROSC was achieved in only 6% of cases, a lower proportion compared with international studies [15, 30]. However, it is important to note that our analysis considered only ROSC documented in the pre-hospital context. It is therefore plausible that additional cases of ROSC occurred after hospital admission, which would not have been captured in our data.

Interestingly, we observed a higher frequency of bystander CPR initiation in rural areas compared with urban settings, a trend consistent with previous reports [39, 40]. This phenomenon may be partly explained by longer EMS response times in less populated areas, which provide bystanders with greater opportunity to intervene, as well as by psychological factors such as a reduced bystander effect and lower “urban overload” in low-density settings [39].

Similarly, we found that witness intervention was more frequent in public spaces such as streets or other non-residential locations, whereas bystander CPR rates were significantly lower in private homes. This finding is consistent with Ting *et al.* [41], who also reported a lower incidence of bystander intervention in residential settings. Such differences may reflect the greater availability of potential witnesses in public areas, while arrests occurring at home are disadvantaged by fewer bystanders and limited access to life-saving devices. The findings highlight the need to strengthen community preparedness and system-level strategies to increase bystander CPR rates. From a clinical and educational perspective, integrating BLS training into school curricula, workplaces, and community programs could improve public responsiveness and confidence in emergency situations. Organizationally, enhancing dispatcher-assisted CPR through structured protocols, continuous dispatcher education, and the use of real-time digital tools may further optimize early intervention. For policymakers, the results support the establishment of standardized regional or national cardiac arrest registries and the implementation of coordinated awareness campaigns to promote a “culture of rescue”, ultimately improving survival outcomes after OHCA.

Although the composition of EMS crews was not captured in our dataset, emerging evidence suggests that prehospital models incorporating advanced nurs-

ing roles may influence clinical outcomes. Recent studies report that the presence of emergency nurse practitioners within EMS teams can increase ROSC rates up to fourfold, highlighting the potential contribution of advanced nursing practice in the early phases of cardiac arrest management [42]. In addition, airway management performed by ambulance nurses has been shown to be safe and effective, with high first-pass success rates for supraglottic devices and improving endotracheal intubation performance over time [43].

While the use of data collected over an entire year ensures a comprehensive and representative picture of OHCA cases in the region, some study limitations should be considered. First, the retrospective nature of the data may have introduced inaccuracies. Second, the information on the use of AEDs and data on initial cardiac rhythm were not available, preventing us from assessing their contribution to resuscitation outcomes. Third, survival data beyond the pre-hospital phase were not collected, which restricted our ability to evaluate longer-term outcomes such as survival to hospital discharge or neurological status. Finally, the registry did not allow us to distinguish between interventions performed by lay bystanders and those initiated by trained lay-responders, limiting our capacity to explore potential differences between these groups. These gaps underscore the importance of adopting more detailed and standardized data collection frameworks, to ensure consistent definitions, reduce heterogeneity, and improve the comparability of outcomes across registries [44].

CONCLUSIONS

This study offers an updated overview of OHCA in the Piedmont region, with an incidence comparable to national and European data. Bystander CPR, although associated with higher ROSC rates, was initiated in fewer than half of cases, particularly in the home setting and rural context. These findings stress the importance of strengthening public training and optimizing dispatcher-assisted CPR to improve outcomes.

Conflicts of interest statement

The Authors declare no conflicts of interest related to this study.

Generative AI declaration statement

During the preparation of this work the Authors used ChatGPT (OpenAI) in order to improve language and readability. After using this tool/service, the Authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Received on 9 December 2025.

Accepted on 28 April 2026.

REFERENCES

- Gräsner JT, Lefering R, Koster RW, Masterson S, Böttiger BW, Herlitz J, et al. EuReCa ONE-27 Nations, ONE Europe, ONE Registry: a prospective one month analysis of out-of-hospital cardiac arrest outcomes in 27 countries in Europe. *Resuscitation*. 2016;105:188-95.
- Böttiger BW, Becker LB, Kern KB, Lippert F, Lockey A, Ristagno G, Semeraro F, Wingen S. BIG FIVE strategies for survival following out-of-hospital cardiac arrest.

- Eur J Anaesthesiol. 2020;37(11):955-8. doi: 10.1097/EJA.0000000000001247
3. Gräsner JT, Herlitz J, Tjelmeland IBM, Wnent J, Masterson S, Lilja G, et al. European Resuscitation Council Guidelines 2021: epidemiology of cardiac arrest in Europe. *Resuscitation*. 2021;161:61-79.
4. Kiguchi T, Okubo M, Nishiyama C, Maconochie I, Ong MEH, Kern KB, Wyckoff MH, McNally B, Christensen EF, Tjelmeland I, Herlitz J, Perkins GD, Booth S, Finn J, Shahidah N, Shin SD, Bobrow BJ, Morrison LJ, Salo A, Baldi E, Burkart R, Lin CH, Jouven X, Soar J, Nolan JP, Iwami T. Out-of-hospital cardiac arrest across the world: first report from the International Liaison Committee on Resuscitation (ILCOR). *Resuscitation*. 2020;152:39-49. doi: 10.1016/j.resuscitation.2020.02.044
5. Zheng J, Lv C, Zheng W, Zhang G, Tan H, Ma Y, Zhu Y, Li C, Han X, Yan S, Pan C, Zhang J, Hou Y, Wang C, Bian Y, Liu R, Cheng K, Ma J, Zheng Z, Song R, Wang M, Gu J, McNally B, Ong MEH, Chen Y, Xu F; BASIC-OHCA Coordinators and Investigators. Incidence, process of care, and outcomes of out-of-hospital cardiac arrest in China: a prospective study of the BASIC-OHCA registry. *Lancet Public Health*. 2023;8(12):e923-32. doi: 10.1016/S2468-2667(23)00173-1
6. Scquizzato T, Gamberini L, D'Arrigo S, Galazzi A, Babini G, Losiggio R, et al. Incidence, characteristics, and outcome of out-of-hospital cardiac arrest in Italy: a systematic review and meta-analysis. *Resusc Plus*. 2022;12:100329.
7. Gräsner JT, Wnent J, Herlitz J, Perkins GD, Lefering R, Tjelmeland I, Koster RW, Masterson S, Rossell-Ortiz F, Maurer H, Böttiger BW, Moertl M, Mols P, Alihodžić H, Hadžibegović I, Ioannides M, Truhlář A, Wissenberg M, Salo A, Escutnaire J, Nikolaou N, Nagy E, Jonsson BS, Wright P, Semeraro F, Clarens C, Beesems S, Cebula G, Correia VH, Cimpoesu D, Raffay V, Trenkler S, Markota A, Strömsøe A, Burkart R, Booth S, Bossaert L. Survival after out-of-hospital cardiac arrest in Europe – Results of the EuReCa TWO study. *Resuscitation*. 2020;148:218-26.
8. Perkins GD, Graesner JT, Semeraro F, Olasveengen T, Soar J, Lott C, Van de Voorde P, Madar J, Zideman D, Mentzelopoulos S, Bossaert L, Greif R, Monsieurs K, Svavarsdóttir H, Nolan JP; European Resuscitation Council Guideline Collaborators. European Resuscitation Council Guidelines 2021: executive summary. *Resuscitation*. 2021;161:1-60. doi: 10.1016/j.resuscitation.2021.02.003. Erratum in: *Resuscitation*. 2021;163:97-8. doi: 10.1016/j.resuscitation.2021.04.012
9. Song J, Guo W, Lu X, Kang X, Song Y, Gong D. The effect of bystander cardiopulmonary resuscitation on the survival of out-of-hospital cardiac arrests: a systematic review and meta-analysis. *Scand J Trauma Resusc Emerg Med*. 2018;26(1):86. doi: 10.1186/s13049-018-0552-8
10. Sasson C, Rogers MAM, Dahl J, Kellermann AL. Predictors of survival from out-of-hospital cardiac arrest: a systematic review and meta-analysis. *Circ Cardiovasc Qual Outcomes*. 2010;3(1):63-81.
11. Semeraro F, Greif R, Böttiger BW, Burkart R, Cimpoesu D, Georgiou M, et al. European Resuscitation Council Guidelines 2021: systems saving lives. *Resuscitation*. 2021;161:80-97.
12. Schroeder DC, Semeraro F, Greif R, Bray J, Morley P, Parr M, Kondo Nakagawa N, Iwami T, Finke SR, Malta Hansen C, Lockey A, Del Rios M, Bhanji F, Sasson C, Schexnayder SM, Scquizzato T, Wetsch WA, Böttiger BW; International Liaison Committee on Resuscitation. KIDS SAVE LIVES: basic life support education for schoolchildren: a narrative review and scientific statement from the International Liaison Committee on Resuscitation. *Circulation*. 2023;147(24):1854-68. doi: 10.1161/CIR.0000000000001128. Erratum in: *Circulation*. 2023;148(1):e1. doi: 10.1161/CIR.0000000000001166
13. Böttiger BW, Lockey A, Aickin R, Castren M, De Caen A, Escalante R, et al. All citizens of the world can save a life – The World Restart a Heart (WRAH) initiative starts in 2018. *Resuscitation*. 2018;128:188-90.
14. Yu Y, Meng Q, Munot S, Nguyen TN, Redfern J, Chow CK. Assessment of community interventions for bystander cardiopulmonary resuscitation in out-of-hospital cardiac arrest: a systematic review and meta-analysis. *JAMA Netw Open*. 2020;3(7):e209256.
15. Gräsner JT, Wnent J, Lefering R, Herlitz J, Masterson S, Maurer H, Perkins GD, Ortiz FR, Tjelmeland IBM, Kamishi D, Moertl M, Mols P, Alihodžić H, Ioannides M, Truhlář A, Baert V, Nikolaou N, Molnar N, Jonsson BS, Semeraro F, Kriksionaitiene A, Clarens C, Giordimaina C, Stieglis R, Zadlo A, Correia VH, Cimpoesu D, Raffay V, Trenkler S, Strnad M, Ruiz JI, Strömsøe A, Wilmes A, Booth S, Bossaert L; EuReCa-THREE collaborator group. European registry of cardiac arrest study THREE (EuReCa-THREE) – EMS response time influence on outcome in Europe. *Resuscitation*. 2026;223:110704. doi: 10.1016/j.resuscitation.2025.110704
16. Aldridge ES, Perera N, Ball S, Finn J, Bray J. A scoping review to determine the barriers and facilitators to initiation and performance of bystander cardiopulmonary resuscitation during emergency calls. *Resusc Plus*. 2022;11:100290. doi: 10.1016/j.resplu.2022.100290
17. Farquharson B, Dixon D, Williams B, Torrens C, Philpott M, Laidlaw H, et al. The psychological and behavioural factors associated with laypeople initiating CPR for out-of-hospital cardiac arrest: a systematic review. *BMC Cardiovasc Disord*. 2023;23(1):19.
18. Van Nieuwenhuizen BP, Oving I, Kunst AE, Daams J, Blom MT, Tan HL, et al. Socio-economic differences in incidence, bystander cardiopulmonary resuscitation and survival from out-of-hospital cardiac arrest: a systematic review. *Resuscitation*. 2019;141:44-62.
19. Shekhar A, Narula J. Globally, GDP per capita correlates strongly with rates of bystander CPR. *Ann Glob Health*. 2022;88(1):36. doi: 10.5334/aogh.3624
20. Uny I, Angus K, Duncan E, Dobbie F. Barriers and facilitators to delivering bystander cardiopulmonary resuscitation in deprived communities: a systematic review. *Perspect Public Health*. 2023;143(1):43-53. doi: 10.1177/17579139211055497
21. Cheng Y, Zhang C, Chen L, Liu H, He W, Shi Z. Public participation willingness in out-of-hospital cardiopulmonary resuscitation: a systematic review and meta-analysis. *Int J Nurs Sci*. 2025;12(2):192-9. doi: 10.1016/j.ijnss.2025.02.012
22. Siddiqui FJ, McNally B, Gräsner JT, Ho AFW, Tjelmeland I, Iwami T, Bray J, Wnent J, Lynn Lim S, Pin Pek P, Do Shin S, Ong MEH; GOHCA Group. Towards advancing out-of-hospital cardiac arrest (OHCA) registries globally: considerations from the global OHCA registry (GOHCA) collaborative. *Resusc Plus*. 2024;18:100615. doi: 10.1016/j.resplu.2024.100615
23. Azienda Zero. La storia di Azienda Zero. Torino: Regione Piemonte. [last access 22/09/2025]. Available from: <https://www.aziendazero.piemonte.it/azienda/la-storia-di-azienda-zero/>
24. Tabi M, Perel N, Taha L, Amsalem I, Hitter R, Maller T, Manassra M, Karmi M, Zacks N, Levy N, Shrem M, Marmor D, Gavriel D, Jarjoui A, Shuvy M, Asher E; Jeru-

- salem Platelets Thrombosis and Intervention in Cardiology (JUPITER-9) Study Group. Out of hospital cardiac arrest – new insights and a call for a worldwide registry and guidelines. *BMC Emerg Med.* 2024;24(1):140. doi: 10.1186/s12873-024-01060-4
25. von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP; STROBE Initiative. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *J Clin Epidemiol.* 2008;61(4):344-9. doi: 10.1016/j.jclinepi.2007.11.008
26. Eurostat. Population structure and ageing. Luxembourg: Eurostat; 2026. Available from: https://ec.europa.eu/eurostat/statistics-explained/index.php?title=Population_structure_and_ageing
27. Istituto Nazionale di Statistica. Il Censimento permanente della popolazione in Piemonte. Roma: ISTAT; 2025. Available from: https://www.istat.it/wp-content/uploads/2025/04/Censimento-permanente-popolazione_Anno-2023_Piemonte.pdf
28. Parvez SS, Parvez S, Ullah I, Parvez SS, Ahmed M. Systematic review on the worldwide disparities in the frequency and results of Emergency Medical Services (EMS) and response to out-of-hospital cardiac arrest (OHCA). *Cureus.* 2024;16(6):e63300. doi: 10.7759/cureus.63300
29. SCORE2 Working Group and ESC Cardiovascular Risk Collaboration. SCORE2 risk prediction algorithms: new models to estimate 10-year risk of cardiovascular disease in Europe. *Eur Heart J.* 2021;42(25):2439-54. doi: 10.1093/eurheartj/ehab309
30. Yan S, Gan Y, Jiang N, Wang R, Chen Y, Luo Z, Zong Q, Chen S, Lv C. The global survival rate among adult out-of-hospital cardiac arrest patients who received cardiopulmonary resuscitation: a systematic review and meta-analysis. *Crit Care.* 2020;24(1):61. doi: 10.1186/s13054-020-2773-2
31. Ng JYX, Sim ZJ, Siddiqui FJ, Shahidah N, Leong BS, Tiah L, Ng YY, Blewer A, Arulanandam S, Lim SL, Ong MEH, Ho AFW. Incidence, characteristics and complications of dispatcher-assisted cardiopulmonary resuscitation initiated in patients not in cardiac arrest. *Resuscitation.* 2022;170:266-73. doi: 10.1016/j.resuscitation.2021.09.022
32. Kern M, Jansen G, Strickmann B, Kerner T. Advancements in public first responder programs for out-of-hospital cardiac arrest: an updated literature review. *Rev Cardiovasc Med.* 2025;26(1):26140. doi: 10.31083/rcm26140
33. Hansen CM, Kragholm K, Granger CB, Pearson DA, Tyson C, Monk L, Corbett C, Nelson RD, Dupre ME, Fosbøl EL, Strauss B, Fordyce CB, McNally B, Jollis JG. The role of bystanders, first responders, and emergency medical service providers in timely defibrillation and related outcomes after out-of-hospital cardiac arrest: Results from a statewide registry. *Resuscitation.* 2015;96:303-9. doi: 10.1016/j.resuscitation.2015.09.002
34. El-Zein RS, Kennedy KF, Chang P. Out-of-hospital cardiac arrest survival when CPR is initiated by first responders. *Resuscitation.* 2023;190:109914. doi: 10.1016/j.resuscitation.2023.109914
35. Nikolaou N, Dainty KN, Couper K, Morley P, Tijssen J, Vaillancourt C; International Liaison Committee on Resuscitation's (ILCOR) Basic Life Support and Pediatric Task Forces. A systematic review and meta-analysis of the effect of dispatcher-assisted CPR on outcomes from sudden cardiac arrest in adults and children. *Resuscitation.* 2019;138:82-105. doi: 10.1016/j.resuscitation.2019.02.035
36. Italia. Legge 4 agosto 2021, n. 116. Disposizioni in materia di utilizzo dei defibrillatori semiautomatici e automatici esterni. *Gazzetta Ufficiale – Serie Generale n. 193*, 13 agosto 2021. Available from: <https://www.gazzettaufficiale.it/eli/id/2021/08/13/21G00126/sg>
37. Imbriaco G, Canova GS, Righi L, Tararan S, Di Mario G, Ramacciati N. Dispatcher-assisted CPR in Italy: a nationwide survey of current practices and future challenges in emergency medical communication centers. *J Clin Med.* 2025;14(2):637. doi: 10.3390/jcm14020637
38. Dainty KN, Debaty G, Waddick J, Vaillancourt C, Malta Hansen C, Olasveengen T, Bray J; International Liaison Committee on Resuscitation Basic Life Support Task Force. Interventions to optimize dispatcher-assisted CPR instructions: a scoping review. *Resusc Plus.* 2024;19:100715. doi: 10.1016/j.resplu.2024.100715
39. Nikonowicz P, Huebinger R, Al-Araji R, Schulz K, Gill J, Villa N, McNally B, Bobrow B. Rural cardiac arrest care and outcomes in Texas. *Am J Emerg Med.* 2024;78:57-61. doi: 10.1016/j.ajem.2023.12.033
40. Gregers MCT, Møller SG, Kjoelbye JS, Jakobsen LK, Grabmayr AJ, Kragh AR, Hansen CM, Torp-Pedersen C, Andelius L, Ersbøll AK, Folke F. Association of degree of urbanization and survival in out-of-hospital cardiac arrest. *J Am Heart Assoc.* 2023;12(10):e8322. doi: 10.1161/JAHA.122.028449
41. Ting PZY, Ho AFW, Lin X, Shahidah N, Blewer A, Ng YY, Leong BS, Gan HN, Mao DR, Chia MYC, Cheah SO, Ong MEH. Nationwide trends in residential and non-residential out-of-hospital cardiac arrest and differences in bystander cardiopulmonary resuscitation. *Resuscitation.* 2020;151:103-10. doi: 10.1016/j.resuscitation.2020.03.007
42. Kaewpaengchan W, Wongtanasarasin W, Khampeera W, Wittayachamnankul B. The impact of emergency nurse practitioners on short-term survival in out-of-hospital cardiac arrest. *Australas Emerg Care.* 2025;28(3):163-6. doi: 10.1016/j.aucc.2025.02.002
43. Doleman LC, de Jong JFF, van Eeden VGM, Schober P, Hollmann MW, van Schuppen H. Airway management by ambulance nurses during out-of-hospital cardiac arrest. *Resusc Plus.* 2025;25:100999. doi: 10.1016/j.resplu.2025.100999
44. Gräsner JT, Bray JE, Nolan JP, Iwami T, Ong MEH, Finn J, McNally B, Nehme Z, Sasson C, Tijssen J, Lim SL, Tjelmeland I, Wnent J, Dicker B, Nishiyama C, Doherty Z, Welsford M, Perkins GD; International Liaison Committee on Resuscitation. Cardiac arrest and cardiopulmonary resuscitation outcome reports: 2024 update of the Utstein out-of-hospital cardiac arrest registry template. *Resuscitation.* 2024;201:110288. doi: 10.1016/j.resuscitation.2024.110288

Leptospirosis outbreak in an urban setting: epidemiological and clinical characteristics of the 2025 epidemic in Sarajevo Canton, Bosnia and Herzegovina

Anes Jogunčić¹, Asaf Salčinović¹, Agnesa Porović Hodžić², Anisa Bajramović¹, Zlatan Hamza², Aida Pošković-Bajraktarević¹, Aysun Tekin³ and Lal Amos³

¹Department of Epidemiology, Public Health Institute of Canton Sarajevo, Sarajevo, Bosnia and Herzegovina

²Department for Environmental Protection and Improvement, Public Health Institute of Canton Sarajevo, Sarajevo, Bosnia and Herzegovina

³Division of Pulmonary and Critical Care Medicine, Department of Internal Medicine, Mayo Clinic, Rochester, MN

Abstract

Background. Leptospirosis is an emerging public-health concern in urban settings, but data from temperate European cities remain limited. This study describes the 2025 leptospirosis outbreak in Sarajevo Canton, Bosnia and Herzegovina.

Methods. A descriptive observational study was conducted using mandatory surveillance data and a supplementary epidemiological questionnaire based on the WHO Leptospirosis Surveillance Tool. Cases were classified according to the ECDC case definition. Epidemiological, clinical, laboratory, and exposure characteristics were analysed.

Results. A total of 119 cases were recorded; 63.9% were male and the mean age was 45.8±13.2 years. The overall incidence was 2.84 per 10,000 population, highest in Novi Grad municipality. Common symptoms included weakness, fever, chills, myalgia, and headache. Elevated liver enzymes were the only independent predictor of hospitalisation.

Conclusion. The outbreak indicates a shift toward urban leptospirosis, likely linked to environmental contamination and rodent activity rather than traditional rural or flood-related exposure.

Key words

- leptospirosis
- outbreak
- urban environment
- Sarajevo
- epidemiology

INTRODUCTION

Leptospirosis is a globally distributed zoonotic disease caused by pathogenic spirochaetes of the genus *Leptospira*, which colonise the renal tubules of animal hosts and are excreted in urine. Numerous species act as intermediates, including rodents, livestock, and companion animals, while humans are incidental hosts, typically infected through direct or indirect contact with contaminated water or soil [1]. The genus *Leptospira* comprises more than 60 species grouped into 24 serogroups and over 300 serovars, reflecting substantial genetic diversity [2]. The pathogen can survive for prolonged periods in warm, humid environments, explaining its predominance in tropical and subtropical regions. However, leptospirosis is increasingly recognised as a re-emerging infection in

temperate and urban settings, where exposure may occur through environmental contamination and rodent infestation [3-5].

Globally, more than one million human cases are estimated to occur annually, resulting in approximately 59,000 deaths [3, 6]. The clinical presentation ranges from mild influenza-like illness to severe forms such as Weil's disease, characterised by jaundice and renal failure, and leptospirosis pulmonary haemorrhagic syndrome, which carries a fatality rate exceeding 50% [7]. In its most severe form, leptospirosis may rapidly progress to multiorgan dysfunction, including circulatory collapse, acute respiratory distress syndrome (ARDS), and hepatic failure. Patients with critical disease often require intensive care, including vasopressor support, mechanical ventilation, and renal replacement therapy,

and mortality remains high despite optimal management [8]. Owing to its non-specific early symptoms—commonly fever, myalgia, headache, and chills leptospirosis is frequently misdiagnosed, delaying appropriate antibiotic treatment [7].

In Europe, leptospirosis incidence declined during the latter half of the twentieth century; however, recent studies indicate a recrudescence, particularly in urban environments [4, 5]. This trend highlights the growing importance of urban reservoirs and suboptimal environmental sanitation in sustaining transmission [9]. Nevertheless, comprehensive epidemiological data for Europe remain limited, and the true burden of urban and peri-urban transmission is likely underestimated [10]. Environmental conditions play a central role in leptospirosis transmission by influencing the survival and persistence of pathogenic *Leptospira* spp. The bacteria remains viable for extended periods in fresh water and moist soil under moderate temperatures and neutral-to-alkaline pH conditions [11]. Several studies report higher recovery rates from soil than from surface water, suggesting soil as an important long-term reservoir [9–11]. During heavy rainfall, re-suspension of contaminated soil particles may increase human exposure [12]. Distinct ecological transmission pathways have been described, with environmental distribution varying by setting [11]. Hydro-meteorological factors are consistently associated with leptospirosis risk. Periods of intense rainfall or flooding are commonly followed by increased human incidence, particularly when drainage, sanitation, and waste-management systems are disrupted [12–15]. Urban infrastructure further shapes leptospirosis dynamics. Blocked drains, accumulated waste, open sewage channels, and unplanned urban development create favourable conditions for bacterial survival and rodent proliferation [12]. Environmental sampling has identified pathogenic *Leptospira* in soil and water even in areas without reported clinical cases, with environmental reservoirs often harbouring greater strain diversity than human infections [1, 16]. Rodents and dogs serve as key maintenance hosts, with intensified environmental contamination often following heavy rainfall or flooding [17]. Evidence from Bosnia and Herzegovina shows that leptospirosis circulates among dogs, with higher seropositivity in older animals and during spring and autumn, supporting the role of domestic and stray animals as potential reservoirs and contributors to seasonal transmission patterns [18].

METHODOLOGY

The 2025 outbreak in Sarajevo Canton provides an opportunity to characterise leptospirosis in a temperate urban European setting and to better define environmental risk factors contributing to this re-emerging zoonosis. Epidemiological surveillance of leptospirosis was conducted through mandatory notifications from primary care physicians and infectious disease specialists at the Clinical Centre of the University of Sarajevo. Cases were classified as probable or confirmed according to the European Centre for Disease Prevention and Control (ECDC) case definition. Harmonised case definitions are essential for effective surveillance and

comparability across data collected in EU/EEA Member States. Through Commission Implementing Decision (EU) 2018/945, the ECDC provides standardised clinical, laboratory, and epidemiological criteria to ensure consistent reporting and timely outbreak detection [19]. The EU case definition reflects the broad clinical spectrum of leptospirosis. A case meets clinical criteria if fever is present or if at least two of the following symptoms occur: chills, headache, myalgia, conjunctival suffusion, rash, mucocutaneous bleeding, jaundice, myocarditis, meningitis, renal impairment, or acute respiratory symptoms. These manifestations range from mild febrile illness to severe disease, including Weil's disease and pulmonary haemorrhage [19]. Laboratory confirmation requires evidence of infection with pathogenic *Leptospira* spp., including isolation of the organism, detection of *Leptospira* DNA by polymerase chain reaction (PCR), identification in tissue by immunofluorescence, or serological confirmation of specific antibodies [19]. A probable case fulfilled clinical criteria and had a relevant epidemiological link—such as exposure to contaminated freshwater, animal contact, or high-risk environments—without laboratory confirmation. A confirmed case required clinical criteria plus laboratory confirmation, regardless of documented exposure. This classification framework supports rapid public health action while ensuring diagnostic accuracy [19]. In Canton of Sarajevo serological testing for leptospirosis was performed using chemiluminescent immunoassay (CLIA) in a referral laboratory. In addition, PCR testing was conducted to exclude other infectious diseases that could potentially lead to cross-reactivity in serological assays. Data on *Leptospira* serogroups were not available, as serogroup identification was not performed.

In the Federation of Bosnia and Herzegovina, communicable disease surveillance is based on mandatory reporting under the Law on Health Care [20], the Law on the Protection of the Population from Infectious Diseases [21], and the Rulebook on the Procedure for Reporting Communicable Diseases [22]. Although routine epidemiological interviews for leptospirosis are not formally required under the Rulebook, the unusually high number of reported cases prompted the development of a targeted survey. This survey was adapted from the WHO Leptospirosis Surveillance Tool and used to collect additional data on environmental exposures, occupational risks, and potential transmission routes. In accordance with national legislation, anti-epidemic measures were implemented for each reported case, including rodent control (deratisation) and disinfection of public transport vehicles. The outbreak was formally reported to the veterinary inspection to support coordinated animal and environmental surveillance. Following a rapid increase in case numbers and on the recommendation of epidemiologists from the Institute of Public Health of Sarajevo Canton, a leptospirosis epidemic was officially declared on 15 May 2025 and lifted on 29 September 2025. Municipal services were instructed to intensify sanitation measures, and systematic deratisation was conducted across the entire canton.

Communication

Effective communication was a central component of the outbreak response. After the initial notification of cases presenting as fever of unknown origin, the Public Health Institute of Sarajevo Canton issued an official alert to all healthcare institutions, calling for heightened vigilance, diagnostic consideration of leptospirosis, and timely reporting. In line with ECDC guidance on outbreak risk communication [23] and WHO recommendations for clear, timely, and transparent public communication [24], particular emphasis was placed on accuracy, consistency, and evidence-based messaging, especially in the post-COVID-19 context where public trust is fragile. Once epidemic conditions were formally met, a press conference was organised to inform the public about the disease, current case numbers, geographical distribution, clinical severity based on hospital data, transmission routes, and recommended preventive measures. Communication strategies prioritised clear, factual, and reassuring messages to reduce misinformation and unnecessary social concern. Daily situation updates were disseminated using infographics published on the Institute's website and distributed through established media mailing lists. Preventive guidelines were issued for educational institutions, nursing homes, and pet owners, alongside recommendations related to outdoor activities and contact with potentially contaminated environments.

Statistical analysis

All statistical analyses were performed using RStudio (version 2024.12.0+467, Posit Software, Boston, MA, USA). Descriptive statistics were used to summarise demographic, clinical, laboratory, and exposure characteristics. Continuous variables (e.g., age) were expressed as mean $\pm$ standard deviation (SD) and compared between groups using the Student's t-test for independent samples. Categorical variables were presented as absolute numbers and percentages (N, %), and differences between groups (male vs female) were assessed using the chi-square (χ^2) test or Fisher's exact test when expected cell counts were below five. The level of statistical significance was set at $p < 0.05$. Incidence rates were calculated per 10,000 inhabitants for each municipality within Sarajevo Canton using 2025 population estimates from the Agency for Statistics of Bosnia and Herzegovina. Spatial distribution of cases was visualised using a choropleth map, with municipalities colour-coded according to incidence levels.

RESULTS

A total of 119 individuals were included, of whom 76 (63.9%) were male and 43 (36.1%) female. The mean age was 45.8 ± 13.2 years, with males significantly younger than females (43.4 ± 12.5 vs 50.1 ± 13.5 years, $p = 0.007$). Most cases occurred in the 30-65-year age group (76.5%), followed by 20-29 years (13.4%), ≥ 66 years (8.4%), and 5-19 years (1.7%). Age distribution did not differ significantly by sex ($p = 0.090$) (Table 1). Confirmed cases accounted for 37.8% and probable cases for 62.2%, with no significant sex difference ($p = 0.281$).

Clinical manifestation

The most frequently reported symptoms were general weakness (87.4%), fever (79.0%), chills (63.0%), muscle pain (61.3%), and headache (61.3%). Confusion occurred in 11.8% of cases, more often in males than females (15.8% vs 4.7%, $p = 0.07$). Other symptoms included vomiting (3.4%), diarrhoea (0.8%), neck stiffness (7.6%), jaundice (1.7%), cough (10.9%), and shortness of breath (7.6%), with no significant sex differences. Laboratory abnormalities were frequently observed, particularly elevated liver enzymes, with 48.7% of patients showing mild to moderate AST/ALT elevation and 11.8% exhibiting severe elevations ($\geq 5 \times$ upper normal limit). Elevated GGT was observed in 21.8% of patients, while bilirubin elevation was rare (1.7%). No significant biochemical differences were found between sexes ($p > 0.05$). The vertical timeline illustrates the typical clinical course during the first 10 days of illness (Figure 1). Early symptoms included fever, chills, general weakness, headache, and occasional cough, often leading to misdiagnosis as a viral illness. A transient improvement around days 3-4 was followed by recurrent high fever, myalgia, and cough on days 5-6. Days 6-7 were characterised by severe myalgia and marked liver enzyme elevation, while bilirubin levels generally remained normal. Most patients sought medical care during this phase, and antibiotic therapy was initiated. Symptoms peaked around days 7-8, with clinical improvement typically observed within 48 hours of treatment initiation.

Risk factors

Overall, 40 participants (33.6%) reported at least one identifiable risk factor, with similar prevalence among males (34.2%) and females (32.6%) ($p = 0.855$). The most frequent exposures were working the soil (9.2%), cleaning basements (4.2%), and recent skin wounds (4.2%). No respondents reported direct contact with flood waters. Outdoor recreational activities were reported by 5.0%. Animal contact was reported by 14.3% of participants, most commonly cats (46%) and parrots (33%), followed by dogs (13%) and other pets. No association was found between pet ownership and sex ($p = 0.533$).

The full logistic regression model significantly improved fit compared with the null model ($\Delta\chi^2 = 18.243$, $p = 0.032$), with moderate explanatory power (Nagelkerke $R^2 = 0.343$; Tjur $R^2 = 0.167$). Elevated AST-ALT values were the only significant predictor of hospitalization (OR = 11.58, $p = 0.039$), indicating substantially increased odds of admission (Table 2). Other symptoms, including chills, general weakness, headache, and cough, were not significant predictors. Very large coefficients for recent skin injury and reported risk factors suggested quasi-separation, resulting in unstable estimates. Age ≥ 50 years and sex were not associated with hospitalization risk.

Incidence of leptospirosis by municipality, Canton Sarajevo, 2025

A total of 119 leptospirosis cases were recorded in Sarajevo Canton, corresponding to an overall incidence of 2.84 per 10,000 population. Incidence varied markedly

Table 1

Demographic, clinical, and laboratory characteristics of patients with leptospirosis, Canton Sarajevo, 2025

Variable	Total N of patients (n=119)	Male patients (n=76)	Female patients (n=43)	p
Demographic characteristics				
N of subjects, n (%)	119 (100%)	76 (63.9%)	43 (36.1%)	
Age (Mean ± SD)	45.8±13.2	43.4±12.5	50.1±13.5	0.007
Age groups	N (%)	N (%)	N (%)	
5-19 years	2 (1.7%)	0 (0%)	2 (2.6%)	0.090
20-29 years	16 (13.4%)	5 (11.6%)	11 (14.5%)	
30-65 years	91 (76.5%)	31 (72.1%)	60 (78.9%)	
66+ years	10 (8.4%)	7 (16.3%)	3 (3.9%)	
Confirmed or probable case				
Confirmed case	45 (37.8%)	26 (34.2%)	19 (44.2%)	0.281
Probable case	74 (62.2%)	50 (65.8%)	24 (55.8%)	
Clinical characteristics				
Hospitalization	9 (7.6%)	7 (8.9%)	2 (4.6%)	0.368
Symptoms				
Vomiting	4 (3.4%)	1 (1.3%)	3 (7.0%)	0.100
Diarrhoea	1 (0.8%)	0 (0%)	1 (2.3%)	0.182
Fever	94 (79.0%)	58 (76.3%)	36 (83.7%)	0.341
Chills	75 (63.0%)	48 (63.2%)	27 (62.8%)	0.968
General weakness	104 (87.4%)	66 (86.8%)	38 (88.4%)	0.809
Confusion	14 (11.8%)	12 (15.8%)	2 (4.7%)	0.070
Conjunctival hyperaemia	10 (8.4%)	7 (9.2%)	3 (7.0%)	0.673
Headache	73 (61.3%)	50 (65.8%)	23 (53.5%)	0.186
Neck stiffness	9 (7.6%)	4 (5.3%)	5 (11.6%)	0.207
Muscle pain	73 (61.3%)	45 (59.2%)	28 (65.1%)	0.525
Jaundice	2 (1.7%)	1 (1.3%)	1 (2.3%)	0.681
Cough	13 (10.9%)	10 (13.2%)	3 (7.0%)	0.299
Shortness of breath	9 (7.6%)	6 (7.9%)	3 (7.0%)	0.856
Laboratory findings				
Hepatocellular injury markers (AST/ALT), n (%)				
None/non-reported elevation	47 (39.5%)	28 (36.8%)	19 (44.2%)	0.791
Mild to moderate elevation ≤5 ULN*	58 (48.7%)	38 (50.0%)	20 (46.5%)	
Acute liver injury >5 ULN*	14 (11.8%)	10 (13.2%)	4 (9.3%)	
Significantly elevated GGT values	26 (21.8%)	19 (25.0%)	7 (16.3%)	0.269
Elevated bilirubin	2 (1.7%)	1 (1.3%)	1 (2.3%)	0.681
Risk factors				
Any risk factor	40 (33.6%)	26 (34.2%)	14 (32.6%)	0.855
Skin injury or wound in the past month	5 (4.2%)	2 (2.6%)	3 (7%)	0.256
Walking barefoot in the past month across meadows or similar areas	2 (1.7%)	1 (1.3%)	1 (2.3%)	0.681
Working the soil (cultivation/gardening)	11 (9.2%)	9 (11.8%)	2 (4.7%)	0.193
Cleaning the basement	5 (4.2%)	3 (3.9%)	2 (4.7%)	0.854
Contact with flood waters	0	0%	0%	x
Activities: hiking, kayaking, rafting, swimming, or similar	6 (5%)	4 (5.3%)	2 (4.7%)	0.883
Having pets	14.3 (%)	12 (15.8%)	5 (11.6%)	0.533

*ULN: upper limit of normal.

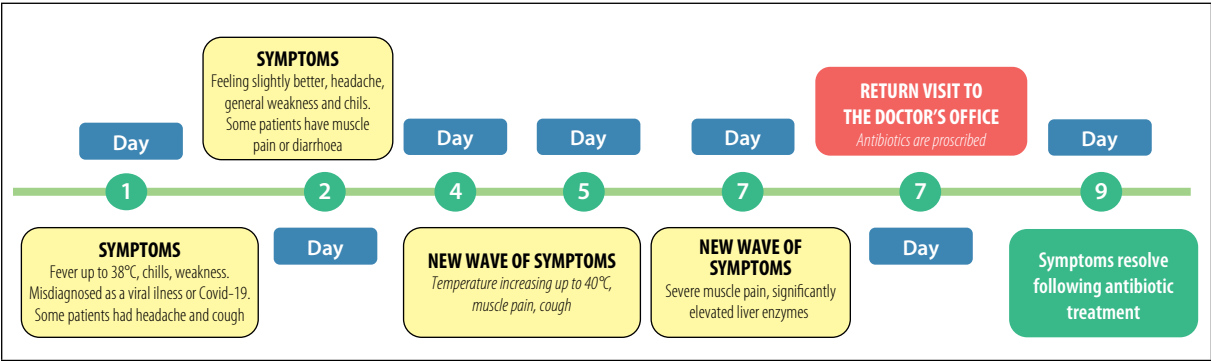


Figure 1
Timeline of symptom progression among patients with leptospirosis in Canton Sarajevo, 2025.

Table 2
Factors contributing to hospitalization

Model*	Deviance	AIC	BIC	df	$\Delta\chi^2$	p	McFadden R ²	Nagelkerke R ²	Tjur R ²	Cox & Snell R ²
M ₀	63.78	65.776	68.55	118	—	—	0	0	0	0
M ₁	45.53	65.533	93.32	109	18.243	0.032	0.286	0.343	0.167	0.142
Predictor	Estimate			Standard Error		Odds Ratio		z	Wald χ^2	p
Intercept	-3.651			2.324		0.026		-1.571	2.468	0.116
Chills	-1.554			1.025		0.212		-1.516	2.297	0.130
General weakness	0.494			1.406		1.638		0.351	0.123	0.726
Headache	-0.817			1.041		0.442		-0.785	0.617	0.432
Cough	1.285			1.416		3.614		0.908	0.824	0.364
Skin injury in past month	20.195			2654.172		5.899×10 ⁸		0.008	5.79×10 ⁻⁵	0.994
Risk factors	-17.885			2654.171		1.708×10 ⁻⁸		-0.007	4.54×10 ⁻⁵	0.995
Age ≥50	-0.164			0.829		0.849		-0.198	0.039	0.843
Sex	0.561			0.849		1.752		0.660	0.436	0.509
Significant increase of liver enzymes	2.449			1.184		11.581		2.069	4.281	0.039

*M₀ denotes the null logistic regression model including only the intercept, whereas M₁ denotes the full multivariable model including all candidate predictors. $\Delta\chi^2$ indicates the likelihood ratio test comparing M₁ to M₀.

by municipality, with the highest rate in Novi Grad (6.52/10,000), accounting for 67.2% of cases (n=80). Moderate rates were observed in Centar (2.48/10,000) and Stari Grad (1.76/10,000), while Novo Sarajevo (1.11/10,000) and Ilidža (1.26/10,000) had lower rates (Figure 2). Peripheral municipalities showed the lowest incidence, and no cases were reported in Trnovo. The spatial pattern demonstrates a clear concentration of cases in the urban core, particularly Novi Grad, suggesting a potential association with population density, sanitation conditions, and rodent exposure.

DISCUSSION

This study provides the first detailed description of a leptospirosis outbreak in an urban setting in Bosnia and Herzegovina, highlighting a change in the epidemiological pattern of this traditionally rural zoonosis. The concentration of cases in densely populated municipalities, highlights the growing role of urban environ-

mental factors-such as sanitation, waste management, and rodent activity-in sustaining transmission. Clinical presentation was largely non-specific in the early phase, frequently resulting in diagnostic delays and misclassification as viral illnesses, a challenge well documented in leptospirosis literature. Notably, classic transmission pathways were largely absent. Most patients denied recent skin wounds, and despite the presumed role of rodents in urban transmission, no cases of haemorrhagic fever or other rodent-borne viral infections were detected in Sarajevo Canton during 2025 up to 15 November, nor were any suspected cases reported. Interestingly, the municipality with the highest incidence was Novi Grad Sarajevo, a highly urbanized area with developed infrastructure and complex environmental and municipal management systems. Water supply is predominantly provided through the central public system, with most residents supplied from the Sarajevsko polje source and an extensive distribution network. Sewerage

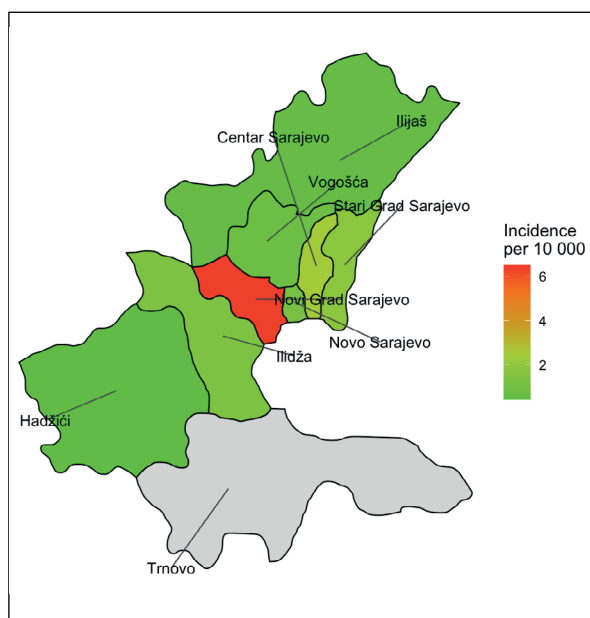


Figure 2
Incidence of leptospirosis by municipality, Canton Sarajevo, 2025.

infrastructure is also widely present, and the municipality hosts the central wastewater treatment plant in Butile, which treats wastewater before discharge into the Bosna River. The area is also exposed to flood risks, particularly following major events in 2014 and 2021 that prompted river regulation and flood protection measures. However, most reported cases were identified in urban residential blocks where full municipal infrastructure is present [25].

