

REVIEW

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Early warning systems for chemical risks in Europe: methodological components for signal detection, prioritization, uncertainty management and follow-up

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Abstract

Chemical pollution can affect ecosystems and human health, highlighting the need for approaches that identify, evaluate, and prioritize emerging chemical risks before they become established public-health issues requiring regulatory actions. This review and perspective article examines methodological components required for Early Warning Systems (EWSs) for chemical risks, with particular attention to the European policy context. It examines methodological components for chemical EWSs rather than proposing a fully implemented system. It considers how state-of-the-art and emerging methods can support signal generation, signal strengthening, prioritization, uncertainty assessment, communication, and follow-up. The reviewed components include matrices and sampling strategies; chemical monitoring, suspect screening, and non-target screening; effect-based methods, New Approach Methodologies, and effect-directed analysis; exposure and hazard modelling; QSAR, read-across, AI-supported and data-mining tools; expert evaluation; and governance processes that link scientific signals to proportionate follow-up. The conceptual workflow is used as an example of how these components may be organized into a signal-handling process, while EU-level developments provide the broader policy and governance context. The actionable value of a chemical EWS does not depend on any single method, but on structured integration of complementary evidence streams. An effective EWS should combine sensitive weak-signal detection with transparent prioritization, explicit uncertainty assessment, FAIR and interoperable data infrastructures, and clearly assigned responsibilities for communication and follow-up.

Keywords Early warning system, EWS, Chemical risk management, Real-time data, Decision support, Hazard prioritization, Early warning signals, Computational tools, Risk governance

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Graphical abstract



Background

A large and growing number of chemicals are used across industry, agriculture, medicine, and consumer products, creating a monitoring and prioritization challenge across environmental, food, occupational, and human-exposure matrices [1]. Transformation and degradation processes further expand this chemical space by generating products that are often less well characterized than their parent compounds [2]. Chemical pollution can contribute to ecosystem disruption, soil and water degradation, and biodiversity loss, supporting the need for earlier detection and prioritization of emerging chemical signals [3, 4]. The number of chemicals relevant for monitoring and risk assessment is large but uncertain, because chemical-count estimates refer to different categories, including inventory listings, regulatory registrations, market-use estimates, low-volume substances, polymers, UVCBs, mixtures, and disclosed chemical structures. Wang et al. [1] reported more than 350,000 chemicals and mixtures listed across national and regional chemical inventories worldwide, whereas European estimates depend on the regulatory perimeter considered, for example, more than 26,600 substances were registered under REACH by December 2020, excluding substances below the one-tonne-per-year threshold, polymers, and active substances regulated under sector-specific legislation [5].

Inventory- and market-based estimates should be distinguished from broader chemical-identity databases such as the CAS Registry, which catalogue disclosed substances but do not indicate whether a substance is currently produced, marketed, emitted, or relevant for exposure. Production and consumption volumes should also not be equated with emissions, exposure, or risk, because chemicals may be used in closed systems, transformed during use, incorporated into products, or released at different life-cycle stages. Nevertheless,

the scale and diversity of chemical production and use create many opportunities for releases to air, water, soil, biota, food, drinking water, indoor environments, and workplaces. For example, Eurostat reports that EU production of chemicals hazardous to human health was 172 million tonnes in 2024 and that production of chemicals hazardous to the environment was 66 million tonnes, while these hazard-based volume indicators should not be interpreted as direct measures of exposure or risk [6]. For an Early Warning System (EWS), the central challenge is therefore to identify which substances are released, transformed, transported, accumulated, or associated with emerging exposure or hazard signals before they are routinely monitored or fully assessed, rather than to define a single total number of chemicals.

Chemicals of emerging concern may result from new chemical uses, changing production and consumption patterns, regrettable substitution, transformation during use or in the environment, new exposure pathways, or improved recognition of hazards and susceptibilities [5]. At the design or development stage of chemicals, materials, formulations, or products, prospective approaches such as safe-and-sustainable-by-design (SSbD) can help prevent hazardous substances, materials, or product systems from entering use, or reduce their potential for harmful exposure. The revised JRC SSbD framework provides a structured European approach for applying safety and sustainability considerations to chemicals and materials during innovation, including scoping, safety assessment, life-cycle-based sustainability assessment, evaluation of trade-offs, and documentation [7, 8]. An EWS fulfils a different and complementary role: it supports the early detection, prioritization, and follow-up of signals from substances, mixtures, materials, products, and transformation products that are already in use,

already released, insufficiently characterized, or outside routine monitoring and regulatory assessment.

For substances, mixtures, materials, products, and transformation products already in use or already released, primary prevention at the design stage may no longer be possible. However, an EWS can still support risk prevention and reduction by identifying, contextualizing, and prioritizing early warning signals across complementary evidence streams. Here, identification is used broadly. It may include analytical detection, annotation, or confirmation of chemicals in environmental, food, occupational, or human-exposure matrices; recognition of chemicals or mixtures in products and materials; identification of transformation products; and interpretation of information from monitoring datasets, regulatory databases, use and emission data, or product-related information systems. Such information can support targeted follow-up, source tracing, prioritization, substitution, restriction, emission control, or other risk-management actions.

In the food-safety area, emerging risks have been systematically assessed in Europe for more than a decade, and EFSA's activities provide an established reference point for emerging risk identification. EFSA defines an emerging risk as a "newly identified hazard to which a significant exposure may occur, or an already known hazard with an unexpected new or increased significant exposure and/or susceptibility" [9, 10]. When this definition is applied to chemicals, an EWS should be able to detect both exposure-related and hazard-related changes. Exposure-related signals may include rising concentrations, occurrence in new matrices, new exposure routes, transformation products, mixture patterns, or increased exposure of susceptible populations or ecosystems. Hazard-related signals may include newly observed bioactivity, toxicological effects, mechanistic alerts, or evidence of increased susceptibility. Monitoring and effect-based evidence can also be used to evaluate concerns that are initially predicted rather than observed, for example from exposure modelling, hazard modelling, horizon scanning, expert judgement, or analogy with structurally related chemicals. In such cases, empirical follow-up can support, refine, or deprioritize an early warning signal before it is escalated for further assessment or risk-management action.

EWS design should also account for large-scale societal and technological transitions that can alter chemical production, use, substitution, waste flows, recycling pathways, and exposure patterns. For example, shifts in food systems may change the use of pesticides, processing aids, packaging materials, and contaminants of concern; hazardous-chemical substitution may introduce replacement substances with insufficiently characterized

hazard or exposure profiles; transport electrification may increase the relevance of battery-related chemicals, metals, electrolytes, flame retardants, and recycling streams; and circular-economy initiatives may recirculate legacy contaminants or transformation products in secondary materials [9, 11]. These transition-related changes may generate weak signals that are not yet represented in routine monitoring schemes, regulatory lists, or established risk assessments. An effective chemical EWS should therefore combine horizon scanning, chemical and effect-based monitoring, exposure and hazard modelling, and expert evaluation to detect and contextualize such signals at an early stage. In the present study, an early warning (EW) signal is defined as a "potential manifestation of an emerging chemical issue or risk by a measurement or observation, providing information on its nature, sources, and causes" (WHO Workshop, 2019). We use the term EWS to refer to an organized process that detects, evaluates, prioritizes, strengthens, communicates, and follows up such signals before they develop into fully characterized or widespread risks. This distinction is important because an EWS should allow weak or low-confidence signals to be registered and tracked without treating them as confirmed risks.

The aim of this manuscript is to review how state-of-the-art and emerging methodological approaches can contribute to chemical EWSs and to identify implementation conditions and challenges relevant to their use in European chemical-risk governance. The manuscript does not propose a fully specified new EWS, nor does it describe an already implemented system. Instead, it examines how complementary evidence streams can support signal generation, signal strengthening or deprioritization, prioritization for follow-up, uncertainty assessment, communication, and proportionate follow-up. It also discusses practical implementation requirements, including transparent prioritization criteria, uncertainty management, interoperable data infrastructures, stakeholder communication, and clear responsibilities for weak and strengthened signals. The intended audience includes researchers developing monitoring and assessment methods, risk assessors evaluating uncertain evidence, regulatory and policy actors responsible for follow-up, and data-infrastructure developers supporting interoperable signal exchange.

The novelty of the perspective lies not in treating each method as individually new, but in clarifying how existing and emerging methods can be combined into an operational EWS logic. Specifically, it provides practical guidance on how to select and sequence methods within a signal-handling workflow, specifying quality and uncertainty requirements at each stage before a signal is escalated for regulatory follow-up. Chemical monitoring,

suspect screening, non-target screening (NTS), effect-based monitoring (EBMs), new approach methodologies (NAMs), effect-directed analysis (EDA), computational tools, and expert evaluation each contribute to different types of evidence. Briefly, suspect screening searches for expected or suspected chemicals using prior information, whereas NTS detects and annotates chemical features without restricting the analysis to a predefined analyte list; subsequent prioritization determines which features merit confirmation or follow-up. EBMs measure biological responses in samples, NAMs include non-animal *in vitro*, *in chemico*, and *in silico* approaches, and EDA links observed biological effects to chemical fractions or compounds through fractionation, bioassays, and chemical analysis. Computational or *in silico* tools include QSARs, read-across, exposure and hazard modelling, machine learning, literature mining, and database mining. Their value for an EWS depends on where they enter the workflow: initial weak-signal detection, signal confirmation or strengthening, prioritization, uncertainty assessment, or communication to decision-makers.

To avoid ambiguity, three levels are distinguished throughout the manuscript. First, the review discusses methodological building blocks that are generally relevant for chemical EWSs. Second, the workflow developed in the context of the European Partnership for the Assessment of Risks from Chemicals (PARC) is used as a conceptual example of how these components may be organized into a signal-handling process. This workflow should not be interpreted as a fully implemented system or as identical to the EU-level EWS. Third, Regulation (EU) 2025/2455 establishes a common data platform on chemicals and a monitoring and outlook framework for chemicals, providing the broader EU policy and governance context for early warning and action on emerging chemical risks [12]. The manuscript is therefore intended to support this policy context by clarifying which methodological components can contribute to signal generation, strengthening, prioritization, uncertainty assessment, communication, and follow-up.

