Organisation for Economic Co-operation and Development

ENV/CBC/MONO(2025)11

Unclassified

English - Or. English

30 September 2025

ENVIRONMENT DIRECTORATE
CHEMICALS AND BIOTECHNOLOGY COMMITTEE

Guiding principles to advance occupational mixture risk assessment with effect biomarkers

Series on Testing and Assessment No 413

JT03571933

Please cite this publication as:

OECD (2025), *Guiding principles to advance occupational mixture risk assessment with effect biomarkers,* OECD Series on Testing and Assessment, No 413, OECD Environment, Health and Safety, Paris, https://one.oecd.org/official-document/ENV/CBC/MONO(2025)11/en

Contact us

OECD Environment Directorate,
Environment, Health and Safety Division
2 rue André-Pascal
75775 Paris Cedex 16
France

E-mail: ehscont@oecd.org

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Executive Summary

Traditional occupational health risk assessments often rely on external exposure measurements, such as air monitoring, which may not fully capture the complexities of workplace exposures. This guidance describes the integration of effect biomarkers and the use of Similar Exposure Groups (SEGs) to enhance the accuracy and efficiency of occupational mixture risk assessments. SEG refers to a group of workers having the same general exposure profile for the agent(s) being studied because of similarity and frequency of the tasks they perform, the materials and processes with which they work and the similarity in the way they perform the tasks (Bullock et al. 2006). Effect biomarkers account for individual variations in the responses to chemicals exposure, considering all exposure routes, cumulative effects over time, and impacts of chemical mixtures (see **Table 1**). While human biomonitoring for exposure biomarkers at the individual level is recommended, the use of the SEG approach is advised when conducting human biomonitoring of effect biomarkers. This approach improves risk assessments by focusing on group-level exposures, thereby improving data interpretation through statistical methods. Additionally, the SEG approach facilitates targeted interventions for groups rather than individuals making it a more effective strategy for managing and mitigating risks associated with occupational mixture exposures. SEGs are derived by evaluating work processes and exposure determinants - factors that influence the intensity, frequency, and duration of chemical exposure in the workplace. Collecting appropriate exposure information is a crucial step in human biomonitoring of effect biomarkers. This helps create homogeneous groups regarding exposure potential, develop an appropriate sampling strategy, and define possible exposure scenarios (see **Figure 1**). Information on individual worker characteristics, such as socio-demographics and health status, should also be collected to adjust for factors that influence disease susceptibility or exposure probability. Interpreting effect biomarker data employs a tiered framework with four assessment levels (see **Table 2**). This framework translates effect biomarker data into actionable insights for managing workplace risks. By monitoring effect biomarkers with the SEG approach, this framework offers a more comprehensive, worker-centric method for assessing and managing combined exposure to multiple chemicals, promoting worker health and safety through a more focused understanding of the biological impact of workplace hazards.

1 Objective

The OECD work on effect biomarkers represents a critical step forward in the development of guidance for the use of effect biomarkers in occupational biomonitoring when evaluating combined exposures to multiple chemicals (chemical mixtures) from all routes of exposure. It is important to note that physical/biological agents are not considered in this document. The forthcoming OECD guiding principle document has case studies on seven selected effect biomarkers for occupational effect biomonitoring of, Genotoxicity, Oxidative Neurotoxicity/Developmental Neurotoxicity (NT/DNT) and Reproductive Toxicity and Endocrine Disruption assessment.

As part of this initiative, the present document outlines guiding principles on assessing occupational exposure to chemicals using effect biomarkers, and includes guidance on biological effect monitoring sampling strategies using the Similar Exposure Groups (SEGs) approach. The use of SEGs approach allows for comparison of the results with suggested compliance levels when such levels are available, ensuring that the measured biomarkers align with established safety guidelines.

2 Background

Occupational exposure data encompass external and internal exposures measurements. Occupational exposure may occur via inhalation, skin contact, or ingestion. Total internal exposures (the amount of an agent that enters the organ system by crossing an absorption barrier (Heinemeyer et al. 2022)) can be estimated by human biomonitoring, accounting for all routes of exposure. Human biomonitoring refers to the measurement of chemical agents and/or their metabolites (known as exposure biomarkers), or markers of subsequent early health effects (known as effect biomarkers), in organisms and biological matrices such as tissues, cells or fluids.