Findings from Okinawa Prefecture align closely with our observations [26]. In their 18-year review, 46.3% of 531 clinically suspected cases were laboratory-confirmed, with a strong male predominance and most cases occurring in young adults. Fever, myalgia, and conjunctival hyperaemia predominated, reflecting non-specific febrile illness at presentation. Importantly, traditional agricultural exposures were uncommon, while recreational and occupational river-water exposure emerged as the main risk factors in this urban-peri-urban setting [26]. These findings are consistent with global evidence that leptospirosis is increasing an urban disease. A systematic review by Mutalip *et al.* identified a growing burden in urban areas, driven by inadequate waste management, rodent proliferation, and deteriorating sanitation [5]. Similarly, Obels *et al.* reported a rising national incidence in the Netherlands between 2010 and 2021, linking urban cases to environmental exposure and climatic variability [9]. At a broader scale, Costa *et al.* estimated over one million cases and nearly 59,000 deaths annually worldwide, underscoring the major public health impact of leptospirosis and its frequently under-recognised clinical presentation [7]. Beauté *et al.* further highlighted substantial heterogeneity across EU/EEA countries, noting that urban outbreaks are likely under-detected because of non-specific symptoms and misdiagnosis [27].

Evidence from Western Europe further supports the urban transmission pattern observed in Sarajevo. Dupouey *et al.* described the re-emergence of leptospirosis in Europe, with an increasing proportion of cases linked to urban transmission rather than traditional rural exposures. Expansion of wild rodent populations in cities was identified as a key driver, and although overall EU incidence remains low (0.13 per 100,000), urban outbreaks are likely underestimated, mirroring the diagnostic challenges seen in our setting [4]. Global modelling by Costa *et al.* estimated an annual incidence of 14.77 cases per 100,000 population and 0.84 deaths per 100,000 worldwide [7]. In contrast, Sarajevo Canton experienced an incidence of 2.84 per 10,000 (≈ 28.4 per 100,000), with one municipality reaching 6.52 per 10,000 (≈ 65.2 per 100,000), substantially exceeding global averages. This disparity suggests either an unusually intense local outbreak or enhanced surveillance and diagnostic awareness, or both. It also reinforces the likelihood that leptospirosis remains under-estimated elsewhere, partly due to empirical antibiotic treatment prior to confirmation. Environmental and occupational risk factors related to water, infrastructure, landscape, agriculture, and animal exposure play an essential role in leptospirosis transmission. The magnitude of these risk factors varies across geographical regions and is influenced by climate, urbanization, population growth, and socioeconomic conditions [28]. These risk factors are particularly relevant for Bosnia and Herzegovina, where seasonal precipitation, flood events, and mixed urban-rural environments create favourable conditions for transmission, especially in areas with inadequate infrastructure and close human-animal interaction [29].

Although leptospirosis is considered uncommon in the continental United States, surveillance data indicate persistent underdiagnosis and periodic outbreaks too. National data linked most cases to freshwater exposure and rodent contact, with frequent hospitalisation and case-fatality rates up to 6% [30]. Waterborne outbreaks among athletes, including triathlon- and adventure-race-associated events, further emphasise the role of environmental exposure following rainfall [31]. More recent CDC surveillance confirmed ongoing sporadic transmission, with Puerto Rico experiencing recurrent clusters of severe disease [32]. Hawai'i continues to report the highest endemic incidence in the US, driven by heavy rainfall, abundant rodent reservoirs, and occupational exposure, with long-term surveillance confirming persistent environmental circulation of pathogenic *Leptospira* [33].

Collectively, these findings support our conclusion that leptospirosis in Sarajevo Canton during 2025 followed an urban transmission pattern, characterised by non-specific clinical presentation, absence of classic exposure pathways, and risk factors consistent with rodent-associated environmental contamination rather than agricultural or flood-related exposure.

CONCLUSION

The 2025 leptospirosis outbreak in Sarajevo Canton demonstrates that this traditionally rural zoonosis can

represent a significant urban public health challenge. The concentration of cases in densely populated areas highlights the importance of urban environmental determinants, including sanitation, waste management, and rodent control, in sustaining transmission. Although clinical outcomes were generally favourable, diagnostic delays were observed, largely due to non-specific early symptoms and initial misclassification as viral infections, underscoring the need for improved clinical awareness and timely laboratory confirmation.

This outbreak emphasises the importance of integrated surveillance and intersectoral collaboration between public health, veterinary, and municipal services. Strengthening routine environmental monitoring, systematic rodent control, and targeted risk communication should be prioritised in urban preparedness strategies. The findings indicate that leptospirosis is not confined to rural or post-flood settings but can emerge in urban environments through complex interactions between humans, animals, and the urban ecosystem.

Strengths and limitations

Strengths

This study represents the largest systematically documented leptospirosis outbreak in an urban setting in Bosnia and Herzegovina and the surrounding region, providing novel insight into the epidemiology of a traditionally rural zoonosis within a densely populated European environment. Integration of clinical, laboratory, environmental, and epidemiological data enabled comprehensive characterisation of the outbreak and its determinants. Use of the standardised ECDC case definition ensured comparability with European surveillance systems, while the epidemiological questionnaire adapted from the WHO Leptospirosis Surveillance Tool allowed detailed assessment of exposure pathways beyond routine reporting. Complete inclusion of all diagnosed cases and high-resolution spatial analysis strengthened the understanding of urban transmission dynamics in temperate settings.

Limitations

Reliance on routine diagnostic pathways resulted in a high proportion of probable cases, reflecting limited access to confirmatory laboratory testing, which was mainly available early in the outbreak or through sero-

logical analysis. The non-specific clinical presentation, overlapping with common viral illnesses, may have contributed to delayed diagnosis and underreporting. Exposure data were based on self-reported questionnaires, introducing potential recall bias for minor environmental contacts. Although all cases were included, the small number of hospitalisations limited statistical power in regression analyses, leading to unstable estimates. As an observational outbreak investigation, causal relationships between environmental conditions and transmission cannot be definitively established.

Ethical approval

In November 2025, the joint Ethics Committee of several healthcare institutions in the Canton of Sarajevo granted approval for the conduct of this study (05.5.46-9055-1/25). It should be noted that the epidemiological questionnaire used in this research is part of the Institute's routine daily work. The study was carried out in accordance with the principles of good clinical and epidemiological practice and in full compliance with data protection standards.

Funding

The Authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

Authors' contributions

AJ performed the statistical analyses and wrote the Results and Discussion sections. ZH contributed to the Introduction and Methodology. APB was responsible for data collection and the legal framework of the study. AS contributed to the Results and portions of the Discussion. AB drafted part of the Introduction. AT contributed to sections of the Discussion and performed final paper revision. APH wrote the environmental factors section and contributed to the Methodology. LA provided final review, critical revisions, and overall manuscript finalisation.

Conflict of interest statement

None

Received on 1 January 2026.

Accepted on 30 April 2026.

REFERENCES

- Bradley EA, Minett C, Hartwig A, et al. Leptospirosis and the environment: a review and future directions. *Pathogens*. 2023;12:1167. doi: 10.3390/pathogens12091167
- Guglielmini J, Bourhy P, Schiettekatte O, et al. Genus-wide *Leptospira* core genome multilocus sequence typing for strain taxonomy and global surveillance. *PLoS Negl Trop Dis*. 2019;13(4):e0007374.
- European Centre for Disease Prevention and Control. Leptospirosis – annual epidemiological report. Stockholm: ECDC; 2024.
- Dupouey J, Faucher B, Edouard S, et al. Human leptospirosis: an emerging risk in Europe? *Comp Immunol Microbiol Infect Dis*. 2014;37:77-83. doi: 10.1016/j.cimid.2013.12.002
- Mutalip MHA, Mohd Rosly MM, Othman TF, et al. Environmental risk factors of leptospirosis in urban settings: a systematic review. *BMJ Open*. 2019;9:e023359. doi: 10.1136/bmjopen-2018-023359
- Sykes JE, Haake DA, Galloway RL, et al. A global One Health perspective on leptospirosis in humans. *J Am Vet Med Assoc*. 2022;260:1523-33. doi: 10.2460/javma.22.06.0258
- Costa F, Hagan JE, Calcagno J, et al. Global morbidity and mortality of leptospirosis: a systematic review. *PLoS*

- Negl Trop Dis. 2015;9:e0003898. doi: 10.1371/journal.pntd.0003898
8. Haake DA, Levett PN. Leptospirosis in humans. *Curr Top Microbiol Immunol*. 2015;387:65-97. doi: 10.1007/978-3-662-45059-8_5
 9. Obels I, van den Kerkhof J, Koopmans M, et al. Increased incidence of human leptospirosis and the effect of temperature and precipitation, the Netherlands, 2010-2021. *Euro Surveill*. 2024;29:240015. doi: 10.2807/1560-7917.ES.2024.29.240015
 10. Muñoz-Zanzi C, Dreyfus A, Limothai U, et al. Leptospirosis – improving healthcare outcomes for a neglected zoonotic disease. *Open Forum Infect Dis*. 2025;12:ofaf035. doi: 10.1093/ofid/ofaf035
 11. Bierque E, Thibeaux R, Girault D, et al. A systematic review of *Leptospira* in water and soil environments. *PLoS One*. 2020;15:e0227055. doi: 10.1371/journal.pone.0227055
 12. Sayanthi Y, Gunasekara TD, Fernando N. Pathogenic *Leptospira* contamination in the environment: a systematic review. *Infect Ecol Epidemiol*. 2024;14:2324820. doi: 10.1080/2008686.2024.2324820
 13. Douchet L, Menkes C, Herbreteau V, et al. Climate-driven models of leptospirosis dynamics in tropical islands from three oceanic basins. *PLoS Negl Trop Dis*. 2024;18(4):e0011717. doi: 10.1371/journal.pntd.0011717
 14. Ranieri TM, de Souza FN, Vieira ML, et al. Leptospirosis cases during catastrophic flooding in southern Brazil. *Pathogens*. 2025;14:393. doi: 10.3390/pathogens14040393
 15. Rohmah NS, Aryanto S, Wiratama BS, Ibrahim AH. Examining the impact of rainfall patterns on leptospirosis cases in Bantul District, Indonesia: a four-year ecological study 2020-2023. *BIO Web Conf*. 2024;132:03002. doi: 10.1051/bioconf/202413203002
 16. Ling BL, Saleh R, Ahmad N. Detection of *Leptospira* in environmental samples of wet markets and paddy fields in Penang, Malaysia. *Trop Biomed*. 2025;42:51-7. doi: 10.47665/tb.42.1.009
 17. Davignon G, Bierque E, Goarant C. Leptospirosis: toward a better understanding of the environmental cycle. *Front Water*. 2023;5:1195094. doi: 10.3389/frwa.2023.1195094
 18. Maksimović Z, Babić S, Zahirović A, Rifatbegović M. Seroprevalence of leptospirosis among stray dogs in Bosnia and Herzegovina. *Comp Immunol Microbiol Infect Dis*. 2024;109:102171.
 19. European Union. Commission Implementing Decision (EU) 2018/945 of 22 June 2018 on communicable diseases and related special health issues to be covered by epidemiological surveillance. *Off J Eur Union*. 2018;170:1-74.
 20. Federation of Bosnia and Herzegovina. Law on health care. *Official Gazette of the Federation of BiH*, n. 46/10 and 75/13; 2010.
 21. Federation of Bosnia and Herzegovina. Law on the protection of the population from infectious diseases. *Official Gazette of the Federation of BiH*, n. 29/05, 2005.
 22. Federation of Bosnia and Herzegovina. Rulebook on the procedure for reporting communicable diseases. *Official Gazette of the Federation of BiH* n. 101, 2012.
 23. European Centre for Disease Prevention and Control. Effective risk communication for the prevention and control of communicable diseases in Europe. Stockholm: ECDC; 2013. doi: 10.2900/64747
 24. World Health Organization. Guidance on risk communication. Geneva: WHO; 2021.
 25. Municipality of Novi Grad Sarajevo. Development strategy of the municipality of Novi Grad Sarajevo 2021-2027. Sarajevo: Municipality of Novi Grad Sarajevo; 2022.
 26. Kakita T, Okano S, Kyan H, et al. Laboratory diagnostic, epidemiological, and clinical characteristics of human leptospirosis in Okinawa Prefecture, Japan, 2003-2020. *PLoS Negl Trop Dis*. 2021;15:e0009993. doi: 10.1371/journal.pntd.0009993
 27. Beauté J, Innocenti F, Aristodimou A, et al. Epidemiology of reported cases of leptospirosis in the EU/EEA, 2010 to 2021. *Euro Surveill*. 2024;29(7):2300266. doi: 10.2807/1560-7917.ES.2024.29.7.2300266
 28. Baharom M, Ahmad N, Hod R, et al. Environmental and occupational factors associated with leptospirosis: a systematic review. *Heliyon*. 2024;10:e23473. doi: 10.1016/j.heliyon.2023.e23473
 29. Bećirović A, Trnacević A, Dubinović-Rekić A, Džafić F. Floods associated with environmental factors and leptospirosis: experience from Tuzla Canton, Bosnia and Herzegovina. *Mater Sociomed*. 2022;34(3):193-6.
 30. Atherstone C, Galloway R, Schafer I, et al. Epidemiological, temporal, and geographic trends of leptospirosis in the United States, 2014-2020. *PLoS Negl Trop Dis*. 2025;19:e0013427. doi: 10.1371/journal.pntd.0013427
 31. Morgan J, Bornstein SL, Karpati AM, et al. Outbreak of leptospirosis among triathlon participants and community residents in Springfield, Illinois, 1998. *Clin Infect Dis*. 2002;34:1593-9. doi: 10.1086/340615
 32. Jones FK, Medina AG, Ryff KR, et al. Leptospirosis outbreak in the aftermath of Hurricane Fiona – Puerto Rico, 2022. *MMWR Morb Mortal Wkly Rep*. 2024;73:763-8. doi: 10.15585/mmwr.mm7335a2
 33. McMillan IA, Kallas GN, McMillan JKP, et al. Analysis of confirmed leptospirosis cases in Hawai'i identifies spatial and environmental patterns of incidence. *Am J Trop Med Hyg*. 2025;113:980-9. doi: 10.4269/ajtmh.25-0267

Current background human exposure to polybrominated diphenyl ethers: a review

Annalisa Abballe, Isotta Cursi, Valentina Marra, Noemi Milia, Roberto Miniero, Silvia Valentini and Anna Maria Ingelido

Unità Esposizione Umana a Contaminanti Ambientali, Dipartimento Ambiente e Salute, Istituto Superiore di Sanità, Rome, Italy

Abstract

Introduction. Polybrominated diphenyl ethers (PBDEs) have been used as flame retardants for years, until they were banned following their inclusion in the Stockholm Convention on Persistent Organic Pollutants (2001). The chemical and physical properties of PBDEs determine their ability to spread and accumulate in the environment and bioaccumulate along food chains. More than 90% of total human exposure to PBDEs comes from food. Chronic exposure to PBDEs can cause hepatotoxic, thyrotoxic, neurotoxic, genotoxic, immunotoxic, developmental toxic and potential carcinogenic effects in humans. Although the ban began decades ago, PBDEs are still present in various environmental matrices.

Method. This review aims to assess the current picture of human exposure to PBDEs by collecting and evaluating data from human biomonitoring studies available worldwide over the last 10 years.

Results and conclusions. The analysis of chosen papers suggests that levels of BDE-47, BDE-99, BDE-100 and BDE-153 have a global unclear trend, depending on the geographical area in question in the last ten years.

Key words

- human exposure
- PBDEs
- flame retardants
- general population
- concentration levels in biological fluids

INTRODUCTION

Polybrominated diphenyl ethers (PBDEs) are a family of 209 congeners that differ in the number and position of bromine atoms attached on the two aromatic rings, produced by the bromination reaction of diphenyl oxide. They are stable compounds characterized by boiling temperatures ranging from 310 to 425 °C and low vapour pressures with values ranging from approximately 10^{-1} Pa for mono-brominated to 10^{-13} Pa for decaBDE (BDE-209) at 298.15 K; they are also highly lipophilic substances whose solubility in water is extremely low and decreases with increasing bromination degree. The n-octanol-water partition coefficient, log (K_{OW}), varies between 4.28 and 9.9 [1].

The most important industrial use of PBDEs has been as flame retardants, with the aim of reducing the flammability of various materials used in everyday products. PBDEs have been widely incorporated into items like foam cushioning and fabrics in sofas/chairs and plastics for electrical and electronic equipment such as TV monitors, computers, and printers [2-4].

The most widely used flame retardants for years were mixtures of PBDEs containing mainly tetra-, penta-,

octa- and deca-brominated congeners, until they were banned following their inclusion in the Stockholm Convention on Persistent Organic Pollutants [5]. Among the aforesaid congeners, deca-PBDE was the most widely produced one, accounting for approximately 75% of total PBDEs production [6]. Due to their wide use, PBDEs were released into the environment during industrial manufacturing, use of end products, and nowadays, they can be still liberated in the environment during waste management operations [7-9].

With the implementation of Directive 2002/95/EC (known as RoHS – Restriction of Hazardous Substances) [10], PBDEs use in electrical and electronic equipment has been discontinued; various measures have been adopted to regulate or phase out PBDEs in several countries, leading to their ban in the EU in 2004 [11]. The replacement of PBDEs with other flame retardants primarily driven by concerns regarding their persistence, bioaccumulation capacity and toxicity [12].

The chemical and physical properties of PBDEs determine their ability to spread and accumulate in the environment and bioaccumulate along food chains. Bioaccumulation capacity is congener-specific, and

generally, congeners with a lower degree of bromination can bioaccumulate to a greater extent than those with a higher degree of bromination. Highly brominated congeners, such as deca-BDE, are found mainly in environmental matrices such as sediments and soils, while they are difficult to detect in biota [13]. This indicates that the bioaccumulation of highly brominated PBDEs congeners (particularly deca-BDE) is relatively low. In mammals, including humans, PBDEs accumulate mainly in adipose tissue, the gastrointestinal tract and the liver.

The general population's exposure to PBDEs (Figure 1) occurs mainly through the diet, especially from animal sources such as fish, meat, eggs and fats. Specifically, it has been estimated that in the general population, more than 90% of total human exposure to PBDEs comes from food [14, 15]. Also, inhalation of household dust seems to be an important route of exposure to these contaminants [4, 16, 17]. In addition, for newborn and infants, relevant exposure sources are breast milk consumption [18-20], and exposure through the placenta during the prenatal life period [21-23], and household dust [4]. Four congeners (PBDE-47, -99, -153, and -209) were most frequently found in the environment and in human biological media: for them toxicological studies suitable for dose response assessments were available [24]. For the identical reason, the same ones were also taken into consideration by the European Food Safety Authority [25, 26] to develop advice for human consumption. Chronic exposure to PBDEs can cause hepatotoxic, thyrotoxic, neurotoxic, genotoxic, immunotoxic, developmental toxic and potential carcinogenic effects in humans [15, 27]. The International Agency for Research on Cancer (IARC) classified PBDEs as "probably carcinogenic to humans" (Group 2A) based on mechanistic similarities to polychlorinated biphenyls [28].

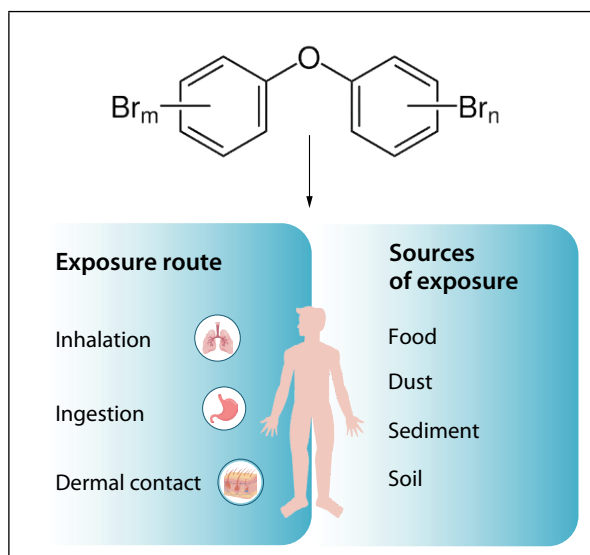


Figure 1
Routes and sources of human exposure to polybrominated diphenyl ethers (PBDEs).

Despite the restrictions, PBDEs are now mainly found in bulky waste and municipal waste consisting of electrical and electronic equipment and plastics, for which recovery and recycling activities can promote their re-release into the environment [4, 7-9]. For this reason, it is extremely important to continue monitoring the presence of PBDEs in the environment and in humans. The best way to assess the extent of human exposure to PBDEs is through human biomonitoring, i.e., by measuring their concentration in tissues or biological fluids. The best matrix for assessing the internal dose of this type of compound is human blood/serum, although they can also be measured in other matrices, including breast milk. Among all matrices, blood is widely used and is considered the ideal matrix for most chemicals due to its contact with the whole organism and its equilibrium with organs and tissues where chemicals are stored. In the human body, PBDEs accumulate mainly in adipose tissue from which they can pass into plasma or serum lipids and from there reach tissues and target organs [29]. PBDEs have been measured in human serum in various countries around the world. This review aims to assess the current picture of human exposure to PBDEs by collecting and evaluating data available worldwide over the last 10 years.

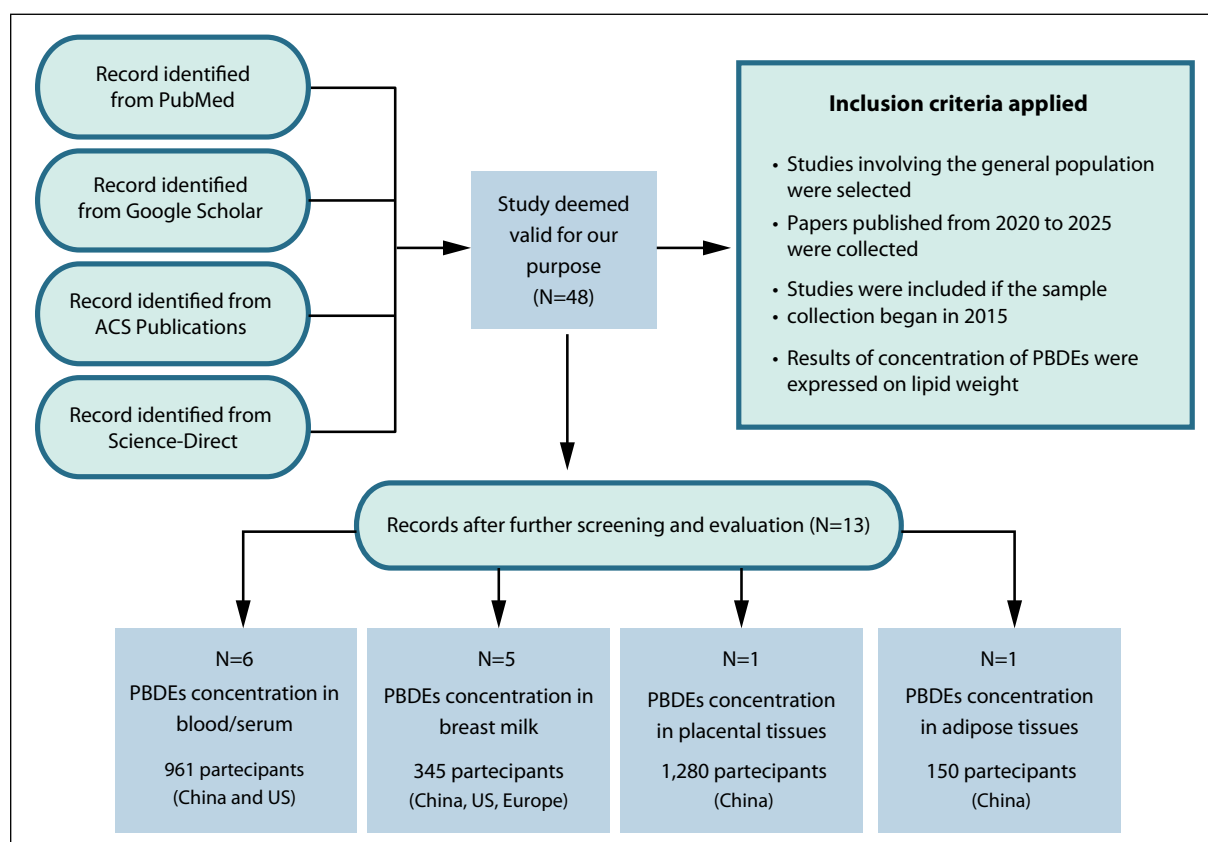
METHOD

Bibliographic research and study selection

In this review, PubMed, Google Scholar, ACS Publications, and Science-Direct databases were consulted. The search results were updated on November 10, 2025. Keywords including "human exposure, PBDEs or polybrominated diphenyl ethers, flame retardants, general population, levels, women, men, breast milk or human milk, blood, serum, pregnant women, PBDE-47, PBDE-99, PBDE-100, PBDE-153, PBDE-209" were considered involving 2-3 keywords for each search.

This review aims to assess the current exposure to PBDEs of the general population worldwide. Due to restrictions on the use of PBDEs, the number of exposure assessment studies is declining. The phase-out, which began with voluntary manufacturer actions in the early 2000s [30] and was later reinforced by regulations (the Stockholm Convention and the EU's POPs Regulation) [5], is expected to have led to a decrease in human exposure to PBDEs in recent years. For this reason, we have considered all geographical areas of the planet, from the USA to China, from India to Europe, from Africa to Oceania. We also included studies that assessed exposure to multiple contaminants (e.g., polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins and -furans (PCDDs/PCDFs), hexabromocyclododecane (HBCDD), per- and polyfluoroalkyl substances (PFASs), etc.). Further inclusion criteria were: (1) papers published from 2020 to 2025 in scientific journals; (2) studies on general population; (3) studies with a sampling year from 2015 onwards; (4) studies reporting results on lipid weight.

Upon initial review, a total of 48 studies were deemed valid for our purposes. After further screening and evaluation, 13 final studies were selected. The workflow was also presented in Figure 2. Most of the selected stud-

**Figure 2**

Reference scheme for selecting polybrominated diphenyl ethers (PBDEs) biomonitoring studies.

ies were from China. We found that human serum and breast milk were the most analyzed matrices. PBDEs were also detected in placenta and adipose tissue samples. Unfortunately, no articles relating to exposure of the male population were found that met our selection criteria. Data relating to the male population only were usually included in studies on the general population. However, in this paper we consider the study conducted by [16] on males, despite it was published in 2019, to collect some indicative information about this group of population.

In our study, we selected PBDE-47, -99, -100, and -153 for analysis because they were the most frequently quantified and reported as individual congeners in the available literature. No quantitative meta-analysis was conducted in this review paper.

RESULTS

Due to the heterogeneity of the papers (samples, countries, laboratories, detection method and data processing), we present a summary of the collected data. The PBDEs levels, year and country of sampling, sample size, and sampling period were reported in Table 1, where studies are listed in ascending order by sampling date. Only studies carried out by methods based on high sensitivity and specificity techniques, and in which analytical reliability was specifically warranted, were taken into consideration. The arithmetic mean, median, and geometric mean concentrations of PBDEs were summarized and compared to assess the presence

of these compounds in human matrices. Minimum and maximum values, along with concentration ranges, were also reported when available. The median concentration was used for comparison to reduce the influence of skewed distributions. When the median value was unavailable, the geometric mean or arithmetic mean was used instead.

All studies were published from 2020 to 2025, except for [16], and samples were collected between 2015-2023. Eight of the studies exclusively sampled women of reproductive age [31-38]. Mixed male and female studies generally had wider age ranges [39-41]. As mentioned above, only one study concerned male exposure [16]. In collected data, PBDEs concentrations in blood/serum were reported in six studies [16, 32, 38-41], which included 961 participants from four countries (three from Asia and one from USA); levels in breast milk were reported in five studies [31, 33-36], which included 345 participants from five countries. In one study [36], PBDEs levels were measured in 1,280 Chinese placental tissues, while in another research [41] 150 adipose tissue samples were analyzed in female patients undergoing plastic surgery from Hospital in China.

Globally, the total concentrations of the four selected PBDEs Σ_4 [PBDE-47, -99, -100, -153] ranged from 0.10 to 26.12 ng/g on lipid weight (lw) in blood and from 0.03 to 3.90 ng/g lw in breast milk. Within these totals, PBDE-153 was the highest contributing congener in both blood and breast milk while the lowest contributing congeners were PBDE-99 in blood and PBDE-100

Table 1

Summary of PBDEs biomonitoring data from international studies. The following information is reported: article source, year and country of sampling, matrix and sample size, age of participants and their BMI, and concentration of PBDE-47, -99, -100, and -153

Year of sampling	Country	Matrix and number of samples	Age of participants (years)	BMI	PBDE-47 (ng/g lw)	PBDE-99 (ng/g lw)	PBDE-100 (ng/g lw)	PBDE-153 (ng/g lw)	Ref.
2014-2015	Colombia	Breast milk, 60	26.1±7.0	24.4±4.1	Mean±SD: 0.78±0.91 Range: 0.06-5.5 25th 50th 75th 95th: 0.28 0.50 0.94 2.26	Mean±SD: 0.28±0.47 Range: <LOD-2.28 25th 50th 75th 95th: 0.04 0.13 0.27 1.50	Mean±SD: 0.24±0.44 Range: <LOD-2.23 25th 50th 75th 95th: <LOD 0.09 0.20 1.25	Mean±SD: 0.14±0.2 Range: <LOD-1.12 25th 50th 75th 95th: 0.04 0.06 0.16 0.50	Torres-Moreno <i>et al.</i> 2023 [31]
2016	Laizhou Bay, north China	Serum, 172 adults (118 males and 54 females)	21-58 Mean: 36	17.4-34.1 Mean: 24.2	Median: 0.482 Mean: 1.84 GM: 0.607 Range: 5.50x10 ⁻² -85.8	Median: 0.434 Mean: 2.53 GM: 0.600 Range: 3.10 x10 ⁻² -66.8	Median: 0.251 Mean: 1.69 GM: 0.333 Range: 2.20 x10 ⁻² -104	Median: 2.25 Mean: 4.65 GM: 2.62 Range: 0.147-104	Zhao <i>et al.</i> , 2021 (a) [39]
2014-2018	USA (California)	Serum, 506 pregnant women	32±5.4	–	Median: 10.9 Max: 364.3	Median: 4.1 Max: 141.3	Median: <LOQ Max: 90.2	Median: 9.59 Max: 232.1	Eick <i>et al.</i> , 2020 [32]
2016-2017	Qingyuan People's Hospital, China	Serum, 76 adult males living in an e-waste area	37.5±8.6 20-50 years	23.74±2.81	GM: 0.95±2.30	GM: 0.55±1.64	GM: 0.28±0.62	GM: 3.32±4.93	Yu <i>et al.</i> 2019 [16]
2016-2018	Germany	Breast milk, 100	20-45 Median: 34	–	Median: 0.204 Mean: 0.314 Max: 0.122	Median: 0.062 Mean: 0.086 Max: 2.42	Median: 0.054 Mean: 0.076 Max: 0.419	Median: 0.378 Mean: 0.464 Max: 0.453	Fromme <i>et al.</i> , 2022 [33]
2018	Beijing, China	Breast milk, 105	20-40	–	Median: 0.0759 Mean: 0.137 Min.: 0.0336 Max: 1.71	Median: 0.0344 Mean: 0.0468 Min.: 0.0091 Max: 0.296	Median: 0.0336 Mean: 0.0513 Min.: 0.00739 Max: 0.643	Median: 0.389 Mean: 0.527 Min.: 0.114 Max: 5.67	Zhao <i>et al.</i> , 2021 (b) [34]
2019	China	Adipose tissue of women, 150	35.91 ± 10.61 (21-84)	–	Median: 2.01 Mean: 2.72	Median: 0.43 Mean: 0.72	Median: 0.69 Mean: 1.03	Median: 26.12 Mean: 30.44	Zhang <i>et al.</i> , 2022 [41]
2019	USA	Breast milk, 50	24-42; mean: 35	–	Median: 3.17 Mean: 14.1 Range: <0.46-347	Median: 1.44 Mean: 7.5 Range: <0.3-207	Median: 1.73 Mean: 14.2 Range: <0.3-313	Median: 3.90 Mean: 10.8 Range: <0.68-131	Schreder <i>et al.</i> 2023 [35]
2019-2022	Zagreb, Croatia	Breast milk, 30	26-42	–	Median: <LOQ Max: 1.5	Median: 0.04 Max: 3.4	Median: <LOQ Max: 0.98	Median: 0.23 Max: 0.83	Jagic <i>et al.</i> , 2024 [36]
2020-2021	Zunyi, southwest China	Placental tissue, 1,280	27.3 ± 5.1	22.5±3.5	Median: 0.34 Min.: 0.02 Max: 67.5	Median: 0.53 Min.: 0.06 Max: 86.7	Median: 0.67 Min.: 0.06 Max: 57.6	Median: 1.00 Min.: 0.11 Max: 74.5	Liu <i>et al.</i> , 2024 [37]
2020-2022	Jiangsu province, China	Blood, 104 control group of pregnant women	30.1±3.1	22.5±3.7	Median: 4.39 Range: 0.45-153.99	Median: 0.10 Range: 0.06-7.55	Median: 0.11 Range: 0.06-4.27	Median: 0.17 Range: 0.10-7.17	Ma <i>et al.</i> , 2023 [38]
2022	Thailand	Blood, 26 control group of adults (7 men and 19 women)	46-68	–	Median: <LOQ Range: <LOQ-0.83	Median: <LOQ Range: <LOQ-2.33	Median: <LOQ Range: -	Median: <LOQ Range: <LOQ-0.38	Walaska <i>et al.</i> , 2024 [40]
2023	Mongolia, China	Serum, 77 agro-pastoralists (37 females and 40 males)	>64 Median: 72	Median: 26.6	Median: 0.38 Mean: 1.24 Range: ND-19.8	Median: 1.88 Mean: 3.44 Range: ND-17.5	Median: 1.87 Mean: 3.04 Range: ND-23.0	Median: 0.66 Mean: 1.25 Range: ND-6.8	Dalutai <i>et al.</i> , 2025 [42]

PBDEs: polybrominated diphenyl ethers; BMI: body mass index; SD: standard deviation; LOD: limit of detection; LOQ: limit of quantification; GM: geometric mean; ND: non-detect.

in breast milk, respectively. The selected studies reported data collected from 2,736 biological samples (serum/blood, breast milk, placental tissue, and adipose tissue) from the general population, aged 20 to 50, across various countries. Only in [39] and [40] an older population (46-80 years) was selected.

Figure 3 provides indication of relative differences between matrices and geographical regions: median blood/serum and breast milk PBDEs concentrations were reported in studies carried out in Asia, USA and Europe (Germany and Croatia). The four studies [33-36] analyzing breast milk showed PBDE-47 and -PBDE-153 to be the predominant PBDEs congeners. In milk samples PBDE-153 concentration was the highest value among all four congeners considered except for breast milk samples from Colombian women where PBDE-47 was the main congener [31].

A similar pattern was observed in two studies carried out in blood/serum samples: PBDE-153 was the major congener in early studies in China [16, 39]; subsequently, PBDE-47 was the major congener in other studies carried out in China by [38] and in USA by [32], respectively. Results showed that human exposure in male population seems to be like that of women [16]. In Figure 3 we showed results in Mongolia [42] and Thailand [40] also. Patterns were different with a predominance of penta-PBDEs (PBDE-99 and -100). Despite the limited number of papers available, some considerations may be made: a decrease in PBDE-153 over time appears to be common to all matrices analyzed, except for adipose tissue in Chinese women where hydrophobic environmental chemicals, as PBDEs, tend to accumulate [43-45].

PBDEs total concentrations and proportions varied between Asia, USA, and EU regions as might be expected based on varied national usage of PBDEs flame retardants. In USA penta-BDE technical mix was more

heavily used, whereas in Europe penta-BDE use was lower than that in the USA and penta- and octa-BDE were banned in 2004. These findings indicate that many factors such as regional PBDEs regulations, time of phase out of PBDE use, previous quantity of PBDE used, and half-life of congeners can be key factors in distribution and levels of analytes in human matrices. China implemented some restrictions on penta-BDE and octa-BDE in 2014 (https://www.mee.gov.cn/gkml/hbb/bgg/201404/t20140401_270007.htm), but deca-BDE products are not subject to any regulations.

DISCUSSION

This review aimed to assess the current picture of human exposure to PBDEs by collecting and evaluating data available worldwide over the last 10 years. A total of 12 studies met all our criteria; one study published before 2020 [16] was also considered to have information on male population. Despite the limited number of papers available, the number of samples analysed was substantial. Four congeners were selected because they are more frequently reported in the literature. China is the country with the highest number of studies: environmental contamination is a particular concern due to the production and utilization of deca-BDE products and to the concentration of PBDEs in certain areas near electronic waste disposal sites.

Several studies suggest that PBDE-47 and -99 have a decreasing trend in Asia, Europe and Oceania since 2004, except the North American region where a continuous increase was registered [46]. Although the three main commercial mixtures (penta-, octa-, and deca-PBDE) were included in the Stockholm Convention in 2004 [5], human exposure to PBDEs varied depending on congener-specific differences in metabolism and persistence in the body: the half-lives of deca- to

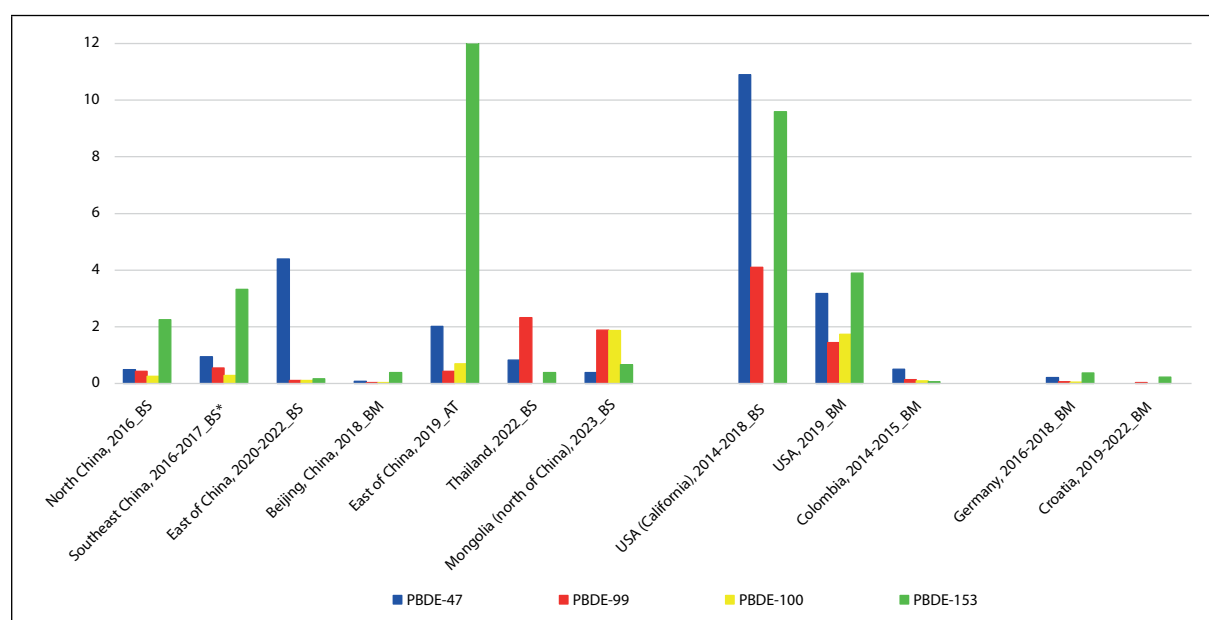


Figure 3

Median polybrominated diphenyl ethers (PBDEs) concentrations (ng/g lw) in blood/serum (BS), breast milk (BM), and adipose tissue (AT) from China, USA, and Europe studies over the period 2015-2022. Values * are geometric means.

hepta-BDEs in serum increased with decreasing number of bromine substituents [47]. In particular, PBDE-47 and -99 have human elimination half-lives of approximately 0.37-3 and 0.77-8 years, respectively [48-50], while the human elimination half-life of PBDE-153 is between 3.5 and 11.7 years [48-50]. The longer persistence suggests why PBDE-153 usually has higher levels than other congeners, especially after 2015. One of the main metabolic pathways is reductive debromination in which higher-brominated congeners are transformed into lower-brominated congeners. For the fully brominated BDE-209, the apparent half-life was as short as 15 days [47]. PBDE-209 undergoes reductive debromination to form lower-brominated congeners, including nona- and octa-BDEs [47]. Mörrck *et al.* [51] found that up to 90% of a single oral dose of PBDE-209 is excreted via feces, and a portion is metabolized resulting in hydroxylated metabolites that may be more toxic than the parent compound. It should also be noted that human exposure to PBDEs has continued over time, as many countries have only ratified the Stockholm Convention in recent years. Furthermore, PBDEs are still present in furniture, building materials, e-waste, and waste electrical and electronic equipment. In some regions of the world, such as China and Nigeria, the disposal of electrical waste containing PBDEs represent a major problem [8, 9, 52]: in some Chinese regions in the vicinity of the e-waste recycling area remains elevated [53]. Several studies in China suggest an important human exposure to PBDEs from house dust ingestion, especially in people residing in rural areas, southern regions, or near e-waste-contaminated areas [4, 8, 54, 55].

Although the industrialized nations had gradually implemented restrictive measures, the temporal trend of

PBDEs concentrations remained ambiguous. In human biomonitoring studies the rate of decline is often divergent between congeners and age groups of population. In NHANES study [56] it was observed that PBDEs reported in serum samples from the years 2005-06 to 2013-14 decreased in concentration, except PBDE-153, for which concentrations increased by 12.0% and 8.4% for the age groups 40-59 and ≥ 60 years, respectively.