Accordingly, after this Background section, the Main text is organized around six functional components of a chemical EWS considered in this review. This structure follows operational EWS logic rather than a purely method-by-method description. First, matrices and sampling strategies are discussed as the EWS input layer because they define the observation space: the types of chemicals, mixtures, effects, exposure pathways, spatial patterns, and temporal trends that can be detected. Second, chemical monitoring, suspect screening, and NTS are discussed as tools for detecting known, suspected, and previously unrecognized chemicals. Third, EBMs, NAMs, and EDA are considered as approaches

for adding biological and mechanistic relevance. Fourth, exposure and hazard modelling, QSAR/read-across, AI-supported tools, and data mining are discussed as approaches for hypothesis generation and prioritization, especially where empirical evidence is incomplete. Fifth, acceptance, prioritization, and uncertainty management are discussed to explain how signals can be archived, strengthened, escalated, or communicated. Sixth, governance, communication, and follow-up are discussed to show how strengthened signals may be transferred to actors responsible for monitoring, assessment, regulatory evaluation, or risk-management consideration. Table 1 summarizes these components, their operational contribution to a chemical EWS, and their potential value for follow-up and decision-making.

Matrices and sampling strategies for EWS

Traditional (bio)monitoring programs are highly effective for measuring specific, targeted analytes, but are inherently limited in their ability to identify unknown or insufficiently monitored chemicals of emerging concern. In an EWS, the role of matrices and sampling strategies is not simply to expand analytical coverage, but to determine which weak signals can be detected early, interpreted correctly, and followed up efficiently. Sampling design should therefore be matched to the expected signal type. Source-proximal and high-frequency sampling can detect new emissions, spills, or short-lived contamination events; archived samples and specimen banks can reveal temporal trends and provide retrospective baselines; spatial sampling can identify hotspots and support source tracing; time-integrated samplers and accumulative matrices can capture episodic or low-level contamination; and exposure-relevant matrices such as food, indoor dust, workplace air, and human biomonitoring samples can connect environmental occurrence to potential human exposure. The added value for an EWS lies in combining these strategies so that signals can be detected across the environment-food-human nexus and strengthened through convergence between independent evidence streams.

Table 2 summarizes the main matrix and sampling strategies relevant for a chemical EWS, the types of early warning signals they support, and their potential value for follow-up and decision-making.

The effectiveness of an EWS depends on the sample matrix, the chemicals analysed, and the measured endpoints. The combination of chemical domain and sample matrix is crucial for interpreting chemical signals [13]. The detectable chemical domain is shaped by the sampling strategy, preparation protocols, analytical systems, and data processing workflows. Sampling parameters, such as matrix type, proximity to potential sources,

Table 1 Operational contribution of the reviewed components to a chemical EWS

EWS component	Operational contribution to the EWS	Actionable value for decision-making
Matrices and sampling strategies	Define where, when, and how signals can be detected across environmental, biological, food, indoor, occupational, and archived matrices	Supports early detection of spatial hotspots, temporal trends, emission events, persistent contamination, and exposure-relevant signals
Chemical monitoring, suspect screening, and NTS	Detect known, suspected, and previously unrecognized chemicals, including transformation products and chemicals outside routine target lists	Triggers confirmation, source tracing, trend analysis, and prioritization of chemicals not yet covered by standard monitoring or regulatory lists
EBMs, NAMs, and EDA	Detect biological activity, mixture effects, and toxicologically relevant fractions, including cases where the responsible chemicals are unknown	Helps prioritize signals by biological relevance and can guide targeted identification of hazardous compounds or mixtures
Exposure and hazard modelling, QSAR/read-across, AI, and data mining	Provide evidence for chemicals or scenarios where monitoring data are limited, and identify potential concern from structure, use, occurrence, literature, patent, or trend data	Supports early hypothesis generation, prioritization of data-poor chemicals, and targeted follow-up before full empirical evidence is available
Acceptance, prioritization, and uncertainty management	Apply transparent quality, confidence, exposure, hazard, and weight-of-evidence criteria to decide whether signals should be archived, strengthened, escalated, or communicated	Reduces signal overload, makes uncertainty explicit, and helps balance the risk of false positives against the risk of missing emerging hazards
Governance, communication, and follow-up	Connect scientific signal generation with regulatory screening, stakeholder communication, and risk-management processes	Ensures that EWS outputs are understandable, traceable, and linked to responsibilities for confirmation, monitoring, mitigation, or regulatory action

Table 2 EWS value of matrices and sampling strategies for early chemical-risk detection

Sampling or matrix strategy	Type of early warning signal supported	EWS value and possible follow-up
Specimen banks and archived samples	Retrospective trends, baseline shifts, delayed recognition of emerging contaminants	Enables re-analysis when new chemicals, hazards, or analytical methods become available; helps distinguish new signals from long-standing background contamination
Source-proximal sampling, e.g., industrial zones, WWTPs, product-related sources, workplace air	New emissions, spills, intermittent releases, concentrated exposure sources	Increases probability of detecting weak or short-lived signals; supports source tracing, targeted confirmation, and rapid follow-up monitoring
High-frequency or near-real-time monitoring	Temporal spikes, daily variation, episodic contamination events	Enables earlier alerts than periodic grab sampling; supports responsive investigation of industrial emissions, accidental releases, or changing use patterns
Spatial transects and catchment-scale sampling	Hotspots, dispersion patterns, upstream–downstream gradients	Helps locate sources, define affected areas, and prioritize sites for confirmatory sampling or mitigation
Time-integrated sampling, including passive samplers, active samplers, flow-proportional pooling, and accumulative matrices	Episodic, low-level, persistent, or bioaccumulative contamination	Reduces the risk of missing transient signals; improves temporal representativeness and supports prioritization of chemicals for targeted analysis
Food, drinking water, indoor dust, workplace matrices, and human biomonitoring samples	Exposure-relevant signals and internal exposure trends	Links environmental occurrence to potential human exposure; helps prioritize signals by relevance for public health, occupational health, or vulnerable populations
Cross-nexus sampling across environment, food, and humans	Converging evidence across sources, pathways, and receptors	Strengthens weak signals by showing consistency across independent matrices; supports escalation from occurrence detection to exposure assessment and risk-management follow-up
Matrix- and chemical-domain-matched analytical coverage	Recognition of method blind spots and potential missed signals	Supports selection of LC-HRMS, GC-HRMS, class-based screening, targeted methods, EBMs, and other approaches according to the expected chemical domain, matrix, and EWS question

spatial coverage, and sampling frequency, affect signal strength and statistical reliability. Because NTS often has higher detection limits and greater semi-quantitative uncertainty than optimized target analysis, source-proximal sampling near industrial zones, wastewater treatment plants (WWTPs), concentrated product-related sources, or workplaces can increase the probability of detecting early signals; sentinel species may complement this by integrating exposure over biologically relevant time scales. Environmental matrices complement biological ones, such as urine and blood, in human biomonitoring by providing broader information on sources, pathways, and external exposure. Challenges such as chemical instability, degradation, matrix interferences, and low signal levels can hinder detection and should be considered before signals are interpreted, prioritized, or communicated.

Examples from different matrices and sampling strategies illustrate how the EWS input layer can support different signal functions, including retrospective trend detection, source tracing, short-term event detection, and exposure assessment. Analysis of per- and polyfluoroalkyl substances (PFAS) using extractable organofluorine (EOF), target analysis, and screening approaches in suspended particulate matter (SPM) from the German Environmental Specimen Bank demonstrates the value of combining archived matrices with complementary analytical methods for regional exposure assessment and retrospective trend analysis [14]. SPM, which consists of fine mineral and organic particles transported within the water column, can provide information on pollutant loads in aquatic systems, while specimen banking programmes provide temporal baselines that allow new signals to be compared with historical occurrence patterns.

High-frequency or automated monitoring provides a different EWS function. For example, NTS-based water monitoring at the Rhine station near Basel has identified industrial contaminants through daily signal variation [15]. This approach is now used at additional Rhine stations to support cross-site screening and responsive alarm systems for emission or spill detection [16]. Spatial sampling along rivers or across wastewater-treatment plants within a catchment can further support source tracing, hotspot identification, and distinction between local and diffuse signals [17]. Wastewater-based epidemiology (WBE), using raw sewage or pooled urine, can provide population-level information on chemical exposure and changing use patterns [18, 19]; for example, WBE at music festivals has revealed new psychoactive substances and supported intervention planning [20].

To date, many screening efforts have focused on aquatic matrices, such as wastewater, surface water, drinking water, suspended particulate matter (SPM),

and sediments [21]. Liquid chromatography-high resolution mass spectrometry (LC-HRMS) is frequently used in these applications because it provides broad coverage for polar to semi-polar chemicals relevant to waterborne exposure pathways [22–24]. In this section, LC-HRMS is therefore used as an example of current method coverage, not as an EWS recommendation in itself. For an EWS, the key requirement is that the analytical platform is matched to the matrix and chemical domain of interest, and that blind spots are explicitly recognized. LC-HRMS-based approaches should therefore be complemented, where relevant, by Gas chromatography-high resolution mass spectrometry (GC-HRMS) or other methods for volatile, semi-volatile, hydrophobic, and medium-to-non-polar compounds, by class- or total-fraction approaches such as EOF analysis, and by EBM when biological relevance needs to be assessed.

Aqueous matrices typically provide limited temporal integration for polar contaminants compared with adsorbing matrices for non-polar compounds, but this limitation can be addressed through increased sampling frequency, flow-proportional pooling, passive or active time-integrated sampling, or near-real-time monitoring [25]. These strategies are particularly useful for identifying irregular exposure scenarios, such as recent pesticide applications, industrial emissions, or short-lived contamination events [26, 27]. In situ active samplers, such as TIMFIE [28, 29], can support time-integrated monitoring of chemicals and toxins in water, while passive samplers using, for example, PDMS can support water, biota, and seafood monitoring [30–34].