Analysis of selected studies for this paper shows an unclear trend in the last ten years for the four considered congeners of PBDEs. This tendency is also evident in Figure 4 where our results are shown with that obtained in studies carried out in women sera in the previous decade [57]. The data indicates that it is difficult to accurately determine the trend in exposure. Indeed, due to the different patterns of use and periods of restriction, the available data cannot be considered as a whole, but they must be examined with specific geographical and policy area. In fact, except for European data, which shows a certain downward trend, others do not appear to follow a characteristic pattern. Of the four selected PBDEs, PBDE-47 and PBDE-153 appear to be the predominant congeners, alternating discontinuously in time and space. This is particularly evident in the US and China. Further data are essential in order to accurately assess human exposure to PBDEs, particularly in regions such as the USA and China, where large-scale industrial production, the size and characteristics of the territory and its contamination can significantly influence the results. Conclusions on selected PBDEs are limited because of the lack of papers. This is the main limitation of our review: the lack of recent human biomonitoring studies on PBDEs limits the evaluation of current population exposures. Due to global

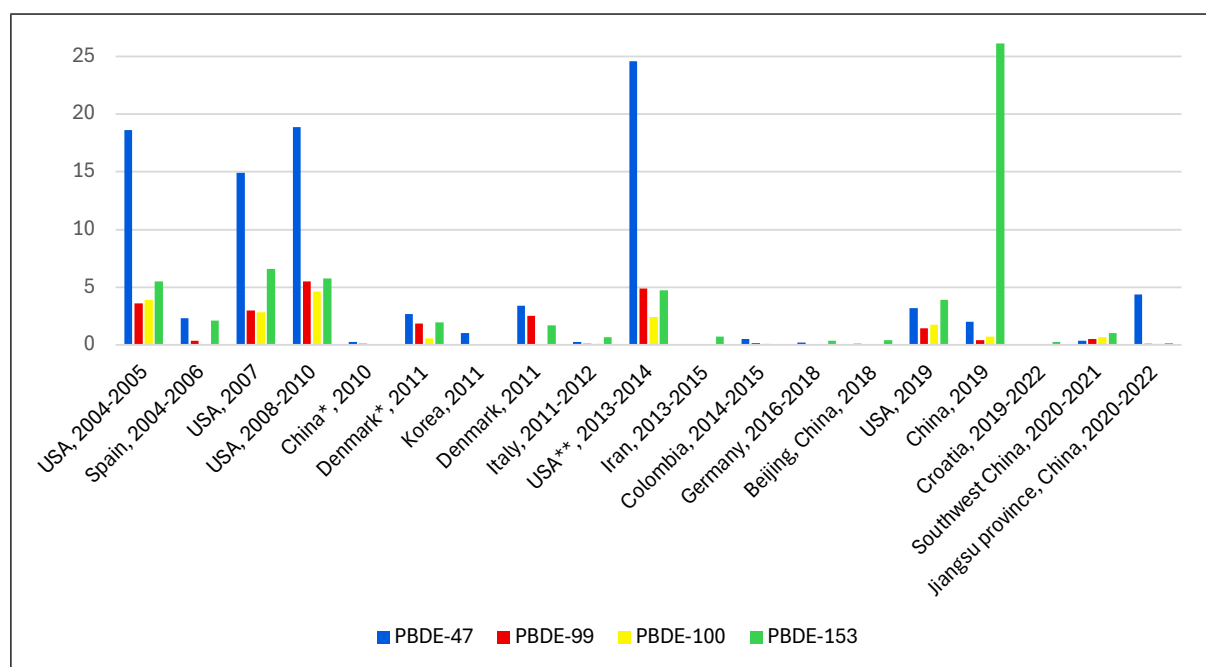


Figure 4

Polybrominated diphenyl ethers (PBDEs) concentrations (ng/g lw) in biological samples from women of various countries over the period 2004-2022. Median, mean *, and geometric mean ** estimates are reported.

restrictions on the production and use of PBDEs, research has shifted to newer or emerging substances. In our opinion, due to the chemical and physical properties of persistence, bioaccumulation, and toxicity of PBDEs, continuous studies are necessary to monitor these substances both in the environment and in humans. It is essential to understand trends in human exposure to PBDEs, especially in regions where the production or improper disposal of products containing these substances is significant in order to understand the mobility, transformation, and impact of PBDEs-containing products still in use (e.g., in old furniture, electronics).

Despite the above commented limitations, the current review represents a comprehensive effort to synthesize the knowledge on human exposure to PBDEs in the last decade.

CONCLUSION

This review aims to assess the current exposure to PBDEs of the general population worldwide. Because of the lack of recent studies, the analysis of chosen papers suggests that levels of PBDE-47, -99, -100 and -153 have a global unclear trend in the last ten years. Due to different patterns of use and banning period, the available data cannot be considered as a whole, but they must be examined with specific geographical and policy area. As PBDEs are still present in furniture, building materials, electronic equipment and e-waste despite recent restrictions, it is essential to continue assessing human exposure to these contaminants through biomonitoring studies.

REFERENCES

1. World Health Organization, United Nations Environment Programme, International Labour Organisation. Brominated diphenyl ethers. Geneva: WHO; 1994. (Environmental Health Criteria, 162).
2. Portet-Koltalo F, Guibert N, Morin C, de Mengin-Fondragon F, Frouard A. Evaluation of polybrominated diphenyl ether (PBDE) flame retardants from various materials in professional seating furnishing wastes from French flows. *Waste Manag.* 2021;131:108-16. doi: 10.1016/j.wasman.2021.05.038
3. Charbonnet JA, Weber R, Blum A. Flammability standards for furniture, building insulation and electronics: benefit and risk. *Emerg Contam.* 2020;6:432-41. doi: 10.1016/j.emcon.2020.05.002
4. Cai K, Song Q, Yuan W, Ruan J, Duan H, Li Y, Li J. Human exposure to PBDEs in e-waste areas: a review. *Environ Pollut.* 2020;267:115634. doi: 10.1016/j.envpol.2020.115634
5. United Nations Environment Programme UNEP. Draft guidance on preparing inventories of polybrominated diphenyl ethers (PBDEs) listed under the Stockholm Convention on Persistent Organic Pollutants. Geneva: Secretariat of the Basel, Rotterdam and Stockholm conventions, UNEP; 2021. Available from: <https://www.gefislands.org/sites/default/files/downloads/tools/10.%20UNEP-POPS-NIP-GUID-04-Inventory-PBDE-2021.English.pdf>
6. Risk & Policy Analysts. Support to an Annex XV Dossier on Bis-(pentabromophenyl) ether (DecaBDE). RPA; 2014.
7. Yu Y, Lin B, Liang W, Li L, Hong Y, Chen X, Xu X, Xiang M, Huang S. Associations between PBDEs exposure from house dust and human semen quality at an e-waste areas in South China – A pilot study. *Chemosphere.* 2018;198:266-73. doi: 10.1016/j.chemosphere.2018.01.150
8. Lin C, Zeng Z, Xu R, Liang W, Guo Y, Huo X. Risk assessment of PBDEs and PCBs in dust from an e-waste recycling area of China. *Sci Total Environ.* 2022;803:150016. doi: 10.1016/j.scitotenv.2021.150016
9. Oloruntoba K, Sindiku O, Osibanjo O, Herold C, Weber R. Polybrominated diphenyl ethers (PBDEs) concentrations in soil and plants around municipal dumpsites in Abuja, Nigeria. *Environ Pollut.* 2021;277:116794. doi: 10.1016/j.envpol.2021.116794
10. European Union. Direttiva 2002/95/CE del Parlamento Europeo e del Consiglio del 27 gennaio 2003 sulla restrizione dell'uso di determinate sostanze pericolose nelle apparecchiature elettriche ed elettroniche. *Gazzetta Ufficiale dell'Unione Europea L 037, 13/02/2003.* (in Italian). p.19-23.
11. United Nations Environment Programme. Manual on compliance with and enforcement of multilateral environmental agreements. Nairobi, Kenya: UNEP; 2006.
12. Gibson EA, Stapleton HM, Calero L, Holmes D, Burke K, Martinez R, Cortes B, Nematollahi A, Evans D, Anderson K, A, Herbstman JB. Differential exposure to organophosphate flame retardants in mother-child pairs. *Chemosphere.* 2019;219:567-73. doi: 10.1016/j.chemosphere.2018.12.008

Authors' contributions

AA was involved in literature search, data collection, data analysis and interpretation, writing; IC contributed to the literature search, data collection and revising the manuscript; VM contributed to the literature search and revising the manuscript; NM was involved in literature search, visualisation of charts and figures and revising the manuscript; RM contributed to data analysis and interpretation, and revising the manuscript; SV contributed to the literature search and revising the manuscript; AMI contributed to writing and revising the manuscript, charts and figures.

All Authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Funding

The Authors declare that no financial support was received for this paper.

Availability of data and materials

This manuscript used data from published studies and public databases; therefore, no original data are available for sharing.

Conflicts of interest statement

All Authors declared that there are no conflicts of interest.

Received on 25 February 2026.

Accepted on 6 May 2026.

13. Bemporad E, Berardi S, Campanari S, Ledda A, Napolitano P; Istituto Nazionale per l'Assicurazione contro gli Infortuni sul Lavoro. Polibromodifenileteri (Pbde). Microinquinanti organici – Informazioni e dati per una gestione sicura. Roma: INAIL; 2020.
14. Linares V, Bellés M, Domingo JL. Human exposure to PBDE and critical evaluation of health hazards. *Arch Toxicol*. 2015;89:335-56. doi: 10.1007/s00204-015-1457-1
15. Gao J, Xie, Wang Z, Yu Y, Qi Z, Yu X, Zhong T, Wang L, Feng K, Peng Y, Xiao Y. Human toxicity of polybrominated diphenyl ethers (PBDEs) and their derivatives: a comprehensive review. *Curr Res Food Sci*. 2024;9:100918. doi: 10.1016/j.crfs.2024.100918
16. Yu Y, Lin B, Chen X, Qiao J, Li L, Liang Y, Zhang G, Jia Y, Zhou X, Chen C, Kan H. Polybrominated diphenyl ethers in human serum, semen and indoor dust: effects on hormones balance and semen quality. *Sci Total Environ*. 2019;671:1017-25. doi: 10.1016/j.scitotenv.2019.03.319
17. Bramwell L, Glinianaia S, V, Rankin J, Rose M, Fernandes A, Harrad S, Pless-Mulolli T. Associations between human exposure to polybrominated diphenyl ether flame retardants via diet and indoor dust, and internal dose: a systematic review. *Environ Int*. 2016;92:3:680-94. doi: 10.1016/j.envint.2016.02.017
18. Guo W, Holden A, Smith SC, Gephart R, Petreas M, Park J-S. PBDE levels in breast milk are decreasing in California. *Chemosphere*. 2016;150:505-13. doi: 10.1016/j.chemosphere.2015.11.032
19. Romanić S, H, Miličević T, Jovanović G, Sarić M, M, Mendaš G, Fingler S, Jakšić G, Popović A, Relić D. Persistent organic pollutants in Croatian breast milk: an overview of pollutant levels and infant health risk assessment from 1976 to the present. *Food Chem Toxicol*. 2023;179:113990. doi: 10.1016/j.fct.2023.113990
20. Sanguos C, L, Suárez O, L, Martínez-Carballo E, Luz Couce M. Postnatal exposure to organic pollutants in maternal milk in north-western Spain. *Environ Pollut*. 2023;318:120903. doi: 10.1016/j.envpol.2022.120903
21. Jeong Y, Lee S, Kim S, Park J, Hai-Joong K, Choi G, Choi S, Kim S, Kim S, Y, Kim S, Choi K, Moon H, B. Placental transfer of persistent organic pollutants and feasibility using the placenta as a non-invasive biomonitoring matrix. *Sci Total Environ*. 2018;612:1498-505. doi: 10.1016/j.scitotenv.2017.07.054
22. Vizcaino E, Grimalt J, O, Fernández-Somoano A, Tardon A. Transport of persistent organic pollutants across the human placenta. *Environ Int*. 2014;65:107-15. doi: 10.1016/j.envint.2014.01.004
23. Zhao Y, Song Q, Ge W, Jin Y, Chen S, Zhao Y, Xiao X, Zhang Y. Associations between in utero exposure to polybrominated diphenyl ethers, pathophysiological state of fetal growth and placental DNA methylation changes. *Environ Int*. 2019;133(B):105255. doi: 10.1016/j.envint.2019.105255
24. United States Environmental Protection Agency. An alternatives assessment for the flame retardant decabromodiphenyl ether (DecaBDE). EPA; 2012.
25. EFSA Panel on Contaminants in the Food Chain, Schrenk D, Bignami M, Bodin L, et al. Update of the risk assessment of polybrominated diphenyl ethers (PBDEs). *EFSA J*. 2024;22(1):e8497. doi: 10.2903/j.efsa.2024.8497
26. EFSA Panel on Contaminants in the Food Chain. Scientific opinion on emerging and novel brominated flame retardants (BFRs) in food. *EFSA J*. 2012;10(10):2908. doi: 10.2903/j.efsa.2012.2908
27. Xue J, Xiao Q, Zhang M, Li D, Wang X. Toxic effects and mechanisms of polybrominated diphenyl ethers. *Int J Mol Sci*. 2023;24:13487. doi: 10.3390/ijms241713487
28. International Agency for Research on Cancer. Polychlorinated biphenyls and polybrominated biphenyls. Lyon: IARC; 2015. (IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, 107).
29. World Health Organization Regional Office for Europe. Human biomonitoring: facts and figures. Copenhagen: WHO Europe; 2015. Available from: <https://iris.who.int/server/api/core/bitstreams/7bf97eec-1f9e-47c4-a2d0-7607afc73771/content>
30. United States Environmental Protection Agency. Polybrominated diphenyl ethers (PBDEs) project plan. EPA; 2006.
31. Torres-Moreno AC, Mejia-Grau K, Puente-DelaCruz L, Codling G, Villa A, L, Ríos-Marquez O, Patequiva-Chauta L, Cobo M, Johnson-Restrepo B. Polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs) in human breast milk from Colombia: a probabilistic risk assessment approach. *Chemosphere*. 2023;339:139597. doi: 10.1016/j.chemosphere.2023.139597
32. Eick SM, Hom Thepaksorn EK, Izano MA, Cushing LJ, Wang Y, Crispo Smith S, Gao S, Park JS, Padula AM, DeMicco E, Valeri L, Woodruff TJ, Morello-Frosch R. Associations between prenatal maternal exposure to per- and polyfluoroalkyl substances (PFAS) and polybrominated diphenyl ethers (PBDEs) and birth outcomes among pregnant women in San Francisco. *Environ Health*. 2020;19:100. doi: 10.1186/s12940-020-00654-2
33. Fromme H, Fuchs V, Albrecht M, Aschenbrenner B, Röhl C, Janitzki N, Herber-Jonat S, Wöckner M, Völkel W, Flemmer AW, Schober W. Polychlorinated dioxins and dibenzofurans (PCDD/F), polybrominated dioxins and dibenzofurans (PBDD/F), polychlorinated biphenyls (PCB), polybrominated diphenyl ethers (PBDE), and per- and polyfluoroalkyl substances (PFAS) in German breast milk samples (LUPE 8). *Sci Total Environ*. 2022;825:154066. doi: 10.1016/j.scitotenv.2022.154066
34. Zhao X, Shi Z. Legacy brominated flame retardants in human milk from the general population in Beijing, China: biomonitoring, temporal trends from 2011 to 2018, and nursing infant's exposure assessment. *Chemosphere*. 2021;285:131533. doi: 10.1016/j.chemosphere.2021.131533
35. Schreder E, Zheng G, Sathyanarayana S, Gunaje N, Hu M, Salamova A. Brominated flame retardants in breast milk from the United States: first detection of bromophenols in US breast milk. *Environ Pollut*. 2023;334:122028. doi: 10.1016/j.envpol.2023.122028
36. Jagić K, Dvoršćak M, Lovaković B, T, Klinčić D. Polybrominated diphenyl ethers in paired dust-breast milk samples: levels, predictors of contamination, and health risk assessment for infants and mothers. *Environ Toxicol Pharmacol*. 2024;111:104547. doi: 10.1016/j.etap.2024.104547
37. Liu Y, Xie Y, Tian Y, Liao J, Fang D, Wang L, Zeng R, Xiong S, Liu X, Chen Q, Zhang Y, Yuan H, Li Q, Shen X, Zhou Y. Exposure levels and determinants of placental polybrominated diphenyl ethers in Chinese pregnant women. *Environ Res*. 2024;241:117615. doi: 10.1016/j.envres.2023.117615
38. Ma J, Li Y, Qian L, Geng S, Yao X, Gao X, Yan Y, Wen J. Serum levels of polychlorinated biphenyls and polybrominated diphenyl ethers in early pregnancy and their associations with gestational diabetes mellitus. *Chemosphere*. 2023;339:139640. doi: 10.1016/j.chemosphere.2023.139640
39. Zhao X, Yang X, Du Y, Li R, Zhou T, Wang Y, Chen T, Wang D, Shi Z. Polybrominated diphenyl ethers in serum from residents living in a brominated flame re-

- ardant production area: occurrence, influencing factors, and relationships with thyroid and liver function. *Environ Pollut.* 2021;270:116046. doi: 10.1016/j.envpol.2020.116046
40. Walaska H, Dvorska A, Petrlik J, Boontongmai T, Bubphachat N, Strakova J, Thowsakul C, Teebthaisong A, Jelinek N, Grechko V, Saetang P, Jeungsmarn P, Phanphet P, Pulawun S, Sykороva A, Gramblicka T, Pulkrabova J, Carpenter DO. PBDEs and dechlorane plus contamination in community e-waste recycling: environmental and health implications in Northeastern Thailand. *Toxicology.* 2024;509:153972. doi: 10.1016/j.tox.2024.153972
 41. Qiong Zhang, Jiajun Peng, Anyan Huang, Shukai Zheng, Xiaoling Shi, Boyu Li, Wenlong Huang, Wei Tan, Xin Wang, Kusheng Wu. Associations between polybrominated diphenyl ethers (PBDEs) levels in adipose tissues and blood lipids in women of Shantou, China. *Environ Res.* 2022;214(Pt 3):114096. doi <https://doi.org/10.1016/j.envres.2022.114096>
 42. Dalutai M, An H, Sun J, Zheng J, Wang Y, Hu J, Jin J. Polybrominated diphenyl ethers (PBDEs) exposure in elderly agro-pastoralists of inner Mongolia: ethnic disparities, dietary drivers, and metabolic-inflammatory implications. *Ecotoxicol Environ Saf.* 2025;306:119314. doi: 10.1016/j.ecoenv.2025.119314
 43. Pan S, Li Z, Walker DI, Baumert BO, Wang H, Goodrich JA, Rock S, Inge TH, Jenkins TM, Sisley S, Bartell SM, Xanthakos S, Lin X, McNeil B, Robuck AR, Mullins CE, La Merrill MA, Garcia E, Aung MT, Eckel SP, McConnell R, Conti DV, Ryder JR, Chatzi L. Metabolic signatures in adipose tissue linking lipophilic persistent organic pollutant mixtures to blood pressure five years after bariatric surgery among adolescents. *Environ Sci Technol.* 2025;59:4364-75. doi: 10.1021/acs.est.4c13902
 44. Moriceau MA, Cano-Sancho G, Kim M, Coumoul X, Emond C, Arrebola JP, Antignac JP, Audouze K, Rouselle C. Partitioning of persistent organic pollutants between adipose tissue and serum in human studies. *Toxics.* 2023;11:41. doi: 10.3390/toxics11010041
 45. Rolle-Kampczyk U, Gebauer S, Haange SB, Schubert K, Kern M, Moulla Y, Dietrich A, Schön MR, Klötting N, Bergen MV, Blüher M. Accumulation of distinct persistent organic pollutants is associated with adipose tissue inflammation. *Sci Total Environ.* 2020;748:142458. doi: 10.1016/j.scitotenv.2020.142458
 46. van der Schyff V, Kalina J, Abballe A, Iamiceli A, L, Goverts E, Melymuk L. Has regulatory action reduced human exposure to flame retardants? *Environ Sci Technol.* 2023;57:19106-24. doi: 10.1021/acs.est.3c02896
 47. Thuresson K, Höglund P, Hagmar L, Sjödin A, Bergman A, Jakobsson K. Apparent half-lives of hepta- to decabrominated diphenyl ethers in human serum as determined in occupationally exposed workers. *Environ Health Perspec.* 2006;114:176-81.
 48. Wong F, Cousins IT, MacLeod M. Bounding uncertainties in intrinsic human elimination half-lives and intake of polybrominated diphenyl ethers in the North American population. *Environ Internat.* 2013;59:168-74. doi: 10.1016/j.envint.2013.05.004
 49. Trudel D, Scheringer M, von Goetz N, Hungerbühler K. Total consumer exposure to polybrominated diphenyl ethers in North America and Europe. *Environ Sci Technol.* 2011;45(6):2391-7. doi: 10.1021/es1035046
 50. Geyer H, J, Schramm K, W, Darnerud P, O, Aune M, Feicht E, A, Fried K, W, Henkelmann B, Lenoir D, Schmid P, McDonald T, A. Terminal elimination half-lives of the brominated flame retardants TBBPA, HBCD, and lower brominated PBDEs in humans. *Organohalogen Compound.* 2004;66:3820-5.
 51. Mörck A, Hakk H, Örn U, Klasson Wehler E. Decabromodiphenyl ether in the rat: absorption, distribution, metabolism, and excretion. *Drug Metab Dispos.* 2003;31:900-7.
 52. Chen Z, Luo X, Zeng Y, Tan S, Xu Z. Polybrominated diphenyl ethers in indoor air from two typical E-waste recycling workshops in Southern China: emission, size-distribution, gas-particle partitioning, and exposure assessment. *J Hazard Mater.* 2021;402:123667. doi: 10.1016/j.jhazmat.2020.123667
 53. Zheng K, Zeng Z, Lin Y, Wang Q, Tian Q, Huo X. Current status of indoor dust PBDE pollution and its physical burden and health effects on children. *Environ Sci Pollut.* 2023;30:19642-61. doi: 10.1007/s11356-022-24723-w
 54. Zhang X, Zhang W, Liu T, Song F, Chen C, Wang C, Zhang W, Han X, Wang X. Levels, distribution, sources and children's health risk of PBDEs in household dust from 2010 to 2022 in China. *Indoor Environ.* 2025;2:100114. doi: 10.1016/j.indenv.2025.100114
 55. Niu D, Qiu Y, Li L, Zhou Y, Du X, Zhu Z, Chen L, Lin Z. Occurrence of polybrominated diphenyl ethers in floor and elevated surface house dust from Shanghai, China. *Environ Sci Pollut Res.* 2018;25:18049-58. doi: 10.1007/s11356-018-1968-4
 56. Sjödin A, Jones R, S, Wong L, Y, Caudill S, P, Calafat A, M. Polybrominated diphenyl ethers and biphenyl in serum: time trend study from the national health and nutrition examination survey for years 2005/06 through 2013/14. *Environ Sci Technol.* 2019;53(10):6018-24. doi: 10.1021/acs.est.9b00471
 57. Abballe A, De Felip E, Dellatte E, Iacovella N, Marra V, Miniero R, Valentini S, Ingelido AM. Concentrations of polybrominated diphenyl ethers (PBDEs) in women of reproductive age in Italy: a human biomonitoring study. *Toxics.* 2026;14(1):72. doi: 10.3390/toxics14010072

Human-horse interactions as complementary strategies for mental health promotion

Barbara Collacchi^{1*}, Chiara Ciacchella^{1*}, Nadia Francia¹, Stefania Cerino²
and Francesca Cirulli³

¹*Centro di Riferimento per le Scienze Comportamentali e la Salute Mentale, Istituto Superiore di Sanità, Rome, Italy*

²*Centro di Riabilitazione Equestre "Girolamo de Marco" Onlus (CRE), Rome, Italy*

³*Centro Nazionale per la Ricerca e la Valutazione Preclinica e Clinica dei Farmaci, Istituto Superiore di Sanità, Rome, Italy*

**These Authors contributed equally to this work*

Abstract

Background. Animal-assisted interventions (AAI) represent complementary rehabilitative approaches designed to address a range of physical and mental health conditions. Among these, Equine-assisted interventions (EAI) offer structured opportunities for engagement through diverse activities conducted within naturalistic settings, thereby integrating therapeutic processes with ecological contexts.

Methods. Over the past years, our group has developed and implemented standardized AAI methodologies that are now embedded within comprehensive intervention planning. This overview summarizes the application of different rehabilitative programs based on EAI.

Results. Our findings suggest that EAI can be effectively implemented across a heterogeneous spectrum of psychiatric disorders, contributing to recovery processes and social integration across the lifespan, from childhood to old age.

Conclusions. EAI represent innovative, ecologically grounded interventions with promising clinical potential. Further research involving larger, well-characterized samples is warranted to strengthen the evidence base and to expand the applicability of these promising complementary interventions.

Key words

- human-animal interactions
- horses
- rehabilitation
- mental health
- complementary therapies

INTRODUCTION

During the past 20 years, growing attention has been directed toward the contribution of companion animals to individual well-being and mental health [1-3], promoting their introduction into a variety of social, educational, and, in some cases, therapeutic contexts [4]. Indeed, pets can be a source of emotional stimuli, offering opportunities for social engagement and physical activity relevant both in daily life and in institutionalized settings [4-9]. Interaction with animals can promote affiliative behaviors and reduce arousal, as documented in clinical and non-clinical populations (e.g., patients with mental disorders, animal owners, and non-owners) [10-12]. As for the underlying mechanisms, physical contact with a companion animal activates physiological systems specifically involved in emotional/affiliative behaviors, and arousal (e.g., beta-endorphin, cortisol, oxytocin, dopamine) [8, 11].

Collectively, this scientific evidence has been used as the basis to develop Animal-assisted interventions (AAI), which represent recreational, educational, or therapeutic activities in which companion animals are intentionally engaged to promote the physical and mental health of individuals [4-7, 13-18]. Structured and goal-oriented AAI have proven to be an effective integrative approach that can complement traditional pharmacological and non-pharmacological treatments [4, 6, 14, 18]. Grounded in the biopsychosocial model, AAI exert their effects through integrated biological, psychological, and social pathways [3, 8]. This multidimensional approach is also reflected in the distinction of AAI into three areas: Animal-assisted activities (AAA), Animal-assisted education (AAE), and Animal-assisted therapies (AAT) [19-21].

In Italy, the Ministry of Health's National Guidelines define the planning and monitoring of AAI, as well as

the role and specific training of clinical professionals, and of the veterinarians and the experts in animal management and behavior, which are part of the multidisciplinary team managing these interventions [4, 20, 21]. The multi-professional background of the team is essential to managing the complexity of the AAI and ensuring the well-being of the participants involved, both humans and animals [21, 22]. The choice of the clinical professionals involved depends upon the objectives to be achieved, which may be recreational (AAA), educational (AAE), or therapeutic (AAT) [4, 20, 21]. According to the Italian National Guidelines on AAI, the intervention team must also include animal experts (veterinarians and handlers). In every AAI program, veterinarians assess the animal's suitability for participation through a multimethod evaluation of health status, living conditions, workload, and equipment requirements [22]. Moreover, the handler is responsible for regularly monitoring the animal's well-being during intervention sessions through direct observation of its behaviors. The animal's characteristics should always be matched to the rehabilitation goals [21, 23-26]. The animal species primarily involved in AAI are those that have undergone a long process of domestication by humans (e.g., dogs, horses, donkeys, cats, and rabbits), which has led to the selection of physical and behavioral characteristics that are best suited to human needs, such as temperament traits, communicative and cognitive skills [4, 21]. Dogs share a long history of co-evolution with humans and have developed a highly complex system of nonverbal communication with our species. As a result, they are currently recognized as one of the most versatile species for AAI across a wide range of mental health conditions and settings [4, 6, 7, 22]. Regular interactions with dogs can provide significant benefits in both clinical and educational contexts, contributing to the reduction of symptoms such as anxiety, depression, and loneliness at both young and older ages. Small companion animals, like cats and rabbits, are becoming popular in AAI targeting institutionalized older adults, such as individuals diagnosed with dementia living in nursing homes. Although the evidence is still limited, interacting with cats appears to improve mobility, interpersonal relationships, and daily activities such as personal hygiene and self-care [22]. In addition, AAI implemented in natural and de-medicalized environments, primarily involving horses and donkeys, might be a good addition to conventional therapies. In this regard, horses are particularly well-suited as they demonstrate significant cognitive and interspecific abilities that allow them to discriminate human communicative behaviors and emotional states [27]. For instance, horses respond to humans pointing at food, indicating that they can associate gestures with relevant situations and develop encoding and interpretation strategies based on human cues [28]. Horses are likewise capable of distinguishing human emotional cues – such as vocal tones, posture, and, more generally, nonverbal communication – and modulating their behavior through mechanisms of emotional transfer [23, 24, 26, 29-33]. Additionally, horses form long-term memories about humans based on previous experiences and the quality of interactions,

extending those memories to new contexts or unfamiliar subjects [27]. In educational and therapeutic contexts, donkeys represent a valuable resource: their calm temperament and reduced size help mitigate the fear commonly elicited by horses, supporting their employment in AAI targeting individuals with cognitive and psychiatric disorders [22].

From a clinical/rehabilitative perspective, Equine-assisted interventions (EAI) integrate the beneficial effects of the emotional relationship with an animal with the opportunity to engage in physical activity, ultimately activating multiple functional domains. Participation in equestrian activities, in fact, provides a significant degree of motor stimulation, which improves coordination, balance, agility, and flexibility, at the same time providing cognitive enrichment and opportunities for the development of social skills [34-40]. This approach is in line with a large body of scientific evidence highlighting the benefits of prescribing sports and physical activities to promote mental health [1, 41-43]. Such benefits are further enhanced by the recreational and natural environment in which EAI are provided, which places the individual away from standard medicalized settings, increasing motivation for intervention adherence [39].

EAI IN DIFFERENT REHABILITATIVE SETTINGS

EAI are an extremely versatile approach that can be tailored to specific individual needs [5, 39, 44-48]. A recent scoping review has distinguished EAI programs into two major categories: unmounted EAI, which includes exclusively ground-based activities (grooming, stable work, and horse-handling exercise), and mounted EAI (riding, driving, or vaulting), which borrows technical elements of equestrian sports, adapting them to the rehabilitative goal [39]. Ground-based activities involve a predefined sequence of actions affecting neurobehavioral components. These are aimed at caring for the horse, facilitating skill development, cooperation, and teamwork. In addition, grooming activities can help to build trust and confidence between the horse and the rider, and to strengthen their mutual relationship over time. Horse handling and stable management activities further enhance confidence in interacting with the animal while supporting the development of self-esteem, autonomy, and self-efficacy. Neuromotor components are mostly engaged during horseback riding, the rhythmic movement of the horse inducing pelvic motions in the rider that closely resemble those of human gait. This neuromotor input contributes to the normalization of muscle tone, including reductions in spasticity, and supports overall improvements in motor function. Concurrently, mounted activities engage key cognitive neurobehavioral processes, such as attention, memory, executive functions, and spatial-temporal perception, while fostering self-regulation and effective coping strategies.

A correct balance between ground-based and mounted activities should be an integral part of the intervention methodology and should be matched with the behavioral characteristics of the rider-horse dyad

to ensure well-being and the achievement of tailored rehabilitation goals [49]. Equestrian activities offer a wide range of tools that can bring broad multifunctionality to the intervention methodology and influence the outcome of the rehabilitative process (Table 1). Over the years, our group has developed standardized protocols and methodologies that have become an integral part of the rehabilitation of children, adolescents, and adults with mental and/or neurodegenerative disorders (Table 2).

Applying EAI in children and adolescents

A substantial number of mental disorders have their onset in childhood or adolescence and can influence growth trajectories and social functioning [50]. Several studies have demonstrated that EAI can be effective in supporting young people facing mental health challenges [39, 44, 47]. Particularly with children and adolescents, the non-judgmental and calming presence of the horse within the setting is an important support for health professionals seeking to improve social behavior and address emotional problems [47, 51]. During EAI,

young people are facilitated in taking an active role in the healing process, with an improvement in their communication, cognitive, and social skills, also reducing the stigma associated with mental health conditions [44, 52]. Regular interactions with the horse promote positive attitudes towards another living being, fostering a sense of care and attention and providing a valuable opportunity to nurture self-esteem and enhance one's sense of responsibility [47]. Lastly, the recreational approach and the nature-based environment of EAI can elicit positive emotions and interest, thereby promoting adherence to the intervention plan [39, 51].

Children with autism spectrum disorder

Autism spectrum disorder (ASD) constitutes a heterogeneous diagnostic category of neurodevelopmental disorders [53]. Characteristic features include persistent difficulties in the social use of verbal and non-verbal communication, impairment in social interactions (social-emotional reciprocity; development, maintenance, and understanding of relationships), as well as restricted and repetitive patterns of behavior [53].

Table 1

Description of ground and mounted activities in Equine-assisted intervention sessions

	ACTIVITY	DESCRIPTION	OBJECTIVE AND CLINICAL IMPLICATION
GROUND	Grooming and rewarding	It involves a predefined sequence of actions aimed at caring for the horse and requires the use of specific tools (e.g., curry comb, soft brush, mane and tail brush, hoof pick). Observation of the horse's behaviour and reactions. When mounted activities are scheduled, the horse is also harnessed according to the type of activity (riding, vaulting, driving). Reward the horse with snacks at the end of the session (e.g., carrots, sugar, hay)	Facilitate non-verbal (tactile, visual, olfactory) contact and communication with the horse. Facilitate metacognitive understanding of the horse. Promote affiliative behaviours and the opportunity to exhibit prosocial behaviour. Promote emotional connection and trust. Learn the importance of being grateful
	Horse handling	Use a rope attached to the horse's halter to get the horse to move with clients. Learn the basic elements of leading a horse, such as stopping, turning, walking, tension and the proper way to hold the rope. More complex activities consist of hand leading the horse inside an arena following a maze with pre-determined elements (e.g., leading the horse over barriers, around poles and cones) and longing the horse in a circle	Experience the horse's responsiveness and make requests properly. Increase the comfort level with the horse. Gain confidence, self-esteem, self-efficacy and mastery
	Stable work	It includes stable management activities such as box and paddock cleaning, feeding and minor facility maintenance activities	Strengthen autonomy and self-efficacy. Promote responsibility, routine and reliability. Facilitate skill development, cooperation, teamwork and social growth
MOUNTED	Horse riding	More sophisticated communication with the horse based on physical contact that involves riding the horse, independently or assisted by a handler, inside and/or outside an arena. Learn and perform basic riding figures (e.g., circles, vaults, half vaults, serpentines). More complex activities include riding independently in an outdoor arena or completing gymkhana and dressage exercises	Improve motor skills (muscle strength, balance and coordination), cognitive skills (memory, attention, problem-solving executive functions and spatial-temporal perception), behavioural and emotional skills (modulate physiologic arousal levels, self-regulation behaviours, coping strategies). Enhance self-esteem, independence and self-efficacy
	Carriage driving	This modality facilitates access for people unable to ride due to weight, balance and physical limitations (e.g., severe obesity, lower limb disabilities, spinal cord injuries, back injuries, spina bifida) and consists of driving a carriage	
	Equestrian vaulting	More sophisticated communication with the horse is based on physical contact and involves performing gymnastic exercises to the rhythm of music and coordinating body movements with the horse, which is led in a circle by an experienced equestrian trainer. It also includes warm-up exercises and body-awareness exercises. Vaulting sessions are usually group-based	Improve motor skills (muscle strength, coordination, balance, agility and rhythm), cognitive skills (memory, attention and executive functions) and interpersonal skills through group-based activities. Promote creativity, body awareness and expressiveness

Table 2
Examples of standardised Equine-assisted intervention (EAI) methodologies and monitoring applied to populations with different clinical conditions

Population and clinical condition	INTERVENTION METHODOLOGY				MONITORING	
	Grooming and rewarding	Horse handling	Horse riding	Equestrian vaulting	Quantitative evaluation (outcome)	Qualitative evaluation (outcome)
Children with autism spectrum disorder	X		X	X	TOL (executive functioning and problem-solving) VABS (adaptive functioning) Video recording (problem behaviours, spatial relationships, social interactions, and communication displayed by the child toward the horse)	
Adolescents with eating disorders	X			X	BMI and BIA (body weight and composition) EDI-3 (eating disorder symptoms) SCL-90-R (psychopathological dimensions) STAI (anxiety)	Observation (participants' attitudes, emotionality, behaviour during the EAI sessions, and relationship with horse and team members)
Adolescents at risk for psychopathologies	X	X	X	X		Observational schedules (participants' socialisations, attitudes, emotionality, behaviour, and motor performance during the EAI sessions)
Adults with stress-related disorders	X	X	X		HAM-D (depression) SCL-90-R (psychopathological dimensions) STAI form Y (anxiety) WEMWBS (psychophysical well-being)	Observation (participants' attitudes, emotionality, behaviour during the EAI sessions, and relationship with horse and team members) Group discussions guided by a psychiatrist (psychological themes elicited by the EAI)
Adults with schizophrenia	X	X	X		BPRS (negative symptoms) PANSS (positive symptoms)	
Adults with Parkinson's disease	X		X		AES (apathy) Gait analysis and UPDRS (motor assessment) GDS (depression) PD-39 (quality of life) STAI form Y Stroop test (cognitive performance)	

TOL: Tower of London test; VABS: Vineland Adaptive Behavior Scale (VABS); BMI: Body Mass Index; BIA: Bioimpedance Analysis; EDI-3: Eating Disorder Inventory-3; SCL-90-R: Symptom Checklist-90-Revised Scale; STAI: State-Trait Anxiety Inventory; HAM-D: Hamilton Depression Rating Scale; WEMWBS: Warwick Edinburgh Mental Well-Being Scale; BPRS: Brief Psychiatric Rating Scale; PANSS: Positive and Negative Syndrome Scale; AES: Apathy Evaluation Scale; UPDRS: Unified Parkinson's disease Rating Scale; GDS: Geriatric Depression Scale; PD-39: Parkinson's Disease Questionnaire-39.

ASD affects more than 78 million people in the world, and approximately 1 out of 100 children receives a diagnosis [54, 55]. The complex nature of ASD calls for a multidimensional treatment approach, and EAI can complement traditional treatments, including educational/behavioral interventions and pharmacotherapy [51, 56, 57]. It has been shown that EAI can have a positive effect on the social and behavioral skills of children with ASD, as well as on the well-being of their caregivers, reducing parental stress and improving family functioning [51, 52, 58, 59].

The distinctive characteristics of ASD highlight the importance of tuning the intervention protocol to promote the child's optimal adaptation and involvement during the sessions. Individuals with ASD do not cope

well with abrupt changes and intense multisensory stimulation. Indeed, in the initial stages of EAI, it may be appropriate to plan short sessions with a maximal duration of 30 minutes, and then gradually lengthen the activities to around 60 minutes. This approach promotes a gradual adaptation to non-familiar situations, removing an excessive burden coming from multiple novel sensory and emotional inputs. According to the evidence gathered from our projects, each session should incorporate an initial ground-based activity, including horse grooming (i.e., brushing the horse's coat, mane, and tail with a comb or brush), during which children are educated on basic safety rules and learn the practical aspects necessary for a positive interaction with the animal [58, 60, 61]. Images and drawings depicting

grooming tools (comb, brush, and hoof pick) and horse facial expressions are commonly used as visual aids to further facilitate the child during this phase [58].

Subsequently, sessions can include mounted activities, such as equestrian vaulting (EV) or riding. EV is a valuable initial approach, which is particularly well-suited to younger children and those with difficulties with impulse control. Indeed, during vaulting activities, the child does not have to deal with the responsibility of leading the horse and is therefore able to focus more on the tasks proposed by the therapist. Furthermore, vaulting activities facilitate collaborative learning in small groups, allowing therapists to integrate children with ASD into peer-based activities. This approach has the additional benefit of creating a supportive relational network and improving opportunities for social interactions. By contrast, riding activities are designed to facilitate cognitive rehabilitation through the acquisition of essential skills for independently managing a horse within an arena. The setting can be further enriched with appropriate stimuli (music in the case of EV; letters, cones, barriers, cup games in the case of horseback riding), increasing involvement and promoting treatment adherence. At the end of each session, the intervention typically entails a further ground-based phase, which represents an opportunity for children to engage in prosocial and affiliative behaviors by providing the horse with rewards (e.g., carrots, sugar, hay), as well as verbal and non-verbal expressions of gratitude and farewell [58, 62]. When developing an intervention program for children with ASD, it is important to adapt the activity to their individual functional levels. As mounted activities require concentration and compliance with professional instructions to ensure safety, they may be less suitable for individuals with low-functioning ASD. In these cases, grooming, horse handling exercises, and stable work are generally preferred, as they offer greater adaptability and fewer safety constraints.

To reliably assess the efficacy of the EAI, a multi-method approach is suggested, which incorporates different tools, such as caregiver questionnaires, standardized tests, and video recording and coding (see Table 2) [63, 64]. Quantitative tools can be used to evaluate the effects of EAI, monitoring improvements in adaptive and executive functioning and problem-solving skills. Additionally, quantitative evaluation can be complemented with direct observation and analysis of child behavior through video recordings [61]. This method, which relies on an ethological approach, is considered the best way to assess the quality of human-horse interaction in children with ASD.

Results from multiple projects show that, starting from the grooming phase, the presence of the horse contributes to creating a rehabilitative space in which the child learns to relate with adults and peers and performs specific tasks that stimulate cooperation, attention, and memory skills [58, 60, 61]. Additionally, the soothing influence of equine interaction and the proprioceptive input elicited during mounted activities – both equestrian vaulting (EV) and riding – allows modulation of the levels of sensory and affective arousal [62], as well as positively influencing problem-

solving abilities and executive functions [52, 58, 65]. Horse stimulation can also improve gross motor skills such as balance, coordination, and posture [66]. Lastly, the unspoken communication with the horse enables children with ASD to establish and gradually reinforce an emotional connection with the animal, overcoming the communication and social skills difficulties [52, 61]. From a psychotherapeutic perspective, the child's relationship with the animal can catalyze the expression of internal states and experiences, thereby creating a foundation for therapeutic work [60].

Adolescents with eating disorders

Eating disorders (ED) are mental health disorders characterized by persistent disturbances in eating or feeding behaviors that significantly impair physical health or psychosocial functioning [53]. ED typically have a chronic course with long-term remission rates and an increased risk of suicide; among these, anorexia nervosa (AN) has limited recovery and the highest mortality [67, 68]. Individuals with AN exhibit an intense fear of gaining weight or a persistent pattern of behavior that interferes with it, as well as disturbances in body weight or shape perception [53]. Treatment plans require the intervention of a multidisciplinary team, and they may include individual, group, or family psychotherapy, nutritional counselling, and medical care [69]. As treatment failure and relapses are common after completion of therapy [70], developing supplementary rehabilitative interventions to enhance the efficacy of established treatments is a specific unmet need [71].

Our group has recently developed a pilot project named "Horses and Butterflies", to test the feasibility and effectiveness of an EAI based on EV that exploited the physical and emotional synergy between horse and participant for the psychosocial rehabilitation of young patients with AN [72, 73]. The intervention included a grooming phase designed to shift participants' attention from obsessive thoughts about their own bodies toward the care of another living being, thereby fostering the development of a positive relationship with the horse [73]. This was followed by warm-up and vaulting exercises aimed at restoring bodily awareness. The movement is no longer used to sustain anorexic symptoms related to weight reduction but instead becomes a means of expression and interaction, initially with the horse and subsequently with other participants, promoting social engagement. EV involves a substantial physical effort, encompassing muscular strength, postural control, and coordination. Access to EV-based programs for individuals with AN should therefore be conditional upon a multidisciplinary clinical evaluation, aimed at ensuring participant safety and an appropriate risk-benefit balance. In particular, it is essential to confirm that body mass index (BMI) is not critically low (e.g., BMI < 16) and that sufficient skeletal and muscular integrity is present, considering the increased risk of osteoporosis and reduced muscle mass commonly associated with the disorder.

Seven girls (aged between 13 and 20 years old), in outpatient treatment for anorexia, were enrolled in the project and randomly assigned to two groups: 3 to the

EV group and 4 to the control group. The intervention lasted 3 months and comprised 10 weekly sessions (each 60-90 minutes long) carried out in small groups [72].

Longitudinal evaluations of anthropometric indices and body composition were complemented by psychological tests (Table 2) [74-78]. Participants' attitudes, emotions, and general behaviors were observed by a psychologist during the session, as well as their relationship with the horses and the other team members. This intervention has shown good feasibility and a high level of acceptability by the participants and their families [72]. Preliminary results indicate a promising improvement in some of the core psychological symptoms associated with anorexia (e.g., perfectionism, interoceptive deficits, overcontrol, and affective problems), anxiety management, and physical condition (an increase in fat mass and a reduction in lean mass weight) [72, 73] suggesting that it can be employed effectively as an integrative approach to promote recovery of body awareness and social reintegration in AN.

Vulnerable adolescents

As many mental disorders have their onset during adolescence, a proactive approach is needed to prevent mental illness and to promote the well-being of individuals in this vulnerable age group [79-81]. Adolescents in residential treatment facilities often fail to adapt and exhibit strong opposition to social norms, showing difficulties in social relationships, reduced school attendance, a propensity towards self-harm, and an inability to regulate their emotions and behavior effectively in the face of frustration or competition [82].

Previous studies have shown the potential for EAI on the socio-emotional functioning of at-risk adolescents in residential care [82, 83]. We have set up the pilot project *Horses & Friends* to evaluate the suitability and potential benefits of an EAI program as a complementary tool to promote the psycho-physical well-being and socio-emotional functioning of adolescents undergoing psychiatric treatment in a therapeutic residential setting [84]. The project was conducted over five months, with weekly sessions lasting 70 minutes. The intervention was led by a multidisciplinary team of qualified professionals in mental health and EAI. Each session included a 1st ground activity phase (grooming and horse handling), a 2nd mounted activity phase (both riding and vaulting activities), and a 3rd ground activity phase (final grooming and rewarding of the horse). The psychologists involved in the project employed qualitative evaluations through observational schedules to monitor participants' socializations (interactions with horses, peers, and team members) and progress in terms of attitudes, emotionality, behavior, and motor performance during the activities.

During the sessions, the participants established a strong emotional connection with the horses, which was a motivating factor and a catalyst for interpersonal relationships. This is relevant as rehabilitation work with adolescents living in a residential facility is largely focused on positively redirecting aggressive behavior, replacing alienation and hostility with feelings of trust,

safety, and acceptance [82]. When implementing EAI for vulnerable adolescents, it is crucial to address motivation from the outset to foster active engagement and reduce the risk of dropout. The interaction with the horse was perceived as fun, stimulating, and engaging, promoting good adherence to the intervention and improvements in the adolescents' attitudes and behavior during the session. Moreover, a social gathering was organized after each session, which proved to be a valuable experience, facilitating deeper connections between participants and reinforcing the interpersonal relationships established during the project.

The integration of two distinct sporting activities, such as horse riding and vaulting, has been particularly beneficial for adolescents, enabling them to simultaneously develop a range of functional competencies. Autonomous horse riding fostered an empowering process, strengthening self-esteem and anxiety management. Additionally, vaulting exercise improved physical skills, including coordination, balance, and agility, as well as concentration, attention, and courage. These qualitative findings are in line with recent evidence about the positive effects of physical activity as a rehabilitative tool for young people with mental disorders [85].

Applying EAI in adults with mental and neurodegenerative disorders

Mental health disorders in adulthood have been associated with an increased prevalence of unhealthy lifestyles, including smoking, weight gain, sedentary behaviors, and decreased physical activity. Additionally, social withdrawal and the impoverishment of emotional relationships can contribute negatively to mental health and quality of life in at-risk individuals [86, 87]. In this overview, we report some examples of EAI applied to adults with stress-related (e.g., military veterans), psychotic (e.g., schizophrenia), and neurodegenerative (e.g., Parkinson's disease) disorders.

EAI in adults with stress-related disorders

In the last decade, EAI has emerged as a promising treatment opportunity for trauma and stressor-related disorders [88, 89]. Most studies on the topic focus on the population of military veterans who, compared with the general population, have a higher risk of being challenged with acute or chronic stressors [90-92]. A recent systematic review and meta-analysis on EAI for military veterans underlines the great heterogeneity of the intervention protocols, emphasizing the need for standardized procedures and tools that may allow evaluation of the effectiveness of this type of intervention [88]. In a preliminary study, Arnon *et al.* [91] developed a standardized EAI protocol for a sample of US military veterans, which appears promising, particularly in terms of short-term symptomatic benefits, improved quality of life, acceptance, and satisfaction with the type of intervention.

Over the past two years, our group has developed an evidence-based, standardized EAI program to support the mental health of military veterans. The intervention's primary focus was on the promotion of effective coping strategies, the alleviation of psychological

symptoms associated with adjustment difficulties, and the enhancement of socio-emotional skills. Additionally, the intervention sought to facilitate a re-evaluation of one's military identity [93]. Longitudinal evaluations were carried out using standardized psychological scales to measure anxiety and depressive symptoms, psychopathological dimensions, and psychophysical well-being (Table 2) [74, 77, 94-96]. The progress of the participants was also assessed through qualitative observations of their attitudes, behaviors, and emotional responses during the EAI sessions.

The project revealed a high degree of adherence by veterans who enjoyed the activities proposed, a relevant result considering the resistance toward traditional therapies and the high dropout rate among this population [91, 97]. During the project, participants had the opportunity to enjoy contact with the natural environment and to challenge themselves with activities they had never tackled before, such as horse handling and riding, which require leadership, benefiting veterans by allowing them to regain their autonomy and sense of agency in a safe and less stressful natural environment [93]. In addition, the activity with the horse brought out relevant identity issues that were shared by the entire group of participants during discussions guided by a psychiatrist. Given the considerable heterogeneity of stress-related and physical conditions observed among military veterans, it is essential to adapt both the intervention and the associated assessment methods through personalized treatment plans. In our experience, the overall intervention included the successful adaptation of the rehabilitation protocol to integrate two participants with physical disabilities, one veteran in a wheelchair and another with blindness, into the EAI sessions, modulating the activities to their specific needs [93].

EAI in adults with schizophrenia

Schizophrenia is a complex psychopathological condition, and the combination of positive (e.g., delusions and hallucinations) and negative (e.g., blunted affect, alogia, social withdrawal, anhedonia, and avolition) symptoms contributes to significant alterations in individual's functioning [98, 99]. In addition, cognitive deficits lead to difficulties in understanding the mental states of others (e.g., beliefs, intentions) and using this information to interpret and predict their behavior [100]. Therefore, the intervention should focus not only on reducing psychotic symptoms but also on promoting social functioning.

Over the past 20 years, many studies have supported the benefits of EAI as an integrative rehabilitative approach for the mental and physical health of patients with psychotic disorders [5, 35, 46, 101, 102]. The multicenter *Pindar* project is an example of good practice demonstrating the effectiveness of a year-long EAI that followed a structured methodology designed for 24 patients diagnosed with schizophrenia [5]. Involving individuals with psychotic disorders in EAI can present significant challenges: acute positive symptoms can interfere with safe interaction with the horse and the therapeutic environment. For this reason, the EAI

should be implemented when positive symptoms are in remission or adequately stabilized through pharmacological treatment. Under these conditions, EAI can be effectively oriented toward relapse prevention, functional recovery, and the targeted improvement of negative symptoms.

In the *Pindar* project, a total of 40 EAI weekly sessions were held for 24 months, after a few introductory meetings planned to help the participants gradually familiarize themselves with horses. Each session included a ground phase, during which participants learned about horses (behavior and body parts) and equipment (saddle and bridle), as well as horseback riding activities aimed at developing nonverbal communication strategies for interacting with the animal [5].

To evaluate the improvements in positive and negative psychiatric symptoms, psychological scales were administered at the beginning and the end of the project (Table 2) [103, 104].

Overall results indicate that EAI allowed patients to take an active role in their rehabilitation process, with significant improvements in negative symptomatology (social isolation and apathy), pharmacological compliance, and hospitalization rates [5]. In particular, the grooming and horse handling activities have allowed participants to experience the animal's calming presence, which became a starting point for simplified interaction and to regain contact with the external world [5]. In this regard, the horse acted as a facilitator by fostering communication: horse-related activities help divert attention away from the disease and provide a topic of conversation within and outside the intervention setting, helping participants in social integration and overcoming self-stigmatization [35, 101]. In addition, observing horses' behavior and reactions during ground activities favored the rehabilitation of cognitive abilities [105].

The rehabilitative work set up during ground activities can be strengthened by horse riding [105]. During riding activities, the communication process is mediated by bodily contact, which allows concretely tackling the difficulties of perception and management of reality; the correct use of reins and legs allows the patients to interact directly with the animal, and body balance adjustments allow the exchange of information between horse and rider. Moreover, horse riding contributes to increasing interest in physical activity, leading to improvements in motor skills and counteracting obesity, a highly common condition in these patients, mainly due to side effects of pharmacological therapies [105].

In conclusion, the integration of EAI into usual care is particularly suited for patients with schizophrenia, showing the potential to foster disease remission, social contact, and the acquisition of skills transferable to other areas of the participant's life [5].

EAI in adults with Parkinson's disease

Parkinson's disease (PD) is a progressive and neurodegenerative disease affecting motor function. This condition is also accompanied by a range of non-motor symptoms, including mood and cognitive disorders [106], impacting patients' quality of life. Current treat-

ment involves a combination of pharmacological and rehabilitative therapies. Patients with PD usually attend highly medicalized treatment programs, whereas the natural context that characterizes EAI may favor higher compliance with the intervention [107, 108]. Indeed, EAI is a suitable approach to tackle PD as it allows synergistic intervention on motor (e.g., balance, posture, gait, rigidity, and involuntary movements), cognitive, and socio-emotional functioning [107, 108].

We report an example of EAI that has followed a standardized 5-week protocol, including weekly sessions of 60 minutes [108]. Several challenges may occur when including individuals with PD in EAI. Motor symptoms raise safety concerns and may increase the fear of falling, negatively affecting confidence and participation. Grooming was planned at the beginning and the end of each session to progressively help patients with PD work on gross-motor and coordination skills. Riding activities constituted the core component of the intervention methodology, given the strong stimulation of the patient's proprioception skills by the horse's gait, which closely resembles that of human walking. This is of particular benefit to patients with difficulties in ambulation [108]. The execution of exercises on the horse (rotation, flexion, extension of the upper arms, and bending/torsion of the trunk) facilitated the stimulation of the vestibular system, thereby assisting in the enhancement of balance maintenance, both in the saddle and following dismount [108]. During mounted activities, reduced tolerance to fatigue should be carefully considered, modulating the intensity of exercises to maintain physical exertion throughout the session. These difficulties are often compounded by depressive states and anhedonia, which are common in this population and can further reduce motivation. However, the interaction between humans and horses has been observed to elicit positive emotional responses that may have a beneficial impact on mood disturbances and quality of life [108]. Gait analysis, motor assessment, and psychological evaluations of cognitive performance, depressive symptoms, apathy, and quality of life were conducted before and after the EAI (Table 2) [95, 109-115]. A comparison of the pre- and post-intervention scores revealed a general improvement in motor skills, as well as a positive change in gait, including an increase in stride length and a decrease in stance and double stance rates [108]. Cognitive and psychological assessments indicated improvements in attention, quality of life, and a reduction in anxiety and apathy levels [108]. These results have been further confirmed by a more recent study [107], indicating that a structured EAI methodology can be integrated with other protocols in the rehabilitation of PD individuals.

DISCUSSION AND CONCLUSIONS

EAI are professional-led interventions aimed at enhancing health and well-being by actively involving individuals in structured and nature-based experiences, stimulating multiple functional domains at the same time (social, emotional, and physical). The interaction between motor, cognitive, and socio-emotional skills has been a particular area of interest for researchers

studying both typical and atypical development across the lifespan [116]. Motor functioning has been demonstrated to have clear associations with the development of communication and object knowledge; moreover, training motor skills and acquiring additional patterns of actions (e.g., those related to different sports techniques) have been proven to have a positive impact on socialization and interpersonal relationships in both children and adults [117, 118]. As evidenced by the experiences presented in this overview, incorporating riding or vaulting activities into EAI enables targeted intervention to improve balance, coordination, and agility. Concurrently, these activities foster the development of cognitive abilities (executive functions, memory, and attention), social skills, and emotional regulation strategies, underscoring their multifaceted benefits [34-40, 119].

From a mechanistic perspective, the rehabilitation process can have important effects on brain plasticity by activating those brain circuits that are dysfunctional as a consequence of the disease, especially after a chronic phase [120]. Another fundamental notion is the existence of periods of vulnerability/opportunity in which the brain can be influenced by stimuli that can promote plasticity of brain areas affected by the pathology. Among the periods of greatest vulnerability to mental illness, we can list the perinatal stages and adolescence [120]. These considerations point to the need to carry out early interventions for greater functional and behavioral recovery.

It has also been proposed that EAI can impact physiological mechanisms underlying emotional regulation and arousal, which are dysfunctional in affective disorders. Tactile contact and direct eye contact with dogs during human-animal interaction are associated with increased oxytocin release, which plays a central role in the regulation of affiliative behaviors within intraspecific relationships [11, 121]. Preliminary data on human-horse contact indicate a significant increase in oxytocin during the first and seventh week of intervention, and a recent study investigated changes in human plasma oxytocin levels after engaging with cows, hypothesizing that interactions with equine and farm animals can elicit the same physiological response [122, 123]. It is widely recognized that the physical contact experienced during grooming activity represents a significant aspect of the EAI. Such contact can facilitate relaxation, a calming response in the participants, and the human-horse emotional connection, thereby contributing to the overall rehabilitation outcome.

In addition, the non-judgmental and welcoming presence of the horse can facilitate the acquisition of a greater awareness of the emotional internal states and the external world [93]. The horse can represent a powerful motivational stimulus and can act as a bridge in establishing trust with the mental health professional [93] who should support the individual in identifying emotions, memories, thoughts, and representations related to their personal experience.

Notwithstanding the promising findings reported in this overview, further research is needed to improve knowledge on the effectiveness of EAI, particularly as

it relates to the underlying mechanisms. Future studies should involve larger samples, appropriate control groups, balanced gender representation, and standardized assessment tools tailored both to the clinical characteristics of the target population and to the specific components of the intervention. A recent paper highlighted the importance of harmonizing the terminology, proposing the umbrella term of Animal-assisted services (AAS), to increase transparency about the key features of the different approaches in the field [124]. In this project's collection, we have maintained the Italian standard terminology as requested by the Italian Ministry of Health [20]. As the field progresses, a common terminology must be agreed upon by health institutions and scientific societies. Nonetheless, strengthening methodological rigor is crucial to allow AAI, or AAS, to be upscaled to "best practices" which are fundamental to innovate the mental health sector (<https://ja-mentor.eu/>).

Acknowledgements

We acknowledge the precious help of Marta Borgi, Antonio Maione, Irene Pistella, and Stella Falsini for technical support.

REFERENCES

- Ciacchella C, Veneziani G, Garenna SA, Lai C. Interpersonal and pet bonding: a meta-analytic review of attachment dimensions. *J Soc Pers Relat.* 2025;42:337-64. doi: 10.1177/02654075241285440
- Brooks HL, Rushton K, Lovell K, Bee P, Walker L, Grant L, et al. The power of support from companion animals for people living with mental health problems: a systematic review and narrative synthesis of the evidence. *BMC Psychiatry.* 2018;18(1):31. doi: 10.1186/S12888-018-1613-2
- Gee NR, Rodriguez KE, Fine AH, Trammell JP. Dogs supporting human health and well-being: a biopsychosocial approach. *Front Vet Sci.* 2021;8:630465. doi: 10.3389/FVETS.2021.630465
- Cirulli F, Borgi M, Berry A, Francia N, Alleva E. Animal-assisted interventions as innovative tools for mental health. *Ann Ist Super Sanità.* 2011;47(4):341-8. doi: 10.4415/ANN.11_04_04
- Cerino S, Cirulli F, Chiarotti F, Seripa S. Non conventional psychiatric rehabilitation in schizophrenia using therapeutic riding: the FISE multicentre Pindar project. *Ann Ist Super Sanità.* 2011;47(4):409-14. doi: 10.4415/ANN.11_04_13
- Correale C, Borgi M, Collacchi B, Falamesca C, Gentile S, Vigeveno F, et al. Improving the emotional distress and the experience of hospitalization in children and adolescent patients through animal assisted interventions: a systematic review. *Front Psychol.* 2022;13:840107. doi: 10.3389/FPSYG.2022.840107
- Berry A, Borgi M, Terranova L, Chiarotti F, Alleva E, Cirulli F. Developing effective animal-assisted intervention programs involving visiting dogs for institutionalized geriatric patients: a pilot study. *Psychogeriatrics.* 2012;12(3):143-50. doi: 10.1111/J.1479-8301.2011.00393.X
- Friedman E, Krause-Parello CA. Companion animals and human health: benefits, challenges, and the road ahead for human-animal interaction. *Rev Sci Tech.* 2018;37(1):71-82. doi: 10.20506/RST.37.1.2741
- Wells DL. The state of research on human-animal relations: implications for human health. *Anthrozoos.* 2019;32:169-81. doi: 10.1080/08927936.2019.1569902
- Khalid A, Dildar S. Effect of pet interaction on stress reduction and positive mood enhancement among pet-owners and non-owners. *Human-Animal Interact Bull.* 2019;7(1):77-104. doi: 10.1079/HAI.2019.0005
- Liu H, Lin J, Lin W. Cognitive mechanisms and neurological foundations of companion animals' role in enhancing human psychological well-being. *Front Psychol.* 2024;15:1354220. doi: 10.3389/FPSYG.2024.1354220
- Plett O, Flasbeck V, Brüne M. Effects of human and animal-assisted skills training on oxytocin und cortisol levels in patients with borderline personality disorder. *J Psychiatr Res.* 2023;162:156-60. doi: 10.1016/J.JPSYCHIRES.2023.05.004
- Chang SJ, Lee J, An H, Hong WH, Lee JY. Animal-assisted therapy as an intervention for older adults: a systematic review and meta-analysis to guide evidence-based practice. *Worldviews Evidence-Based Nurs.* 2021;18(1):60-7. doi: 10.1111/WVN.12484
- Berry A, Borgi M, Francia N, Alleva E, Cirulli F. Use of assistance and therapy dogs for children with autism spectrum disorders: a critical review of the current evidence. *J Altern Complement Med.* 2013;19(2):73-80. doi: 10.1089/ACM.2011.0835
- Kruger KA, Serpell JA. Animal-assisted interventions in mental health: definitions and theoretical foundations. In: Fine AH (Ed). *Handbook on animal-assisted therapy: foundations and guidelines for practice.* Academic Press; 2010. p. 33-48. doi: 10.1016/B978-0-12-381453-1.10003-0
- Rehn AK, Caruso VR, Kumar S. The effectiveness of animal-assisted therapy for children and adolescents with autism spectrum disorder: a systematic review. *Complement Ther Clin Pract.* 2023;50:101719. doi: 10.1016/J.CTCP.2022.101719
- Tyssedal MK, Johnsen E, Brønstad A, Skrede S. Dog-as-

This work was conceptually supported by the Joint Action "MENTOR – Mental Health Together" grant agreement: 101162928.

Declaration of artificial intelligence (AI) use

During the preparation of this work, the Authors used ChatGPT to revise the English language. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

Authors' contributions

BC, CC: conceptualization, writing – original draft, writing – review and editing; NF: review and editing; SC: writing – review and editing; FC: conceptualization, writing – review and editing.

Conflict of interest statement

The Authors declare that they have no conflict of interest.

Received on 4 March 2026.

Accepted on 11 May 2026.

- sisted interventions for adults diagnosed with schizophrenia and related disorders: a systematic review. *Front Psychiatry*. 2023;14:1-21. doi: 10.3389/FPSYT.2023.1192075
18. Veilleux A. Benefits and challenges of animal-assisted therapy in older adults: a literature review. *Nurs Stand*. 2021;36(1):28-33. doi: 10.7748/NS.2020.E11625
 19. Jegatheesan B, Beetz A, Ormerod E, Johnson R, Fine F, Yamazaki K, Dudzik C, Garcia RM, Winkle M, Choi G. The IAHAIO definitions for animal assisted intervention and guidelines for wellness of animals involved in AAI. *IAHAIO White Paper*; 2018. p. 499-504.
 20. Ministero della Salute. Interventi assistiti con gli animali. Roma: Ministero della Salute; 2015.
 21. Simonato M, Santis M De, Contalbrigo L, Benedetti D, Mahne EF, Santucci VU, et al. The Italian agreement between the Government and the Regional Authorities: national guidelines for AAI and institutional context. *People Anim Int J Res Pract*. 2018;1(1):1-11.
 22. Francia N, Borgi M, Collacchi B, Cirulli F. Metodologie per la valutazione dell'idoneità e del benessere animale negli interventi assistiti con gli animali. Roma: Istituto Superiore di Sanità; 2019. (Rapporti ISTISAN, 19/4).
 23. Grandgeorge M, Hausberger M. Human-animal relationships: from daily life to animal-assisted therapies. *Ann Ist Super Sanità*. 2011;47(4):397-408. doi: 10.4415/ANN_11_04_12
 24. Contalbrigo L, Borgi M, De Santis M, Collacchi B, Tuozi A, Toson M, et al. Equine-assisted interventions (EAIs) for children with autism spectrum disorders (ASD): behavioural and physiological indices of stress in domestic horses (*Equus caballus*) during riding sessions. *Anim*. 2021;11(6):1562. doi: 10.3390/ANI11061562
 25. De Santis M, Contalbrigo L, Borgi M, Cirulli F, Luzi F, Redaelli V, et al. Equine-assisted interventions (EAIs): methodological considerations for stress assessment in horses. *Vet Sci*. 2017;4(3):44. doi: 10.3390/VETSCI4030044
 26. Rochais C, Lerch N, Gueguen L, Schmidlin M, Bonamy O, Grandgeorge M, et al. Horses' tactile reactivity differs according to the type of work: the example of equine-assisted intervention. *Vet Sci*. 2023;10(2):130. doi: 10.3390/VETSCI10020130
 27. Hausberger M, Stomp M, Sankey C, Brajon S, Lunel C, Henry S. Mutual interactions between cognition and welfare: the horse as an animal model. *Neurosci Biobehav Rev*. 2019;107:540-59. doi: 10.1016/J.NEUBIOREV.2019.08.022
 28. Fureix C, Jegou P, Sankey C, Hausberger M. How horses (*Equus caballus*) see the world: humans as significant "objects". *Anim Cogn*. 2009;12(4):643-54. doi: 10.1007/S10071-009-0223-2
 29. Baba C, Kawai M, Takimoto-Inose A. Are horses (*Equus caballus*) sensitive to human emotional cues? *Anim*. 2019;9(9):630. doi: 10.3390/ANI9090630
 30. Rochais C, Stomp M, Sébilleau M, Houdebine M, Henry S, Hausberger M. Horses' attentional characteristics differ according to the type of work. *PLoS One*. 2022;17(7):e0269974. doi: 10.1371/JOURNAL.PONE.0269974
 31. Scopa C, Contalbrigo L, Greco A, Lanatà A, Scilingo EP, Baragli P. Emotional transfer in human-horse interaction: new perspectives on Equine assisted interventions. *Animals (Basel)*. 2019;9(12):1030. doi: 10.3390/ANI9121030
 32. Lerch N, Cirulli F, Rochais C, Lesimple C, Guilbaud E, Contalbrigo L, et al. Interest in humans: comparisons between riding school lesson equids and assisted-intervention equids. *Animals (Basel)*. 2021;11(9):2533. doi: 10.3390/ANI11092533
 33. Nakamura K, Takimoto-Inose A, Hasegawa T. Cross-modal perception of human emotion in domestic horses (*Equus caballus*). *Sci Rep*. 2018;8(1):8660. doi: 10.1038/S41598-018-26892-6
 34. Guindos-Sanchez L De, Lucena-Anton D, Moral-Munoz JA, Salazar A, Carmona-Barrientos I. The effectiveness of hippotherapy to recover gross motor function in children with cerebral palsy: a systematic review and meta-analysis. *Children (Basel)*. 2020;7(9):106. doi: 10.3390/CHILDREN7090106
 35. Jormfeldt H, Carlsson IM. Equine-assisted therapeutic interventions among individuals diagnosed with schizophrenia. A systematic review. *Issues Ment Health Nurs*. 2018;39(8):647-56. doi: 10.1080/01612840.2018.1440450
 36. Palomar-Ciria N, Bello HJ. Equine-assisted therapy in post-traumatic-stress disorder: a systematic review and meta-analysis. *J Equine Vet Sci*. 2023;128:104871. doi: 10.1016/J.JEVS.2023.104871
 37. Prieto A, Martins Almeida Ayupe K, Nemetala Gomes L, Saúde AC, Gutierrez Filho P. Effects of Equine-assisted therapy on the functionality of individuals with disabilities: systematic review and meta-analysis. *Physiother Theory Pract*. 2022;38(9):1091-106. doi: 10.1080/09593985.2020.1836694
 38. Sebalj M, Lakhani A, Grindrod A, Stuckey R. Equine-assisted services for people living with dementia: a systematic review. *Alzheimers Res Ther*. 2024;16(1):76. doi: 10.1186/S13195-024-01453-4
 39. Ward J, Hovey A, Brownlee K. Mental health benefits of mounted equine-assisted therapies: a scoping review. *Health Soc Care Community*. 2022;30(6):e4920-35. doi: 10.1111/HSC.13904
 40. White-Lewis S. Equine-assisted therapies using horses as healers: a concept analysis. *Nurs Open*. 2019;7(1):58-67. doi: 10.1002/NOP2.377
 41. Eime RM, Young JA, Harvey JT, Charity MJ, Payne WR. A systematic review of the psychological and social benefits of participation in sport for children and adolescents: informing development of a conceptual model of health through sport. *Int J Behav Nutr Phys Act*. 2013;10:98. doi: 10.1186/1479-5868-10-98
 42. Firth J, Solmi M, Wootton RE, Vancampfort D, Schuch FB, Hoare E, et al. A meta-review of "lifestyle psychiatry": the role of exercise, smoking, diet and sleep in the prevention and treatment of mental disorders. *World Psychiatry*. 2020;19(3):360-80. doi: 10.1002/WPS.20773
 43. Ley C, Rato Barrio M, Koch A. "In the sport i am here": therapeutic processes and health effects of sport and exercise on PTSD. *Qual Health Res*. 2018;28(3):491-507. doi: 10.1177/1049732317744533
 44. Carlsson I-M, Bräutigam Ewe M, Nymberg P, Jormfeldt H. Building up bit by bit, parent's experiences of equine-assisted intervention among children and adolescents with mental illness: a grounded theory study. *Int J Qual Stud Health Well-Being*. 2024;19(1):2354945. doi: 10.1080/17482631.2024.2354945
 45. Fridén L, Hultsjö S, Lydell M, Jormfeldt H. Experiences of a Therapeutic Equine-Assisted Group Intervention for People with Common Mental Disorders on Sick-Leave. *Issues Ment Health Nurs*. 2024;45(3):344-51. doi: 10.1080/01612840.2023.2301611
 46. Fridén L, Hultsjö S, Lydell M, Jormfeldt H. Relatives' experiences of an equine-assisted intervention for people with psychotic disorders. *Int J Qual Stud Health Well-Being*. 2022;17(1):2087276. doi: 10.1080/17482631.2022.2087276

47. Punzo K, Skoglund M, Carlsson IM, Jormfeldt H. Experiences of an Equine-assisted therapy intervention among children and adolescents with mental illness in Sweden - A nursing perspective. *Issues Ment Health Nurs*. 2022;43(12):1080-92. doi: 10.1080/01612840.2022.2126571
48. Fine AH, Andersen SJ. A Commentary on the contemporary issues confronting Animal assisted and Equine assisted interactions. *J Equine Vet Sci*. 2021;100:103436. doi: 10.1016/J.JEVS.2021.103436
49. Grandgeorge M, Lerch N, Delarue A, Hausberger M. From human perception of good practices to horse (*Equus caballus*) welfare: example of equine-assisted activities. *Animals (Basel)*. 2024;14(17):2548. doi: 10.3390/ANI14172548
50. Ferrie J, Miller H, Hunter SC. Psychosocial outcomes of mental illness stigma in children and adolescents: a mixed-methods systematic review. *Child Youth Serv Rev*. 2020;113:104961. doi: 10.1016/J.CHILDYOUTH.2020.104961
51. Fan MSN, Li WHC, Ho LLK, Phiri L, Choi KC. Nature-based interventions for autistic children: a systematic review and meta-analysis. *JAMA Netw Open*. 2023;6(12):e2346715. doi: 10.1001/JAMANETWORKOPEN.2023.46715
52. Gabriels RL, Pan Z, Dechant B, Agnew JA, Brim N, Mesibov G. Randomized controlled trial of therapeutic horseback riding in children and adolescents with autism spectrum disorder. *J Am Acad Child Adolesc Psychiatry*. 2015;54(7):541-9. doi: 10.1016/J.JAAC.2015.04.007
53. American Psychiatric Association (Ed). *Diagnostic and statistical manual of mental disorders*. Fifth edition, text revision (DSM-5-TR). Washington, DC: APA; 2022.
54. Lord C, Charman T, Havdahl A, Carbone P, Anagnostou E, Boyd B, et al. The Lancet Commission on the future of care and clinical research in autism. *Lancet*. 2022;399(10321):271-334. doi: 10.1016/S0140-6736(21)01541-5
55. Zeidan J, Fombonne E, Scorah J, Ibrahim A, Durkin MS, Saxena S, et al. Global prevalence of autism: a systematic review update. *Autism Res*. 2022;15(5):778-90. doi: 10.1002/AUR.2696
56. Trzmiel T, Purandare B, Michalak M, Zasadzka E, Pawlaczyk M. Equine assisted activities and therapies in children with autism spectrum disorder: a systematic review and a meta-analysis. *Complement Ther Med*. 2019;42:104-13. doi: 10.1016/J.CTIM.2018.11.004
57. Stergiou A, Tzoufi M, Ntzani E, Varvarousis D, Beris A, Ploumis A. Therapeutic effects of horseback riding interventions: a systematic review and meta-analysis. *Am J Phys Med Rehabil*. 2017;96(10):717-25. doi: 10.1097/PHM.0000000000000726
58. Borgi M, Loliva D, Cerino S, Chiarotti F, Venerosi A, Bramini M, et al. Effectiveness of a standardized equine-assisted therapy program for children with autism spectrum disorder. *J Autism Dev Disord*. 2016;46(1):1-9. doi: 10.1007/S10803-015-2530-6
59. Xiao N, Shinwari K, Kiselev S, Huang X, Li B, Qi J. Effects of Equine-assisted activities and therapies for individuals with autism spectrum disorder: systematic review and meta-analysis. *Int J Environ Res Public Health*. 2023;20(3):2630. doi: 10.3390/IJERPH20032630
60. Cerino S, Borgi M, Fiorentini I, Correale C, Lori A, Cirulli F. Equine-assisted intervention in a child diagnosed with autism spectrum disorder: a case report. *Riv Psichiatri*. 2016;51(6):270-4. doi: 10.1708/2596.26730
61. Collacchi B, Pinchaud N, Borgi M, Cirulli F. Equine-assisted activities (EAAs) for children with autism spectrum disorder (ASD): positive effects revealed using an ethological approach. *Appl Sci*. 2023;13(13):7417. doi: 10.3390/APPI3137417/S1
62. Peters BC, Pan Z, Christensen H, Gabriels RL. Self-regulation mediates therapeutic horseback riding social functioning outcomes in youth with autism spectrum disorder. *Front Pediatr*. 2022;10:884054. doi: 10.3389/FPED.2022.884054
63. Cicchetti DV, Carter AS, Gray SAO. Vineland Adaptive Behavior Scales. In: Volkmar FR (Ed). *Encyclopedia of Autism Spectrum Disorders*. New York: Springer; 2013. doi: 10.1007/978-1-4419-1698-3_255
64. Shallice T. Specific impairments of planning. *Philos Trans R Soc Lond B Biol Sci*. 1982;298(1089):199-209. doi: 10.1098/RSTB.1982.0082
65. Srinivasan SM, Cavagnino DT, Bhat AN. Effects of equine therapy on individuals with autism spectrum disorder: a systematic review. *Rev J Autism Dev Disord*. 2018;5(2):156-75. doi: 10.1007/S40489-018-0130-Z
66. Ruggeri A, Dancel A, Johnson R, Sargent B. The effect of motor and physical activity intervention on motor outcomes of children with autism spectrum disorder: a systematic review. *Autism*. 2020;24(3):544-68. doi: 10.1177/1362361319885215
67. Allen KL, Mountford VA, Elwyn R, Flynn M, Fursland A, Obeid N, et al. A framework for conceptualising early intervention for eating disorders. *Eur Eat Disord Rev*. 2023;31(2):320-34. doi: 10.1002/ERV.2959
68. Van Eeden AE, Van Hoeken D, Hoek HW. Incidence, prevalence and mortality of anorexia nervosa and bulimia nervosa. *Curr Opin Psychiatry*. 2021;34(6):515-24. doi: 10.1097/YCO.0000000000000739
69. Crone C, Fochtmann LJ, Attia E, Boland R, Escobar J, Fornari V, et al. The American Psychiatric Association practice guideline for the treatment of patients with eating disorders. *Am J Psychiatry*. 2023;180(2):167-71. doi: 10.1176/APPI.AJP.23180001
70. Frostad S, Bentz M. Anorexia nervosa: outpatient treatment and medical management. *World J Psychiatry*. 2022;12(4):558-79. doi: 10.5498/WJP.V12.I4.558
71. Zakers A, Cimolai V. Complementary and integrative medicine and eating disorders in youth: traditional yoga, virtual reality, light therapy, neurofeedback, acupuncture, energy psychology techniques, art therapies, and spirituality. *Child Adolesc Psychiatr Clin N Am*. 2023;32(2):421-50. doi: 10.1016/J.CHC.2022.08.014
72. Cerino S, Fiorentini I, Collacchi B, Borgi M, Cirulli F. Sport e salute mentale: dall'approccio dimensionale alla metodologia operativa. *Knowl Psychiatry J*. 2021;2:16-21.
73. Collacchi B, Francia N, Borgi M, Cirulli F. La riabilitazione equestre per i disturbi del comportamento alimentare: uno studio pilota. *Notiziario Istituto Super Sanità*. 2021;34:7-11.
74. Derogatis LR. *SCL-90-R: symptom checklist-90-R: administration, scoring & procedures manual*. 3rd ed. Minneapolis: NCS Pearson; 1994.
75. Garner DM. *EDI-3, Eating disorder inventory-3: professional manual*. Odessa, FL: Psychological Assessment Resources; 2004.
76. Giannini M, Pannocchia L, Dalle Grave R, Muratori F. *Adattamento italiano dell'EDI-3. Eating Disorder Inventory-3*. Firenze: Organizzazioni Speciali; 2008.
77. Prunas A, Sarno I, Preti E, Madeddu F, Perugini M. Psychometric properties of the Italian version of the SCL-90-R: a study on a large community sample. *Eur Psychiatry*. 2012;27(8):591-7. doi: 10.1016/J.EURPSY.2010.12.006

78. Spielberger, CD, Gorsuch RL, Lushene RE. Manual for the state-trait anxiety inventory. Palo Alto, CA: Consulting Psychologists Press; 1970.
79. Christie D, Viner R. Adolescent development. *BMJ*. 2005;330(7486):301-4. doi: 10.1136/BMJ.330.7486.301
80. Guddal MH, Stensland SØ, Småstuen MC, Johnsen MB, Zwart JA, Storheim K. Physical activity and sport participation among adolescents: associations with mental health in different age groups. Results from the Young-HUNT study: a cross-sectional survey. *BMJ Open*. 2019;9(9):e028555. doi: 10.1136/BMJOPEN-2018-028555
81. Hirot F, Huas C, Durand D, Godart N. The evaluation of therapeutic residential care for adolescents and young adults in France: a systematic review. *Front Psychiatry*. 2021;12:609365. doi: 10.3389/FPSYT.2021.609365
82. Bachi K, Terkel J, Teichman M. Equine-facilitated psychotherapy for at-risk adolescents: the influence on self-image, self-control and trust. *Clin Child Psychol Psychiatry*. 2012;17(2):298-312. doi: 10.1177/1359104511404177
83. Weiss-Dagan S, Naim-Levi N, Brafman D. Therapeutic horseback riding for at-risk adolescents in residential care. *Child Adolesc Psychiatry Ment Health*. 2022;16(1):90. doi: 10.1186/S13034-022-00523-5
84. Francia, N, Borgi M, Cirulli F, Collacchi, B, Calamandrei G. Lo sport per la promozione della salute mentale negli adolescenti. *Notiziario Istituto Super Sanità*. 2023; 36(7-8):3-7.
85. Anthony J, Papathomas A, Annandale A, Breen K, Kinnaick FE. Experiences of physical activity for adolescents in secure psychiatric care: staff and patient perspectives. *Ment Health Phys Act*. 2023;24:1-8. doi: 10.1016/J.MHPA.2023.100506
86. Connell J, Brazier J, O'Cathain A, Lloyd-Jones M, Paisley S. Quality of life of people with mental health problems: a synthesis of qualitative research. *Health Qual Life Outcomes*. 2012;10:138. doi: 10.1186/1477-7525-10-138
87. Vancampfort D, Rosenbaum S, Schuch F, Ward PB, Richards J, Mugisha J, et al. Cardiorespiratory fitness in severe mental illness: a systematic review and meta-analysis. *Sports Med*. 2017;47(2):343-52. doi: 10.1007/S40279-016-0574-1
88. Provan M, Ahmed Z, Stevens AR, Sardeli AV. Are equine-assisted services beneficial for military veterans with post-traumatic stress disorder? A systematic review and meta-analysis. *BMC Psychiatry*. 2024;24(1):1-14. doi: 10.1186/S12888-024-05984-W
89. Buck PW, Bean N, De Marco K. Equine-Assisted Psychotherapy: An emerging trauma-informed intervention. *Adv Soc Work*. 2017;18:387-402. doi: 10.18060/21310
90. Burton LE, Qeadan F, Burge MR. Efficacy of equine-assisted psychotherapy in veterans with posttraumatic stress disorder. *J Integr Med*. 2019;17(1):14-9. doi: 10.1016/J.JOIM.2018.11.001
91. Arnon S, Fisher PW, Pickover A, Lowell A, Turner JB, Hilburn A, et al. Equine-assisted therapy for veterans with PTSD: manual development and preliminary findings. *Mil Med*. 2020;185(5-6):e557-64. doi: 10.1093/MILMED/USZ444
92. Fisher PW, Lazarov A, Lowell A, Arnon S, Turner JB, Bergman M, et al. Equine-assisted therapy for post-traumatic stress disorder among military veterans: an open trial. *J Clin Psychiatry*. 2021;82(5):e1-11. doi: 10.4088/JCP.21M14005
93. Collacchi B, Ciacchella C, Borgi M, D'Onofrio A, Fanelli G, Merolla S, et al. Promoting mental health in military veterans. Methodology and evaluation tools in a pilot study involving an Equine-assisted intervention. *Riv Psichiatria*. 2024;59(6):298-305. doi: 10.1708/4386.43838
94. Hamilton M. A rating scale for depression. *J Neurol Neurosurg Psychiatry*. 1960;23(1):56-62. doi: 10.1136/JNPN.23.1.56
95. Pedrabissi L, Santinello M. Verifica della validità dello STAI forma Y di Spielberger. Firenze: Giunti Organizzazioni Speciali; 1989.
96. Tennant R, Hiller L, Fishwick R, Platt S, Joseph S, Weich S, et al. The Warwick-Edinburgh Mental Well-being Scale (WEMWBS): development and UK validation. *Health Qual Life Outcomes*. 2007;5(1):63. doi: 10.1186/1477-7525-5-63
97. Marchand WR. Potential mechanisms of action and outcomes of equine-assisted services for veterans with a history of trauma: a narrative review of the literature. *Int J Environ Res Public Health*. 2023;20(14):1-34. doi: 10.3390/IJERPH20146377
98. Bègue I, Kaiser S, Kirschner M. Pathophysiology of negative symptom dimensions of schizophrenia – Current developments and implications for treatment. *Neurosci Biobehav Rev*. 2020;116:74-88. doi: 10.1016/J.NEUBIOREV.2020.06.004
99. McCutcheon RA, Reis Marques T, Howes OD. Schizophrenia-an overview. *JAMA Psychiatry*. 2020;77(2):201-10. doi: 10.1001/JAMAPSYCHIATRY.2019.3360
100. Bora E, Gökçen S, Kayahan B, Veznedaroglu B. Deficits of social-cognitive and social-perceptual aspects of theory of mind in remitted patients with schizophrenia: effect of residual symptoms. *J Nerv Ment Dis*. 2008;196(2):95-9. doi: 10.1097/NMD.0B013E318162A9E1
101. Hultsjö S, Jormfeldt H. The role of the horse in an Equine-Assisted Group Intervention-as Conceptualized by persons with psychotic conditions. *Issues Ment Health Nurs*. 2022;43(3):201-8. doi: 10.1080/01612840.2021.1975332
102. Peraile-Huerta AM, Jiménez-López E, Díaz-Goni V, Martins-de-Passos TO, Peral-Martínez F, Herráiz-Garrote S, et al. Animal-assisted therapy in patients with psychotic disorders: a systematic review and meta-analysis. *Complement Ther Med*. 2026;96:103322. doi: 10.1016/j.ctim.2026.103322
103. Kay SR, Fiszbein A, Opler LA. The positive and negative syndrome scale (PANSS) for schizophrenia. *Schizophr Bull*. 1987;13(2):261-76. doi: 10.1093/SCH-BUL/13.2.261
104. Overall JE, Gorham DR. The Brief Psychiatric Rating Scale. *Psychol Rep* 1962;10:799-812. doi: 10.2466/PRO.1962.10.3.799
105. Cerino S. La riabilitazione equestre con pazienti psicotici. Roma: Aracne; 2015.
106. Reich SG, Savitt JM. Parkinson's disease. *Med Clin North Am*. 2019;103(2):337-50. doi: 10.1016/J.MCNA.2018.10.014
107. Berardi A, Di Napoli G, Ernesto M, Fabbrini G, Conte A, Ferrazzano G, et al. The effectiveness of equine therapy intervention on activities of daily living, quality of life, mood, balance and gait in individuals with Parkinson's disease. *Healthcare (Basel)*. 2022;10(3):561. doi: 10.3390/HEALTHCARE10030561
108. Peppe A, Costa A, Cerino S, Caltagirone C, Alleve E, Borgi M, et al. Targeting gait and life quality in persons with Parkinson's disease: potential benefits of Equine-assisted interventions. *Parkinsonism Relat Disord*. 2018;47:94-5. doi: 10.1016/J.PARKRELDIS.2017.12.003
109. Galeoto G, Colalelli F, Massai P, Berardi A, Tofani M, Pierantozzi M, et al. Quality of life in Parkinson's disease: Italian validation of the Parkinson's Disease Questionnaire (PDQ-39-IT). *Neurol Sci*. 2018;39(11):1903-9. doi: 10.1007/S10072-018-3524-X

110. Martínez-Martín P, Gil-Nagel A, Gracia LM, Gómez JB, Martínez-Sarriés J, Bermejo F. Unified Parkinson's Disease Rating Scale characteristics and structure. The Cooperative Multicentric Group. *Mov Disord.* 1994;9(1):76-83. doi: 10.1002/MDS.870090112
111. Marin RS. Apathy: a neuropsychiatric syndrome. *J Neuropsychiatry Clin Neurosci.* 1991;3(3):243-54. doi: 10.1176/JNP.3.3.243
112. Peppe A, Chiavalon C, Pasqualetti P, Crovato D, Caltagirone C. Does gait analysis quantify motor rehabilitation efficacy in Parkinson's disease patients? *Gait Posture.* 2007;26(3):452-62. doi: 10.1016/J.GAIT-POST.2006.11.207
113. Antonini A, Abbruzzese G, Ferini-Strambi L, Tilley B, Huang J, Stebbins GT, et al. Validation of the Italian version of the Movement Disorder Society – Unified Parkinson's Disease Rating Scale. *Neurol Sci.* 2013;34(5):683-7. doi: 10.1007/s10072-012-1112-z
114. Stroop JR. Studies of interference in serial verbal reactions. *J Exp Psychol.* 1935;18:643-62. doi: 10.1037/H0054651
115. Yesavage JA, Brink TL, Rose TL, Lum O, Huang V, Adey M, et al. Development and validation of a geriatric depression screening scale: a preliminary report. *J Psychiatr Res.* 1982;17(1):37-49. doi: 10.1016/0022-3956(82)90033-4
116. Holloway JM, Long TM. The interdependence of motor and social skill development: influence on participation. *Phys Ther.* 2019;99(6):761-770. doi: 10.1093/PTJ/PZZ025
117. Cella M, Roberts S, Pillny M, Riehle M, O'donoghue B, Lyne J, et al. Psychosocial and behavioural interventions for the negative symptoms of schizophrenia: a systematic review of efficacy meta-analyses. *Br J Psychiatry.* 2023;223(1):321-31. doi: 10.1192/BJP.2023.21
118. Wilson A, Piek JP, Kane R. The mediating role of social skills in the relationship between motor ability and internalizing symptoms in pre-primary children. *Infant Child Dev.* 2013;22(2):151-64. doi: 10.1002/ICD.1773
119. McDaniel Peters BC, Wood W. Autism and equine-assisted interventions: a systematic mapping review. *J Autism Dev Disord.* 2017;47(10):3220-42. doi: 10.1007/S10803-017-3219-9
120. Cirulli F, Alleva E. Early environmental manipulations and long-term effects on brain neurotrophin levels. In: Sale A (Ed). *Environmental experience and plasticity of the developing brain.* Hoboken, NJ: Wiley Online Library; 2016. p. 139-60. doi: 10.1002/9781118931684.ch7
121. Nagasawa M, Kikusui T, Onaka T, Ohta M. Dog's gaze at its owner increases owner's urinary oxytocin during social interaction. *Horm Behav.* 2009;55(3):434-541. doi: 10.1016/J.YHBEH.2008.12.002
122. Hoagwood K, Vincent A, Acri M, Morrissey M, Seibel L, Guo F, et al. Reducing anxiety and stress among youth in a CBT-Based Equine-Assisted Adaptive Riding Program. *Animals (Basel).* 2022;12(19):2491. doi: 10.3390/ANI12192491
123. Berget B, Vas J, Pedersen G, Uvnäs-Moberg K, Newberry RC. Oxytocin levels and self-reported anxiety during interactions between humans and cows. *Front Psychol.* 2023;14:1-9. doi: 10.3389/FPSYG.2023.1252463
124. Binder AJ, Parish-Plass N, Kirby M, Winkle M, Skwerer DP, Ackerman L, et al. Recommendations for uniform terminology in animal-assisted services (AAS). *Hum-Anim Interact.* 2024;12:1. doi: 10.1079/hai.2024.0003

A mixed-methods study of facilitators and barriers of an audit and feedback intervention using key performance indicators for the Emergency Medical System in Northeastern Italy

Laura Deroma¹, Annarita Tullio², Sabrina Licata² and Francesca Valent¹

¹Dipartimento di Prevenzione, SOC Igiene e Sanità Pubblica, Azienda Sanitaria Universitaria Friuli Centrale, Udine, Italy

²Azienda Sanitaria Universitaria Friuli Centrale, Udine, Italy

Abstract

Background. Audit and feedback (A&F) interventions are strategies used in different health care settings to monitor and improve professional practice and quality of assistance. However, when professional practice needs to be changed, barriers and enablers should be taken into account.