Matrices such as soil, sediment, and biota integrate exposure over a longer timeframe, making them better suited for assessing contamination by persistent and bioaccumulative chemicals rather than short-term events [35, 36]. For these matrices, GC-HRMS screening approaches covering volatile, semi-volatile, hydrophobic, and medium-to-non-polar chemicals can complement widely used LC-HRMS-based approaches. Indoor environments are also important exposure settings because chemical profiles are influenced by building materials, consumer products, indoor activities such as smoking and cooking, and infiltration of outdoor pollutants. Indoor dust can act as an exposure-relevant integrative matrix for chemicals that partition to particles and can be contacted through ingestion, inhalation, or dermal pathways. Targeted and NTS analyses of indoor dust have shown that this matrix can reveal both legacy contaminants and less routinely monitored chemicals, making it useful for identifying exposure-relevant signals that may require confirmation in air, product, or human biomonitoring matrices.

Food and drinking-water analyses can support early warning when chemicals enter or accumulate in the food chain or migrate from food-contact materials and production processes. Systems such as the EU's Rapid Alert System for Food and Feed (RASFF) and EFSA's SCREENER [37] illustrate how food-related occurrence data can be used to flag contamination issues and support follow-up. Relevant matrices include drinking water, vegetables, fruits, grains, and animal products such as meat, milk, and fish. Retrospective analysis of stored food baskets can add temporal context by allowing emerging signals to be compared with historical dietary-exposure patterns [37].

Human biomonitoring uses matrices such as blood, urine, hair, placenta, cord blood, and human milk to estimate internal exposure. Among these, blood and urine are the most widely employed because they are accessible and informative for many exposure biomarkers. Hair can be useful for evaluating longer-term exposure to selected chemicals and may provide a retrospective exposure timeline, whereas placenta, cord blood, and human milk can provide information on foetal, early-life, or lactational exposure [38–43]. When combined with targeted, suspect, or non-target screening, these matrices can support an EWS by identifying emerging contaminants and exposure trends in the general population, vulnerable groups, or occupational settings.

Biomarker discovery can further strengthen this EWS function when human biomonitoring is combined with epidemiology, phenotyping, metabolomics, and other omics or clinical data. In practice, this involves linking measured or estimated exposures with molecular, physiological, clinical, or health-related endpoints in well-characterized populations, occupational studies, or biobanks. Candidate exposure biomarkers may indicate internal exposure to a chemical or mixture; candidate effect biomarkers may indicate early biological responses, such as oxidative stress, inflammation, endocrine activity, genotoxicity, or metabolic perturbation; and candidate susceptibility biomarkers may help identify population subgroups with increased vulnerability. Such candidate biomarkers can be identified through association analyses, repeated-sampling designs, comparison of exposed and reference populations, or integration of chemical exposure data with metabolomic or phenotypic profiles. Before candidate biomarkers are used for signal strengthening, prioritization, or escalation in an EWS, they should be evaluated for analytical reliability, biological plausibility, temporal relationship with exposure, reproducibility, and relevance for the EWS decision context.

In an EWS, validated biomarkers can help connect external occurrence data with internal exposure and early biological response. For example, biomarkers of

occupational exposure to air pollution have complemented traditional measurements of workplace air pollutants when assessing exposure to xenobiotics [44]. However, several limitations affect the use of biomarker information for early warning. For many emerging chemicals, no validated exposure biomarker is available, and candidate metabolites may be non-specific, short-lived, or influenced by metabolism, timing of sampling, lifestyle, disease status, or co-exposures. Reference standards may also be unavailable for suspected biomarkers, transformation products, or metabolites, limiting confirmation and quantitative interpretation. In addition, knowledge gaps on environmental fate, toxicokinetics, and toxic effects can make it difficult to determine whether a biomarker reflects current exposure, past exposure, a specific source or pathway, or an early biological response that is relevant for health risk. These limitations do not preclude the use of biomarkers in an EWS, but they require transparent reporting of uncertainty and, where possible, signal strengthening or confirmation through repeated sampling, complementary matrices, external exposure data, chemical monitoring, EBMs, NAMs, or epidemiological evidence.

Before a signal is communicated or escalated, the chemical-domain coverage and limitations of the sampling and measurement strategy should be described explicitly. This includes expected chemical classes and effects, matrix suitability, extraction and clean-up procedures, chromatographic separation, ionization efficiency, feature detection, annotation confidence, and data-evaluation workflows [13, 22, 45, 46]. Such documentation does not eliminate false negatives, but it makes blind spots visible and supports proportionate follow-up. Sampling strategies should be matched to suspected sources, chemical properties, and emission or transformation patterns. For example, aquatic systems are relevant for persistent, mobile, and toxic (PMT) substances, while remote sites can support baseline assessment and detection of chemicals with long-range transport potential. For PFAS, volatile precursors may be detected in air, whereas more persistent and less volatile transformation products may be detected in aquatic systems or accumulated in organisms [47, 48]. Integrating environmental monitoring with food, workplace, indoor, and human biomonitoring data can therefore strengthen signals by linking occurrence, exposure pathways, and internal exposure [49].

Once the relevant matrices, sampling design, and analytical coverage have been defined, the next step in an EWS is to determine whether the collected evidence generates or supports a potential early warning signal. The following section therefore focuses on approaches that can generate or strengthen such signals, including

chemical screening, EBMs, NAMs, EDA, exposure and hazard modelling, and data-driven horizon scanning.

Approaches for identifying and strengthening early warning signals

Building on the matrices and sampling strategies described above, early warning signals can be generated from several complementary evidence streams. These include unexpected chemical occurrence, increasing concentrations or distribution patterns, biological activity, toxicological or mechanistic alerts, model-based exposure or hazard predictions, and information from literature, regulatory databases, patents, product data, or expert evaluation. This section focuses on the methods that can contribute to the signal generation and signal strengthening steps of the conceptual EWS workflow shown in Fig. 1. It does not present a separate workflow; rather, it explains how different methodological approaches can feed signals into the broader EWS process, where they can then be screened, prioritized, strengthened, archived, or communicated.

Established workflows for non-targeted analysis and quantitative NTA provide important procedures for chemical feature detection, annotation, prioritization, quantification, and connection to chemical safety evaluation [50, 51]. These workflows are not replaced here and should not be interpreted as equivalent to a complete EWS. Instead, they are considered methodological components that can support specific steps within an EWS, particularly the detection, documentation, and strengthening of chemically derived EW signals.

In this broader context, a signal may be triggered by different types of evidence depending on the purpose of the EWS, the signal source, and the available data. For example, detection of an unexpected compound in a sensitive or source-proximal matrix may be sufficient to trigger internal follow-up, whereas escalation to a stronger EW signal may require additional evidence such as recurrence over time, occurrence across locations or matrices, biological activity, predicted persistence, mobility, bioaccumulation or toxicity, source plausibility, or relevance to human or ecological exposure [50, 51]. The added

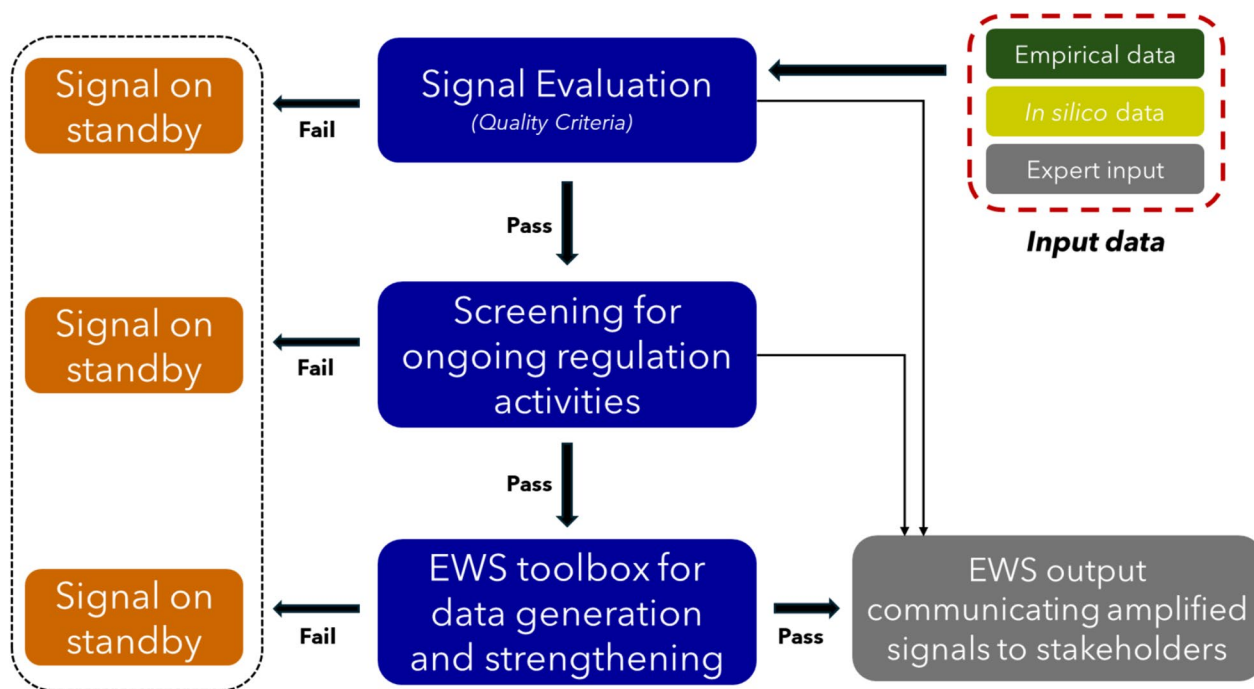


Fig. 1 Conceptual workflow for operationalizing a chemical Early Warning System (EWS) in the PARC context. The workflow links the evidence-generation methods with subsequent prioritization, uncertainty, and communication steps. The process begins with an early warning signal, defined here as an initial indication of potential concern based on empirical data, computational predictions, expert input, or other documented evidence. Signals are first evaluated against predefined quality and relevance criteria. Signals that do not yet meet these criteria are not discarded, but are archived or placed on standby until additional evidence becomes available. Signals that meet the criteria proceed to screening for ongoing regulatory or assessment activities to determine whether the concern is already being addressed within existing frameworks. If the concern is already covered, the signal may return to standby or be monitored for new evidence. If not, it enters an EWS toolbox, where additional data generation and signal strengthening can be performed using targeted chemical analysis, EBMs, NAMs, exposure or hazard modelling, EDA, expert evaluation, or other fit-for-purpose methods. A strengthened signal can then become an EWS output that is communicated to stakeholders to support further assessment, monitoring, source tracing, risk-management consideration, or regulatory follow-up

value of the EWS perspective is therefore the structured integration of multiple signal sources into a process that includes quality screening, regulatory-context screening, signal strengthening, prioritization, uncertainty assessment, communication, and follow-up. Emerging chemical risks may be driven by persistence and bioaccumulation, changing environmental conditions, new production or use patterns, substitution processes, circular-economy practices, technological transitions, or changes in human behaviour and exposure patterns [5, 9, 11, 52–54]. A proactive chemical EWS should therefore combine empirical observations with predictive, knowledge-based, and expert-driven approaches, so that potential risks can be investigated before they are fully established or widely distributed.

Within this workflow, the identification and strengthening of EW signals relies on a combination of advanced chemical and bioanalytical techniques, effect-based and NAM approaches, and data-driven or model-based methods. State-of-the-art chemical analysis is crucial for the detection of previously unknown contaminants, where NTS can be used to identify novel or unexpected chemicals without the constraints of predefined analytical standards. EBM play a complementary role by detecting and characterizing biological activity that may be relevant for hazard prioritization and signal strengthening [55]. In an EWS context, this role is not limited to chemicals already recognized as emerging contaminants. EBMs can also reveal unexpected effects from known chemicals, replacement substances, transformation products, complex mixtures, or chemicals previously considered to be of low concern. Such findings may generate an EW signal by indicating newly recognized bioactivity, altered mixture effects, or a need for renewed assessment, but they do not by themselves establish toxicological risk; this requires integration with exposure, occurrence, potency, and contextual information. The growing recognition of EBMs in EWSs has led to the development of structured workflows for their integration, incorporating bioassays targeting diverse modes of action [56]. Within the broader EBM framework, NAMs can provide non-animal evidence on chemical effects in biological systems. These approaches include *in vitro*, *in chemico*, and *in silico* methods that can provide mechanistic insights and support interpretation within adverse outcome pathway frameworks [57]. In an EWS context, NAM-derived evidence can help strengthen or prioritize EW signals by linking chemical occurrence or predicted exposure to plausible biological mechanisms, hazard alerts, or endpoints requiring further investigation.

Integration of NAM-generated data with NTS should be performed in a tiered and bidirectional manner within an EWS. First, NTS can identify chemical features,

suspected compounds, or confirmed substances that show recurrence, increasing trends, unusual spatial distribution, or occurrence in exposure-relevant matrices. For features that can be annotated or confirmed with sufficient confidence, NAM data can then be retrieved or generated to evaluate biological activity, mechanistic alerts, potency, toxicokinetic relevance, or links to adverse outcome pathways. This allows NTS-derived exposure evidence to be combined with NAM-derived hazard evidence when prioritizing signals for follow-up.

Second, NAM results can guide NTS by helping to define suspect lists or chemical domains of concern. For example, chemicals associated with endocrine activity, developmental toxicity, neurotoxicity, immunotoxicity, persistence, mobility, or bioaccumulation alerts can be prioritized for suspect screening in relevant matrices. Third, when NTS features remain unidentified, direct linkage to substance-specific NAM data is not yet possible. In such cases, prioritization should rely on feature-level evidence, such as recurrence, trend, abundance, isotope or adduct patterns, occurrence across matrices, association with bioactivity, or presence in toxic fractions after EDA. These features can then be selected for further annotation, confirmation, targeted analysis, or effect-directed investigation.

In practical EWS terms, combined NTS-NAM evidence can support tiered decision-making: low-confidence or isolated signals may be archived and tracked; recurrent or exposure-relevant signals may be prioritized for confirmation; signals with both exposure evidence and NAM-based hazard concern may be escalated for further assessment; and signals associated with measured bioactivity may trigger EDA, source tracing, or targeted monitoring. Thus, the value of integrating NTS and NAMs lies not only in broader chemical profiling, but in linking occurrence, exposure relevance, biological plausibility, and uncertainty to transparent EWS follow-up decisions [51, 58, 59].

Considering signals in an integrative manner across the environment-food-human nexus can strengthen EWSs. For instance, when a signal amplifies in human tissues, it may indicate bioaccumulation [60–63]. For rapidly metabolized compounds, signals detected in environmental media or food may hold little relevance for human health, especially when no bioactivation occurs; in this case metabolites formed in humans serve as more meaningful indicators. This approach is most effective when dealing with known compounds and does not directly apply to unidentified mass spectrometric features. However, the recurrence of unknown signals in biomonitoring data should prompt their prioritization for later identification. The incorporation of human biomonitoring into an EWS offers significant advantages, particularly

in identifying metabolites from rapidly metabolized compounds.

Since EBM is not always sufficient to establish cause-effect relationships, EDA can be applied as a higher-tier approach to identify toxic drivers in complex environmental, food, or human-exposure-related samples [55, 64]. EDA supports the prioritization of chemicals for identification by linking bioanalytical responses to chromatographic fractionation and instrumental analysis [63]. For an EWS, its main value is not routine screening of all samples, but targeted signal strengthening: EDA can help determine whether an observed biological effect is associated with one or more chemical fractions and can guide the identification of responsible compounds or mixtures.

More recent developments, including high-throughput and high-resolution fractionation, microfractionation, downscaled *in vitro* bioassays, miniaturized and automated bioassay platforms, biosensors, and computational workflows for feature prioritization, are increasing the potential scalability of EDA [65–68]. These approaches can improve the detection and localization of biological effects in complex samples, especially when only low enrichment levels or limited sample volumes are available. However, the identification of responsible chemicals still requires integration with chromatographic fractionation, sensitive HRMS or complementary chemical analysis, and transparent data-processing workflows. Routine and consistent large-scale application in an EWS remains challenging because of limited endpoint coverage, fractionation and bioassay reproducibility, sample-volume constraints, compound losses during preparation, uncertainty in linking effects to chemical features, and the need for harmonized data-processing and reporting workflows. EDA should therefore be considered a targeted follow-up tool for biologically relevant or otherwise prioritized EW signals, rather than a first-tier method for all EWS samples. To boost performance, integrating high-throughput fractionation, biotesting systems, and biosensors, along with sensitive HRMS and modern data processing, significantly enhances the detection and identification of signals at low enrichment levels [69]. Since sample preparation, separation and fractionation, the performance of bioassays and analytical methods and data processing algorithms all influence the detection of toxic drivers [13], gaps between effect and chemical signals must be carefully considered and addressed in the workflow for triggering and interpreting EW signals.

For a robust EWS, early warning evidence should be interpreted in both temporal and spatial dimensions and, where possible, supported by modelling and retrospective analysis [70]. Temporally resolved sampling can reveal increasing trends, episodic releases, seasonal patterns, or short-lived contaminant spikes, for example

through high-frequency river or wastewater sampling [13, 17]. Spatially resolved analysis can identify hotspots, trace dispersion pathways, distinguish local from diffuse sources, and select sites for confirmatory sampling or mitigation [15, 17]. Archived matrices and specimen banks add a retrospective dimension by allowing re-analysis when new chemicals, hazards, or analytical methods become relevant. Case studies using temporal sample series, including Swedish maternal serum samples [71] and eagle tissue archives [72], illustrate how long-term sample collections can reveal contaminants with significant temporal trends.

Fate and exposure modelling can strengthen this interpretation when used as a hypothesis-generating and prioritization tool rather than as a confirmed signal by itself. Depending on the EWS question, models can use physicochemical properties, use and emission information, release assumptions, degradation or transformation data, environmental parameters, and human activity or product-use data to estimate likely compartments, transport pathways, receptor matrices, exposure routes, and potential source areas. Multimedia fate and regulatory exposure-assessment models can help evaluate distribution among air, water, sediment, soil, and biota; catchment, hydrological, or site-specific models can support spatial source attribution and transport assessment; and high-throughput human exposure models can prioritize chemicals, products, or use scenarios for follow-up when empirical exposure data are limited [73–76]. Comparative models such as USEtox may also be useful when EWS questions relate to product systems, circular-economy scenarios, or substitution choices, where relative exposure and toxicity potentials need to be screened across many chemicals [77].

The EWS value of integrating temporal analysis, spatial analysis, archived samples, and modelling is that weak signals can be contextualized before escalation. For example, an isolated chemical occurrence may be archived and tracked; a recurring temporal trend may trigger targeted confirmation; a spatial cluster may support source tracing; and a model-predicted pathway may guide sampling in additional compartments or receptor matrices. Where biological relevance is part of the EWS question, chemical trends can be complemented by EBM or bioassays to evaluate whether changes in occurrence are accompanied by changes in biological activity. The uncertainty of model inputs and assumptions, including emissions, use volumes, partitioning, degradation, transformation products, environmental parameters, and human activity patterns, should be documented and considered during signal prioritization. Advanced approaches, such as NTS methodologies, offer new opportunities to characterize these trends and detect signals at an early stage

with bioactivity potential [78]. High-throughput screening, multiparametric analyses, and computational (eco-) toxicology empower EWSs to process large datasets in real time, enhancing their specificity and sensitivity [79]. The integration of these advanced technologies ensures timely detection of contaminants, thereby supporting public health and environmental protection efforts.