Material and methods. We conducted an online survey, a focus group, and a few interviews after disseminating two reports illustrating 61 key performance indicators (KPIs) describing the management of trauma, acute myocardial infarction and stroke in the Emergency Medical System of a Northeastern Italy Region. The study was conducted in the framework of the EASY-NET Project (NET-2016-02364191).

Results. Novelty, perception, communication, change, data, and pandemics are the primary themes describing the attitude of health care professionals towards an A&F intervention using KPIs.

Conclusions. Improving collaborations and providing specific education should be a primary concern before investing further resources in developing indicators and disseminating results.

Key words

- audit
- feedback
- barriers
- facilitators

INTRODUCTION

Audit and feedback (A&F) interventions are used to monitor and improve professional practice and quality of assistance [1, 2]. In the *audit phase*, key performance indicators (KPIs) measure the performance of health care professionals and compare it to specific targets. In the *feedback phase*, professionals receive the measurement and benchmarking results [3]. A&F usually produces little but beneficial improvement, allows cumulative incremental gains through repeated cycles [4, 5], and is potentially cost-effective [6, 7].

When professional practice needs to be changed, interventions should be tailored considering all possible barriers and enablers [8].

A number of factors are related to recipients: the different health care professionals [5, 9, 10]; their reactions and perspectives [10], specific skills and education on data interpretation [5, 11]; attitude, motivation

and intention [5, 9, 10]; capability for change and improvement [5]; competing priorities [9-12]; awareness of being self-effective [10]; the degree their attention is engaged [10] and their defensive reactions are avoided [10]; engagement in A&F design [4, 5, 10, 11, 13-15].

Specific characteristics of the A&F content are also paramount: its cognitive load should not be excessive [4, 9, 10, 13, 15]; the comparators should be appropriate [4, 5, 10, 13, 15, 16], shared [17], and encourage motivation to change [5, 16, 18]; the identification of well-defined goals should be challenging [13] and perceived as relevant [9, 10, 14], achievable and under the recipient's control [4, 9, 11, 13, 14]; actions and coping strategies to achieve the goals should be well described [10]; a justification for the need of change should be provided [10]; what was achieved rather than what is still missing should be emphasized (cognitive influence) [10]. The nature of data shown also plays an important role [10],

since A&F effectiveness depends on the characteristics of those available for feedback [5, 13, 19], especially reliability [5, 9, 10, 20, 21]. Indicators should be valid, accurate [9, 11] and tailored to the context [5, 15, 22].

Effectiveness is also influenced by feedback timing and frequency [4, 5, 10, 13, 17, 21], being more effective when provided multiple times [10]. The way it is delivered and displayed is also crucial [4, 5, 9, 10, 13, 15, 21], since the credibility of the sender affects the results [4, 5, 9, 10, 13, 14]. Effectiveness also increases when it creates learning opportunities, encouraging reflection and improving memory [10]; when information is structured to allow the recipient to eventually get more afterward [10]; if recipients are engaged in discussions about the A&F. Functional clinical and managerial relationships are also facilitating factors [4, 17], especially when they allow supportive top-down feedback [4], avoiding performance anxiety [18].

Other determinants are costs [10] and an encouraging environment [10].

In the framework of the EASY-NET Project (NET-2016-02364191), we developed and calculated 61 KPIS on trauma, acute myocardial infarction, stroke to describe the Emergency Medical System (EMS) performance in Friuli Venezia Giulia (FVG; 1.2 million inhabitants), Italy [23]. We disseminated the results through: a report (November 2019; 2018 data); a report and a webinar (December 2020; 2019 data); a report and a workshop (April 2022; 2020 data). We e-mailed doctors and ward managers of two local Health Care Authorities (HCAs) a first survey in February 2020 (n=457) and a second in May 2021 (n=497) to explore their attitude towards the KPI implementation and use in the emergency area. We asked for top-down dissemination and collected 71 and 34 answers, respectively. Among the responders to the first survey, 60/71 consider feedback on their performance beneficial.

Engagement varied according to different ways to provide feedback, with multimodality proving better than the text only (1st feedback, only text report: 21/71 responders knew the report, 4 had read it; 2nd feedback, text report and webinar: up to 40 attendees; 3rd feedback, text report and workshop: 100 participants) [24].

This mixed methods study aims to describe the attitudes of health care professionals towards KPIS as quality improvement tools and to analyze the determinants of their implementation and use through an online survey, a focus group (FG), and ten interviews.

MATERIALS AND METHODS

The mixed-methods design

We used a convergent parallel design, comprising a parallel collection of quantitative and qualitative data, an analysis conducted after data collection, and a final comparison of the results. Quantitative data was collected through an online questionnaire, while qualitative data originated from one FG and ten interviews.

The rationale for integration was complementarity, since we aimed to provide a comprehensive description of the research problem through the analysis of multiple sources of evidence. At the interpretation and results level, integration was performed through narra-

tive, both with a contiguous approach, presenting the qualitative and quantitative findings separately, and using a joint display where data is brought together and organised to facilitate comparison.

The reports

The 2020 KPI Report (2020 data; published and presented during the Workshop in 2022) describes the KPIS regarding the EMS (n=6), Emergency Room (ER; n=12), cardiology (n=20), neurology (n=4), major traumas (n=12), and Emergency Operation Centre (n=7).

The KPI Comparison Report (published in 2022) compares the second semesters of 2019 and 2020.

All sections of both reports consist of a description of the KPI calculation method (datasheets), a brief plain explanation of the main results, and a variable number of tables and graphs.

The online survey

In May 2023, we contacted the three local HCA, two Scientific Institutes for Research, Hospitalization and Health care, and the Regional Health Coordination Authority of the FVG Region, asking to send to all health care professionals a predefined brief e-mail illustrating the project and asking for collaboration, and including links to both reports and to an online survey about the attitude towards those reports and KPIS. The email reached all the employees through the generic "Mailbox Users" recipient, including all employees with an institutional email address. No reminders were sent.

To define its questions, we conducted a literature search focusing on the barriers and facilitators encountered during A&F programs using performance indicators [25-32].

The survey (estimated compilation time: 7-10 minutes) consisted of 50 multiple-choice and 23 open questions (D1-D73): D1 asked whether the compiler had read the report or not; the readers had to define if it was engaging, easy to read, complete, understandable, beneficial, and to express their opinion about the number of indicators, tables, and graphs (D2-D30). D31 asked whether the compiler had read the KPI Comparison Report and readers could compile the related questions (D32-D36). All compilers could answer questions on the mode the indicators' results were provided (D37-D38) and their attitudes and opinions towards indicators (D39-D58). D59-D67 focused on the EASY-NET Project, while D68-D73 collected general data about the compiler.

We created the questionnaire using the online survey-management system EUSurvey (<https://ec.europa.eu/eusurvey/>), pre-tested it on a small sample of the final recipients, modified it according to their suggestions and kept online for 40 days (May-June 2023). The survey was anonymous, but compilers could choose to leave their name when they finished. The translation of this tool is available online as *Supplementary Material*.

The focus group

In June 2023, we emailed all 33 physicians who led the units involved in the emergency health care services, both in-hospital and out-of-hospital, in the FVG Region (ERs, EMS, cardiology, and neurology units),

asking them to provide the names of a physician and a nurse who were both working in their unit and interested. We sent an invitation reminder one week apart. We used a multiple-choice doodle to schedule the FG session among twenty session choices (6 am, 2 pm) in 16 possible dates (14 weekdays, 2 Saturdays), from July 10th to August 1st. We asked professionals to select all the sessions they were available to attend, and we scheduled the FG to balance two objectives: ensuring the best representativeness of the emergency-urgency disciplines and geographic areas, and maximizing attendance. Six head physicians of the following units from two out of three of the FVG HCA participated in the FG: the Helicopter Rescue Unit (CL1), a spoke hospital ER (CL2), a local EMS (CL3), two cardiology units (CL4, CL5), and a neurology unit (CL6). Two researchers of the EASY-NET Project conducted the FG. CL2 could not participate in the FG and was interviewed separately.

We conducted both the FG and the interview according to Krueger and Casey [33]. The FG and the interview lasted about 60 and 50 minutes, respectively, and were all recorded and transcribed.

The interviews

Between April and June 2023, two researchers conducted, recorded and transcribed ten interviews with all the staff involved in the study: seven research team members, five co-workers of the EASY-NET Project, and two administrative assistants from the Azienda Sanitaria Universitaria Friuli Centrale (ASUFC) Research Office. The mean interview duration was 43 minutes.

Statistical analysis

Survey data: we described categorical variables as absolute frequencies and percentages.

FG and interviews: after repeatedly listening to the recordings and reading the transcriptions, we performed a thematic analysis, as described by Braun and Clarke [34].

RESULTS

The online survey

In the study period, 316 professionals compiled the questionnaire (Table 1): 22 knew the EASY-NET Project, and 10 had received at least one of its reports. Five professionals had received the invitation to join the 2020 webinar where we presented the 2019 KPI results but none of them participated due to shift overlap, day off or sick leave; 145 had not received the invitation (102 would have liked to, 43 were not interested). When asked whether they had received the invitation to join the 2022 workshop where we presented the overall project, including the 2020 KPI results, eight answered they did (one of them participated), 159 answered they did not (111 would have liked to, 48 were not interested), 141 did not remember.

When asked if they read the reports received in this study framework, 30 (9.5%) answered they read only the 2020 KPI report, 6 (1.9%) only the KPI Comparison Report, and 49 (15.5%) both. The majority (73.1%;

Table 1

Characteristics of the online survey respondents

#	Question	n	%
D68	Sex (n=307)		
	Male	89	29.0
	Female	216	70.4
	Not binary	2	0.7
D69	Age (n=310)		
	<25	2	0.7
	25-34	48	15.5
	35-44	77	24.8
	45-54	102	32.9
	55-64	74	23.9
	>=65	7	2.3
D70	Profession (n=311)		
	Physician	61	19.6
	Nurse	142	45.7
	Social worker in public health care service	23	7.4
	Clerk	12	3.9
	Other*	73	23.5
D72	Do you work in the emergency-urgency setting? (n=307)		
	Yes	65	21.2
	No	175	57.0
	No, but I used to	67	21.8
D73	What local health care authority do you belong to?		
	ASUGI (5954 employees)**	71	22.8
	ASUFC (8500 employees)**	165	52.9
	ASFO (3537 employees)**	52	16.7
	ARCS (182 employees)**	1	0.3
	IRCCS CRO (744 employees)**	23	7.4
	IRCCS Burlo Garofolo (753 employees)**	-	-

*9 laboratory technicians, 5 radiology technicians, 2 physical therapists, 1 biologist, 1 midwife, 1 professional educator, 1 psychologist, 1 speech therapist; **number of employees on 31st December 2022; ASUGI: Azienda Sanitaria Universitaria Giuliano Isontina; ASUFC: Azienda Sanitaria Universitaria Friuli Centrale; ASFO: Azienda Sanitaria Friuli Occidentale; ARCS: Azienda Regionale di Coordinamento per la Salute; IRCCS CRO: Centro di Riferimento Oncologico, Oncology Reference Centre.

n=231) reported having read none. When asked about the 2020 KPI report, only 23 answered they knew it before receiving our e-mail (17 being interested, eight being not), 283 they did not (232 being interested), and eight did not answer.

Readers were 42.7% among males, 21.8% among females, none among binary persons, while according to the age group, readers ranged from 22.0% (<34 years) to 30.4% (45-54 years). Among physicians, nurses and other professionals, 37.7%, 23.2% and 25.9%, respectively, had read the report. The readers were 30.3% of those who ever worked in the emergency-urgency set-

ting (23.9% of past workers, 36.9% of current workers). The percentage of readers according to the local HCA was very similar, ranging from 25.0% (13/52) among the employees of the Azienda Sanitaria Friuli Occidentale (ASFO) to 27.3% (45/165); the only employee of the Azienda Regionale di Coordinamento per la Salute (ARCS) has read the report, as well.

Report readers

Among those who read the 2020 KPI Report (n=79), 81.0% found it very easy or easy enough to read and 86.1% found its subjects very/enough interesting.

The numbers of indicators, tables and graphs included in the 2020 KPI Report were considered “fair”. Table 2 reports a more detailed analysis.

We obtained similar results from those who read the KPI Comparison Report (n=55; data not shown).

All respondents

The majority of respondents (67.1%; n=212) reported they would be more interested if the presentation modality were different: 126 suggested an interactive presentation (education courses, congresses, conferences), 12 non-interactive tools (e-mail updates, n=5; audio or

Table 2

The 2020 KPI Report, according to those who read it (n=79): readability, interest, and usefulness; number of indicators, tables and graphs. Results are expressed as absolute numbers (percentages)

READABILITY		Not at all	Not very	Enough	Very
How easy do you find it to...?					
D2	Read a report like this (n=79)	4 (5.1)	11 (13.9)	63 (79.8)	1 (1.3)
How clear did you find the...?					
D6	Datasheets describing the calculation of the indicators (n=77) ^{D7}	2 (2.6)	9 (11.7)	57 (74.0)	9 (11.7)
D8	Text (n=78)	2 (2.6)	5 (6.4)	54 (69.2)	17 (21.8)
D10	Tables (n=75) ^{D11}	2 (2.7)	10 (13.3)	51 (68.0)	12 (16.0)
D12	Graphs (n=77) ^{D13}	2 (2.6)	13 (16.9)	40 (52.0)	22 (28.6)
INTEREST		Not at all	Not very	Enough	Very
How interesting did you find...?					
D3	The subjects it describes (n=79)	1 (1.3)	10 (12.7)	46 (58.2)	22 (27.9)
USEFULNESS		Not at all	Not very	Enough	Very
How useful did you find the...?					
D14	Datasheets describing the calculation of the indicators (n=77)	1 (1.3)	13 (16.9)	41 (53.3)	22 (28.6)
D16	Text (n=78)	-	8 (10.3)	45 (57.7)	25 (32.1)
D18	Tables (n=76)	2 (2.6)	11 (14.5)	41 (54.0)	22 (29.0)
D20	Graphs (n=76)	-	14 (18.4)	37 (48.7)	25 (32.9)
NUMBER OF KPIS		Scarce	Fair	Excessive	
How did you find the number of ...?					
D22	EMS KPIS (n=76)	9 (11.8)	62 (81.6)	5 (6.6)	
D23	Hospital Emergency System KPIS (n=76)	8 (10.5)	65 (85.5)	3 (4.0)	
D24	Cardiological KPIS (n=76)	4 (5.3)	66 (86.8)	6 (7.9)	
D25	Neurological KPIS (n=75)	8 (10.7)	64 (85.3)	3 (4.0)	
D26	Major traumas KPIS (n=73)	8 (11.0)	64 (87.7)	1 (1.4)	
D27	EMS regional operating centre KPIS (n=74)	8 (10.8)	59 (79.7)	7 (9.5)	
NUMBER OF TABLES		Scarce	Fair	Excessive	
D29	Tables (n=76)	2 (2.6)	56 (73.7)	18 (23.7)	
NUMBER OF GRAPHS		Scarce	Fair	Excessive	
D30	Graphs (n=75)	3 (4.0)	59 (78.7)	13 (17.3)	

Additional hints from open questions; D7: better explain captions and acronyms; D11: use ascending/descending rather than alphabetical order; better explain captions and acronyms; highlight significant results; D13: use more contrasting colours, number graphs; KPI: key performance indicator; EMS: Emergency Medical System.

video recordings, n=7), and one a daily update on social networks. Twenty-two respondents expressed the need to receive a summary of the results.

The majority of respondents reported indicators improve their performance (very, 26%; enough, 57.3%) and 72.8% think that receiving feedback through indicators increases their job quality. Professionals declared performance indicators show professionals the improvable aspects in everyday tasks (73.7%), are useful to improve pathways (55.4%), allow comparisons with other organizations (52.8%), are objective (21.5%), increase weaknesses and strengths awareness (2.8%), reveal the need to introduce improvement strategies and allow to measure their effect (3.2%), help to quantify the resources amount (0.3%). The respondents also reported several negative aspects: they are calculated by people who are not directly involved in the care pathways and may not know essential practical details (45.9%), they're not used to improve pathways (36.4%), they're often calculated on old

data (18.0%), and they are perceived as somehow judging their job (3.5%), they need to be interpreted (1.9%).

Most respondents (82.6%) believe discussing KPI results is beneficial, especially at the unit (59%), department (61.1%), and local HCA (61.7%) level (Table 3).

Most respondents reported they would find it convenient to have an update of KPI results annually (42.4%) or biannually (36.7%), but some of them would require it quarterly (13.9%) or monthly (3.2%). Consistently, the majority would find it beneficial to discuss the KPI results yearly (50.3%) or every six months (34.8%), while some of them would require a higher frequency (every three months: 9.8%; every month: 1.6%).

The large majority of respondents think the usefulness of KPI depends on whether data is recent (yes: 59.5%; sometimes: 27.2%); data is considered as suitable when not older than 3, 6, 12 and 24 months by 36 (11.4%), 100 (31.6%), 143 (45.3%) and 29 (9.2%) respondents, respectively.

Table 3

Professionals to be engaged in KPI discussion and discussion level, according to all respondents (n=316)

#	Question	n	%
D44	What doctors should be engaged in KPI discussion? (Multiple choice is allowed) ^{D45}		
	Department leads	191	60.4
	Unit leads	222	70.3
	Physicians	224	70.9
	None	20	6.3
D46	What health care professionals other than doctors should be engaged in KPI discussion? (Multiple choice is allowed) ^{D47}		
	Department nurse leads	178	56.3
	Unit nurse leads	240	75.9
	Nurses	275	87.0
	Professionals other than nurses	147	46.5
	None	6	1.9
D48	What administrative/organizational professionals should be engaged in KPI discussion? (Multiple choice is allowed) ^{D49}		
	Professionals from the Medical Directorate – hospital level	191	60.4
	Professionals from the Medical Directorate – health care service level	206	65.2
	Professionals from the Regional Health Directorate	168	53.2
	Professionals who identified indicators and defined how to calculate them	193	61.1
	Professionals who calculated the indicators	136	43.0
	None	12	3.8
D50	At what level would KPI discussion be beneficial? (Multiple choice is allowed)		
	Unit	185	58.5
	Department	193	61.1
	Local health care authority	195	61.7
	Region	134	42.4
	National	70	22.2
	International	25	7.9

Additional hints from open questions; D45: KPI discussion should include doctors working in health care districts and/or General Practitioners (n=2); D47: other professional figures cited are health care assistants (n=1) and nurses involved in quality and risk management (n=1); one respondent also suggested multidisciplinary audits that go beyond hierarchical concepts, also taking into account the patients' families' and social environment's position; in this context, a psychologist could be useful in settling relational dynamics among professionals; D49: management engineers (n=1), the Chief medical officer (n=1), local and regional health care authorities (n=1); KPI: key performance indicator.

The focus group (clinicians, CL)

Four main themes emerged from the thematic analysis: perception, communication, change, and data.

CL2/CL3/CL5 expressed their *perception*, one of them considering KPIS as essential, especially when dealing with time-dependent pathologies in the pre-hospital setting, where collecting data is not easy, and tools are less available than in the hospital setting (CL3). On the one hand, CL2/CL3 identify KPIS as a starting point for enhancement, allowing one to take standardized pictures of reality and to improve it. On the other hand, CL5 declared feeling disheartened, thinking KPIS are not useful for adjusting behaviors. CL2 also described the negative perception of the physicians and nurses working in the same unit: they think KPIS have no value, sometimes being felt as a school report that may be important to the head physician and it is only necessary to achieve the budget's objectives and earn benefits.

All clinicians discussed their points of view about *communication*; CL2/CL3 believe a close clinicians-researchers collaboration would allow clinicians to know which KPIS researchers can calculate, and researchers to better understand data and its limits and to identify KPIS that are relevant for clinicians and that may have an impact on the patients' pathways.

CL2/CL4/CL5/CL6 think that meetings could be beneficial for understanding data, should be organized at a regional and at local level and include both the head physicians and their collaborators, both doctors and nurses. CL2 also described the need for education on KPIS, which is paramount to learning how to contextualize KPIS and appreciate their value.

As for *change*, CL3 described the hard past unification of the provincial EMSs in a Regional Operations Centre, while CL2/CL5 are more future-oriented, with a strong willingness to change, CL2 believing the identification of barriers and hurdles should not prevent from changing actively, step by step, CL5 asking how to improve the quality of data and to obtain KPIS clinicians can rely on.

Data was deeply discussed: all clinicians described data collection as inadequate, since it sometimes derives from hardcopy archives or is input by health care personnel for specific purposes when information technology is insufficient; moreover, KPIS show inconsistencies when resulting from different databases or analyses. CL1/CL3/CL4 reported difficult or impossible access to data due to privacy and data ownership issues.

In addition, CL2/CL3/CL4 believe data is underused by organizations and policymakers, although adopting an A&F strategy using KPIS to measure clinical systems could help improvement; CL3/CL4 read this attitude as a lack of interest, preventing specific ventures from being sporadic and leaving clinicians isolated, while CL2 suggests that urgency outclasses planning and KPIS do not represent a priority for policymakers.

Of note, CL3 appreciated the novelty of analyzing and interpreting emergency KPIS in FVG and declared to be positively astonished, interested and enthusiastic about it.

The interviews (researchers)

Researchers debated the themes of novelty, perception, communication, change, data and pandemics.

Having calculated KPIS on the FVG emergency activities for the first time led to the enthusiastic participation of several health care professionals (*novelty*). Nevertheless, KPIS were sometimes interpreted as marks or judgements (*perception*).

KPIS interpretation was difficult for health care professionals not used to them; the lack of mediators between clinicians and researchers caused misunderstandings and hindered the comprehension of KPIS and their use for improvement, at times. In addition, a laboured collaboration between researchers and institutions did not help the project's success. Researchers found it hard to give feedback to health care professionals, especially when results had to be shared hierarchically; in particular, the unit's heads hardly involved their collaborators (*communication*).

Moreover, the context where researchers conducted the project was not stable in time (*change*): FVG political government and health care top management changed; modifications in health care districts and local HCA occurred both geographically and organizationally; the Regional Committee for Urgency and Emergency was dismissed; several EMS heads succeeded one another; the regional triage system and the urgency-emergency software changed. Therefore, it was not feasible for researchers to have stable institutional and technical reference figures aware of the system and trusted by professionals; moreover, systematically monitoring indicators in different periods was demanding.

Change also affected *data*, since the database used changed during the study period (number of records; name, number and coding of variables), often making reproducibility difficult when not impossible; moreover, a manual with metadata was not available, sometimes making not even possible to define whether changes were retrospective or prospective. However, data available in the FVG Region, although difficult to obtain, is considered a treasure by the researchers and the collaboration with colleagues working in institutional units represented a value.

Last but not least, *pandemics* could have been a barrier which may have lowered the interest in KPIS.

Table 4 shows a joint display which summarizes the barriers and facilitators emerging from the online survey, FG, and interviews we conducted.

DISCUSSION

This mixed-methods study investigated the barriers and facilitators of using KPIS, as reported by health care professionals and researchers. Six themes emerged from the online survey, FG and interviews: a) novelty: describing for the first time the emergency setting in FVG through KPIS led to the enthusiastic participation of health care professionals; b) perception: although KPIS are described as objective tools allowing improvement through comparison, they are also perceived as a judging method; c) communication: a fluid information flow, a close clinicians-researchers

Table 4
Joint display of barriers and facilitators emerging from the online survey, focus group, and interviews

Theme	Online survey Quantitative results	Online survey Open questions (excerpts)	Focus group	Interviews
1 NOVELTY				
Barriers				
Facilitators			• First experience with kpis in emergency setting	• Enthusiastic participation of several health professionals emergency kpis are calculated for the first time
2 PERCEPTION				
Barriers	D52. What do you think are the negative aspects of the indicators? Indicators are a form of judgement of my job 3.5% Indicators are calculated by external people who don't know reality 45.9% Indicators are not used to improve pathways 36.4%	<i>Healthcare and everyday tasks are much more complex than simple a priori indicators (D42)</i> <i>[Indicators] could be only useful if they were contextualized and the results were used for a serious project for improvement (unfortunately, this is not what I saw in my experience) (D42)</i> <i>I don't think [indicators] are useful because most of those who should interpret them are not even basically educated to be able to modify them (D42)</i> <i>Those who judge do not work and those who judge work in an office (D53)</i>	• KPIs interpreted as marks or judgements kpis are not useful to adjust behaviors • KPIs are only necessary to achieve the budget's objectives and earn benefits	• KPIs interpreted as marks or judgements
Facilitators	D39. Do you think indicators could be beneficial to improve performance? very much 26.6% somewhat 57.3% D40. Do you think receiving feedback such as the indicators results in your working environment is beneficial to improve the quality of your job? Yes 72.8% D41. How do you think performance indicators could be beneficial? Indicators reveal the aspects that need improvement in everyday work 73.7% Indicators are useful for top and middle management to improve pathways 55.4% Indicators allow comparison with other organisations 52.9% Indicators are objective, thus non-interpretable 21.5%	<i>It should be asked those working on the field what are the actually useful indicators (D42)</i> <i>[Indicators] increase awareness and, thus, the motivation for change (D42)</i> <i>[Indicators are useful because] measuring performance means not only where we can improve but also where we stand out (D42)</i> <i>[Indicators are useful to] guide our own professional behaviour (D42)</i> <i>[Indicators are useful to] align operating modes (D42)</i> <i>[Indicators are useful] to commit appropriate human and instrumental resources (D42)</i> <i>[Indicators] allow me to share possible criticalities to improve performance (D42)</i>	• KPIs are essential, especially in time-dependent pathologies in the emergency setting • KPIs are a starting point for improvement	

Continues

Table 4
Continued

Theme	Online survey Quantitative results	Online survey Open questions (excerpts)	Focus group	Interviews
3 COMMUNICATION				
Barriers	D37. Would you be more interested if the results presentation were different from a written document (e.g., a meeting of professionals, a conference, a training course)? Yes 67.1%	<i>I have the problem of finding the time to read a long and complex text; I am not used to reading such texts, and my reading is slower than it should be (D38)</i>		• Hard to give feedback hierarchically • Sharing from leads to collaborators was difficult • Lack of mediators between clinicians and researchers • Labored collaboration between researchers and institutions • KPis interpretation was difficult due to scarce education
Facilitators	D43. Do you think it is useful to discuss the results of the indicators? Yes 82.6% D38. What KPI results sharing mode would you appreciate? Can you explain why? Interactive presentation (education courses, congresses, conferences) 39.9% Summary of the results 7.0%	<i>Dialogue between those professionals who prepared the report/analysed the data and the report recipients could be a great opportunity for discussion (D38)</i> <i>Sharing data makes data knowledge less boring (D38)</i>	• A close collaboration between clinicians and researchers is paramount • Meetings are beneficial for understanding data, discussing and working together for improvement • Education on kpis is beneficial	
4 CHANGE				
Barriers			• Change of the EMS organizational assessment	• Change of political government • Geographical and organizational modifications in healthcare districts • Dismission of the regional committee for urgency and emergency • EMS heads turnover • Change of the regional triage system • Change of the urgency-emergency software • Absence of stable institutional and technical reference figures
Facilitators		<i>[Indicators] are feedbacks that allow to guide necessary changes (D42)</i> <i>If interpreted and tackled with intellectual honesty, KPis allow us to overcome self-referential and localist reasoning, to offer a service much more oriented to the actual needs of the "stakeholders" (D42)</i>	• Willingness to change	

Continues

Table 4
Continued

Theme	Online survey Quantitative results	Online survey Open questions (excerpts)	Focus group	Interviews
5 DATA				
Barriers	D52. What do you think are the negative aspects of the indicators? They are often calculated using too old data 18.0% D56. Do you think the usefulness of the indicators also depends on how old the data used to calculate them is? Yes 59.5% Sometimes 27.2%	<i>Without interpretation, the data is for the sake of it (D53)</i> <i>The data need to be explained (D53)</i> <i>Limitations: actual reliability of data sources, confounding factors, personal interpretation of specific indicators from uneducated professionals, et cetera (D53)</i> <i>The data is often not easily classified in specific categories (D53)</i>	• Inadequate data collection • Insufficient information technology • Privacy and data ownership issues • Data is underused by organizations and policymakers	• Database change (number of records; name, number, and coding of variables) • Absence of a metadata manual • Data is difficult to obtain
Facilitators		<i>The data in itself is neutral. They need to be collected, analyzed, and interpreted by highly qualified professionals. After publication, dissemination and discussion with collaborators, a debate should origin, open to criticism, motivated doubts, suggestions [omissis]. This data should also be divulged to the general population because, in the end, society itself benefits from the performance improvement. (D53)</i>		• Data is a treasure • Collaboration with colleagues working in institutional units
6 PANDEMICS				
Barriers		<i>The negative aspect is that the compared data also describes a pandemic year and the emergency-urgency system had this additional load (D53)</i> <i>[Indicators] suffer from bias needs to take into account (e.g., comparison between 2019, before pandemics, and 2020, pandemic year) (D53)</i> <i>Pandemics made data irregular and caused several outliers in many research fields; thus, it is paramount to gradually start collecting data regularly to obtain a picture of the new "norm" (D58)</i>		• Pandemics may have lowered the interest in KPIs

KPI: key performance indicator; EMS: Emergency Medical System.

collaboration, dedicated meetings to discuss KPIs and specific education allowing their correct interpretation are crucial; d) change: many organizational changes were described as barriers but clinicians also showed a strong willingness to change; e) data: although much data is collected, data and IT infrastructure are often described as old, inadequate and hardly available due to privacy policies; f) pandemics may have lowered the interest on KPIs.

Among recipient-related factors, specific skills and education on data interpretation are described as facilitators [5, 11, 35]; consistently, in our study, clinicians reported the need for education on KPIs, which would help health care professionals to interpret their significance and appreciate their value. This is also in line with

the suggestion, provided by some authors, to provide simplified key messages to minimize cognitive overload [2, 10, 13], and with the need for support in understanding and interpreting data [36].

Several authors reported that engaging the recipients when designing A&F interventions may improve effectiveness [4, 5, 10, 11, 13, 15]. Similarly, during the FG and the interviews both clinicians and researchers expressed the need for collaboration and debate to exchange information and build appropriate and meaningful indicators, also suggesting structured and well-timed meetings involving all the emergency actors, in line with the suggested participatory feedback construction [2, 13] and social engagement and interaction [10, 37]; similarly, Snider *et al.* reported the success of

collaboration, allowing to tailor the report's contents and structure [20].

Consistently with both our previous survey [24] and the FG, the online survey showed that receiving feedback on performance was perceived as beneficial because indicators increase awareness of both weaknesses and strengths, they reveal improvable aspects and allow to measure the effect of improvement strategies and to estimate the necessary amount of resources. Moreover, they allow comparisons with other organizations. However, we recorded contrasting opinions among the interviewees and the FG clinicians about the KPIS' benefit in improving pathways. Moreover, some respondents considered KPIS objective, while others underlined that their interpretation needed contextualization. This is consistent with previous literature, suggesting the effectiveness of A&F varies according to data credibility and trustworthiness, as perceived by recipients [10].

Despite an overall positive attitude towards KPIS, a few interviewees declared they perceive their job being judged by KPIS, consistently with the researchers and one of the FG clinicians. This is in line with other studies, reporting that clinicians may suffer from performance anxiety [18] and feel threatened rather than supported by top-down feedback [4]. Some authors similarly described the role of attitude, motivation and intention of the recipients [5, 9, 10].

In our organization, this perception can be explained because KPIS are almost always linked to budget goals, thus KPIS may be interpreted as tools used to force behaviours.

As in other studies [10, 38], data quality was of concern. Most interviewees reported that the usefulness of KPIS depends on whether the data is recent, being less beneficial when calculated using old data. Data was also one of the themes widely discussed during the FG, when clinicians agreed that the quality of emergency data is not satisfactory, possibly causing inconsistencies when using different sources and databases. This confirms that data available for feedback should be appropriate [5, 13, 19] and tailored on the specific context [5, 15, 22], other than reliable [5, 9, 10, 20, 21], valid and accurate [9, 11]. Almost half of the interviewees reported that researchers who calculate KPIS do not have knowledge of the context they describe; for this reason, health care professionals often disagree with calculation methods and suggest clinicians and researchers should collaborate to increase the acceptance of KPIS results through the trust in data sources [10, 14, 20]. However, in our study, data represented not only a treasure but also a troublesome problem for the researchers, due to database changes and because they are difficult to obtain. Clinicians appreciated data value, as well, and some of them believe data is underused by organizations and policymakers, probably because KPIS do not represent a priority.

Our study revealed the difficulty of communicating the KPIS results to our target recipients. On the one hand, sending personal e-mails to each health care professional allowed us to enrol many more subjects than in the previous surveys [24]; on the other hand, only

2.5% of the interviewees reported they received the invitation to the workshop where we presented the KPIS results. This issue was unsurprisingly due to top-down dissemination and in line with the scientific literature, reporting that the effectiveness is influenced by the way feedback is delivered and displayed [4, 5, 9, 10, 13, 15, 21]. Unfortunately, a different dissemination method was not permitted, preventing a better engagement through multimodality feedback [2, 13, 20].

Another issue concerned the expected low percentage of the interviewees who read at least one of the KPI Reports (26.9%). This was not unexpected, since some Canadian authors reported that opening feedback reports only slightly improved even with different behavioural nudges [39].

In our study, the poor result could possibly be explained because most respondents would have leaned towards a different presentation modality, preferably interactive. Moreover, they would have preferred a summary of the results, since other studies already identified time [35, 38, 40] and competing priorities [9-12] as the most important barriers. Unfortunately, even though our reports consisted of text, tables and graphs, as suggested by the literature [2], they did not include short key messages that could encourage a first reading [2, 13].

Most online survey respondents reported they would find it convenient to have an update of KPI results and discuss them annually or biannually, especially at the unit, department, and local HCA level. Similarly, the FG clinicians requested meetings to discuss KPIS. This is in line with the scientific literature affirming A&F is more effective if recipients are engaged in discussions about the A&F [10] and that supervisors or colleagues are more reliable than other sources [13]. Moreover, it is consistent with the role of facilitator played by feedback timing and frequency [4, 5, 10, 13, 17, 21], suggesting A&F is more effective when delivery is provided multiple times [10].

An encouraging environment affects the effectiveness of A&F interventions [10], functional clinical and managerial relationships being needed [4]; consistently, we found as a major hurdle the political and organizational changes occurred in recent years, which prevented stable and informed institutional and technical reference figures professionals could trust.

Strengths and limitations

The main strength of this study is novelty: although KPIS are regularly used in FVG to monitor health care performance, this was the first research conducted to explore the point of view of health care professionals and to investigate the barriers and facilitators when using these tools. Another strength is the choice of a mixed-methods design, which allows us to provide a comprehensive description of the research problem through the analysis and integration of multiple sources of evidence. This allowed us to collect the viewpoints of: a) the health care professionals, who are those asked to change their behaviour to improve KPI estimates, b) the emergency-urgency area head physicians, who are directly involved in the budgeting process, where KPIS are used to measure the performance of each clinical

unit, and c) the researchers, who are often asked to calculate KPIs but just as often are not involved in the clinical pathways. In addition, the survey's open questions allowed health care professionals to express their point of view without constraint, not being forced to choose among predefined answers. Moreover, although the FG engaged only 6 out of the 33 invited physicians, these represent all the emergency-urgency disciplines and work in two of the three local HCA. This should also be considered a strength because the choice is derived from the predefined objective of reaching the best representativeness of disciplines and geographical areas.

However, this study also suffers from several limitations. First of all, only about three hundred health care professionals filled out the online survey, with response rates across the Authorities ranging from 0% (IRCCS Burlo Garofolo) to 3.1% (IRCCS Centro di Riferimento Oncologico, CRO, Oncology Reference Centre). One possible explanation is that the only possible way to reach health care professionals was a massive email dissemination from the Directorate of each local HCA, and for this reason, no reminders could be sent. Moreover, the email itself could have been considered as routine communication, and possibly ignored. Last but not least, the intended survey recipients were all health care professionals, but the email explicitly mentioned the emergency-urgency KPIs report; thus, it is likely that many health care professionals not engaged in that field simply ignored the invitation. This, possibly, led to a selection bias, where only more engaged and interested professionals, especially if working in the emergency-urgency field, decided to participate and provided their point of view and opinions. A similar limitation may have affected the FG results; in fact, although we asked each head physician to provide the names of a physician and a nurse, only leading physicians joined the FG, and we missed the point of view of other physicians and nurses, although the head physicians often reported their collaborators' perceptions. However, the results are both internally consistent and in line with the evidence from the literature; thus, the sample is likely representative of the entire population and, accordingly, the study results reasonably reflect the actual attitudes of health care professionals.

CONCLUSIONS

Novelty, perception, communication, change, data, and pandemics are the primary themes describing the attitude of health care professionals towards an A&F intervention using KPIs. The facilitators and barriers identified, consistent with the scientific literature, are primarily related to education, feedback strategies and the relationships among management, researchers and recipients. Middle and top management should treasure this evidence to reinforce facilitators and remove or minimize hurdles, thus leading to a better acceptance of the KPIs as a method to measure and improve the efficiency of the system. First of all, all health care professionals should be given specific basic tools which allow them: to discriminate when data is reliable, valid and accurate, and when they're not; to understand what

numerator and denominator are used to calculate a specific KPI; to read a KPI estimate and its variability; to interpret simple tables and graphs; to figure out when different KPIs and data can be compared and when comparison is not appropriate. Education would allow health care professionals to adopt a common language and shared knowledge would lead them to be engaged and to play on a common ground with researchers, as well.

Close collaboration between researchers and health care professionals should be ensured when calculation methods are decided, in order to build appropriate and meaningful KPIs, to contextualize them, to understand their significance beyond the numeric value, and to interpret them correctly. Moreover, KPIs should be mostly used to measure the efficiency of processes, procedures and pathways with a planned frequency and only as an afterthought as a budgeting tool. Last but not least, KPIs results should be presented in dedicated interactive meetings where all the actors are invited and involved, and the moderators are preferably colleagues or supervisors; health care professionals should be actively engaged in discussions where data is presented and support for interpretation is given, allowing for discussion and debate. When published materials are also provided, they should be short, easy to read and understand, focused on clear key messages.

Funding

This work was funded by the Italian Ministry of Health and by the Region Friuli Venezia Giulia, in the framework of the EASY-NET research program, under Grant NET-2016-02364191.

Conflict of interest statement

The Authors have no conflicts of interest to declare.

Ethical statement

Ethical approval was not required for this study because the online survey collected anonymous data and the entire research collected opinions about organizational issues.

Authors' contributions

LD: conceptualization, methodology, formal analysis, investigation, data curation, writing – original draft, writing – review and editing, visualization, supervision, project administration; AT: formal analysis, investigation, writing – review and editing; SL: methodology, validation, investigation, writing – review and editing; FV: conceptualization, resources, writing – review and editing, project administration, funding acquisition.

Acknowledgements

We would like to express our sincere gratitude to the clinicians who accepted to participate in the FG or to be interviewed to share their ideas, perceptions and suggestions. We are also grateful to Cinzia Maria Bellettato, Francesco Carli, Letizia Cialli, Paola Corazza, Jessica De Biasi, Antonio Di Capua, Gennaro Di Ruberto, Valentino Facca, Elisa Franco, Nicola Leonardo Galizia, Federica Maria Celeste Gallizia, Elisabetta

Gasparotto, Roberta Grando, Cristina Grilli, Luca Giovanni Locatello, Maristella Colette Lo Cicero, Sergio Muiesan, Consuelo Nadalin, Mario D'Oria, Belinda Prelli, Donatella Righini, Enrico Scarpis, Andrea Tandelle, Raffaello Tosolini, and all the health care pro-

fessionals who decided to help our research by compiling the online questionnaire.

Received on 26 November 2025.

Accepted on 24 June 2026.