Machine learning is advancing (eco-)toxicological assessment by enabling hazard prediction through molecular fingerprints from mass spectral data and algorithms. Using databases like Tox21 and ToxCast, these models accelerate hazard identification and reduce dependence on bioassays [78, 80–82]. However, integrating *in silico* tools with chemical monitoring data, EBM results, NAM-derived hazard information, and exposure data remains challenging because each evidence stream has distinct applicability domains, uncertainty sources, and quality criteria. Quantitative Structure–Activity Relationship (QSARs), machine-learning models, read-across, and chemical grouping approaches can contribute to an EWS by generating hypotheses and prioritizing chemicals, features, or use scenarios for further investigation, particularly when empirical monitoring or toxicity data are limited. For example, fit-for-purpose QSARs can be used to screen large chemical inventories for specific endpoints, physicochemical properties, or mechanistic alerts, provided that the endpoint is clearly defined and the prediction is supported by information on model performance, input quality, applicability domain, uncertainty, and fitness for the intended EWS decision context.

Read-across and chemical grouping can also support early warning by identifying data-poor target substances that may share relevant structural, physicochemical, metabolic, mechanistic, or toxicological properties with better-characterized source substances. In this context, machine-learning-based clustering or similarity analysis can help identify potential analogues or chemical categories, but the EWS signal should not rely on clustering alone. The read-across hypothesis should be transparent, biologically or mechanistically plausible where possible, and accompanied by an assessment of uncertainty and data gaps [83]. Thus, computational predictions are best used as tiered evidence within an EWS: they can generate suspect lists, identify chemicals for targeted or suspect screening, prioritize NTS features for annotation, support selection of NAMs or EBMs, and guide expert review, but they should normally be strengthened by empirical occurrence, exposure, bioactivity, or toxicological evidence before escalation.

Machine learning has also been applied to detect EW signals using diverse data sources—from lab toxicity results and HRMS to macro-scale drivers like climate, trade, and regulation [84]. Bouzembrak and Marvin [85]

used a Bayesian Network on RASFF cases to predict chemical food safety hazards based on climatic, agricultural, and economic factors. Patent and literature trends can further highlight chemicals of emerging or regional concern [34, 86–88].

Criteria for acceptance and prioritization of early warning signals

Acceptance and prioritization criteria are needed to decide whether a candidate's EW signal should be archived, monitored, strengthened, escalated, or communicated (Fig. 1). Because chemical EWSs draw on heterogeneous evidence sources, signals should be screened against predefined quality and relevance criteria rather than treated as confirmed hazards or risks. Methods for quantifying false positives and false negatives can support this screening under defined experimental-design assumptions [89], but the relevant criteria and parameter values will vary by signal source, application, user requirements, and decision context. Universal quality assurance/quality control (QA/QC) acceptance criteria have not yet been established for all EWS evidence streams [34, 90]. Data supporting candidate signals should, where possible, follow FAIR principles [91], because archived weak signals may later be used for trend analysis, re-evaluation, and signal strengthening. The purpose of acceptance and prioritization criteria is therefore not to minimize false positives and false negatives simultaneously, but to manage this trade-off through tiered thresholds, transparent uncertainty reporting, and proportionate follow-up. An overview of example evidence-quality considerations that can support the acceptance, strengthening, and prioritization of signals from different types of input data is given in Table 3. Weight-of-Evidence (WoE) is not treated here as a stand-alone acceptance criterion, but as a transparent approach for integrating evidence across signal sources after the reliability, relevance, uncertainty, and fitness-for-purpose of the individual evidence streams have been assessed.

Expert input to an EWS may originate from individual research groups, scientific expert panels and communities, research projects, trade organizations, non-governmental organizations (NGOs), national or international agencies, and competent authorities. Examples include expert-based emerging-risk identification processes, network-based prioritization activities, and national or EU-level monitoring or alert systems that combine expert judgement with monitoring, exposure, hazard, occurrence, or contextual information [5, 92]. Because expert-derived signals can vary in scope, documentation, and evidential strength, they should be recorded with transparent information on the source of the signal, the type and quality of supporting evidence, the expertise

Table 3 Summarizes evidence-quality considerations for prioritizing candidate EW signals

Category	Evidence-quality considerations
Computational tools	Clearly defined predicted endpoint, property, exposure scenario, or hazard alert relevant to the EWS question Correct chemical identity, input structure, and input-data quality documented Model applicability domain assessed for the chemical, endpoint, and decision context Model performance, validation approach, uncertainty, transparency, and reproducibility documented Independent validation or reproducibility demonstrated where possible, preferably using external datasets, independent test sets, or independent applications Concordant predictions from two or more sufficiently independent and fit-for-purpose models, where available Independence of models considered, including potential overlap in training data, descriptors, algorithms, or assumptions Single-model predictions from novel or non-standard methods treated as lower-confidence screening evidence unless supported by independent data Use of models, tools, or frameworks in regulatory contexts documented where relevant, but not treated as a stand-alone indicator of reliability Computational outputs interpreted according to intended EWS use, e.g., watch-listing, suspect-list generation, prioritization for monitoring, or escalation for further assessment Consistency with empirical evidence, such as occurrence, exposure, bioactivity, NAM/EBM results, or expert evaluation, used to strengthen the EW signal
Chemical monitoring methods	Adequate sampling strategy to ensure representativeness Use of sufficiently sensitive and specific analytical technologies (e.g., GC- or LC-(HR)MS/MS) with appropriate QA/QC Minimal compound ID confidence level reached (≤ 3) or equivalent Identification point score (≥ 0.5–0.6) [121] Sufficient signal-to-noise ratio and/or fold change compared to blanks or other types of negative control reference samples (e.g., > 3) High signal in group-based methods [e.g., adsorbable organic halogens (AOX)] above a threshold (e.g. tenfold compared to reference sample) Accessible suspect lists/databases if used for compound annotation Exceedance of experimental toxicity thresholds for known compounds or predicted toxicity thresholds for definitive and tentatively identified signals, respectively [121] Spatial distribution assessed as signal-strengthening evidence, including recurrence across sites, regions, countries, matrices, or monitoring networks, where relevant European-scale relevance considered when the signal occurs across multiple countries or regions, is linked to cross-border transport, widely distributed products or supply chains, broadly distributed exposure, or a potential regulatory or monitoring gap Localized signals retained for prioritization when they indicate high hazard, high exposure, sensitive populations or ecosystems, source-proximal contamination, or a plausible emerging issue Increasing signal intensity over time [e.g., tenfold for short-term (days) and twofold for long-term (months to years)] High levels in single areas (e.g., industry-related) and/or one given specific sub-population
Effect based monitoring methods	Assay relevance and reliability documented using standardized toxicity-test guidelines, where available, or transparent guidance-based reporting templates for non-standardized methods Endpoint relevance for the EWS question, e.g., endocrine activity, genotoxicity, adaptive stress responses, developmental toxicity, neurotoxicity, immunotoxicity, or other hazard-relevant modes of action Assay validity criteria met, including appropriate positive and negative controls, acceptable background response, and documented deviations Specificity, sensitivity, and dynamic range adequate for the expected effect level and enrichment factor Reproducibility demonstrated across independent experiments, samples, or laboratories where feasible Matrix interferences, cytotoxicity, extraction effects, and blank effects assessed and controlled Detection limits or effect detection limits appropriate for relevant exposure concentrations Effect-based trigger values, assessment criteria, or benchmark responses available, where applicable Consistency with chemical monitoring, NAM evidence, exposure information, or repeated observations used to strengthen the EBM-derived EW signal
Expert input/evaluation	Signal source and expertise documented Rationale for expert judgement recorded Conflicts of interest declared where relevant Supporting evidence specified, such as monitoring observations, incident reports, publications, stakeholder alerts, product-use information, expert assessment, or regulatory/contextual information Uncertainties and evidence gaps documented Transparent weight-of-evidence reasoning used to decide whether the signal should be archived, tracked, strengthened, or escalated

Figure 1 illustrates how the evidence-generation approaches discussed above may feed into a conceptual signal-handling process in which candidate signals are screened, checked against ongoing regulatory or assessment activities, strengthened where needed, archived or placed on standby, and communicated for follow-up when sufficiently supported

involved, possible conflicts of interest, and the main uncertainties. Peer-reviewed evidence is valuable when available, but it should not be the only route by which an early signal can enter the EWS, since some relevant signals may first arise from monitoring observations, incident reports, product-use information, patent or literature trends, stakeholder alerts, or expert judgement.

A signal originating from a single location, matrix, or dataset may therefore be registered and tracked as a weak EW signal if it is sufficiently documented, plausible, and relevant for chemical risk assessment. Wider occurrence across multiple sites, matrices, regions, or countries should not be treated as a prerequisite for entry into the EWS. Rather, it is one possible form of signal strengthening that may support escalation to broader follow-up, for example when a signal is considered relevant at European scale. Other forms of strengthening may include recurrence over time, independent confirmation, increasing concentrations, evidence of exposure, biological activity, predicted persistence or toxicity, source plausibility, or relevance for vulnerable populations or ecosystems. Decisions to prioritize or escalate such signals should be made through the defined EWS coordination process, expert panel, or competent authority, using transparent weight-of-evidence reasoning.

Computationally derived signals should be treated as screening and prioritization evidence within an EWS. Natural language processing (NLP) can support extraction, analysis, and interpretation of patterns or anomalies from large datasets and scientific literature, including signals that may not be detected by traditional monitoring approaches [93–95]. QSARs, read-across, machine-learning or deep-learning models, chemical grouping, and scenario-based exposure models can support watch-listing, suspect-list generation, targeted monitoring, or prioritization for NAM/EBM follow-up when empirical evidence is incomplete [79, 96–99]. The confidence assigned to such signals should depend on endpoint clarity, input-data quality, applicability domain, model performance, independence of concordant predictions, uncertainty reporting, transparency, and consistency with empirical or expert evidence. For computational tools, “concordant predictions” refers to agreement between models that are fit for the endpoint and chemical domain under consideration and are sufficiently independent in their training data, descriptors, algorithms, or assumptions. Recent guidance and assessment frameworks, including OECD guidance for QSAR validation and the OECD (Q)SAR Assessment Framework, can support structured evaluation of models, individual predictions, and results based on multiple predictions, but their use should not be equated with regulatory acceptance or reliability of any individual prediction [98, 100]. Tools used in regulatory

contexts, such as the OECD QSAR Toolbox, can support reproducible profiling, chemical grouping, read-across, trend analysis, and data-gap filling, but they do not remove the need to document uncertainty and fitness for the specific EWS decision context [101, 102].