REFERENCES

- Khalid AF, Grimshaw JM. Preparing for audit and feedback: practical considerations. *JBIM Evid Implement*. 2022;20(2):111-2. doi: 10.1097/XEB.0000000000000330
- Di Blasio N, Bramanti P, Licata S, Acampora A, Ciccone G, Venturella R, Agabiti N. Quindici modi per ottimizzare il feedback. *Recenti Prog Med*. 2023;114(2):110-7. doi: 10.1701/3966.39450
- Ivers N, Jamtvedt G, Flottorp S, Young JM, Odgaard-Jensen J, French SD, O'Brien MA, Johansen M, Grimshaw J, Oxman AD. Audit and feedback: effects on professional practice and healthcare outcomes. *Cochrane Database Syst Rev*. 2012;(6):CD000259. doi: 10.1002/14651858.CD000259.pub3
- Foy R, Skrypak M, Alderson S, Ivers NM, McInerney B, Stoddart J, Ingham J, Keenan D. Revitalising audit and feedback to improve patient care. *BMJ*. 2020;368:m213. doi: 10.1136/bmj.m213
- Ivers NM, Sales A, Colquhoun H, Michie S, Foy R, Francis JJ, Grimshaw JM. No more "business as usual" with audit and feedback interventions: towards an agenda for a reinvigorated intervention. *Implement Sci*. 2014;9:14. doi: 10.1186/1748-5908-9-14
- Conombo B, Guertin JR, Hoch JS, Grimshaw J, Bérubé M, Malo C, Berthelot S, Lauzier F, Stelfox HT, Turgeon AF, Archambault P, Belcaid A, Moore L. Implementation of an audit and feedback module targeting low-value clinical practices in a provincial trauma quality assurance program: a cost-effectiveness study. *BMC Health Serv Res*. 2024;24(1):479. doi: 10.1186/s12913-024-10969-2
- Moore L, Guertin JR, Tardif PA, Ivers NM, Hoch J, Conombo B, Antony J, Stelfox HT, Berthelot S, Archambault P, Turgeon A, Gandhi R, Grimshaw JM. Economic evaluations of audit and feedback interventions: a systematic review. *BMJ Qual Saf*. 2022;31(10):754-67. doi: 10.1136/bmjqs-2022-014727
- Baker R, Camosso-Steinovic J, Gillies C, Shaw EJ, Cheater F, Flottorp S, Robertson N, Wensing M, Fiander M, Eccles MP, Godycki-Cwirko M, van Lieshout J, Jäger C. Tailored interventions to address determinants of practice. *Cochrane Database Syst Rev*. 2015;4:CD005470. doi: 10.1002/14651858.CD005470.pub3
- Brown B, Gude WT, Blakeman T, van der Veer SN, Ivers N, Francis JJ, Lorencatto F, Presseau J, Peek N, Daker-White G. Clinical performance feedback intervention theory (CP-FIT): a new theory for designing, implementing, and evaluating feedback in health care based on a systematic review and meta-synthesis of qualitative research. *Implement Sci*. 2019;14(1):40. doi: 10.1186/s13012-019-0883-5
- Colquhoun HL, Carroll K, Eva KW, Grimshaw JM, Ivers N, Michie S, Sales A, Brehaut JC. Advancing the literature on designing audit and feedback interventions: identifying theory-informed hypotheses. *Implement Sci*. 2017;12(1):117. doi: 10.1186/s13012-017-0646-0
- Rouleau G, Reis C, Ivers N, Desveaux L. Characterizing the gaps between best-practice implementation strategies and real-world implementation: qualitative study among family physicians who engaged with audit and feedback reports. *JMIR Hum Factors*. 2023;10:e38736. doi: 10.2196/38736
- Minard LV, Deal H, Harrison ME, Toombs K, Neville H, Meade A. Pharmacists' perceptions of the barriers and facilitators to the implementation of clinical pharmacy key performance indicators. *PLoS One*. 2016;11(4):e0152903. doi: 10.1371/journal.pone.0152903
- Brehaut J, Colquhoun H, Eva KW, Carroll K, Sales A, Michie S, Ivers N, Grimshaw JM. Practice feedback interventions: 15 suggestions for optimizing effectiveness. *Ann Intern Med*. 2016;164:435-41. doi: 10.7326/M15-2248
- Colquhoun HL, Carroll K, Eva KW, Grimshaw JM, Ivers N, Michie S, Brehaut JC. Informing the research agenda for optimizing audit and feedback interventions: results of a prioritization exercise. *BMC Med Res Methodol*. 2021;21(1):20. doi: 10.1186/s12874-020-01195-5
- Shuldiner J, Kiran T, Agarwal P, Daneshvarfar M, Eldridge K, Kim S, Greiver M, Jokhio I, Ivers N. Developing an audit and feedback dashboard for family physicians: user-centered design process. *JMIR Hum Factors*. 2023;10:e47718. doi: 10.2196/47718
- McCleary N, Desveaux L, Presseau J, Reis C, Witteman HO, Taljaard M, Linklater S, Thavorn K, Dobell G, Mulhall CL, Lam JMC, Grimshaw JM, Ivers NM. Engagement is a necessary condition to test audit and feedback design features: results of a pragmatic, factorial, cluster-randomized trial with an embedded process evaluation. *Implement Sci*. 2023;18(1):13. doi: 10.1186/s13012-023-01271-6
- Jolliffe L, Morarty J, Hoffmann T, Crotty M, Hunter P, Cameron ID, Li X, Lannin NA. Using audit and feedback to increase clinician adherence to clinical practice guidelines in brain injury rehabilitation: a before and after study. *PLoS One*. 2019;14(3):e0213525. doi: 10.1371/journal.pone.0213525
- Catlow J, Bhardwaj-Gosling R, Sharp L, Rutter MD, Sniehotta FF. Using a dark logic model to explore adverse effects in audit and feedback: a qualitative study of gaming in colonoscopy. *BMJ Qual Saf*. 2022;31:704-15. doi: 10.1136/bmjqs-2021-013588
- McCabe A, Brenner M, Larkin P, Nic An Fhailí S, Gannon B, O'Sullivan R, Wakai A. Capturing data for emergency department performance monitoring purposes. *HRB Open Res*. 2019;2:18. doi: 10.12688/hrbopenres.12912.1
- Snider MDH, Boyd MR, Walker MR, Powell BJ, Lewis CC. Using audit and feedback to guide tailored implementations of measurement-based care in community mental health: a multiple case study. *Implement Sci Commun*. 2023;4(1):94. doi: 10.1186/s43058-023-00474-8
- Wagner DJ, Durbin J, Barnsley J, Ivers NM. Measurement without management: qualitative evaluation of a voluntary audit & feedback intervention for primary care teams. *BMC Health Serv Res*. 2019;19(1):419. doi: 10.1186/s12913-019-4226-7
- Putnam W, Bower KN, Cox J, Twohig P, Pottier K, Jackson

- L, Burge F. Quality indicators for cardiac care: national standards in a community context. *J Health Serv Res Policy*. 2006;11(1):5-12. doi: 10.1258/135581906775094226
23. Valent F, Di Chiara A, Di Bartolomeo S. Emergency key performance indicators in the Italian Region Friuli Venezia Giulia. *Emerg Care J*. 2020;16(2):8834. doi: 10.4081/ecj.2020.8910
24. Licata S, Valent F, Deroma L. Key performance indicators in the Emergency Medical System: barriers and facilitators. *Eur J Public Health*. 2023;33(S2):ii379. doi: 10.1093/eurpub/ckad160.952
25. Belizán M, Bergh AM, Cilliers C, Pattinson RC, Voce A and the Synergy Group. Stages of change: a qualitative study on the implementation of a perinatal audit programme in South Africa. *BMC Health Serv Res*. 2011;11:243. doi: 10.1186/1472-6963-11-243
26. Reddy ST, Zegarek MH, Fromme HB, Ryan MS, Schumann SA, Harris IB. Barriers and facilitators to effective feedback: a qualitative analysis of data from multispecialty resident focus groups. *J Grad Med Educ*. 2015;7(2):214-9. doi: 10.4300/JGME-D-14-00461.1
27. Sinuff T, Muscedere J, Rozmovits L, Dale CM, Scales DC. A qualitative study of the variable effects of audit and feedback in the ICU. *BMJ Qual Saf*. 2015;24(6):393-9. doi: 10.1136/bmjqs-2015-003978
28. Roberts N, Hooper G, Lorencatto F, Storr W, Spivey M. Barriers and facilitators towards implementing the Sepsis Six care bundle (BLISS-1): a mixed methods investigation using the theoretical domains framework. *Scand J Trauma Resusc Emerg Med*. 2017;25(1):96. doi: 10.1186/s13049-017-0437-2
29. Singh K, Devarajan R, Mohanan PP, Baldrige AS, Kondal D, Victorson DE, Karmali KN, Zhao L, Lloyd-Jones DM, Prabhakaran D, Goenka S, Huffman MD, ACS QUIK Investigators. Implementation and acceptability of a heart attack quality improvement intervention in India: a mixed methods analysis of the ACS QUIK trial. *Implement Sci*. 2019;14(1):12. doi: 10.1186/s13012-019-0857-7
30. Chartier LB, Jalali H, Seaton MB, Ovens H, Borgundvaag B, McLeod SL, Dainty KN, Ostrow O. Qualitative evaluation of a mandatory provincial programme auditing emergency department return visits. *BMJ Open*. 2021;11(4):e044218. doi: 10.1136/bmjopen-2020-044218
31. Pearse BL, Keogh S, Rickard CM, et al. Barriers and facilitators to implementing evidence based bleeding management in Australian Cardiac Surgery Units: a qualitative interview study analysed with the theoretical domains framework and COM-B model. *BMC Health Serv Res*. 2021;21(1):550. doi: 10.1186/s12913-021-06269-8
32. Springer MV, Salesa AE, Islam N, McBride AC, Landis-Lewis Z, Tupper M, Corches CL, Robles MC, Skolarus LE. A step toward understanding the mechanism of action of audit and feedback: a qualitative study of implementation strategies. *Implement Sci*. 2021;16(1):35. doi: 10.1186/s13012-021-01102-6
33. Krueger RA, Casey MA. Designing and conducting focus group interviews. In: *Social analysis: selected tools and techniques*. Paper n. 36. Washington DC: The World Bank; 2001. p. 4-23. [Social Development Papers series].
34. Braun V, Clarke V. Using thematic analysis in psychology. *Qual Res Psychol*. 2006;3(2):77-101. doi: 10.1191/1478088706qp063oa
35. de Vos ML, van der Veer SN, Graafmans WC, de Keizer NF, Jager KJ, Westert GP, van der Voort PH. Implementing quality indicators in intensive care units: exploring barriers to and facilitators of behaviour change. *Implement Sci*. 2010;5:52. doi: 10.1186/1748-5908-5-52
36. Desveaux L, Ivers NM, Devotta K, Ramji N, Weyman K, Kiran T. Unpacking the intention to action gap: a qualitative study understanding how physicians engage with audit and feedback. *Implement Sci*. 2021;16(1):19. doi: 10.1186/s13012-021-01088-1
37. Desveaux L, Nguyen MD, Ivers NM, Devotta K, Upshaw T, Ramji N, Weyman K, Kiran T. Snakes and ladders: a qualitative study understanding the active ingredients of social interaction around the use of audit and feedback. *Transl Behav Med*. 2023;13(5):316-26. doi: 10.1093/tbm/ibac114
38. Wilkinson EK, McColl A, Exworthy M, Roderick P, Smith H, Moore M, Gabbay J. Reactions to the use of evidence-based performance indicators in primary care: a qualitative study. *Qual Health Care*. 2000;9(3):166-74. doi: 10.1136/qhc.9.3.166
39. Daneman N, Lee S, Bai H, Bell CM, Bronskill SE, Campitelli MA, Dobell G, Fu L, Garber G, Ivers N, Kumar M, Lam JMC, Langford B, Laur C, Morris AM, Mulhall CL, Pinto R, Saxena FE, Schwartz KL, Brown KA. Behavioral nudges to improve audit and feedback report opening among antibiotic prescribers: a randomized controlled trial. *Open Forum Infect Dis*. 2022;9(5):ofac111. doi: 10.1093/ofid/ofac111
40. Casey C, Chung CP, Crofford LJ, Barnado A. Rheumatologists' perception of systemic lupus erythematosus quality indicators: significant interest and perceived barriers. *Clin Rheumatol*. 2017;36(1):97-102. doi: 10.1007/s10067-016-3487-3

Bisphenol S-induced inflammation: a comparative insight on inflammatory pathway activation in zebrafish and rodent models

Wan Nur Izzati Wan Azhan¹, Fatin Nadzirah Zakaria¹, Danial Akmal Fuad Al' Arifin¹, Nur-Zulaikha Amlie¹, Evana Kamarudin², Faheze Paiman³, Rio Risandiansyah⁴ and Razif Dasiman¹

¹Department of Basic Sciences, Faculty of Health Sciences, Universiti Teknologi MARA, Selangor, Malaysia

²Centre for Medical Laboratory Technology, Faculty of Health Sciences, Universiti Teknologi MARA, Selangor, Malaysia

³Egze Guppy Sdn Bhd, Jalan Haji Wahab, Kg Olak Lempit, Banting, Selangor, Malaysia

⁴Faculty of Medicine, Islamic University of Malang, Dinoyo, Malang, East Java, Indonesia

Abstract

Background. Bisphenol S (BPS) is a widespread BPA substitute with emerging immunotoxicity; this review synthesizes zebrafish and rodent experimental evidence to clarify BPS-induced inflammation.

Methods. Narrative synthesis of experimental studies in zebrafish and rodent models, focusing on molecular pathways, organ-level effects, and exposure timing.

Results. Across models, BPS consistently activates an oxidative stress-mediated NF- κ B axis with increased ROS, lipid peroxidation, and upregulation of TNF- α , IL-1 β , and IL-6; chronic and developmental low-to-moderate exposures produced stronger inflammatory phenotypes than acute high doses.

Conclusions. Convergent mechanistic and pathological findings strengthen the weight of evidence for BPS immunotoxicity and support targeted monitoring and controls; key research gaps include inflammasome activation, receptor mechanisms, sex differences, mixture effects, epigenetic regulation, and standardization.

Key words

- bisphenol S
- inflammation
- inflammatory pathway
- rodent
- zebrafish

INTRODUCTION

Bisphenol S (BPS) is a widely used substitute for bisphenol A (BPA) analogues in consumer and industrial products such as polycarbonate plastics, epoxy resins, and thermal papers. BPA is a non-perennial anthropogenic compound widely used in recent decades as a primary plastic material for various applications such as food packaging, personal care products, household items, and medical devices [1]. BPA has been recognized as an Endocrine Disruptor Chemical (EDC) that alters endocrine function by imitating, inhibiting, or modifying hormone production, transport, metabolism, and receptor binding [2]. BPA has been banned in consumer products, including all baby bottle production, in the EU and Malaysia since 2012, and the ban has expanded to all materials used in food contact applications in 2024.

BPS, also known as 4,4'-sulphonyldiphenol, is a primary substitute for BPA, sharing a similar chemical

structure with BPA, including two phenol groups connected by a sulphonyl group [3]. Despite being introduced as a safer alternative, BPS has become ubiquitous in the environment and in human populations. Several studies have reported that BPS has been detected in surface water, sewage, and sediment samples globally, indicating widespread environmental pollution [4, 5]. As a result, biomonitoring data show that BPS is detected in the urine in most people, including 89.4% of adults in the US, and broad detection across Asian and European cohorts [6]. Temporary trends suggest that BPA levels are gradually stabilising or declining as a result of global BPA ban regulations. In contrast, BPS concentrations in human samples are gradually increasing, raising serious concerns about the potential health consequences, which may be comparable to or worse than those of BPA. Various consumer products, particularly food and beverage packaging, primarily expose

people to BPS. Furthermore, thermal paper, such as receipts, is a major source of exposure. High levels of BPS were detected in fractional urinary excretion, reaching up to 92% in men and 72% in women [7].

Inflammation represents an essential biological response to harmful stimuli, but its dysregulation is a major cause of disease. Acute inflammation serves as a protective, short-term response to injury or infection, while chronic inflammation indicates a prolonged, low-level condition marker due to ongoing immune activation and tissue damage [8]. Recent research has focused on the recognition of systemic chronic inflammation as a fundamental mechanism in the pathogenesis of major noncommunicable diseases, including cardiovascular disease, type 2 diabetes, obesity, neurodegenerative disorders, and cancer [9]. Pathological processes are mediated by critical intracellular signalling pathways, particularly the Nuclear Factor kappa-B (NF- κ B) pathway, the Mitogen-Activated Protein Kinase (MAPK) cascade, and the NLRP3 inflammasome [10]. These pathways regulated the production of pro-inflammatory cytokines, including TNF- α , IL-1 β , and IL-6 [8].

Well established toxicological evidence links that environmental pollution can act as an endocrine-disrupting chemical (EDC), potentially altering the homeostasis balance between pro-inflammatory and anti-inflammatory signals [1]. The relationship between environmental toxicants is increasingly linked to the induction of sterile inflammation, establishing a mechanistic connection between chemical exposure and the risks associated with chronic diseases [2]. Although BPS has been extensively studied for its ability to induce inflammation via oxidative stress and receptor-mediated pathways, its inflammatory capacity remains poorly understood despite its structural similarities. It is scientifically conceivable that BPS may cause a comparable or different inflammatory response due to its similar phenol groups responsible for receptor binding. Therefore, an extensive assessment of its immunotoxicity potential is required.

A cross-species comparative approach would allow for a thorough examination of the mechanisms of BPS-induced inflammation, providing valuable translational insight. Animal models allow for controlled dosing and exposure timing, which aids in the identification of thresholds and chronic effects relevant to environmental and human exposure [11]. Monitoring and comparing BPS-induced inflammation in different animal models is critical for understanding its health effects and causes, as well as applying the findings to human health. This phenomenon is due to variations in BPS sensitivity, immune response, and metabolic processing among species. By comparing animal model studies, we can determine which findings are likely to be applicable to humans, and some models can highlight different mechanistic diversity, such as gut microbiome involvement, while others focus on immune cell activation or organ-specific damage, providing a comprehensive view of BPS toxicity.

This narrative review provides a novel comparative synthesis of BPS-induced inflammatory pathways in zebrafish and rodent models. By exploring the underexplored existing literature to identify conserved

mechanisms, model-specific responses, and gaps for human risk assessment. Databases consulted included PubMed, Web of Science, and Scopus. Search terms combined "bisphenol S" OR "BPS" with "inflammation" OR "inflammatory pathway" AND "zebrafish" OR "*Danio rerio*" OR "rodent" OR "mouse" OR "rat". Studies from 2015 to 2025 were included if they reported BPS-induced inflammation in these models with mechanistic data. Exclusion: non-English paper, review without primary data, non-peer-reviewed sources.

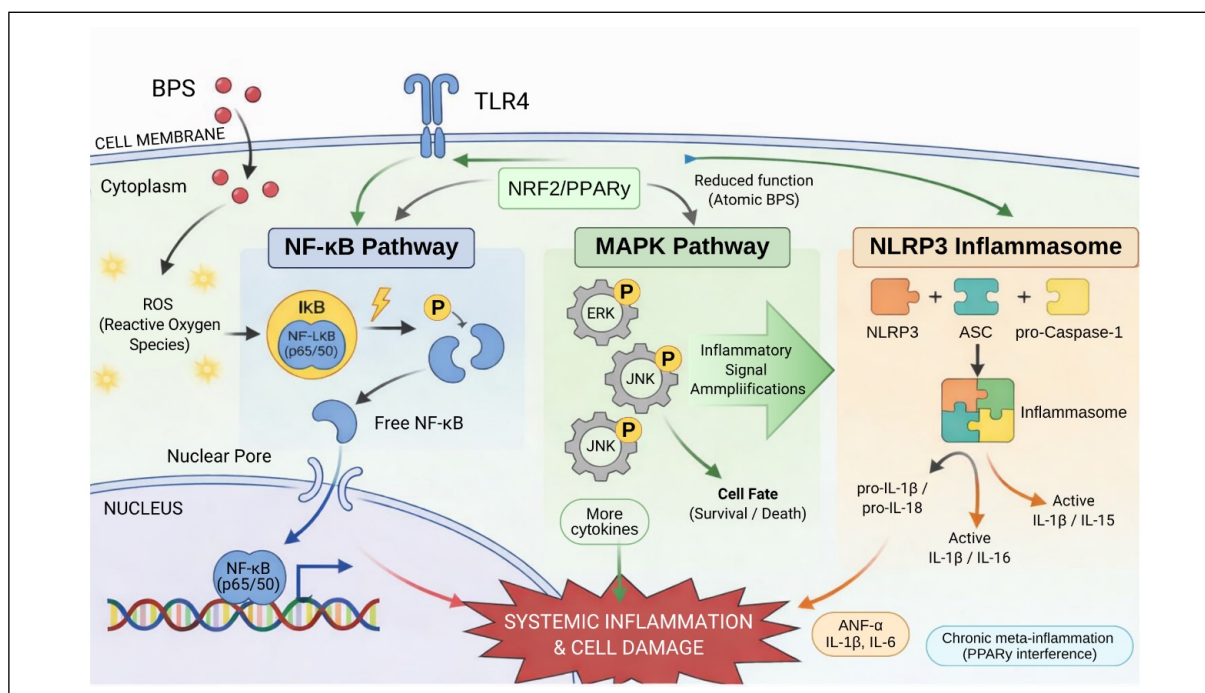
INFLAMMATORY PATHWAYS OVERVIEW

The molecular landscape of BPS toxicity involves a complex network of signaling cascades that bridge initial chemical exposure to systemic pathology. As illustrated in Figure 1, BPS-induced inflammation is characterized by the integrated activation of the NF- κ B pathway, the MAPK cascade, and the NLRP3 inflammasome, all of which are modulated by upstream sensors like TLR4 and metabolic regulators such as PPAR γ .

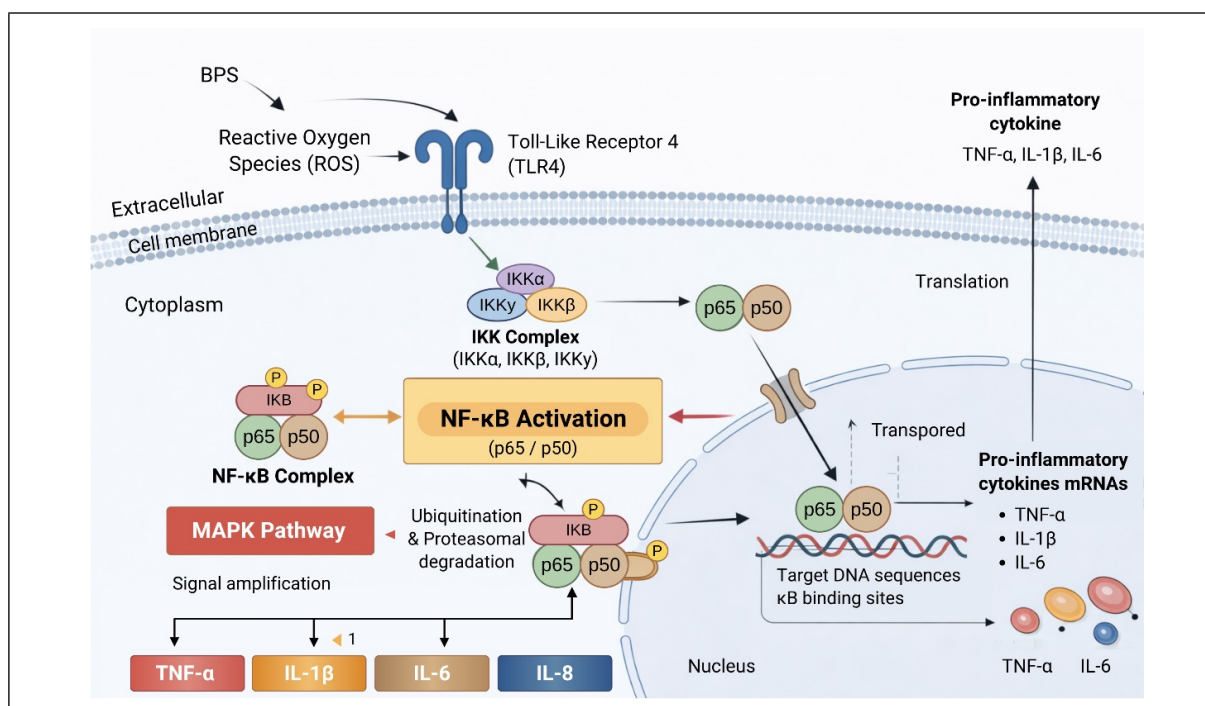
The primary regulator of the inflammatory response is the NF- κ B signalling pathway. Under normal physiological conditions, NF- κ B is sequestered in the cytoplasm by the inhibitory protein I κ B. Figure 2 illustrates a detailed schematic representation of canonical NF- κ B activation sequence following BPS-induced ROS/TLR4 signaling. Research has shown that upon BPS exposure, I κ B undergoes rapid phosphorylation and degradation, facilitating the translocation of the active p65/p50 NF- κ B complex into the nucleus [12]. Upon entering the nucleus, this complex binds to specific DNA promoter regions, leading to the transcription of a diverse array of pro-inflammatory genes. These genes include cytokines like TNF- α , IL-6, and IL-1 β , which are recognised as key biomarkers of BPS immunotoxicity [13]. This pathway acts as a central convergence point for BPS toxicity, integrating signals from oxidative stress and upstream receptors in the chain, initiating a systemic inflammatory response.

The Mitogen-Activated Protein Kinase (MAPK) cascade exhibits a significant relationship with NF- κ B. This pathway serves as a crucial mechanism for the signal transduction pathway that responds to external stressors outside the cells. Exposure to BPS has been proved to activate essential MAPK subfamilies, particularly ERK1/2, JNK, and p38 [13]. The activation of the phosphorylation process in response to Reactive Oxygen Species (ROS) via BPS or receptor binding. This pathway functions as an essential signal transducer, converting stress signals from outside the cell, which are frequently caused by BPS-induced ROS, into a response inside the cell that increases cytokine production and influences cell fate, shifting the equilibrium towards survival and apoptotic cell death [14]. The MAPK pathway is an important mechanism that acts as an amplifier of inflammatory signals, bridging the gap between initial chemical toxicant exposure and the cellular stress response.

The activation of the NLRP3 inflammasome, a multi-protein intracellular complex that serves as a sensor for "danger" signals, represents a distinctive and significant component of BPS-induced inflammation. The expo-

**Figure 1**

Overview of BPS-induced pathways (NF-κB, MAPK, NLRP3, upstream TLR4/Nrf2/PPARγ) leading to cytokine production, cell death, and chronic meta-inflammation.

**Figure 2**

Schematic representation of the canonical NF-κB activation pathway. This Figure illustrates BPS-induced ROS/TLR4 signaling leading to IκB degradation, p65/p50 nuclear translocation, and cytokine (TNF-α, IL-1β, IL-6) transcription conserved across models.

sure of BPS triggers the formation of a complex comprising NLRP3, ASC, and pro-caspase-1 and serves as a molecular mechanism responsible for processing and releasing the inflammatory cytokines IL-1β and IL-18 [15]. This pathway is particularly significant because it

leads to pro-apoptosis, which is a form of programmed cell death that induces significant inflammation [2]. The activation of the NLRP3 inflammasome suggests that BPS may induce sterile inflammation, leading to tissue injury even in the absence of pathogens.

The upstream sensor frequently initiates or modifies the activation of these key pathways. Toll-Like Receptor 4 (TLR4), which is conventionally recognised as a sensor for bacterial endotoxins, may undergo aberrant activation due to cellular damage signals caused by BPS. This activation triggers a signalling cascade that directly interacts with NF- κ B and MAPK pathways [16]. Simultaneously, BPS interferes with the Nrf2/Keap1 pathway, which is the cell's main mechanism for protecting cells against oxidative stress. The initial activation of Nrf2 is a protective response designed to neutralise ROS created by BPS. However, when this pathway is chronically dysregulated, it may lead to persistent oxidative stress and inflammation. Finally, BPS interferes with PPAR γ signalling, a nuclear receptor pathway that links lipid metabolism with inflammation [17]. By disrupting PPAR γ activity, BPS can cause "meta-inflammation", a chronic low-grade inflammatory state linked to metabolic disorders such as obesity and fatty liver disease.

ANIMAL MODELS IN BPS-INDUCED/EXPOSED INFLAMMATION

Zebrafish and rodents were chosen as complementary vertebrate models to capture both mechanistic detail and systemic complexity of BPS-induced inflammation. zebrafish enable high-resolution, real-time analysis of innate immune cell dynamics and early-life responses under controlled waterborne exposure, but underdeveloped adaptive immunity, and lack complex mammalian organs. While rodents provide insights into organ-specific chronic pathology, adaptive immunity, and dose-dependent systemic effects relevant to human exposure scenarios.

ZEBRAFISH MODEL

Zebrafish (*Danio rerio*) have emerged as a popular vertebrate model for studying inflammation, including the effects of toxicants such as BPS, due to their high genetic and physiological similarities to mammals. Over 70% of human genes have a zebrafish counterpart, and more than 80% of genes associated with human disease are conserved [18]. This indicates many essential inflammatory pathways and immune cell types are shared between zebrafish and mammals. This significant degree of conservation is also observed in the innate immune system. Zebrafish macrophages, neutrophils, and various inflammatory mediators and receptors are similar to those found in humans [19].

Zebrafish offer practical and mechanistic advantages for BPS studies. Zebrafish larvae are transparent, facilitating real-time imaging of leukocyte recruitment, barrier damage, and tissue regeneration at single-cell resolution with fluorescent reporter lines for neutrophils, macrophages, NF- κ B activity, inflammasomes, and cytokines [20]. High reproductive rate, low cost, rapid development, and compatibility with microinjection or waterborne exposure make them a valuable model for studying both the mechanisms of inflammation and the effects of environmental toxicants like BPS. However, differences in immune response and susceptibility to specific inflammatory triggers exist due to evolutionary

divergence, and certain aspects of adaptive immunity are less developed compared to mammals [19].

RODENT MODEL (MICE AND RATS)

Rodent models, primarily *Mus musculus* (mice) and *Rattus norvegicus* (rats), serve as the essential foundation for *in vivo* studies of BPS-induced inflammation due to the substantial similarities in the genomes of about 85% of the immune, endocrine, and metabolic systems with humans [21]. Both rats and mice allow regulated oral, inhalation, or parenteral exposure to BPS with precise doses and defined life stages, enabling investigation of acute and chronic low-dose effects on specific organs such as the gastrointestinal tract, liver, kidneys, lungs, brain, heart, and reproductive tissues.

Mice, is a widely used mammalian model for studying inflammation caused by environmental toxicants. This advantage stems from their unique characteristics, small size, rapid reproductive rate, and extensive genetic information readily available for research. Various knockout and transgenic lines, including NF- κ B reporters, cytokine knockouts, AhR, PPAR γ , and microbiota-manipulated lines [22]. This enables the examination of particular BPS-sensitive inflammatory pathways within specific cells or organs. Common strains include C57BL/6, widely used in immunology and metabolism research; BALB/c, preferred for Th2-biased and allergy or asthma models; and outbred Swiss mice, typically used in general toxicology and reproductive studies [23]. These strains facilitate the alignment of the strain background with specific inflammatory phenotype under investigation.

Rats provide larger organ dimensions and improved sampling capabilities for biochemical, histological, and haemodynamic endpoints, which are advantageous for evaluation of organ-level inflammatory injuries associated with BPS, such as effects on the liver, kidney, cardiovascular system, and neuroinflammatory responses [24]. The Wistar and Sprague-Dawley rat strains possess comprehensive historical information on systemic inflammation, organ weights, and clinical chemistry, making them ideal for evaluating BPS-induced injuries in the liver, kidneys, and cardiovascular system.

COMPARATIVE RESULTS OF MODEL-SPECIFIC PATTERNS AND MECHANISTIC CONVERGENCE

The research articles listed in *Table 1* and *Table 2* of the matrix were published over the past five years, with some studies extending back a decade. This selection offers a broad perspective on the effects of BPS on inflammation across widely used vertebrate models, including zebrafish and rodents. It highlights how each model contributes to a deeper understanding of BPS's impact on inflammation at various developmental stages and within different experimental contexts.

CONSERVED INFLAMMATORY MECHANISMS ACROSS MODELS

Across zebrafish and rodent models, BPS consistently activates an oxidative stress driven from the NF- κ B axis with upregulated ROS and classical pro-inflammatory

Table 1
Matrix of rodent BPS-induced toxicity in inflammation

Author(s)	Rodent species/strain	Age/sex	Dose exposure	Route	Duration	Organ/System	Inflammatory pathway/biomarker (findings)	Key inflammatory pathway	Remarks on biomarkers
[8]	Mice C57BL/6	Male (6-7 weeks)	0, 100, 1000 µg/kg bw/day	Daily oral gavage	3 months	Colon/intestine	↑macrophage (F4/80+), ↑TUNEL apoptosis, ↑IL-18, ↓Reg3b/Reg3g	NF-kB	BPS caused crypt distortion, increased macrophage infiltration, apoptosis, pro-inflammatory IL-8, and changes in barrier genes.
[17]	Mice C57BL/6	Both (12 weeks)	25 µg/kg/day	Oral gavage in drinking water	12 weeks	Heart	↑TNF-α, ↑IL-6, ↑CD11c	NF-kB PPARγ	BPS + high-fat diet increases cardiac pro-inflammatory cytokines, leading to chronic low-grade cardiac inflammation.
[26]	Mice C57BL/6	Female (6-7 weeks)	0, 0.05, 5 mg/kg/day	Daily oral gavage	4 weeks	Colon	↑TNFα, ↑IL1β, ↑IL6, ↓IL10, ↑NFκB p65, ↓ZO1, ↓Claudin1, gut dysbiosis	NF-kB	BPS includes colonic injury with increased pro-inflammatory cytokine gene expression. NF-kB activation reduces anti-inflammatory IL-10.
[29]	Mice C57BL/6	Both (12-24 weeks)	25-30 µg/kg/day	Oral gavage in drinking water	12 weeks	Kidney	↑AhR, ↑NFκB, ↑CD31, no change in IL6	NF-kB	BPS increases NF-kB and AhR expression in renal tubules and CD31 in glomeruli. NF-kB is linked to inflammatory signaling despite unchanged IL-6 levels.
[30]	Rat	Female pregnant	12 µg/kg/day	Daily oral gavage in olive oil	3 weeks before GD 0	Brain	↑NF-kB, ↑p53, ↓Bcl2, ↓NeuN, ↓Ki67, ↑GFAP, ↑βamyloid, ↓NO	NF-kB	BPA exposure in the perinatal period activates NF-kB and p53, reducing anti-apoptotic proteins. GFAP and β-amyloid produce striatal neuroinflammation
[31]	ICR Mice	Male adult (6 weeks)	0, 5, 50, 500 mg/kg/day	Daily oral gavage	90 days	Heart	↑P-NFκB (Pp65), ↑ROS	NF-kB	BPS induces oxidative stress. Increased P-NF-kB and ROS indicate activation of NF-kB inflammatory signaling.
[34]	Mice C3H/HeJ	Male (6-11 weeks)	0.04, 0.4, 4 µg/kg/day	Oral gavage in drinking water	6 weeks	Lungs	↑IL-5, ↑IL-13, ↑IL-33, ↑CCL11/eotaxin, ↑OVA-IgE, ↑OVA-IgG1	Indirect NF-kB (Th2 cytokines mediated)	BPS + allergic asthma increase pulmonary inflammation, Th2 cytokines/chemokines.
[35]	Long-Evans rat	Male (21 days)	5 and 20 mg/kg	Daily oral gavage in drinking water	14 days	Liver	↓ROS, no changes in caspase-3, IL-1	NA	No detectable pro-inflammatory or pro-apoptotic liver response compare to control.
[36]	Sprague Dawley rat	Male (8 weeks)	0, 0.05, 20 mg/kg	Daily oral gavage	4 weeks	Intestine	↑pp38 and ↑pERK (MAPKs),	MAPK	BPS damaged intestinal epithelium and increased inflammatory cytokines. Activation of p38 and ERK MAPK leads to impaired sperm quality.
[37]	Mice C57BL/6J	Pregnant female	1.5 mg/kg/day	Oral gavage in drinking water	GD 0 - PND 21	Colon	NA	Other (intestinal immune)	BPS causes persistent intestinal inflammation in F1 and F2 male gut. F3 reduces gut inflammation.
[38]	Mice C57BL/6J	Both (8 weeks)	4 µg/kg/day	Oral gavage by dams	GD 0 – PND 28	Spinal cord (CNS)	↑ inflammatory cell infiltration and glial activation in spinal cord	Other (CNS neuro-inflammation)	BPS shows neurodegeneration with milder inflammatory marker changes.
[39]	Mice C3H/HeN	Pregnant female	5 or 50 µg/kg bw/day	Daily oral gavage	GD 15 – PND 21	Intestine	↑ fecal lipocalin	Other (Barrier-linked inflammation)	Perinatal BPS exposure alters intestinal immune function in offspring and increases fecal inflammatory lipocalin. Low-grade inflammation.
[44]	ICR Mice	Female (4 weeks)	1 mg/kg/bw/day	Daily oral gavage in corn oil	4 weeks	Gut and ovary	↑Lymphocyte infiltration, ↑IL-6,	Other (microbiota-intestinal inflammation)	BPS reshaped the gut microbiota, increased intestinal permeability, and altered the inflammatory, impairing oocyte quality.
[45]	Mice C57BL/6J	Male (8 weeks)	50 mg/kg/day	Daily oral gavage	6 weeks	Liver	↑IL1β, ↑TNFα, ALT, ↑AST; ↑MDA, ↓SOD	PPARγ	BPS caused lipid accumulation & elevated IL-1β and TNF-α, oxidative stress and histologic inflammatory cell infiltration.

BPS: bisphenol S; GD: gestational day; PND: postnatal day; NA: not available; ICR: Institute of Cancer Research; CNS: Central Nervous System; ROS: Reactive Oxygen Species; MDA: malondialdehyde.

Table 2
Matrix of zebrafish BPS-exposed toxicity in inflammation

Author(s)	Fish species/ strain	Age/sex	BPS dose exposure	Route	Duration	Organ/ System	Inflammatory pathway/ biomarker (findings)	Key inflammatory pathway	Remarks on biomarkers
[25]	Zebrafish (<i>Danio rerio</i>)	Adult (5 months)	1, 10, 100, 1000 µg/L	water exposure	14 days	Intestine	↑MDA, ↑8OHdG, ↑IL1β, ↑TNFα	PPARγ	BPS increased intestinal oxidative damage and pro-inflammatory cytokines. Altered PPAR signalling and other metabolic pathways, and disrupted gut microbial.
[27]	Zebrafish (<i>Danio rerio</i>)	Embryo	1, 10, 100 mg/L	semi-static exposure	3 hpf-120 dpf	Liver	↑IL-1β, ↑TNFα, ↑NF-κB, ↑IKK	NF-κB	BPS exposure drove progression from steatosis to steatohepatitis. Marked with IL-1β, TNF-α, and NF-κB indicates chronic hepatic inflammation driven by lipotoxicity.
[33]	Zebrafish (<i>Danio rerio</i>)	Juvenile (2hpf-3dpf)	1, 10, 100 µg/L	water exposure	2hpf-3dpf	Brain	↑Microglia & macrophages, ↑ROS, ↓SOD, ↓CAT, ↑apoptotic cells, ↑caspase8/9/3a/3b mRNA	Other (lipid neuro-inflammation)	BPS reduces brain lipid, increases microglial, increases oxidative stress, and causes neuroinflammatory/apoptotic changes
[43]	Zebrafish (<i>Danio rerio</i>)	Larvae	8.13 mg/L	water exposure	4 hpf – 120 hpf	Immune system	↑IL-6, ↑TNF-α, activation of IKKB	NF-κB	BPS enhances inflammation via IKKB and modulates Ptg2a (COX-2), Nos2a, and antioxidant genes.

BPS: bisphenol S; Hpf: hour after fertilisation; dpf: day after fertilisation.

cytokines like TNF-α, IL-1β and IL-6 across liver, intestine, kidney, heart, brain, and immune tissues [25]. *Figure 3* visually summarizes the conserved oxidative stress NF-κB axis and highlights model-specific organ manifestations in zebrafish and rodent studies. In C57BL/6 mice, colonic exposure models and high-fat diet sensi-

tized cardiac models show increased TNF-α, IL-1β, and IL-6, alongside activation of NF-κB-p65 and barrier disruption, resulting in a pattern of low-grade chronic inflammation in the gut and heart [17, 26]. In zebrafish studies from *Table 2*, prolonged exposure of waterborne BPS results in increased expression of IL-1β and TNF-α

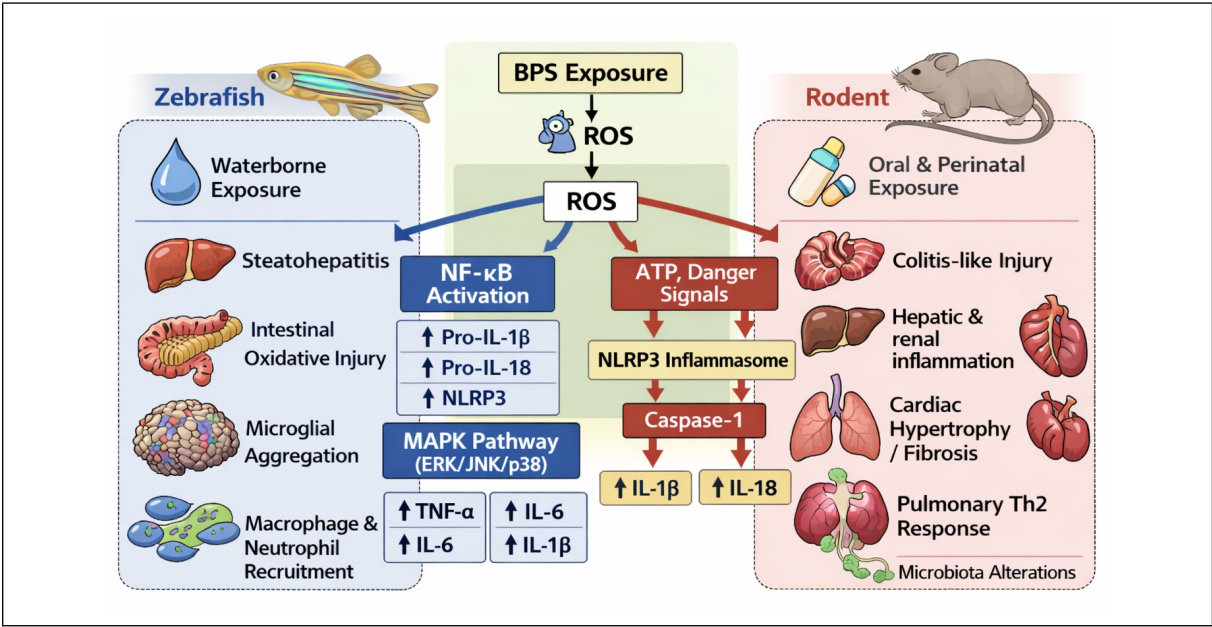


Figure 3
Conserved and model-specific inflammatory responses to BPS. Oxidative stress activates NF-κB with MAPK and NLRP3 branches, producing TNF-α, IL-1β, IL-6, and IL-18. Zebrafish (left): waterborne exposure causes hepatic, intestinal, and neural inflammation. Rodents (right): oral/perinatal exposure leads to gut, hepatic, cardiac, pulmonary, and neuroinflammatory outcomes.

as well as NF- κ B and IKK, thereby promoting the progression from steatosis to steatohepatitis [27]. Comparable responses associated with NF- κ B signaling have been documented in zebrafish models of immunity and intestinal function, demonstrating that BPS promotes intestinal injury or neuroinflammation. Rodent model studied by the [28-31] reveal a consistent pattern involving the colon, heart, kidneys, and liver show elevated levels of TNF- α , IL-1 β , and IL-6, along with nuclear NF- κ B or phospho-p65 and an indicator of oxidative damage, ROS, MDA, and SOD/CAT activity, which reduces antioxidant enzyme activity. This suggests a conserved relationship between redox imbalance and NF- κ B-mediated cytokine production across both species.

Chronic or developmental exposure to low-to-moderate doses of BPS more consistently induces inflammatory phenotypes compared to rapid high-dose exposure. Research involving zebrafish and the application of semi-static waterborne protocols from early developmental phases to the juvenile or adult stage (30-120 days) reveals a significant oocyte-dependent activation of IL-1 β , TNF- α , and NF- κ B. This activation has been linked with notable histological steatohepatitis and immune-organ injury [25, 32, 33]. Meanwhile, in rodent studies, the administration of several μ g-mg/kg/day through drinking or feeding for 4-12 weeks consistently results in elevated levels of cytokines in tissues, activation of the NF- κ B signaling pathway, and histological evidence of inflammation in various organs including the colon [26], liver [20], kidneys [29], lungs [34], and heart [17]. Conversely, shorter rat studies (14-28 days) yield more inconsistent results, with some indicating oxidative alterations but exhibiting limited classical inflammatory markers [35, 36]. Furthermore, perinatal exposure to BPS via dams has a high potential to induce latent or sex-dependent inflammatory responses in the intestines and Central Nervous System (CNS) of adult offspring [37-39].

Cross-species evidence suggests that BPS induces oxidative stress by increasing ROS and MDA levels and decreasing antioxidant enzyme activity, thereby activating the NF- κ B pathway. In some models, interact with PPAR γ , AhR, or other metabolic/xenobiotic pathways to drive organ-specific inflammatory response [25, 29, 32, 40]. Although affected organs may differ between models, for example, zebrafish are primarily in the liver, intestine, and brain, whereas rodents are primarily in the gut, liver, kidney, lung, heart, and central nervous system. The current combination of ROS upregulation, NF- κ B activation, and pro-inflammatory cytokine upregulation is a common inflammatory mechanism of BPS toxicity seen in rodent and zebrafish studies.

Across zebrafish and rodent studies, the exposure route influences the inflammatory outcomes observed in each model. Although there is currently no information on internal BPS concentrations, zebrafish are frequently immersed in water from embryogenesis, resulting in whole body absorption and simultaneous exposure to the liver, gut, and brain [41]. Meanwhile, rodent studies employ BPS doses between mg/kg/day over several weeks to months through oral administration, similarly mimicking possible BPS exposure in the

daily human lifestyle when drinking water or food [42]. These methodologies more accurately replicate human consumption and allow for organ-specific exposure scenarios such as increased cardiac, hepatic, or renal inflammation caused by chronic BPS exposure and lifestyle. The perinatal mouse and rat models add complications because the subject mother is exposed to BPS during pregnancy and lactation. The offspring are indirectly exposed to BPS doses through the placenta and milk, which is important for programming effects but complicates precise dose-response interpretation.

MODEL-SPECIFIC INFLAMMATORY RESPONSES

Zebrafish research focusses on early developmental and organ-specific innate inflammation, making use of transparent larvae and whole organism exposure. Chronic waterborne BPS exposure in zebrafish leads to hepatic IL-1 β /TNF- α upregulation, NF- κ B or PPAR γ involvement, acute protein induction, and intestinal oxidative injury under standardised exposure and genetic background [25]. Further research on the brain reveals that environmental BPS reduces key phospholipids (PC, LPC) and causes microglial aggregation, ROS, and neural apoptosis. This suggests that BPS causes inflammation through lipid changes, which provides greater clarity in zebrafish than traditional models. Research on transgenic zebrafish shows that BPS induces macrophages and neutrophils and elevates ROS and malondialdehyde (MDA), indicating innate immunotoxicity and mixture effects at the LC50-based concentration [43].

Rodent studies, on the other hand, show more complex inflammatory responses involving multiple organs and potentially influenced by external factors such as sex and diet. For instance, chronic oral BPS administration in the gastrointestinal tract alters the gut microbiota, which can cause colitis-like manifestations, including crypt distortion, macrophage accumulation, epithelial apoptosis, and increased IL-18, suggesting that the microbiota influences the response to BPS-induced inflammation [28, 44]. Perinatal BPS exposure induces sex-specific intestinal/metabolic inflammation in F1-F3 offspring [37, 38]. Additionally, perinatal exposure in the Experimental Autoimmune Encephalomyelitis (EAE) model of multiple sclerosis appears to accelerate disease onset and worsen neurodegeneration in adult males [38]. Administration of low-dose BPS in conjunction with a high-fat diet leads to cardiac hypertrophy, fibrosis, and upregulation of TNF- α , IL-1 β , and IL-6 in the left ventricle [17]. In obese mice, AhR and NF- κ B are activated within renal tubules and glomeruli, resulting in the upregulation of CD31 and subsequent structural damage to the kidney. This is frequently accompanied by relatively minor changes in classical cytokines [29]. Variations in rodent observation significantly reflect the differences in strain, sex, nutrition, co-exposure, and developmental stage, in contrast to the more uniform inflammatory responses reported in standard zebrafish larval and juvenile.

In addition to differences in exposure route, the biomarker panels used across studies also vary between

models. Zebrafish studies often use nominal water concentration exposure and heavily rely on mRNA profiles of IL-1 β , TNF- α , IL-6, NF- κ B, and PPAR γ -associated genes, oxidative stress indicators, including ROS, MDA, and antioxidant enzymes, acute-phase proteins, and immune cell or microglia images [25]. Rodent studies typically use dosages ranging from μ g to mg/kg/day, including tissue and serum cytokines, immune cell markers, histopathological evaluations, functional outcomes, and protein pathways like NF- κ B, AhR, P-NF- κ B, or tight junction components. A study conducted by Liu *et al.* [35] on Long Evans rat hepatocellular shows that minimal and brief exposure to BPS can reduce ROS levels without increasing inflammatory markers. This demonstrates how time and exposure degree have a significant impact on the biomarker profile. These methodological differences highlight the need for more standardised internal dose measurement and a minimal overlapping panel of cytokines and pathway markers to improve quantitative cross-model synthesis.

KNOWLEDGE GAPS AND UNDEREXPLORED AREAS

Despite strong evidence supporting the involvement of oxidative stress and NF- κ B pathways, several inflammatory mechanisms in BPS toxicology remain poorly understood. The activation of the NLRP3 inflammasome, detailed TLR4 signalling, and Nrf2/AhR cross-talk have been investigated in a small number of rodent studies, specifically in kidney, liver, and reproductive contexts. However, these mechanisms are still significantly underexplored in zebrafish and extrahepatic tissues [29, 36]. The role of PPAR γ mediated meta-inflammation has been relatively described in hepatic endpoints within both zebrafish and rodent models [32, 45]. However, its implications in cardiovascular, renal, and CNS models remain limited, restricting researchers' ability to understand the specific contributions of various pathways in different organs. Zhu *et al.* [36] documented the involvement of MAPK cascades p38 and ERK in testicular inflammation in rats exposed to BPS, but no systematic evaluation was conducted in other rodent organs or fish inflammatory responses.

Limited research has systematically compared males and females within identical frameworks, but available evidence suggests a sex-dependent effect. For example, perinatal exposure to BPS causes more intestinal inflammation in F1-F2 males than females across generations [37], enhance CNS autoimmune damage in male but not female EAE offspring [38], and show different fecal lipocalin and immune skewing patterns between sexes in perinatal immune studies [39]. This study looks into the effects of real-world BPS exposure scenarios, such as low-dose BPS combined with other toxicants, an unhealthy diet or lifestyle, and long-term environmental exposure. Wang *et al.* [25] and Su *et al.* [43] found that BPS exposure with a high-fat diet causes additive immunotoxicity and synergistic oxidative stress in zebrafish larvae, as well as NF- κ B-mediated neuroinflammation in the rat brain [30]. However, the majority of rodent studies evaluate BPS in isolation, leaving sig-

nificant uncertainty about the relevance of experimental findings to chronic, multi-pollutant human exposure scenarios. Similarly, Brulport *et al.* [37] and Bonaldo *et al.* [38] have documented a transgenerational inflammatory program following perinatal BPS in the mouse intestine and CNS, respectively, whereas the epigenetic mechanisms of microbiota inheritance and the persistence of inflammatory phenotypes beyond F2 are poorly understood.

CONCLUSION

This review demonstrates that bisphenol S-induced inflammation operates through a conserved oxidative stress-NF- κ B axis across evolutionarily divergent animal models. The organ-specific inflammatory manifestations reflect differences in anatomical complexity and immune architecture between fish and mammals. The complementary strengths of zebrafish and rodent systems, namely transparency and mechanistic clarity in fish versus systemic integration and adaptive immunity in rodents, provide powerful tools for understanding how chronic BPS exposure disrupts inflammatory homeostasis. Evidence suggests that low-dose, persistent environmental exposure to BPS can activate inflammatory signalling pathways, disrupt epithelial barriers, alter gut microbiota, and cause transgenerational immune dysfunction, implicating BPS in the pathogenesis of chronic inflammatory diseases. However, the current evidence base is limited by incomplete mechanistic coverage, insufficient standardization of exposure design and biomarker reporting, and a lack of consistent sex-specific and life-stage-specific analyses, which reduces cross-study comparability and translational confidence. In particular, pathways such as NLRP3 inflammasome activation, TLR4 signaling, Nrf2/AhR crosstalk, and PPAR γ -mediated inflammatory regulation remain underexplored in a comparative framework. Future research should therefore prioritize standardized internal dose assessment, harmonized biomarker panels across models, and systematic inclusion of both sexes and multiple developmental windows. In addition, studies investigating combined exposures, dietary or metabolic co-stressors, epigenetic regulation, microbiota-mediated effects, and transgenerational outcomes are needed to better reflect real-world exposure scenarios. Such efforts will strengthen the mechanistic interpretation of BPS immunotoxicity and improve its relevance for human health risk assessment and regulatory decision-making.

Acknowledgments

The Authors would like to thank all staff and contributors at the Faculty of Health Sciences, Optometry, and Visual Sciences Research Groups (iROViS), UiTM Selangor, Puncak Alam Campus, for their assistance, supervision, and insightful suggestions in preparing this article. WNIWA, RD, FNZ, NZA, DAFA, and EK also acknowledge their membership of the Optometry and Visual Science Research Group (iROViS), UiTM Selangor, for their contribution to this study. Thank you to the Tun Abdul Razak Library (PTAR) at UiTM for creating a suitable online database platform for this review.

Authors' contributions

RD designed, administered, and supervised this study; WNIWA, NZA and DAFA conducted sampling and statistical analysis; RD, FNZ, FP and EK provided resources for this study and were responsible for the formal analysis; WNIWA, RD, FNZ, NZA, DAFA, RR, and EK participated in writing original draft, writing-review & editing.

Disclaimer statement

The Authors used ChatGPT, Grammarly, and QuillBot to improve the readability and language of this review. The Authors declare that all Figures are original

and were prepared by the Authors. AI-assisted drafting tools were used in part, with subsequent verification and editing performed in BioRender. Final Figures were validated for accuracy by the Authors. The Authors used these tools to review and edit the content as needed, and they accept full responsibility for the content of the published article.

Conflicts of interest statement

The Authors declare no conflict of interest.

Received on 23 December 2025.

Accepted on 27 May 2026.

REFERENCES

- Meli R, Monnolo A, Annunziata C, Pirozzi C, Ferrante MC. Oxidative stress and BPA toxicity: An antioxidant approach for male and female reproductive dysfunction. *Antioxidants*. 2020;9(5):405. doi: 10.3390/antiox9050405
- Kasitipradit K, Thongkorn S, Kanlayaprasit S, Saeliw T, Lertpeerapan P, Panjabud P, et al. Sex-specific effects of prenatal bisphenol A exposure on transcriptome-interactome profiles of autism candidate genes in neural stem cells from offspring hippocampus. *Sci Rep*. 2025;15(1):2882. doi: 10.1038/S41598-025-86392-2
- Siracusa JS, Yin L, Measel E, Liang S, Yu X. Effects of bisphenol A and its analogs on reproductive health: a mini review. *Reprod Toxicol*. 2018;79:96-123. doi: 10.1016/J.REPROTOX.2018.06.005
- Eladak S, Grisin T, Moison D, Guerquin MJ, N'Tumba-Byn T, Pozzi-Gaudin S, et al. A new chapter in the bisphenol A story: bisphenol S and bisphenol F are not safe alternatives to this compound. *Fertil Steril*. 2015;103(1):11-21. doi: 10.1016/j.fertnstert.2014.11.005
- Thoene M, Dzika E, Gonkowski S, Wojtkiewicz J. Bisphenol S in food causes hormonal and obesogenic effects comparable to or worse than bisphenol A: a literature review. *Nutrients*. 2020;12(2):532. doi: 10.3390/NU12020532
- Lehmler HJ, Liu B, Gadogbe M, Bao W. Exposure to bisphenol A, bisphenol F, and bisphenol S in US adults and children: the national health and nutrition examination survey 2013-2014. *ACS Omega*. 2018;3(6):6523-32. doi: 10.1021/acsomega.8b00824
- Oh J, Choi JW, Ahn YA, Kim S. Pharmacokinetics of bisphenol S in humans after single oral administration. *Environ Int*. 2018;112:127-33. doi: 10.1016/j.envint.2017.11.020
- Abdulkhaleq LA, Assi MA, Abdullah R, Zamri-Saad M, Taufiq-Yap YH, Hezmee MNM. The crucial roles of inflammatory mediators in inflammation: a review. *Vet World*. 2018;11(5):627-35. doi: 10.14202/vetworld.2018.627-635
- Xie Y, Meijer AH, Schaaf MJM. Modeling inflammation in zebrafish for the development of anti-inflammatory drugs. *front. Cell Dev Biol*. 2021;8:620984. doi: 10.3389/fcell.2020.620984
- Bu J, Mahan Y, Zhang S, Wu X, Zhang X, Zhou L, et al. acacetin inhibits inflammation by blocking MAPK/NF- κ B pathways and NLRP3 inflammasome activation. *Front Pharmacol*. 2024;15:1286546. doi: 10.3389/fphar.2024.1286546
- Fang L, Miller YI. Emerging applications for zebrafish as a model organism to study oxidative mechanisms and their roles in inflammation and vascular accumulation of oxidized lipids. *Free Radic Biol Med*. 2012;53(7):1411-20. doi: 10.1016/J.FREERADBIOMED.2012.08.004
- Al-Shami AS, Abd Elkader HTAE, Moussa N, Essawy AE, Haroun M. Early-life bisphenol A exposure causes neuronal pyroptosis in juvenile and adult male rats through the NF- κ B/IL-1 β /NLRP3/caspase-1 signaling pathway: exploration of age and dose as effective covariates using an in vivo and in silico modeling approach. *Mol Cell Biochem*. 2025;480(4):2301-30. doi: 10.1007/s11010-024-05039-4
- Huang F, Chang Y, Lee S, Yang M, Kuan Y. Expression of pro-inflammatory cytokines and mediators induced by bisphenol A via ERK-NF κ B and JAK1/2-STAT3 pathways in macrophages. *Environ Toxicol*. 2019;34(4):486-94. doi: 10.1002/tox.22702
- Son Y, Cheong YK, Kim NH, Chung HT, Kang DG, Pae HO. Mitogen-activated protein kinases and reactive oxygen species: how can ROS activate MAPK pathways? *J Signal Transduct*. 2011;2011:792639. doi: 10.1155/2011/792639
- Xu J, Núñez G. The NLRP3 inflammasome: activation and regulation. *Trends Biochem Sci*. 2023;48(4):331-44. doi: 10.1016/j.tibs.2022.10.002
- Zinatizadeh MR, Schock B, Chalbatani GM, Zarandi PK, Jalali SA, Miri SR. The nuclear factor kappa B (NF- κ B) signaling in cancer development and immune diseases. *Genes Dis*. 2021;8(3):287-97. doi: 10.1016/j.gendis.2020.06.005
- Alexandre-Santos B, Reis G dos S, Medeiros GR, Stockler-Pinto MB, Oliveira NSC, Miranda-Alves L, et al. Bisphenol S exposure induces cardiac remodeling and aggravates high-fat diet-induced cardiac hypertrophy in mice. *Environ Res*. 2024;261:119781. doi: 10.1016/j.envres.2024.119781
- Belo MAA, Oliveira MF, Oliveira SL, Aracati MF, Rodrigues LF, Costa CC, et al. Zebrafish as a model to study inflammation: a tool for drug discovery. *Biomed Pharmacother*. 2021;144:112310. doi: 10.1016/j.biopha.2021.112310
- Phillips JB, Westerfield M. Zebrafish models in translational research: tipping the scales toward advancements in human health. *Dis Model Mech*. 2014;7(7):739-43. doi: 10.1242/DMM.015545
- Wu Y, He Y, Luo H, Jin T, He F. AIEE-active flavones as a promising tool for the real-time tracking of uptake and distribution in live zebrafish. *Int J Mol Sci*. 2023;24(12):10183. doi: 10.3390/ijms241210183

21. Blais EM, Rawls KD, Dougherty BV, Li ZI, Kolling GL, Ye P, et al. Reconciled rat and human metabolic networks for comparative toxicogenomics and biomarker predictions. *Nat Commun.* 2017;8(1):14250. doi: 10.1038/ncomms14250
22. Clough B, Finethy R, Khan RT, Fisch D, Jordan S, Patel H, et al. C57BL/6 and 129 inbred mouse strains differ in Gbp2 and Gbp2b expression in response to inflammatory stimuli in vivo. *Wellcome Open Res.* 2014;4:124. doi: 10.12688/wellcomeopenres.15329.1
23. Lanna MF, Resende LA, Aguiar-Soares RDO, de Miranda MB, de Mendonça LZ, Melo Júnior OAO, et al. Kinetics of phenotypic and functional changes in mouse models of sponge implants: rational selection to optimize protocols for specific biomolecules screening purposes. *Front Bioeng Biotechnol.* 2020;8:538203. doi: 10.3389/fbioe.2020.538203
24. Ellenbroek B, Youn J. Rodent models in neuroscience research: is it a rat race? *Dis Model Mech.* 2016;9(10):1079-87. doi: 10.1242/dmm.026120
25. Wang Y, Wang B, Wang Q, Liu Y, Liu X, Wu B, et al. Intestinal toxicity and microbial community disorder induced by bisphenol F and bisphenol S in zebrafish. *Chemosphere.* 2021;280:130711. doi: 10.1016/j.chemosphere.2021.130711
26. Liu H, Wang Y, Wei L, Xu J, Wang R, Zhao LG, et al. Unveiling BPS-induced colonic inflammatory injury in female mice: integrated evidence from colon microbiome and metabolomic analyses. *Ecotoxicol Environ Saf.* 2025;302:118747. doi: 10.1016/j.ecoenv.2025.118747
27. Qin J, Qi X, Li Y, Tang Z, Zhang X, Ru S, et al. Bisphenols can promote antibiotic resistance by inducing metabolic adaptations and natural transformation. *J Hazard Mater.* 2024;470:134149. doi: 10.1016/j.jhazmat.2024.134149
28. Li S, Ma X, Zhang X, Bai S, Li X, Huang Y, et al. Bisphenol S Exposure induces intestinal inflammation via altering gut microbiome. *Food Chem. Toxicol.* 2024;190:114830. doi: 10.1016/j.fct.2024.114830
29. Li S, Ma X, Zhang X, Bai S, Li X, Huang Y, et al. Exposure to bisphenol S promotes renal damage via aryl hydrocarbon receptor and NF- κ B pathways in a mice model of obesity. *Environ Res.* 2025;285:122240. doi: 10.1016/j.envres.2025.122240
30. Okoh OS, Akintunde JK, Akamo AJ, Akpan U. Thymoquinone inhibits neuroinflammatory mediators and vasoconstriction injury via NF- κ B dependent NeuN/GFAP/Ki-67 in hypertensive dams and F1 male pups on exposure to a mixture of bisphenol-A analogues. *Toxicol Appl Pharmacol.* 2025;494:117162. doi: 10.1016/j.taap.2024.117162
31. Luo H, Yang Y, Zhang H, Ren L, Han X, Lin Y, et al. Toxic effects of bisphenol S on mice heart and human umbilical cord endothelial cells. *Ecotoxicol Environ Saf.* 2023;267:115652. doi: doi.org/10.1016/j.ecoenv.2023.115652
32. Qin J, Ru S, Wang W, Hao L, Ru Y, Wang J, et al. Long-term bisphenol S exposure aggravates non-alcoholic fatty liver by regulating lipid metabolism and inducing endoplasmic reticulum stress response with activation of unfolded protein response in male zebrafish. *Environ Pollut.* 2020;263:114535. doi: 10.1016/j.envpol.2020.114535
33. Du Z, Li S, Peng H, Li J, Li Z, Ru S, et al. Low lipid levels caused by bisphenol S exposure trigger neuroinflammation and apoptosis in the brain of zebrafish. *Aquat Toxicol.* 2025;282:107328. doi: 10.1016/j.aquatox.2025.107328
34. Yanagisawa R, Koike E, Win-Shwe TT, Takano H. Effects of oral exposure to low-dose bisphenol S on allergic asthma in mice. *Int J Mol Sci.* 2022;23(18):10790. doi: 10.3390/ijms231810790
35. Liu K, Kadannagari S, Deruiter J, Pathak S, Abbott KL, Salamat JM, et al. Effects of developmental exposures to bisphenol-A and bisphenol-S on hepatocellular function in male long-evans rats. *Life Sci.* 2023;326:121752. doi: 10.1016/j.lfs.2023.121752
36. Zhu B, Zhang Z, Xie Y, Huang M, Chen Y, Yang Y, et al. Effects of environmental bisphenol S exposure on male rat reproductive health and gut-blood-testicular axis integrity. *Ecotoxicol Environ Saf.* 2025;289:117646. doi: 10.1016/j.ecoenv.2024.117646
37. Brulport A, Lencina C, Chagnon MC, Le Corre L, Guzylack-Pirou L. Transgenerational effects on intestinal inflammation status in mice perinatally exposed to bisphenol S. *Chemosphere.* 2021;262:128009. doi: 10.1016/j.chemosphere.2020.128009
38. Bonaldo B, Casile A, Montarolo F, Bettarelli M, Napoli F, Gotti S, et al. Effects of perinatal exposure to bisphenol A or S in EAE model of multiple sclerosis. *Cell Tissue Res.* 2023;392(2):467-80. doi: 10.1007/s00441-023-03746-w
39. Malaisé Y, Lencina C, Cartier C, Olier M, Ménard S, Guzylack-Pirou L. Perinatal oral exposure to low doses of bisphenol A, S or F impairs immune functions at intestinal and systemic levels in female offspring mice. *Environ Health.* 2020;19(1):14. doi: 10.1186/s12940-020-00614-w
40. Wu X, Tian Y, Zhu H, Xu P, Zhang J, Hu Y, et al. Invisible hand behind female reproductive disorders: bisphenols, recent evidence and future perspectives. *Toxics.* 2023;11(12):1000. doi: https://doi.org/10.3390/TOXICS11121000
41. Fol V Le, Brion F, Hillenweck A, Perdu E, Bruel S, Aït-Aïssa S, et al. Comparison of the in vivo biotransformation of two emerging estrogenic contaminants, BP2 and BPS, in zebrafish embryos and adults. *Int J Mol Sci.* 2017;18(4):704. doi: 10.3390/ijms18040704
42. Li J, Li Z, Zhu Y, Peng H, Du Z, Ru S, et al. Bisphenol S remodels red blood cell membrane lipids by altering plasma lipid levels, causing the risk of venous thrombosis in SD rats and zebrafish embryos. *Environ Int.* 2023;182:108331. doi: 10.1016/j.envint.2023.108331
43. Su X, Kai L, Han X, Wang R, Yang X, Wang X, et al. Equipotent bisphenol S and bisphenol F with widely differing modes of action exhibit additive effects in immunotoxicity: insights based on intrinsic immunity, apoptosis and regeneration, and oxidative stress. *Sci Total Environ.* 2025;977:179405. doi: 10.1016/j.scitotenv.2025.179405
44. Zhang J, Yu X, Li W, Jiang Y, Zhang L, Wang S. Bisphenol S impairs oocyte quality by inducing gut microbiota dysbiosis. *mSystems.* 2025;10(1):00912-24. doi: 10.1128/mSystems.00912-24
45. Wu ZY, Luo L, Kan YQ, Qin ML, Li HT, He QZ, et al. Puerarin prevents bisphenol S induced lipid accumulation by reducing liver lipid synthesis and promoting lipid metabolism in C57BL/6J mice. *Toxics.* 2023;11(9):736. doi: doi.org/10.3390/toxics11090736

Three-year risk of colorectal cancer by faecal haemoglobin concentration in subjects with a negative FIT

Donella Puliti¹, Giuseppe Gorini¹, Adele Caldarella¹, Teresa Intrieri¹, Zhanna Tumanova², Gianfranco Manneschi¹, Carmen Beatriz Visioli¹, Beatrice Mallardi¹, Francesca Battisti¹, Simonetta Bisanzi¹, Cristina Sani¹, Giovanna Masala¹, Marco Zappa¹ and Paola Mantellini¹

¹Istituto per lo Studio, la Prevenzione e la Rete Oncologica (ISPRO), Florence, Italy

²Dipartimento di Sanità Pubblica, Università degli Studi di Firenze, Florence, Italy

Abstract

Introduction and aim. To evaluate whether a risk-stratified colorectal cancer (CRC) screening approach based on a single faecal immunochemical test (FIT) performed better than one using two consecutive tests.

Methods. Approximately 420,000 negative FIT results (positivity cut-off: 20 µg faecal haemoglobin, f-Hb/g) were collected between 2000 and 2015 within the Florence screening program.

Results. The 3-year CRC risk was 15.9‰ in individuals with 15-20 µg/g on a single test, and 9.36‰ for those with a cumulative f-Hb concentration of 20-40 µg/g across two tests.

Discussion. The two-tests approach showed lower discriminatory capacity in identifying the highest-risk subgroup compared to a single test. Screenees with 15-20 µg/g accounted for <1%, but faced a significantly increased 3-year CRC risk.

Conclusion. While increasing screening intensity would require more colonoscopies, extending the screening interval for individuals <60 years with undetectable f-Hb, could allow for the reallocation of colonoscopy resources.

Key words

- colorectal cancer
- faecal haemoglobin concentration
- faecal immunochemical test (FIT)

INTRODUCTION

Colorectal cancer (CRC) is the second most leading cause of cancer-related mortality worldwide, accounting for approximately 900,000 deaths in 2022 [1]. Population-based CRC screening programs using faecal immunochemical tests (FIT) significantly reduce both CRC incidence and mortality and are now widely implemented across Europe [2]. In Italy, as in many other European countries, the positivity cut-off is set at 20 µg of faecal haemoglobin per gram of faeces (µg f-Hb/g). Current screening programmes follow similar protocols: f-Hb concentrations at or above the established threshold are considered positive and warrant a referral for total colonoscopy (TC). Conversely, concentrations below the cut-off are classified as negative, with individuals re-screened every two years in most European countries, or annually in the United States and a few other regions [2].

Accumulating evidence indicates that individuals with a negative FIT but an f-Hb value just below the threshold exhibit increased CRC detection rates in subsequent rounds, and a higher probability of devel-

oping interval cancers (ICs) compared to those with no detectable f-Hb [3-10]. These studies pursued varied objectives, such as estimating IC risk following a negative FIT in single or consecutive screening rounds [4, 7, 10], developing risk-scoring models that integrate f-Hb concentrations from a single FIT with other CRC risk factors [8], or evaluating the predictive value of the first two consecutive FIT rounds [3, 9]. Furthermore, cross-study comparisons remain challenging due to substantial heterogeneity in f-Hb stratification and positivity threshold: for instance, the cut-off was set at ≥47 µg/g in the Netherlands [7, 9], ≥20 µg/g in Denmark and Italy [3, 10], and ≥80 µg/g in Scotland [8]. To address this, a recent systematic review and dose-response meta-analysis synthesized these data, demonstrating that individuals with sub-threshold f-Hb concentrations of 5, 10, and 15 µg/g had a 4-, 6-, and 8-fold higher risk of CRC, respectively, at the subsequent screening round compared to individuals with undetectable f-Hb [11].

These findings suggest that risk-stratified screening strategies – tailoring protocols to individual risk levels – could enhance detection rates, optimize the benefit-

to-harm ratio, and improve the efficiency of endoscopy resource allocation. Conversely, implementing risk-based screening introduces significant organizational challenges and requires robust information technology and communication frameworks. The 2022 Council of the European Union Recommendation affirmed that quantitative FIT is the preferred tool for colonoscopy referral and endorsed its use in risk-tailored strategies, suggesting the introduction of thresholds tailored to sex, age and previous screening history [12].

However, the optimal strategy for designing such a risk-based approach remains poorly defined. Specifically, it is unclear whether a single FIT performs better than two consecutive tests in assessing subsequent CRC risk. Notably, a single-test strategy offers substantial organizational advantages, given that screening adherence fluctuates and many individuals do not consistently participate in every consecutive round.

The objective of this study is to evaluate, within a retrospective cohort from the ongoing FIT-based screening program (20 µg/g cut-off) in the province of Florence, Italy, whether a risk-stratification approach based on a single FIT outperforms one based on two consecutive tests in discriminating the cumulative three-year CRC risk. Discriminatory capacity will be assessed by the ability to differentiate – relative to the average risk of all FIT-negative subjects – a higher-risk subgroup, that warrants a more intensive protocol from lower-risk subgroup eligible for a de-escalated screening interval.

MATERIALS AND METHODS

Screening program and study population

The Florence district provides a population-based, biennial colorectal cancer (CRC) screening program using faecal immunochemical test (FIT) for individuals aged 50-70. Established in the 1980s, the program utilized the OC-Hemodia (Eiken) method from 2000 to 2003, transitioning to the OC-Sensor (Eiken) method in 2004. The positivity threshold is set at 20 µg/g of faeces (equivalent to 100 ng/ml). Negative FIT results are mailed to participants with a recommendation to repeat the test in two years, with no mention of their specific f-Hb concentration. Individuals with a positive FIT are contacted by screening staff and offered a total colonoscopy (TC) at dedicated endoscopy reference centres. All screening services are free of charge and funded by the Regional Health System Program performance is routinely monitored using standard quality indicators [13]; target population participation rates fluctuated between 48.0% and 57.0% during the 2000-2015 study period [14-16]. This analysis was conducted as part of the routine monitoring of program performance. The study protocol was approved by the local Ethics Committee of Florence area (Comitato Etico Regionale per la Sperimentazione Clinica della Regione Toscana, Sezione Area Vasta Centro; reference number: 23723_oss).

We evaluated all negative FIT (index FIT) obtained between January 1, 2000 and December 31, 2015, from individuals aged 50-65 years, for each negative FIT, data on f-Hb level, sex, and age were retrieved. Linkage with the screening database allowed classification

of each test as either a first or subsequent screening round. To compare the 3-year cumulative CRC risk based on a single negative FIT with the predictive value of cumulative f-Hb levels across two consecutive tests, we selected a subset of index FITs that had a preceding test recorded 18 to 60 months prior. Following the categories proposed by Senore *et al.* [3], f-Hb concentrations for a single FIT were stratified into five groups: 0 µg/g or undetectable f-Hb; 0-4 µg/g (≤ 4 µg/g); 4-10 µg/g (from 4.1 to 9.9 µg/g); 10-15 µg/g (from 10 to 14.9 µg/g); and 15-20 µg/g (from 15 to 19.9 µg/g). Cumulative f-Hb concentrations across two consecutive FITs were stratified into six groups: 0 µg/g; 0-4 µg/g; 4-10 µg/g; 10-15 µg/g; 15-20 µg/g; and 20-40 µg/g (from 20 to 39.9 µg/g).

Outcome assessment and data linkage

CRC incidence was tracked via linkage with the regional cancer registry, which was updated through December 31, 2020. Adenocarcinomas of the colon and rectum (ICD-10 codes C18, C19 and C20) [17] diagnosed within 3 years of a negative FIT were included. Disease stage was determined using histology reports or clinical records according to the 7th edition of the TNM classification [18], with Stages III and IV classified as advanced CRC.

CRCs were categorized into: (i) screen-detected (SD) CRCs: cancers diagnosed within 6 months of a positive FIT (including individuals who underwent a TC and non-compliant participants who refused the screening TC); (ii) interval cancers (ICs): CRCs diagnosed between 6 and 36 months following a negative FIT. Cancers diagnosed more than 6 months after a positive FIT underwent individual clinical review to ensure accurate classification as either an SD CRC or an IC.

Statistical analysis

The statistical unit of analysis was the individual test, and differences in proportions were evaluated using the Chi-square test. Person-time was calculated from the date of the index negative FIT to the earliest of the following events: a subsequent negative FIT, a CRC diagnosis, or the end of the 3-year follow-up period. Consequently, a subject diagnosed with CRC following a subsequent positive FIT exited the cohort at the time of diagnosis, whereas a subject with a subsequent positive FIT but no CRC diagnosis remained under observation for the full 3 years.

The association between f-Hb concentration levels and the risk of total or advanced CRC was estimated using multivariate Cox regression models, adjusted for age-group (50-54, 55-59, 60-65 years), sex, and screening history (first vs subsequent test). All statistical tests were two-sided, with significance defined as $p < 0.05$.

RESULTS

From January 2000 to December 2015, 419,822 negative FIT results were obtained from 168,437 individuals aged 50-65 years within the Florence CRC screening program (average 2.5 FITs per person). First examinations accounted for 39.8% of the tests, while 60.2% were subsequent screenings. Faecal haemoglo-

bin (f-Hb) was undetectable (0 µg/g) in 48.9% of the negative FITs, and exceeded 15 µg/g in only 0.9%. Individuals with higher f-Hb levels were predominantly male and older (Table S1 available online as Supplementary Material).

During the 3-year follow-up period after a negative FIT, 554 CRCs were diagnosed, corresponding to an overall risk of 1.32‰ (95% CI: 1.21‰-1.43‰). Of these, 53.8% (n=298) were diagnosed at an early stage (stages I-II), 38.1% (n=211) at an advanced stage (stages III-IV), and 8.1% (n=45) had an unknown stage (Table 1a). Tumour stage distribution did not significantly differ across f-Hb categories (p=0.950). Under the single-test strategy, the 3-year CRC risk increased progressively from 0.65‰ among individuals with undetectable

f-Hb, to 15.9‰ among those with f-Hb level of 15-20 µg/g. After adjusting for age group, sex, and screening history, the hazard of a CRC diagnosis ranged from 60% higher in the <4 µg/g group (adjusted Hazard Ratio – aHR: 1.61; 95% CI: 1.29-2.02), to 21-fold higher in the 15-20 µg/g group (aHR: 21.00; 95% CI: 15.35-28.74) compared to the undetectable f-Hb reference group. Advanced CRC risk followed an identical trend; notably, 10.4% of advanced cancers (22/211) were diagnosed within 3 years among the 0.9% of individuals (3,595/419,822) in the highest sub-threshold f-Hb tier (Table 1a; Figure 1a).

A subset of 241,243 negative FITs (57.5%) had a preceding test recorded within an 18-60 month interval. In this cohort, 318 CRCs were diagnosed within

Table 1

Crude three-years risk (%) of CRC and advanced CRC by f-Hb at a single FIT (a) and by cumulative f-Hb at two consecutive FIT (b) and adjusted hazard ratios (aHR)

a)		CRC		Advanced CRC	
f-Hb (µg/g)	N	N	aHR*	N	aHR*
	FIT (%)	‰	(95% CI)	‰	(95% CI)
0	205,365	134	ref	52	ref
	(48.9%)	0.65‰		0.25‰	
(0-4)	172,312	180	1.61	67	1.52
	(41.0%)	1.04‰	(1.29-2.02)	0.39‰	(1.06-2.19)
[4-10)	31,648	125	5.76	46	5.42
	(7.5%)	3.95‰	(4.51-7.37)	1.45‰	(3.64-8.09)
[10-15)	6,902	58	11.47	24	12.26
	(1.6%)	8.40‰	(8.41-15.65)	3.48‰	(7.52-20.00)
[15-20)	3,595	57	21.00	22	20.91
	(0.9%)	15.9‰	(15.35-28.74)	6.12‰	(12.66-34.55)
Total	419,822	554	-	211	-
	(100%)	1.32‰		0.50‰	
b)		CRC		Advanced CRC	
Cumulative f-Hb (µg/g)	N	N	aHR**	N	aHR**
	FIT (%)	‰	(95% CI)	‰	(95% CI)
0	73,203	43	ref	14	ref
	(30.3%)	0.59‰		0.19‰	
(0-4)	119,685	96	1.35	42	1.82
	(49.6%)	0.80‰	(0.94-1.94)	0.35‰	(0.99-3.34)
[4-10)	35,250	82	3.84	27	3.91
	(14.6%)	2.33‰	(2.65-5.58)	0.77‰	(2.04-7.51)
[10-15)	7,497	47	9.97	19	12.42
	(3.1%)	6.27‰	(6.60-15.08)	2.53‰	(6.24-24.71)
[15-20)	4,113	36	13.70	15	17.55
	(1.7%)	8.75‰	(8.79-21.34)	3.65‰	(8.47-36.38)
[20-40)	1,496	14	13.57	6	17.91
	(0.6%)	9.36‰	(7.39-24.91)	4.01‰	(6.84-46.86)
Total	241,243	318	-	123	-
	(100%)	1.32‰		0.51‰	

*Adjusted for sex, age and previous screening test; **Adjusted for sex and age; CRC: colorectal cancer; FIT: faecal immunochemical test; f-Hb: faecal haemoglobin; CI: confidence interval.

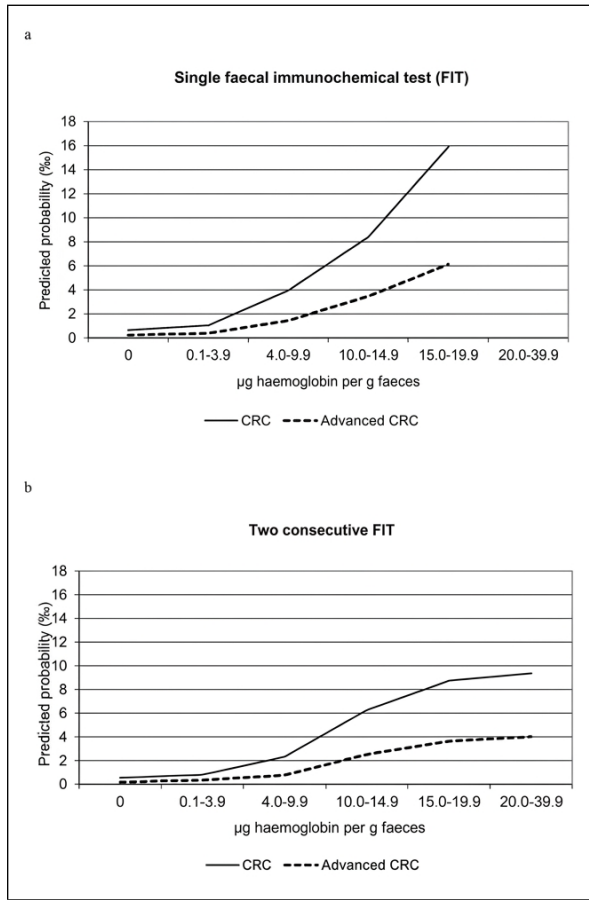


Figure 1
Crude 3-year risk (95% CI) of CRC by f-Hb concentration at a single or a two consecutive FIT.
CRC: colorectal cancer; f-Hb: faecal haemoglobin; FIT: faecal immunochemical test.

3 years, yielding an overall risk of 1.32‰ (95% CI: 1.18‰-1.47‰). Early-stage tumours comprised 52.8% (n=168) of cases, advanced-stage tumours accounted for 38.7% (n=123), and 8.5% (n=27) were of unknown stage, with no significant differences across f-Hb categories (p=0.278) (data not shown). The 3-year CRC risk using the two-test cumulative f-Hb modality ranged from 0.59‰ to 9.36‰ (Table 1b). The 3-year CRC risk was significantly higher in the ≥ 15 µg/g category of the single-test approach than in the ≥ 20 µg/g category of the two-test approach – 15.9‰ (57/3,595) vs 9.36‰ (14/1,496), p<0.001. Furthermore, the single-test approach identified a significantly higher percentage of high-risk individuals than the two-test modality – 0.86% (3,595/419,822) vs 0.62% (1,496/241,243), p=0.001. Conversely, both methods demonstrated comparable performance in identifying the lowest-risk group (0.65 for single FIT vs 0.59% for two consecutive tests, % p=0.549). For the two-test strategy, the adjusted hazard of CRC ranged from a non-significant 35% increase for f-Hb <4 µg/g (aHR:1.35; 95% CI: 0.94-1.94), to 14-fold increase for cumulative levels of 20-40 µg/g (aHR: 13.60; 95% CI: 7.40-24.91). Advanced CRC risk mirrored these trends, with 4.9% of advanced cancers (6/123) occurring within the 0.6% of

Table 2
Predictors of the 3-years CRC and advanced CRC risk (n=241,243)

Predictors	CRC		Advanced CRC	
	aHR	95% CI	aHR	95% CI
f-Hb (µg/g) at the index FIT				
0	ref		ref	
(0-4)	1.69	1.26-2.27	1.66	1.03-2.68
[4-10)	5.22	3.73-7.32	4.22	2.38-7.50
[10-15)	9.64	6.18-15.03	12.07	6.14-23.74
[15-20)	18.65	12.03-28.91	22.21	11.37-43.39
f-Hb (µg/g) at the previous FIT				
0	ref		ref	
(0-4)	1.18	0.92-1.51	1.26	0.84-1.88
[4-10)	2.14	1.52-3.02	2.06	1.17-3.65
[10-15)	2.43	1.39-4.25	1.30	0.41-4.13
[15-20)	1.91	0.83-4.38	2.38	0.72-7.89
Sex				
M	ref		ref	
F	0.80	0.64-1.00	0.74	0.51-1.05
Age class				
50-54	ref		ref	
55-59	0.95	0.65-1.41	1.02	0.56-1.88
60-65	1.46	1.00-2.12	1.53	0.84-2.77

CRC: colorectal cancer; aHR: adjusted hazard ratios; f-Hb: faecal haemoglobin; FIT: faecal immunochemical test; CI: confidence interval.

tests representing the highest cumulative tier (Table 1b; Figure 1b).

Table 2 presents the multivariate aHRs for f-Hb levels of the index and previous FITs, sex, and age-group within the two-test subset (n=241,243). The index (most recent) FIT f-Hb categories carried the strongest predictive weight, with the aHR escalating from 1.69 to 18.65 relative to the reference group. In contrast, f-Hb levels from the previous screening round significantly increased risk only above 4 µg/g, exhibiting a plateau thereafter; the aHR for the highest previous f-Hb level did not reach statistical significance (aHR: 1.91; 95% CI: 0.83-4.38). Females demonstrated a non-significantly lower risk than males, while risk increased progressively with age.