EBMs can provide EWS signals by detecting biological activity in environmental, food, product-related, or human-exposure-related samples. Depending on the EWS question, these methods may include in vitro bioassays, whole-organism or apical bioassays, biomarker-based approaches, and in situ or ex vivo effect measurements. In this paragraph, in vitro bioassays are emphasized because guidance documents, assay-performance criteria, and effect-based trigger values are particularly relevant for interpreting many in vitro EBM results. The confidence assigned to such signals should distinguish between assay-level reliability, biological relevance, and EWS signal quality, and should account for the fact that a positive EBM signal detects that biologically relevant activity is present but does not identify the responsible chemical(s), needing integration with chemical analysis, NTS, or EDA for follow-up. Standardized toxicity-test guidelines and guidance-based reporting templates, including those based on OECD GD211 for non-standardized methods, are useful because they provide transparent criteria for documenting assay validity, endpoint relevance, controls, sensitivity, reproducibility, and reliability. They do not, however, automatically determine whether an observed effect should be accepted as an EW signal.

For EWS purposes, additional signal-quality criteria are needed to determine whether the observed bioactivity is sufficiently robust and interpretable for follow-up. These criteria include control of matrix interferences, extraction and blank effects, cytotoxicity or assay-specific confounding, detection limits at relevant enrichment factors, reproducibility across samples or laboratories, and comparison with effect-based trigger values or other assessment criteria where available. Effect-based trigger values and related benchmarks can support interpretation and prioritization of EBM signals, although they remain unavailable for many assays and endpoints [103, 104]. Therefore, EBM-derived signals should be evaluated as part of a weight-of-evidence process, together with chemical monitoring, exposure information, NAM-derived hazard evidence, and expert evaluation. For chemical monitoring, the challenges of matrix interferences and blank corrections are particularly critical for NTS, where the goal is to identify unknown compounds. Matrix components can cause significant ion suppression or enhancement, distorting signal intensity and complicating both compound identification and, crucially, any semi-quantitative estimates. Similarly, contaminants in blanks can lead to

false positives if not taken into account correctly. Therefore, robust quality control procedures, including the analysis of procedural blanks and matrix-matched calibrants, are essential to distinguish true environmental signals from analytical artifacts [105, 106].

For chemical monitoring and screening, analytical methods must meet established standards for accuracy, sensitivity, and specificity, with sufficient contextual data to link findings to exposure or risk scenarios. Signals that do not meet these initial criteria should undergo efforts to strengthen their validity, such as additional data collection, bioanalytical testing, exposure modeling, or systematic trend analyses. Collaborative efforts among multidisciplinary teams can also help address data gaps or refine hypotheses. Operationally, the challenge in the EW signal strengthening process is to find the best balance in defining the acceptance criteria. These criteria must enable the rapid verification of a signal's validity, making the optimal use of all available data and complementary approaches, especially in data-poor contexts. The goal is to optimize the use of limited resources while ensuring that signals are assessed accurately and efficiently. For example, the NORMAN prioritization framework recommends an automated workflow with priority action categories [92]. In this framework, suspect screening signals—which are inherently associated with higher uncertainty compared to targeted analysis—are supported by tailored quality criteria.

As outlined in Pu et al. [106], these criteria for NTS include confidence levels for identification and considerations for matrix effects, which can trigger direct actions for signal confirmation through targeted analysis. Similarly, low-strength signals from insufficient target monitoring data, which alone would not meet the defined acceptance criteria, can be benchmarked against suspect screening signals to validate the presence or threshold exceedance of an unwanted chemical. Finally, signals that have been sufficiently documented should be prioritized using, for example, a multi-criteria decision framework [92, 107, 108] to guide follow-up actions and communication. In this context, spatial distribution should be interpreted as a signal-strengthening criterion rather than as a minimum requirement for entry into the EWS. A signal observed in a single location, matrix, or dataset may still warrant registration, tracking, or targeted follow-up if it is plausible, well documented, associated with high hazard or exposure concern, or linked to a sensitive population or ecosystem. By contrast, a European-scale signal refers to a signal with demonstrated or plausible relevance beyond a single local event, for example because it occurs in multiple countries or regions, is linked to cross-border transport, appears in widely distributed products or supply chains, affects broadly distributed populations

or ecosystems, or indicates a regulatory or monitoring gap of European relevance.

Implementing defined criteria and prioritization mechanisms enables the EWS to focus on high-quality, actionable signals while systematically archiving and revisiting relevant data, as summarized in Table 3. Figure 1 brings together the methodological elements discussed in the preceding sections by showing how candidate EW signals may move through a conceptual signal-handling process. In this workflow, signals generated by chemical monitoring, NTS, EBMs, NAMs, EDA, modelling, data mining, or expert evaluation are first screened against quality and relevance criteria. Signals that pass this initial screening can then be checked against ongoing regulatory or assessment activities and, where needed, strengthened using an EWS toolbox before being communicated or returned to standby. Here, the EWS toolbox refers to the fit-for-purpose set of methods, data sources, and expert processes used to strengthen or contextualize a candidate EW signal, such as targeted chemical analysis, suspect screening or NTS, EBMs, NAMs, EDA, exposure or hazard modelling, database and literature mining, retrospective analysis, source tracing, or expert review. The figure was developed in the PARC context and is used here as an operational example of signal handling and strengthening, not as a description of an already fully implemented system.

Assessment and management of uncertainties

Uncertainty in a chemical EWS should be assessed in relation to both the evidence source and the decision being supported. To align with the evidence-source categories used in Table 3, this section considers uncertainties related to chemical monitoring and NTS, EBMs and bioassays, computational tools, expert input and evaluation, and cross-stream evidence integration. Exposure-hazard data integration refers here to evaluating whether measured or predicted occurrence, distribution, or exposure information is biologically or toxicologically meaningful when considered together with hazard-relevant evidence, such as bioactivity, NAM results, toxicological thresholds, effect-based trigger values, persistence, mobility, bioaccumulation potential, or mechanistic alerts.

Cross-stream integration can introduce uncertainty when evidence sources differ in chemical-identity confidence, matrix relevance, temporal or spatial scale, end-point relevance, dose or concentration metrics, biological organization, or decision context. A further source of uncertainty is the match between the chemical domain of interest and the selected matrix. Highly polar or mobile chemicals may be poorly represented in lipid-rich tissues, whereas hydrophobic, particle-reactive, or strongly

sorbing chemicals may be underestimated in dissolved water samples if suspended particulate matter, sediment, biota, or passive samplers are not considered. Short-lived or episodically released contaminants may also be missed by low-frequency grab sampling. These limitations should be documented because they affect whether a signal should be archived, monitored, strengthened, prioritized, or communicated.

For an EWS, matrix–chemical-domain mismatches can lead to false negatives, biased trend interpretation, or misleading spatial patterns. These uncertainties can be reduced by using complementary matrices, aligning sampling strategies with expected chemical properties and suspected sources, and explicitly documenting which chemical domains, exposure pathways, and temporal patterns are likely to be covered or missed.

More generally, uncertainty management in an EWS requires fit-for-purpose quality assurance across all evidence streams, not only analytical chemistry. For chemical monitoring and NTS, this includes controls for contamination, matrix effects, feature detection, annotation confidence, and semi-quantification, for example through field blanks, procedural blanks, matrix-specific performance checks, internal standards, reference materials, or reference values where available [22, 109]. For EBM and bioassays, it includes assay-validity controls, blank and extraction controls, checks for matrix interference or cytotoxicity, endpoint-specific performance criteria, reproducibility, and effect-based trigger values where available. For computational tools, it includes correct input data, applicability-domain assessment, model-performance documentation, uncertainty reporting, and transparency of assumptions. For expert-derived signals, it includes documentation of the evidence source, expertise involved, possible conflicts of interest, and the rationale for interpretation. These controls do not by themselves validate a risk signal; rather, they provide the evidence-quality basis for deciding whether a signal should be archived, monitored, strengthened through additional data generation, prioritized, or communicated.

The interpretation of diverse signals, including those derived from NTS and EBMs, introduces additional uncertainty. NTS is particularly susceptible to uncertainties arising from incomplete suspect lists, limited spectral libraries, feature alignment and prioritization choices, and the processing of large and complex datasets, which may lead to misinterpretation or failure to detect novel chemicals absent from existing databases [110, 111]. Furthermore, the lack of reference standards for many compounds complicates definitive identification and hampers accurate quantification or semi-quantification [112]. EBMs can introduce different uncertainties, including matrix interference, cytotoxicity, endpoint specificity,

assay sensitivity, differences between bioassay response and apical toxicity, and limited availability of effect-based trigger values for many endpoints. To address these challenges, harmonized protocols for performance assessment and uncertainty reporting, continued improvement of semi-quantitative NTS methods, transparent reporting of EBM performance, and the use of openly shared, versioned, and regularly updated suspect lists and screening databases are important [22, 88, 113, 114].

Sector-specific regulations and guidance documents may distinguish between screening and confirmatory methods, but they should not be presented as general EWS frameworks. For example, Commission Implementing Regulation (EU) 2021/808 sets performance requirements for analytical methods used in official controls for residues of pharmacologically active substances in food-producing animals, including requirements relevant to screening and confirmatory methods. EFSA, by contrast, provides scientific advice and risk assessment guidance rather than functioning as a regulatory framework. In an EWS context, screening methods can support early signal detection and prioritization, while confirmatory methods or additional lines of evidence are usually needed before regulatory or risk-management action. Where reference standards are unavailable, confirmation may rely temporarily on alternative lines of evidence, such as MS/MS spectral evidence, class- or surrogate-based semi-quantification, repeated occurrence, or targeted follow-up once standards become available; the remaining uncertainty should be explicitly communicated.

For computational tools, uncertainty arises from input-data quality, endpoint definition, training-set representativeness, applicability domain, model performance, uncertainty reporting, transparency, and transferability to novel chemicals, chemical classes, exposure scenarios, or endpoints. More specifically, key sources of uncertainty include chemical-structure and descriptor quality, endpoint variability, class imbalance, missing or inconsistent negative data, uncertainty in experimental labels, and limited transferability across species, exposure durations, biological systems, or chemical classes. Applicability-domain assessment is therefore essential, especially for complex or poorly represented substances, such as ionizable compounds, UVCBs, transformation products, polymers, and PFAS-like chemicals [115].

Large toxicological and ecotoxicological databases are valuable for EWS development, but the existence of many database entries does not necessarily mean that sufficient fit-for-purpose data are available for a specific model or endpoint. Resources such as RTECS, Tox21, and ECTOX [116] contain substantial information across many chemicals, assays, species, and endpoints, but the data may be heterogeneous in quality, unevenly distributed

across chemical classes, sparse for specific endpoints, difficult to harmonize, or unsuitable for extrapolation to data-poor chemicals and emerging exposure scenarios. Thus, the limitation is better described as a lack of sufficiently curated, endpoint-specific, accessible, and representative data for particular EWS questions, rather than a general absence of toxicological data.

Explainable AI (XAI), deep learning, NLP, and other advanced machine-learning approaches may support EWS tasks such as literature mining, signal detection, chemical grouping, feature prioritization, and hypothesis generation. However, their outputs should be interpreted with caution unless they are supported by transparent data curation, external validation, applicability-domain assessment, uncertainty reporting, and evidence that the model is fit for the intended EWS use. No universal minimum number of data points can be specified, because data requirements depend on endpoint complexity, chemical diversity, model architecture, label quality, expected extrapolation, and the decision being supported. For EWS purposes, such methods should therefore be treated as prioritization and hypothesis-generating tools unless their predictions are strengthened by empirical occurrence, exposure, bioactivity, toxicological, or expert evidence.

For expert input and cross-stream evidence integration, uncertainty arises from the source and documentation of expert-derived signals, potential bias or conflicts of interest, differences in evidence quality across data streams, and the challenge of combining occurrence, exposure, bioactivity, hazard, modelling, and contextual information into a transparent weight-of-evidence assessment. Structuring uncertainty assessment in this way helps ensure that each EW signal is interpreted according to the strengths and limitations of the evidence stream from which it originates, while also supporting transparent integration across evidence streams during prioritization and communication.

Actions and communication of the potential risks

The preceding sections reviewed methodological components for generating, strengthening, prioritizing, and interpreting EW signals. This final section considers how such signals may be communicated and followed up within a European chemical-risk governance context. It is important to distinguish three levels discussed in this manuscript. First, the manuscript reviews methodological building blocks that are generally relevant for chemical EWSs, including monitoring, NTS, EBM, NAMs, modelling, uncertainty assessment, and prioritization. Second, Fig. 1 presents a conceptual workflow developed in the PARC context to illustrate how such evidence streams may be organized into a signal-handling process.

Third, Fig. 2 situates these methodological components in relation to the EU-level early warning and action system for emerging chemical risks established under Regulation (EU) 2025/2455. Thus, this section does not introduce a separate EWS but discusses how methodological signals can be made usable for governance, communication, and follow-up.

Operating an EWS is inherently iterative. A signal may first be registered as a weak indication of potential concern, then strengthened through additional evidence, prioritized according to transparent criteria, and communicated to actors able to decide on monitoring, source tracing, data generation, risk assessment, or risk-management action. The information generated by the EWS can therefore support authorities in assessing whether further measures are needed to manage a potential risk. To be useful for such processes, EWS methods should be designed not only for scientific detection, but also for traceability, reproducibility, uncertainty communication, and compatibility with regulatory and policy workflows.

At the EU level, Regulation (EU) 2025/2455 provides the policy context for linking chemical data, monitoring, outlook activities, and early warning and action on emerging chemical risks. Within this context, the methodological components reviewed here can support the scientific basis for signal generation, documentation, prioritization, strengthening, communication, and follow-up. Chemical monitoring, NTS, EBM, NAMs, exposure and hazard modelling, expert evaluation, and FAIR data infrastructures can provide evidence that may be compiled, assessed, and followed up by relevant institutions and competent authorities. Implementation therefore requires clear responsibilities, transparent decision points, and explicit documentation of signal status, uncertainty, evidence gaps, and intended follow-up. These elements are needed so that weak signals can be registered without being overinterpreted, while stronger signals can be communicated to actors able to initiate monitoring, source tracing, data generation, risk assessment, regulatory evaluation, or risk-management consideration. The scientific community plays a key role at various stages of an effective EWS. Prior to the identification of signals (Step 1, Fig. 2), researchers contribute by developing and harmonizing methods for detecting early warning signals or amplifying weak signals. A recent exemplar of such methodological development is the Enabling Non-Targeted AnaLysis for PFAS (ENTAILs) Toolkit, created by the US Environmental Protection Agency to provide a standardized, open-source workflow for the non-targeted analysis of PFAS, thereby supporting state-level monitoring programs [90]. By generating such relevant data and harmonized methodologies, the scientific community aids the EEA and other institutions

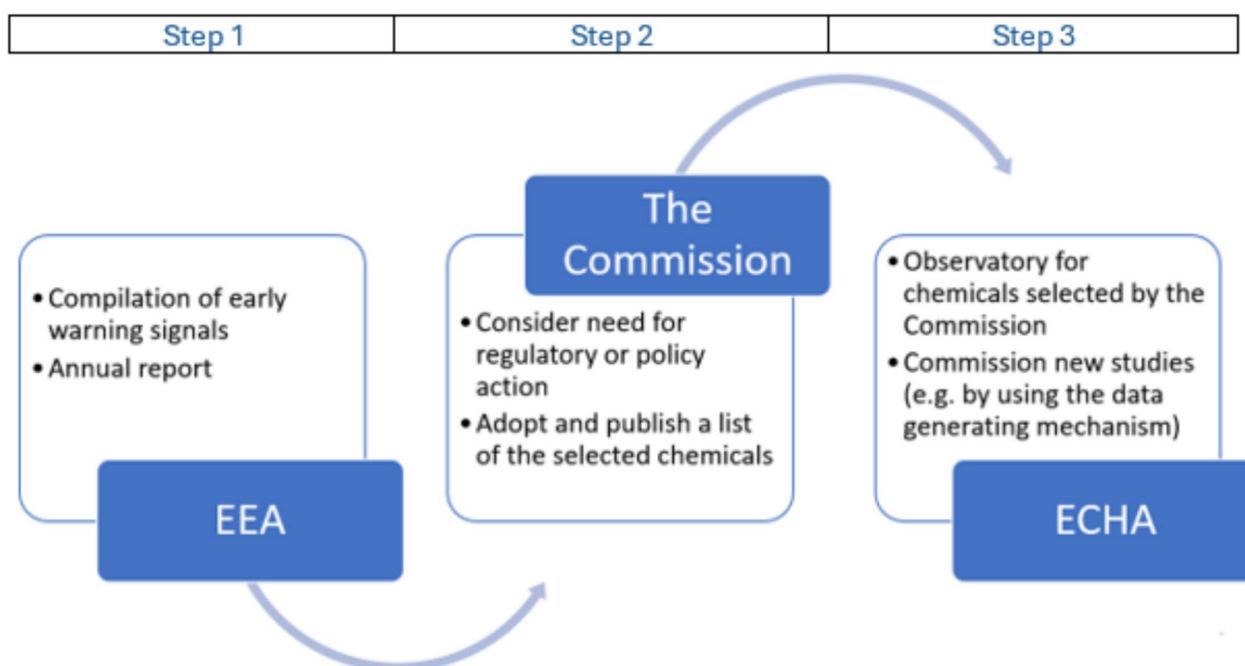


Fig. 2 EU policy context for an early warning and action system for emerging chemical risks. The figure summarizes the governance logic relevant to the Union early warning system established under Regulation (EU) 2025/2455 [12], including signal compilation, communication to relevant authorities, consideration of regulatory, policy, or enforcement follow-up, and linkage to the common data platform and observatory for specific chemicals. The figure is used here to show where methodological components reviewed in this manuscript may contribute to EU-level signal generation, prioritization, and follow-up

in identifying potential risks. Furthermore, the scientific community supports the prioritization process by developing decision-support tools, which are essential for determining which signals require immediate attention. In the next step (Step 2, Fig. 2), the European Commission will need to consider possible regulatory or policy actions and publish a list of prioritized signals to feed information back to the scientific community. At Step 3, scientists contribute by advancing tools for hazard and exposure assessment, clarifying the risks associated with identified signals. The European scientific partnership PARC and the NORMAN network are actively developing innovative tools and methodologies for signal identification and prioritization that can be integrated into the EU EWS [92, 117].

Because the EWS discussed here is a methodological and governance-oriented workflow rather than an already fully operational system, stakeholder communication should be framed as an implementation requirement. Actors involved in signal generation, curation, assessment, prioritization, communication, and follow-up need shared terminology, interoperable data formats, agreed metadata requirements, standardized reporting templates, and clear documentation of signal status, confidence, uncertainty, and recommended next steps. This

is particularly important because signals may originate from different matrices, endpoints, analytical platforms, bioassays, computational tools, monitoring networks, and data repositories. A recent stakeholder survey on NTS methods highlighted barriers to uptake, including difficulties in ensuring data quality and limited comparability between laboratories [118]. Without harmonized documentation and reporting, the same signal may be interpreted differently across research groups, monitoring networks, competent authorities, regulatory agencies, policy actors, and other stakeholders, reducing its reliability, traceability, and usefulness for follow-up.