For the single FIT approach (Table 3a), 191 interval cancers (ICs) were diagnosed within 24 months of a negative test (0.45‰; 95% CI: 0.39‰-0.52‰). The risk gradient across f-Hb categories was more pronounced than for overall 3-year incidence: individuals in the >15 µg/g class exhibited an approximate 30-fold higher risk than those with undetectable f-Hb (aHR: 29.49; 95% CI: 17.41-49.96; data not shown). Between 24 and 36 months, 53 ICs were diagnosed (0.13‰; 95% CI: 0.10‰-0.17‰), showing an attenuated risk gradient (aHR for

>15 µg/g: 12.13; 95% CI: 4.06-36.30). Within 3 years of the index test, 82.2% of individuals (345,176/419,822) underwent a subsequent FIT yielding an overall positivity rate of 3.3% (Table 3a). The subsequent positivity rate scaled from 2.3% for individuals with undetectable index f-Hb to 15.8% for those exceeding 15 µg/g.

Under the two-test strategy (Table 3b), 117 ICs were diagnosed within 24 months (0.49‰), displaying a steeper risk gradient than the overall cohort (aHR for ≥15 µg/g: 22.48; 95% CI: 8.38-60.32; data not shown). An additional 21 ICs were diagnosed between 24 and 36 months (0.09‰), with a risk gradient mirroring the overall incidence. Within 3 years, 88.6% of participants

(213,733/241,243) completed a subsequent FIT, with an overall positivity rate of 3.3% (Table 3b); subsequent round positivity ranged from 2.0% (undetectable cumulative f-Hb) to 19.5% (cumulative f-Hb ≥15 µg/g).

When stratified by age using a single FIT, all age cohorts with an f-Hb ≥15 µg/g experienced a 3-year risk exceeding 10‰, whereas all age groups with undetectable f-Hb maintained a risk below 1‰. Furthermore, the 50-54 and 55-59 age groups retained a risk profile below 1‰ if their f-Hb was <4 µg/g (Table S2a available online as Supplementary Material). Conversely, using the two-test cumulative approach, no age group within the cumulative >15-20 µg/g tier exceeded a 3-year risk of

Table 3

Interval cancers and screen-detected (SD) cancers at subsequent round (within 36 months after the index FIT) by f-Hb level at a single FIT (a) and by cumulative f-Hb at two consecutive FIT (b)

a)		Interval cancers		Next screening round	
f-Hb (µg/g)	N	0-24 months	24-36 months	Subsequent FIT	SD cancer [^]
		N ‰	N ‰	N (% FIT+)	N ‰
0	205,365	39 0.19‰	16 0.08‰	169,654 (2.3%)	79 0.47‰
(0-4)	172,312	52 0.30‰	18 0.10‰	141,708 (3.3%)	110 0.78‰
[4-10]	31,648	51 1.61‰	14 0.44‰	25,566 (7.1%)	60 2.35‰
[10-15]	6,902	27 3.91‰	1 0.14‰	5,405 (11.6%)	30 5.55‰
[15-20]	3,595	22 6.12‰	4 1.11‰	2,843 (15.8%)	31 10.9‰
Total	419,822	191 0.45‰	53 0.13‰	345,176 (3.3%)	310 0.90‰
b)					
f-Hb (µg/g)	N	N ‰	N ‰	N (% FIT+)	N ‰
0	73,203	12 0.16‰	3 0.04‰	65,675 (2.0%)	28 0.43‰
(0-4)	119,684	30 0.25‰	7 0.06‰	105,883 (2.7%)	59 0.56‰
[4-10]	35,250	31 0.88‰	6 0.17‰	30,901 (5.0%)	45 1.46‰
[10-15]	7,497	22 2.93‰	1 0.13‰	6,472 (8.9%)	24 3.71‰
[15-20]	4,113	16 3.89‰	3 0.73‰	3,540 (10.7%)	17 4.80‰
[20-40]	1,496	6 4.01‰	1 0.67‰	1,263 (19.5%)	7 5.54‰
Total	241,243	117 0.49‰	21 0.09‰	213,733 (3.3%)	180 0.84‰

[^]Two cancers diagnosed respectively 10 and 12 months after a FIT positive test, were considered to be screen-detected after evaluation of their screening history. f-Hb: faecal haemoglobin; FIT: faecal immunochemical test.

10‰, while all age cohorts with cumulative f-Hb levels <4 µg/g remained below 1‰. The 50-54 and 55-59 age cohorts consistently demonstrated the lowest risks, except within the highest f-Hb tier (Table S2b available online as Supplementary Material).

DISCUSSION

Quantitative faecal haemoglobin (f-Hb) levels in individuals with negative faecal immunochemical test (FIT) results strongly correlate with the subsequent risk of colorectal cancer (CRC) diagnosis [2-11]. Our findings confirm that among screening participants with a negative FIT, those with sub-threshold f-Hb levels exceeding 15 µg/g on a single test, 20 µg/g across two consecutive tests, represent less than 1% of the population yet face a substantially elevated risk of CRC within three years. These results support the implementation of risk-stratified screening strategies [3-5, 19], such as: (i) extending screening intervals for individuals with consistently undetectable f-Hb (0 µg/g), given that CRC yield remains stable at 2- vs 3-year intervals, (ii) intensifying screening (i.e., shortening intervals to 6-12 months or directly referring to total colonoscopy – TC) for individuals in the highest sub-threshold f-Hb tiers. The resulting increase in endoscopy TC demand could be balanced across subsequent rounds by extending intervals for the low-risk cohort, potentially optimizing resource allocation and reducing interval cancer rates.

The primary novelty of this study lies in determining the optimal approach for identifying elevated f-Hb values among FIT-negative individuals, specifically comparing a single-test strategy against a two-test cumulative approach. Our data indicate that a single test offers superior predictive ability and discriminatory capacity for identifying the highest-risk subgroup compared to two consecutive tests (3-year risk: 15.9% vs 9.36%), while maintaining comparable accuracy for the lowest-risk subgroup (0.65‰ vs 0.59‰). Furthermore, the single-test modality identified a larger absolute number of high-risk individuals (3,595 vs 1,496), whereas f-Hb levels from the preceding screening round did not add robust predictive value. Relying on a single test also provides significant organizational advantages. In the Florentine cohort, approximately 20% of individuals who completed an initial test defaulted from the subsequent round. Capturing these individuals is clinically vital, as those with a first sub-threshold f-Hb ≥15 µg/g, were more likely to present with either an interval cancer or a screen-detected CRC in the following round.

Intensifying screening for individuals with f-Hb levels between 15 and 20 µg/g inevitably demands additional endoscopy capacity. In our screening program, recommending immediate TC for all individuals with an f-Hb ≥15 µg/g on any FIT would increase the baseline positivity rate from 5.0% to 5.9%. However, because roughly 20% of these individuals went on to test positive within the subsequent five years anyway, this initial increase in TC volume would balance out over a 5-year period. Endoscopic resources could be further reclaimed by extending the screening interval (e.g., to 2.5 years) for individuals under 60 years of age with undetectable f-Hb levels. Prior literature confirms that lowering the FIT

positivity threshold increases sensitivity for advanced adenomas in population-based screenings at the expense of specificity, ultimately reducing CRC incidence in subsequent rounds [20-22]. Moreover, baseline sub-threshold f-Hb concentrations have been strongly tied to long-term CRC risk, with an eightfold risk increase observed at an 8-year follow-up among individuals with baseline levels of 8-10 µg/g compared to those with undetectable f-Hb [5].

Relying on cumulative results from two consecutive negative tests present distinct limitations. Within-subject correlation between consecutive f-Hb measurements was weak, and the predictive value of cumulative f-Hb remained similar, regardless of the test sequence [4, 5]. Furthermore, repeated FIT screening can miss a subset of adenomas and CRCs due to systematic false-negative results, typically driven by non-bleeding lesions [23].

From a behavioural perspective, risk-based screening may enhance compliance among high-risk groups, but risk decreasing participation among low-risk individuals due to a false sense of reassurance [24, 25]. Alternatively, low-risk individuals – particularly those of higher socioeconomic status – might reject reduced access to standard services and independently seek more intensive screening elsewhere.

Balancing the precision of a detailed risk classification with real-world implementation remains a challenge. Insights from two ongoing randomized controlled trials evaluating tailored invitation intervals based on single or consecutive FIT results will provide crucial evidence on the scalability of these strategies [26, 27].

Integrating additional risk factors such as age and sex could further refine these models. Some programs already employ sex-specific FIT thresholds or sex-specific starting ages for screening colonoscopy [24]. Similarly, a risk-stratified age for screening cessation – where additional screening is offered to elderly cohorts based exclusively on the f-Hb levels of their last routine round – merits consideration, especially since guidelines generally recommend screening cessation in older populations unless life expectancy exceeds 10 years [28].

Regarding target population expansion, three national and international references indicate that the optimal target population for colorectal cancer screening should be between 50 and 74 years of age, thereby adding individuals aged 70-74 to the standard target population (people aged 50-69 years): the Recommendations of the Council of the European Union (2022/0290 NLE) [12]; the National Italian Prevention Plan 2020-2025 [29]; the National Italian Oncology Plan 2023-2027 (PON) [30].

Our study has certain limitations. Advanced adenomas were not captured because the regional cancer registry tracks malignant neoplasms exclusively. Additionally, lifestyle, socioeconomic variables, anthropometric measures, and family history were unavailable, though such parameters are rarely accessible in routine, population-based screening databases. Finally, historical variations in specimen collection methods, or buffer formulations might have subtly influenced f-Hb concentration distributions [19, 31].

Conversely, key strengths of this work include the

longitudinal analysis across multiple screening rounds, stratification by precise f-Hb levels, and a true population-based setting governed by a highly consistent screening protocol.

CONCLUSION

Among screenees with a negative FIT, those with f-Hb levels ≥ 15 $\mu\text{g/g}$ represent less than 1% of the population but face a significantly higher risk of a subsequent CRC diagnosis. A single-test approach demonstrates superior predictive ability compared to a two-test cumulative approach. While intensifying screening for individuals with elevated sub-threshold f-Hb requires greater colonoscopy capacity, these resources can be successfully reclaimed by extending screening intervals for individuals under 60 years of age with undetectable f-Hb. Future prospective studies and simulation models are warranted to refine the precise logistical and clinical parameters of these risk-stratified strategies.

REFERENCES

- Bray F, Laversanne M, Sung H, et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin.* 2024;74:229-63. doi: 10.3322/caac.21763
- Chen Y, Zhang Y, Yan Y, et al. Global colorectal cancer screening programs and coverage rate estimation: an evidence synthesis. *J Transl Med.* 2025;23:811. doi: 10.1186/s12967-025-06887-4
- Senore C, Zappa M, Campari C, Crotta S, et al. Faecal haemoglobin concentration among subjects with negative FIT results is associated with the detection rate of neoplasia at subsequent rounds: a prospective study in the context of population based screening programmes in Italy. *Gut.* 2020;69(3):523-30. doi: 10.1136/gutjnl-2018-318198
- Buron A, Román M, Augé JM, et al. Changes in FIT values below the threshold of positivity and short-term risk of advanced colorectal neoplasia: results from a population-based cancer screening program. *Eur J Cancer.* 2019;107:53-9. doi: 10.1016/j.ejca.2018.11.004
- Grobbee EJ, Schreuders EH, Hansen BE, Bruno MJ, Lansdorp-Vogelaar I, Spaander MCW, Kuipers EJ. Association between concentrations of hemoglobin determined by fecal immunochemical tests and long-term development of advanced colorectal neoplasia. *Gastroenterology.* 2017;153(3):1251-9.e2. doi: 10.1053/j.gastro.2017.07.034
- Chen LS, Yen AM, Chiu SY, Liao CS, Chen HH. Baseline faecal occult blood concentration as a predictor of incident colorectal neoplasia: longitudinal follow-up of a Taiwanese population-based colorectal cancer screening cohort. *Lancet Oncol.* 2011;12(6):551-8. doi: 10.1016/S1470-2045(11)70101-2
- Breekveldt ECH, Toes-Zoutendijk E, van de Schootbrugge-Vandermeer HJ, et al. Factors associated with interval colorectal cancer after negative FIT: Results of two screening rounds in the Dutch FIT-based CRC screening program. *Int J Cancer.* 2023;152(8):1536-46. doi: 10.1002/ijc.34373
- Digby J, Fraser CG, Clark G, Mowat C, Strachan JA, Steele RJ. Improved use of faecal immunochemical tests for haemoglobin in the Scottish bowel screening programme. *J Med Screen.* 2023;30(4):184-90. doi: 10.1177/09691413231175611
- Meester RGS, van de Schootbrugge-Vandermeer HJ, Breekveldt ECH, et al. Faecal occult blood loss accurately predicts future detection of colorectal cancer. A prognostic model. *Gut.* 2023;72(1):101-8. doi:10.1136/gutjnl-2022-327188
- Deding U, Kobaek-Larsen M, Bøggild H, Kaalby L, Thygesen MK, Baatrup G. Socioeconomic inequalities in interval colorectal cancer are explained by differences in faecal haemoglobin concentration and age: a register-based cohort study. *BMJ Open Gastroenterol.* 2023;10:e001113. doi: 10.1136/bmjgast-2023-001113
- van den Berg DMN, van den Puttelaar R, de Jonge L, Lansdorp-Vogelaar I, Toes-Zoutendijk E. Fecal hemoglobin levels in prior negative screening and detection of colorectal neoplasia: a dose-response meta-analysis. *Gastroenterology.* 2025;168:587-97. doi:10.1053/j.gastro.2024.10.047
- General Secretariat of the European Council. Council Recommendation on strengthening prevention through early detection: a new EU approach on cancer screening replacing Council Recommendation 2003/878/EC. Brussels: European Union; 2022. Available from: <https://data.consilium.europa.eu/doc/document/ST-14770-2022-INIT/en/pdf>
- Zorzi M, Sassoli de' Bianchi P, Grazzini G, et al; Gruppo di Lavoro sugli Indicatori del GISCoR. Indicatori di qualità per la valutazione dei programmi di screening dei tumori colorettali [Quality indicators for the evaluation of colorectal cancer screening programmes]. *Epidemiol Prev.* 2007;31(6 Suppl. 1):6-56.
- Regione Toscana Centro per lo Studio e la Prevenzione Oncologica. I Programmi di screening della Regione Toscana – Quarto rapporto annuale. Firenze: CSPO; 2003. Available from: https://www.ispro.toscana.it/app/uploads/2015/09/04°-Rapporto-Annuale-Programmi-Screening_2003.pdf
- Istituto per lo Studio e la Prevenzione Oncologica. I Programmi di screening della Regione Toscana – 17° rapporto annuale. Firenze: ISPO; 2016. Available from: <https://www.ispro.toscana.it/app/uploads/2015/09/17°>

Data availability

The data have been archived in the Zenodo repository and are available upon request from the corresponding author; doi: 10.5281/zenodo.17788549.

Funding

The study was part of an internal evaluation project of the CRC screening program in Florence. Thus, no fundings were provided for this study.

Acknowledgements

We thank Guglielmo Bonaccorsi, Department of Public Health, Florence University.

Conflict of interest statement

None to declare.

Received on 17 February 2026.

Accepted on 8 June 2026.

- RAPPORTO_x.pdf
16. Zorzi M, Da Re F, Mantellini P, et al. Screening for colorectal cancer in Italy: 2011-2012 survey. *Epidemiol Prev.* 2015;39(3 Suppl. 1):93-107.
 17. World Health Organization. ICD-10: international statistical classification of diseases and related health problems: tenth revision, 2nd ed. Geneva: WHO, 2004.
 18. Sobin LH, Gospodarowicz MK, Wittekind C, Union for International Cancer Control. TNM Classification of malignant tumours, 7th ed. New York: Wiley-Liss; 2009.
 19. van Roon AH, Goede SL, van Ballegooijen M, et al. Random comparison of repeated faecal immunochemical testing at different intervals for population-based colorectal cancer screening. *Gut.* 2013;62(3):409-15. doi: 10.1136/gutjnl-2011-301583
 20. Denters MJ, Deutekom M, Bossuyt PM, Stroobants AK, Fockens P, Dekker E. Lower risk of advanced neoplasia among patients with a previous negative result from a fecal test for colorectal cancer. *Gastroenterology.* 2012;142(3):497-504. doi: 10.1053/j.gastro.2011.11.024
 21. Grazzini G, Visioli CB, Zorzi M, et al. Immunochemical faecal occult blood test: number of samples and positivity cutoff. What is the best strategy for colorectal cancer screening?. *Br J Cancer.* 2009;100(2):259-65. doi: 10.1038/sj.bjc.6604864
 22. Njor SH, Andersen B, Friis-Hansen L, et al. The optimal cut-off value in fit-based colorectal cancer screening: an observational study. *Cancer Med.* 2021;10(5):1872-9. doi: 10.1002/cam4.3761
 23. van der Meulen MP, Lansdorp-Vogelaar I, van Heijningen EM, Kuipers EJ, van Ballegooijen M. Nonbleeding adenomas: Evidence of systematic false-negative fecal immunochemical test results and their implications for screening effectiveness-A modeling study. *Cancer.* 2016;122(11):1680-8. doi: 10.1002/cncr.29952
 24. Lansdorp-Vogelaar I, Meester R, de Jonge L, Buron A, Haug U, Senore C. Risk-stratified strategies in population screening for colorectal cancer. *Int J Cancer.* 2022;150(3):397-405. doi: 10.1002/ijc.33784
 25. Maxwell S, Weller D, Dennison B, et al. Stratified/risk-based screening for colorectal cancer in the UK: an overview. *Colorectal Cancer.* 2025;14(1):2501851. doi: 10.1080/1758194X.2025.2501851
 26. Breekveldt ECH, Toes-Zoutendijk E, de Jonge L, et al. Personalized colorectal cancer screening: study protocol of a mixed-methods study on the effectiveness of tailored intervals based on prior f-Hb concentration in a fit-based colorectal cancer screening program (PERFECT-FIT). *BMC Gastroenterol.* 2023;23(1):45. doi: 10.1186/s12876-023-02670-1
 27. Senore C. Can we analyse stool content to improve the prediction of the risk of developing bowel cancer? 2022. doi: 10.1186/ISRCTN10728933
 28. van Stigt BJ, Lansdorp-Vogelaar I, Spaander MCW, et al. Interval cancer risk after the upper age limit of screening has been reached: Informing risk stratification in FIT-based colorectal cancer screening. *Int J Cancer.* 2025;156(9):1783-90. doi: 10.1002/ijc.35294
 29. Direzione Generale della Prevenzione Sanitaria. Piano nazionale della prevenzione 2020-2025. Roma: Ministero della Salute; 2020. Available from: https://www.salute.gov.it/imgs/C_17_notizie_5029_0_file.pdf
 30. Ministero della Salute. Piano oncologico nazionale: documento di pianificazione e indirizzo per la prevenzione e il contrasto del cancro 2023-2027. Roma: Ministero della Salute; 2023. Available from: https://www.iccp-portal.org/sites/default/files/2025-04/Piano_Oncologico_Nazionale_2023-2027.pdf
 31. Grazzini G, Ventura L, Zappa M, et al. Influence of seasonal variations in ambient temperatures on performance of immunochemical faecal occult blood test for colorectal cancer screening: observational study from the Florence district. *Gut.* 2010;59(11):1511-5. doi: 10.1136/gut.2009.200873

BOOK REVIEWS, NOTES AND COMMENTS

Edited by
Federica Napolitani Cheyne



**LA STRUTTURA
NARRATIVA
DELL'ESPERIENZA UMANA
Un'ipotesi esplicativa
della psicosi**

Vittorio Guidano
Vol. 2 - LA PROCESSUALITÀ
DEL SELF E LE DIMENSIONI
DEL SIGNIFICATO
Giovanni Cutolo and
Adele De Pascale (Eds)
FrancoAngeli; 2026
620 p.
ISBN 9788835174240

*[The processuality of the
Self and the dimensions of
meaning]*

In the field of psychiatry, psychotherapy, and the cognitive and neurosciences, the question of how the human mind constructs meaning remains one of the most compelling and elusive challenges. *The processuality of the Self and the dimensions of meaning*, the second volume of the trilogy *The Narrative structure of human experience: An explanatory hypothesis of psychosis*, edited by Giovanni Cutolo and Adele De Pascale, represents a major contribution to this ongoing inquiry. The work revisits and extends the theoretical legacy of Vittorio F. Guidano, founder of systemic-processual cognitivism, offering a renewed and scientifically grounded vision of the Self as a dynamic, evolving system.

This publication marks a significant intellectual and clinical event for contemporary cognitive psychotherapy, both within Italy and internationally. Rather than merely preserving Guidano's heritage, Giovanni Cutolo and Adele De Pascale re-elaborate his post-rationalist model in light of current developments in cognitive science, neuroscience, and evolutionary theory. Their effort results in an impressive synthesis, at once rigorous, interpretative, and profoundly humanistic, that restores vitality and contemporary relevance to Guidano's thought.

Vittorio F. Guidano's theoretical and clinical work dominated the cognitive psychotherapy landscape from the 1970s through the 1990s. His texts – from *Cognitive processes and emotional disorders* (1983, with Giovanni Liotti) to *Complexity of the Self* (1987) and *The Self in process* (1991) – shaped the contours of post-rationalist cognitivism. Guidano's model advanced a developmental and process-oriented understanding of the Self, grounded in the interplay between emotional regulation, identity formation, and narrative meaning.

Giovanni Cutolo and Adele De Pascale, both long-

time collaborators and students of Guidano, resume and expand this unfinished theoretical trajectory. As the editors emphasize, Guidano's premature death in 1999 interrupted a process of conceptual refinement that he had begun to articulate in lectures and manuscripts. The two authors have collected, systematized, and enriched this material with contemporary scientific findings, constructing a model that integrates biology, psychology, and the philosophy of mind into a unified interpretive framework.

This approach exemplifies what can be described as an *epistemology of complexity*: a vision of human experience as an emergent, self-organizing system whose coherence derives from constant dialogue between its biological, emotional, and relational dimensions. Such an epistemological stance moves beyond reductionist or linear explanations of mental functioning, situating the Self as both a product and an active constructor of its own experiential world.

The second volume of the trilogy focuses on the processual and narrative dimensions of the Self – its development through language, attachment, and interpersonal relationships. Drawing from the biological theories of Maturana and Varela, the authors portray the Self as a system that maintains coherence and continuity through recursive integration of environmental perturbations. The Self emerges, therefore, not as a fixed entity but as a living process of adaptation and self-regulation.

This view finds powerful articulation in the evolutionary account the authors reconstruct from Guidano's insights. From the earliest forms of consensual coordination among primates to the symbolic complexity introduced by language, the Self develops as both a biological and cultural achievement. The text recalls how, through the emergence of narrative and reflective capacities, human beings acquire the ability to organize experience sequentially – to order emotions, memories, and meanings within a coherent personal story.

The volume pays particular attention to the phylogenetic and ontogenetic parallels of this process. Attachment theory, especially as developed by Bowlby, becomes a crucial interpretive key: affective bonds serve as the matrix from which self-organization and identity coherence emerge. The construction of personal meaning is thus inseparable from the quality of early relational experiences, which mediate the individual's capacity for emotional regulation and self-reflection.

Language occupies a central place in this theoretical architecture. It is not merely a communicative tool but a transformative evolutionary innovation that reshapes both external relations and internal experience. Through language, the human mind gains the capacity to step

back from the immediacy of perception, constructing an inner space in which events, emotions, and images are ordered narratively. In this sense, the Self becomes both the narrator and the product of its own story.

Giovanni Cutolo and Adele De Pascale skillfully demonstrate how this process implies a tension between the internal and external dimensions of human experience – between individual subjectivity and the intersubjective contexts of family and society. The Self must remain simultaneously open to shared meaning and faithful to its personal coherence. Psychopathology, in this perspective, arises when this dynamic integration fails – when the reciprocal coordination between inner and outer experience breaks down.

By foregrounding this processual view of identity, the authors contribute an explanatory (rather than merely descriptive) model of psychopathology. They highlight that mental suffering cannot be reduced to isolated trauma or biological dysfunction; rather, it reflects disruptions in the self-organizing process that integrates cognition, affect, and relational experience. This integrative understanding opens new possibilities for psychotherapy, where the aim becomes not simply symptom reduction but the reconstitution of personal meaning.

The processuality of the Self and the dimensions of meaning exemplifies an ambitious attempt to synthesize insights from multiple disciplines – from neuroscience to paleoanthropology, from philosophy of language to systems theory. The breadth of its references is remarkable, yet never dispersive: each citation serves to illuminate the core theme of how the Self maintains coherence amid complexity.

The book's structure reflects this multilevel organization. Early chapters reconstruct the evolutionary emergence of mentalization and narrative thought; later sections examine the developmental trajectory of the individual Self through attachment, emotion regulation, and identity formation. Particularly compelling is the final chapter on *Love and knowledge*, which situates affectivity as the central organizing principle of personal meaning. Love, in this model, is not a sentimental or merely interpersonal phenomenon but a fundamental epistemic category – the affective condition that makes knowledge of self and others possible.

This integration of affectivity and cognition embodies the originality of the systemic–processual approach. The model recognizes the interdependence of emotional, cognitive, and relational domains, conceiving psychotherapy as a dialogical process aimed at restoring coherence within and between them.

From a critical standpoint, one of the most striking qualities of this volume is its refusal to oversimplify. Where much of contemporary neuroscience risks fragmenting human experience into neural correlates, and where some cognitive models tend toward mechanistic reduction, Cutolo and De Pascale reassert the unity of the person. Their writing resists the temptation of premature synthesis, instead cultivating a reflective tension between scientific rigor and phenomenological depth.

Equally noteworthy is the editors' methodological transparency. They do not conceal the fact that their reconstruction of Guidano's thought involves interpre-

tative choices; rather, they make these choices explicit, framing them as part of an ongoing scientific dialogue. The result is a text that is both faithful to its origins and open to innovation – a rare balance in theoretical psychotherapy.

At the same time, the volume's intellectual density may pose a challenge for readers unfamiliar with the broader tradition of Italian cognitivism. Yet this very complexity constitutes one of the book's strengths: it demands active engagement, inviting the reader to inhabit the same processual perspective that it describes. For clinicians, such engagement becomes an exercise in epistemological self-reflection – a reminder that therapeutic practice is itself a narrative and evolutionary endeavor.

Beyond its theoretical richness, *The processuality of the Self and the dimensions of meaning* offers substantial practical relevance. For mental health professionals, it provides a nuanced framework for understanding the intricate interplay of identity, emotion, and relational dynamics underlying severe psychopathology. The systemic–processual perspective does not reduce disorders such as psychosis or personality disturbances to static categories; instead, it situates them within the broader continuum of human experience, emphasizing continuity between so-called normality and pathology.

For students and young researchers, the volume serves as both a guide and an intellectual provocation. It encourages critical engagement with the epistemological foundations of psychotherapy and models a way of thinking that bridges empirical research and philosophical reflection. In an academic climate often dominated by technical specialization, the book stands as a call to restore the unity of scientific and humanistic inquiry.

In conclusion, *The processuality of the Self and the dimensions of meaning* represents a work of extraordinary theoretical and clinical significance. It revitalizes Guidano's systemic–processual model, integrating it with contemporary scientific knowledge while preserving its humanistic spirit. By weaving together evolutionary, neuroscientific, and narrative perspectives, Giovanni Cutolo and Adele De Pascale offer a coherent and deeply original vision of the Self as an ongoing process of meaning-making.

The text's relevance extends well beyond the confines of Italian post-rationalist cognitivism. It contributes to a broader international dialogue on the nature of consciousness, identity, and emotional life – issues that remain central to the future of psychotherapy. For scholars and clinicians alike, this book stands as both a landmark and an invitation: a landmark in the continuity of a tradition that unites theory and practice, and an invitation to explore new directions in the science of mind and the art of healing. The authors thus deliver to the scientific community a work that not only honors the legacy of Vittorio Guidano but renews its vitality, projecting it toward new horizons of research, clinical practice, and epistemological reflection.

Paola Cimbolli
Clinical Psychologist and Psychotherapist
Rome, Italy
pcimbolli@gmail.com

Macroinvertebrates are essential biological indicators for assessing freshwater ecosystem health. Their ecological sensitivity, combined with standardized monitoring methods and strong regulatory frameworks such as the EU Water Framework Directive and the US Clean Water Act, makes them fundamental tools in modern water resource management and for ecosystem health.

A photographic identification manual of freshwater macroinvertebrates, especially including images taken in natural environments, is highly valuable for both scientific monitoring and education. Macroinvertebrates often show subtle morphological differences between species, and many taxa are difficult to distinguish using preserved specimens alone, as colour, posture, and fine anatomical details may be altered during fixation [2]. Field photographs help therefore preserve these diagnostic features in their natural state, improving the accuracy of identification while reducing taxonomic errors.

In addition, images taken directly in aquatic habitats provide essential ecological context, such as substrate type, flow conditions, and microhabitat associations. This information is important because macroinvertebrate distribution is strongly influenced by environmental conditions, and many species are habitat-specific [2]. A photographic guide also supports rapid field identification, increasing efficiency during biomonitoring surveys.

Moreover, such manuals are extremely useful for species identification even in the most complicated cases and for training students and technicians, helping them to develop recognition skills more effectively than using dichotomous keys alone. Overall, photographic manuals enhance data quality, standardisation, and reliability in freshwater biomonitoring programmes, including those required under the EU Water Framework Directive.

Photographic and taxonomic identification manuals of freshwater macroinvertebrates do represent essential tools for biomonitoring and ecological assessment. Traditional keys, such as those developed in many countries provide detailed morphological descriptions and dichotomous identification pathways, structuring the basis of freshwater biomonitoring. At the European level, standardized operational manuals developed under

the Water Framework Directive support harmonized sampling and identification procedures across Member States. In addition, photographic atlases enhance field identification by showing organisms under natural conditions, improving accuracy and reducing taxonomic errors, especially for non-specialists [1]. Together, these resources ensure reliable, repeatable, and standardized assessment of freshwater ecological status.

This up-to-date guidebook on freshwater invertebrates of the central European region is a new guideline for field identification. It is a richly illustrated work, providing a good source of systematic information on freshwater macroinvertebrates. There are 525 illustrations in colour included and additional vector graphic figures that allow readers to identify specific species, genres and taxonomic families. The colour photos of taxa in natural habitat provide a useful support for the taxonomic identification. The book is supplemented by video sequences.

Freshwater invertebrates in Central Europe – A field guide is a must-have for all those interested in the freshwater animals of central Europe such as biologists, ecologists, specialized technicians in biomonitoring of benthos in freshwater as well as students attending courses and classes on freshwater and applied ecology.

Laura Mancini and Enrico Alleva
Istituto Superiore di Sanità, Rome, Italy
laura.mancini@iss.it
enrico.alleva@iss.it

REFERENCES

1. Rosenberg DM, Resh VH. Freshwater biomonitoring and benthic macroinvertebrates. Chapman & Hall; 1993.
2. Hauer FR, Lamberti GA. Methods in stream ecology. Academic Press; 2017.
3. Mancini L. Organization of biological monitoring in the European Union. In: G. Ziglio, M. Siligardi, G. Flaim (Eds). Biological monitoring of rivers: applications and perspectives. John Wiley & Sons; 2006. p. 171-201.
4. US Environmental Protection Agency. National rivers and streams assessment: Field operations manual. Washington, DC: USEPA Office of Water; 2013. (Report n. EPA-841-B-12-009a).

PUBLICATIONS FROM INTERNATIONAL ORGANIZATIONS ON PUBLIC HEALTH

Edited by
Annarita Barbaro

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS (FAO)

Extreme heat and agriculture. FAO-WMO joint report. Rome, Geneva: FAO and WMO 2026; 108 p. ISBN 978-92-5-140656-4. This joint report on extreme heat and agriculture brings together the scientific and operational expertise of the Food and Agriculture Organization of the United Nations (FAO) and the World Meteorological Organization (WMO) to assess how extreme heat is reshaping food production and food security. Drawing on the latest climate observations, agrifood systems analysis, and research, it provides a comprehensive assessment of risks that have too often been under-recognized in climate and development planning and identifies priority areas for policy attention and strategic investment. The findings of this report make clear that building the resilience of agrifood systems requires coordinated and sustained action. Adaptation must be scaled across agrifood systems globally and be carried out at a pace that keeps ahead of the rate at which the damaging changes materialize. Climate-resilient practices, improved water and land management, the development of heat-tolerant crops and breeds and strengthened risk governance can reduce vulnerability and protect livelihoods.

Food Outlook – Biannual report on global food markets. Rome: Food and Agriculture Organization of the United Nations 2026; 137 p. ISBN 978-92-5-140448-5. This new edition of the Food Outlook is released amid heightened economic uncertainty. While the global economy has shown resilience, the growth outlook for 2026 has become more fragile and remains uneven across regions. Inflation remains subject to considerable uncertainty, with geopolitical tensions, volatile energy markets, and fertilizer price developments posing serious risks of renewed cost pressures. At the same time, the interaction between evolving policy settings, shifting trade dynamics and exchange rate movements continues to cloud prospects for global trade and growth. In addition to market assessments, this June 2026 edition features a special article on the use of alternative fuels in international maritime shipping. Additional topics include an analysis of how energy markets influence sugar market dynamics through ethanol linkages, as well as an update on fertilizer markets. The market indicators overview includes recent developments in futures markets, ocean freight rates, the global food import bill, and food price indices.

Karasik R, Nizzetto L, Howell D, Thompson RH. **A review of the international and regional policy landscape for the management of plastics used in agriculture.** Environment and Natural Resources Management Working Paper, No. 112. Rome: Food and Agriculture Organization of the United Nations 2026; 52 p. ISBN 978-92-5-140628-1. Plastics are deeply embedded in modern agriculture, yet global governance frameworks have not kept pace with their growing environmental and health risks. This report provides a comprehensive review of international and regional policy instruments addressing plastics in agrifood systems, identifying critical gaps, emerging trends, and opportunities for transformative action. The policy landscape is fragmented, lacking coherence, coordination, and incentives for national action plans. To close these gaps, the report provides a review of the relevant international regulatory framework for agri-plastics, by describing 50 international and regional policy document or policy document compilations that address, either directly or indirectly, plastics used in agriculture.

UNITED NATIONS EDUCATIONAL, SCIENTIFIC AND CULTURAL ORGANIZATION (UNESCO)

The United Nations world water development report 2026, water for all people: equal rights and opportunities. Paris: UNESCO Publishing 2026; 157 p. ISBN 978-92-3-100853-5. This report presents the latest state-of-the-art knowledge regarding different aspects of water and gender equality. Like in all previous editions, UNESCO endeavoured to produce a balanced, evidence-based and objective account of the current state of knowledge, relying on the latest and most reliable data available. Although primarily targeted at policymakers, decision-makers, water resources managers, academics and the broader development community, this report will also be well received by other specialists and a wider public, including those who are engaged in the alleviation of poverty and humanitarian crises, the pursuit of universal access to water supply and sanitation, and the 2030 Agenda for Sustainable Development. The report also benefited largely from the inputs and contributions of several other UN-Water Members and Partners, as well as from numerous universities, research institutions, scientific associations and non-governmental organizations, who provided a broad range of relevant materials.

JOINT UNITED NATIONS PROGRAMME ON HIV/AIDS (UNAIDS)

Global AIDS Monitoring 2026. Indicators and questions for monitoring progress towards the Global AIDS Strategy 2026-2031 targets. Geneva: Joint United Nations Programme on HIV/AIDS 2026; 224 p. The Global AIDS Monitoring 2026 guidelines provide the core metrics and indicators needed to track country progress against the Global AIDS Strategy 2026–2031. The strategy focuses on reaching 16 top-line targets to end AIDS as a public health threat by 2030 and ensuring sustainability of the HIV response beyond. The indicators and policy questions in this document are designed for use by national AIDS programmes and other government entities and partners to assess a country's HIV response, and to measure progress towards achieving national HIV targets in a standardized way aligned with international standards to enable global aggregation.

UNAIDS Gender Assessment Tool. Towards a gender-transformative HIV response. Geneva: Joint United Nations Programme on HIV/AIDS 2025; 120 p. The Gender Assessment Tool (GAT) for national HIV responses is intended to assist countries in assessing the HIV epidemic; examining the context and response from a gender perspective; and making the HIV responses gender transformative, equitable and rights based to eradicate gender and HIV-related disparities and to ensure an effective response to HIV. It focuses on women, girls and gender diverse people with multiple and intersecting identities that are often marginalized, invisibilized and/or left out.

ORGANISATION FOR ECONOMIC CO- OPERATION AND DEVELOPMENT (OECD)

Improving quality of care through the OECD Patient Reported Indicator Surveys (PaRIS). Using patient-reported data to support health policy. Paris: OECD Publishing 2026; 43 p. The report focusses on the Patient-Reported Indicator Surveys (PaRIS), an OECD programme designed to assess primary care from people's perspective, and to support improvements in quality and in how people centred health services are. The first cycle of PaRIS, which ran between 2018 and 2025, surveyed over 107,000 patients using primary care services and more than 1,800 primary care practices in 19 countries, and provides a large, internationally comparable dataset that countries can use to benchmark performance, identify gaps, and track reforms using measures reported directly by primary care users. Drawing on interviews and written exchanges with country delegates and national project managers of PaRIS in March 2026, this report identifies how PaRIS results have been disseminated, interpreted and used in health policy and practice in the 19

countries that participated in PaRIS Cycle 1 (Australia, Belgium, Canada, Czechia, France, Greece, Iceland, Italy, Luxembourg, the Netherlands, Norway, Portugal, Romania, Saudi Arabia, Slovenia, Spain, Switzerland, the United States and Wales (United Kingdom)). The analysis follows a Knowledge to Action framework, a structured way to examine how evidence is adapted to the local context, communicated, applied, monitored and sustained.

INTERNATIONAL LABOUR ORGANIZATION (ILO)

Rounding N, Arif L, Berg J, et al. **Does a general-purpose large language model improve physicians' clinical reasoning?** ILO Working Paper 175. Geneva: International Labour Organization 2026; 42 p. ISBN 978-92-2-043559-5 (print) ISBN 978-92-2-043560-1 (web PDF). This paper investigates whether access to a general-purpose large language model (LLM) improves physicians' clinical reasoning across diverse healthcare contexts, as well as the possible implications of using an LLM in healthcare settings. Using a randomized controlled trial with 249 physicians in Indonesia, Kenya, and the Netherlands, the study finds that LLM access enhances performance on standardized clinical vignettes in all three countries. The results reveal substantial heterogeneity. Performance distributions overlap, and some physicians with LLM access perform worse than those without, indicating that access alone does not guarantee improvement. Higher usage is associated with better outcomes, and less specialized physicians appear to benefit more, implying that LLMs may help reduce skill gaps. Importantly, the findings emphasize that LLMs function as complements rather than substitutes for clinical expertise. However, the study identifies important risks, including automation bias, hallucinations, and context misalignment, underscoring the importance of careful integration, training, and governance.

WORLD HEALTH ORGANIZATION (WHO)

Guidelines for drinking-water quality: fourth edition incorporating the first, second and third addenda. Geneva: World Health Organization 2026; 647 p. ISBN 978-92-4-012122-5 (electronic version) ISBN 978-92-4-012123-2 (print version). The WHO Guidelines for drinking-water quality provide an authoritative basis for the setting of national regulations and standards for water safety in support of public health. The fourth edition incorporating the first, second, and third addenda of the World Health Organization's (WHO) Guidelines for drinking-water quality (GDWQ) builds on over 65 years of WHO guidance on drinking-water



quality. This guidance has formed an authoritative basis for the setting of national regulations and standards for water safety in support of public health. The Guidelines provide a comprehensive framework for protecting public health through the management of drinking-water quality risks, from catchment to consumer. They support the development of health-based targets, risk-based management approaches and independent surveillance. This latest version incorporates updates that reflect new evidence and substantive implementation experience to better support the effective application of the Guidelines in a range of settings.

Global status report on blood safety and availability 2025. Geneva: World Health Organization 2026; 147 p. ISBN 978-92-4-012154-6 (electronic version) ISBN 978-92-4-012155-3 (print version). The Global status report on blood safety and availability 2025 provides a comprehensive assessment of the status, trends and challenges of national blood systems worldwide, based on data reported by Member States to the WHO Global Database on Blood Safety. Drawing on information from 168 countries representing the vast majority of the global population, the report analyses governance and financing of blood services, blood collection and donation patterns, component preparation, laboratory screening for transfusion-transmissible infections, clinical use of blood and blood components, hemovigilance systems, and the availability of plasma-derived medicinal products. It examines progress towards establishing well-governed, effective and sustainable national blood systems and highlights regional and income-related differences in blood availability, safety and access. This report documents developments in policy, regulation, quality assurance and service organization, while identifying persistent gaps that affect the sufficiency, safety and equitable availability of blood and blood products,

particularly in low- and middle-income countries. It also reviews trends in blood donation, transfusion practices and plasma collection, and includes country case studies illustrating approaches to strengthening blood systems.

Pesticide residues in food: report 2025. Joint FAO/WHO Meeting on Pesticide Residues. Geneva: WHO and FAO 2026; 962 p. ISBN 978-92-4-012296-3 (electronic version) ISBN 978-92-4-012297-0 (print version). This report presents the outcomes of the 2025 Joint FAO/WHO Meeting on Pesticide Residues (JMPR), including evaluations of pesticide residues in food, toxicological assessments and dietary exposure analyses conducted to support international food safety standards and Codex maximum residue levels. The publication documents the review of 38 pesticides, including new compounds and substances under periodic review, covering toxicology, metabolism, residue behaviour, analytical methods and dietary risk assessment. It provides recommendations for acceptable daily intakes, acute reference doses, maximum residue levels, supervised trials median residue values and highest residue estimates for plant and animal commodities. The publication also addresses methodological and regulatory issues relevant to pesticide risk assessment, including dietary exposure methodologies, integration of new approach methodologies, updates to guidance documents, residue definitions, storage stability studies and transparency of dietary burden calculations. Chronic and acute dietary exposure assessments were performed using international dietary intake models to evaluate potential risks to consumers. Annexes include detailed intake estimates, livestock dietary burden calculations, lists of evaluated compounds and supporting technical information intended to assist Codex Alimentarius activities and national regulatory authorities in the assessment and management of pesticide residues in food.

ERRATUM

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

Valeria Aquilina, Francesco Paolo Busardò, Simona Pichini, Giulia Bambagiotti and Silvia Graziano

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

In the originally published version of this manuscript, an error occurred in the third column of Table 1 (Italy drug scheduling section, page 105) reporting the drug scheduling for Italy.

The correct drug scheduling should read:

- "The scheduling system classifies substances into five Tables:
 - Table I: substances with highest abuse potential (e.g., heroin, cocaine, amphetamines)
 - Table II: cannabis products (e.g. marijuana, hashish)
 - Table III: barbiturates
 - Table IV: benzodiazepines
 - Table medicinal products: medicinal products containing narcotic or psychotropic substances"

instead of:

- 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

We apologize for this error and any confusion it may have caused.

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 con-

cisely 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://researchintegrity-journal.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 preceded 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

Extended quotations and illustrative material taken from other publications must be accompanied by the original permission granted by the authors and by the publisher or in accordance with the copyright policy of the original publication and the Creative Commons.

Responsibility for the contents and opinions expressed on this journal rests solely with the author(s).

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/>