For EWS outputs to be useful for follow-up, data infrastructures must support FAIR and traceable storage, curation, reassessment, and transfer of signal-related information. At EU level, the common data platform on chemicals established under Regulation (EU) 2025/2455 is intended to improve access to chemical data Chemicals [12], but methodological and organizational challenges remain, particularly for integrating NTS, EBMs, NAM-derived evidence, exposure modelling, and expert-derived signals into interoperable workflows. PARC should be understood in this context as a contributor to method development, harmonization, and data-workflow coordination, not as the final governance body for the

EU-level EWS. Interoperability between national NTS and EBM databases, NORMAN resources, and infrastructures such as the Research Infrastructure for Environmental Exposure assessment in Europe (EIRENE RI) will be important for signal exchange, reassessment, and follow-up.

To manage the trade-off between false positives and false negatives, an EWS should distinguish between signal registration, archiving, strengthening, prioritization, escalation, communication, and risk-management consideration. A low threshold may be appropriate for registering or archiving a weak signal, because this allows faint, local, or uncertain indications to remain available for later re-evaluation. However, registration of a weak signal should not be equated with confirmation of a risk. Weak signals should therefore be assigned an explicit status, confidence level, uncertainty description, and proposed follow-up need. Signals may then be strengthened through recurrence over time, independent confirmation, occurrence in additional matrices or locations, exposure relevance, biological activity, plausible hazard properties, source information, or consistency with a transparent weight-of-evidence assessment.

Information-sharing strategies should therefore support proportionate follow-up rather than automatic escalation. Low-confidence signals may be archived, monitored, or used to guide suspect lists; stronger signals may trigger targeted confirmation, source tracing, additional monitoring, EBM or NAM testing, exposure modelling, or expert review; and only sufficiently strengthened signals should be communicated for broader regulatory or risk-management consideration. This tiered approach preserves sensitivity for early detection while reducing unnecessary escalation of signals that remain weak, uncertain, or insufficiently documented.

Existing repositories illustrate how this can be operationalized. The NORMAN Digital Sample Freezing Platform (DSFP) supports storage, sharing, retrospective analysis, and trend analysis of chemical signals [119], while the NTS Portal of the German Environment Agency provides secure access for agencies requiring high data confidentiality [120]. Such resources are particularly valuable for revisiting weak or archived signals as new evidence, analytical methods, suspect lists, reference standards, or policy priorities emerge.

The Rapid Alert System for Food and Feed (RASFF), established under Regulation (EC) No. 178/2002, provides a useful analogy for rapid information exchange, but it should not be treated as a direct model for all chemical EWS functions. RASFF focuses on food- and feed-related health risks and supports communication between competent authorities once a concern has reached a level requiring action. A chemical EWS for

emerging risks must also accommodate weaker and more uncertain signals from heterogeneous evidence streams, including monitoring, NTS, EBMs, NAMs, modelling, expert evaluation, and product- or use-related information. The analogy is therefore useful mainly for the communication and traceability function, while the earlier stages of signal registration, strengthening, uncertainty assessment, and prioritization require additional EWS-specific procedures.

Follow-up procedures should distinguish local, national, and EU-level actions. Local or source-proximal signals may require targeted sampling, source tracing, engagement with operators or product suppliers, and communication with local or national authorities. Signals with wider relevance may require coordinated monitoring, hazard or exposure assessment, comparison with existing regulatory or monitoring activities, and consideration by EU-level bodies or competent authorities. For product-related chemical signals, timely access to contextual information, such as product composition, use patterns, supply-chain information, analytical methods, or reference materials where available, can accelerate verification and help determine whether the signal should remain local, be monitored further, or be escalated for broader follow-up.

Conclusions

A robust EWS for emerging chemical risks in Europe is increasingly feasible, but its success will depend on the integration of complementary evidence streams rather than on any single methodological advance. Chemical monitoring, suspect screening and NTS can detect known, suspected, and previously unrecognized chemicals in relevant matrices. EBMs, bioassays, NAMs, and EDA can add biological and mechanistic information that helps determine whether chemical occurrence is associated with hazard-relevant activity. Fate and exposure modelling, hazard modelling, QSARs, read-across, AI-supported tools, literature and database mining, and expert evaluation can support hypothesis generation and prioritization, especially where empirical data are limited. Together, these approaches can contribute to a tiered EWS workflow in which signals are detected, documented, strengthened, prioritized, communicated, and revisited as new evidence becomes available. This includes the need to adapt standards and guidance (e.g. ISO/CEN standards or guidance documents linked to EU directives) to allow the use of suspect screening and NTS, including semi-quantitative data, within environmental monitoring and decision-making frameworks.

The main challenge is therefore not only technical, but also organizational and interpretative. Weak signals need a low threshold for registration and tracking so that early

or localized indications are not missed. However, escalation to broader communication, regulatory follow-up, or risk-management action requires higher evidence standards, including recurrence, independent confirmation, exposure relevance, biological activity, plausible hazard properties, source or pathway information, and transparent weight-of-evidence reasoning. This tiered approach is essential for managing the trade-off between false positives and false negatives and for preventing both signal overload and delayed recognition of emerging risks.

Implementation of a chemical EWS also requires explicit uncertainty management. Each evidence stream has distinct limitations: NTS may suffer from uncertain identification and semi-quantification; EBM may be affected by matrix interference, endpoint specificity, and limited availability of effect-based trigger values; computational models may be constrained by applicability domain, training-set representativeness, and endpoint-specific data gaps; and expert-derived signals may vary in documentation, scope, and potential bias. These uncertainties should not prevent early signal registration, but they must be documented clearly so that signals can be interpreted, strengthened, prioritized, or archived in a transparent and reproducible manner. There is also a need for stronger management and feedback mechanisms linking downstream environmental and product monitoring with upstream regulatory frameworks controlling chemical production, use, and emissions.

A European chemical EWS will also require sustained data and governance infrastructure. FAIR and interoperable repositories, harmonized metadata, common terminology, quality criteria, and secure mechanisms for signal exchange are needed to connect research projects, monitoring networks, national authorities, EU agencies, and policy actors. Existing initiatives and infrastructures can contribute to this development, but practical implementation will require clear responsibilities for signal curation, reassessment, prioritization, communication, and follow-up. Without such coordination, high-throughput monitoring and computational approaches may generate more signals than can be evaluated within available capacity.

In conclusion, the actionable value of an EWS lies in its ability to connect early scientific observations with proportionate follow-up decisions. A well-designed system should not merely detect more chemicals or effects; it should help determine which signals should be archived, which should be monitored, which should be strengthened through additional evidence, and which should be escalated for assessment or risk-management consideration. Achieving this will require harmonized methods, transparent prioritization criteria, explicit uncertainty communication, interoperable data

infrastructures, adaptive regulatory frameworks, and sustained collaboration among scientists, monitoring programmes, regulators, competent authorities, policy actors, and stakeholders. This integrated approach can support earlier identification and mitigation of emerging chemical risks while preserving the flexibility needed to incorporate new methods, data sources, and policy needs.

Abbreviations

AI	Artificial intelligence
AOX	Adsorbable organic halogens
CAS	Chemical abstracts service
DSFP	Digital sample freezing platform
EBM	Effect-based method
EBT	Effect-based trigger
ECHA	European chemicals agency
EDA	Effect-directed analysis
EEA	European Environment Agency
EFSA	European Food Safety Authority
EIRENE RI	Research Infrastructure for EnVIronmental Exposure assessment in Europe
ENTAILS	Enabling Non-Targeted Analysis for PFAS
EU	European Union
EW	Early warning
EWS	Early warning system
FAIR	Findable, accessible, interoperable and reusable
GC-HRMS	Gas chromatography-high-resolution mass spectrometry
GD211	OECD Guidance Document No. 211
HBM	Human biomonitoring
HRMS	High-resolution mass spectrometry
HT-EDA	High-throughput effect-directed analysis
LC-HRMS	Liquid chromatography-high-resolution mass spectrometry
NAMS	New approach methodologies
NLP	Natural language processing
NORMAN	Network of reference laboratories, research centres and related organisations for monitoring of emerging environmental substances
NTS	Non-target screening
OECD	Organisation for Economic Co-operation and Development
PARC	European Partnership for the Assessment of Risks from Chemicals
PFAS	Per- and polyfluoroalkyl substances
PMT	Persistent, mobile and toxic
QA/QC	Quality assurance/quality control
QSAR	Quantitative structure–activity relationship
RASFF	Rapid alert system for food and feed
REACH	Registration, evaluation, authorisation and restriction of chemicals
SCREENER	Screening for emerging chemical risks in the food chain
SPM	Suspended particulate matter
SSbD	Safe and sustainable by design
UVCB	Substance of unknown or variable composition, complex reaction products or biological materials
WBE	Wastewater-based epidemiology
WWTP	Wastewater treatment plant
XAI	Explainable artificial intelligence

Acknowledgements

This work was carried out in the framework of the European Partnership for the Assessment of Risks from Chemicals (PARC) and has received co-funding from the European Union's Horizon Europe research and innovation programme under Grant Agreement No. 101057014. Views and opinions expressed are, however, those of the authors only and do not necessarily reflect those of their affiliation, the European Union or the Health and Digital Executive Agency. Neither the European Union nor the granting authority can be held responsible for them.

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Funding

Open access funding provided by Swedish University of Agricultural Sciences. This project is part of the Partnership for the Assessment of Risk from Chemicals (PARC), co-funded by the European Union's Horizon Europe research and innovation programme under Grant Agreement No. 101057014.

Data availability

No new data were created or analyzed in this study. Thus, data sharing is not applicable to this article.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing of interests

The authors declare no competing interests.

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Received: 3 March 2026 Accepted: 26 May 2026

Published online: 04 June 2026

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