Human biomonitoring can improve exposure assessment and, depending on the selected biomarker(s), provide valuable insights into potential health outcomes, (DeBord et al. 2022). This approach includes the use of exposure biomarkers to evaluate specific environmental or occupational exposures, as well as effect biomarkers to detect early biological changes that may be linked to adverse health effects in individuals and populations (Zare Jeddi et al. 2021). Unlike exposure biomarkers, which indicate the presence of a chemical or its metabolites in the body, effect biomarkers reflect physiological or biochemical changes resulting from exposure. These changes can serve as early warning signals of potential health effects, making them valuable for risk assessment and preventive health and exposure strategies (WHO, 1993; NRC, 2006). Therefore, effect biomarkers may establish exposure-effect relationships to identify possible modes of action (Rodríguez-Carrillo et al. 2023). While biomarkers of exposure tend to be specific for a particular chemical or agent, effect biomarkers are less specific and may reflect the effect of exposure to multiple chemicals or agents, triggering the same 'key event' (i.e. chemicals with a common mode or action) (DeBord et al. 2022; Silins and Högberg 2011; Stanciu et al. 2025). It is also important to note that effect biomarkers may also be triggered by non-chemical stressors, such as biological and physical agents and possibly also psychosocial stressors. Human biomonitoring studies can be designed to assess both exposure and effect biomarkers simultaneously, using either the same matrix or different matrices. Detailed comparison of the advantages and limitations of commonly used monitoring approaches including personal air monitoring, exposure biomarkers, and effect biomarkers in occupational health risk assessment are listed in **Table 1**.

A key element in human health risk assessment is obtaining accurate information on internal exposures within the population of interest (EPA 2019). Currently, data on personal (internal) mixture exposures are largely absent, which significantly limits the effectiveness of mixture risk assessment (MRA). The use of human biomonitoring data may improve this situation (Luijten et al. 2023). In occupational hygiene, human monitoring (or occupational biomonitoring) is commonly used for assessing exposure or to complement other monitoring approaches such as air monitoring and dermal sampling (BOHS 2021, HSE 1997). Occupational biomonitoring is particularly helpful when assessing the effectiveness of exposure controls, developing risk communications, and identifying failures of prevention and control measures already in place. Occupational biomonitoring is also essential following uncontrolled incidents(Viegas et al. 2020).

Table 1. Advantages and limitations of occupational exposure monitoring approaches

Advantages	Limitations
Personal air monitoring	
• Well-established for monitoring large number of agents (e.g., according to EN 689). • Accepted by regulatory bodies. • Used for both long- and short-term exposures. • Measurements directly related to exposure at the workplace. • Many chemical-specific methods available (e.g. HSE MDHSs, ISO standards, OSHA etc.). • Regulatory exposure limits available for many chemicals. • Non-invasive. • Accepted by workers. • Measurement is clearly related to a defined sampling period. • Not associated with privacy concerns and no medical professionals needed.	• Each measure is a point sample. • Only inhalation exposure resulting from air contamination in the workplace is captured. • May underestimate total exposure as it does not assess contribution of dermal or oral exposures. • Reflects potential rather than actual exposure. Does not consider the effectiveness of Personal Protective Equipment (PPE). • Depending on sampling and analytical methods used, may not cover exposure to chemical mixtures. • Sampling errors, including improper handling of sampling equipment by worker.
Exposure biomarkers	
• Account for the physical effort and individual metabolic variability. • Account for all routes of exposure (dermal exposure, inhalation, and oral exposure due to hand-to-mouth contact). • Integrate exposures over time. • Account for unexpected exposures or routes of exposure. • Evaluate the effectiveness of control measures, including personal protective equipment (PPE). • Guidance available (e.g., OECD, many regulatory bodies, and other organizations). • Minimal worker involvement in sampling process reduces the likelihood of errors. • May inform on the longer-term exposure (exposure during the past few days or even months), depending on the chemicals/metabolites and assessed biological matrices.	• Few population reference values available. • Few health-based guidance values (OBL-Occupational biomonitoring level, BLV-Biomonitoring limit values) available • Few sampling and analytical methods available. • Exposure route(s) not known. • Interpretation of data with high individual variability may be challenging. • Biological sample collection must be timed according to the exposure patterns and toxicokinetics of the chemical of interest. • While taking into account all routes of exposure and holistically assess

• Possible to monitor several chemicals in the same biological sample. • Use to reconstruct exposures following acute or accidental events if appropriate biomarkers are available.	the impact on an individual, it is difficult to determine the contribution of non-occupational from occupational exposures, as required by the legal/regulatory frameworks.
Effect biomarkers	
• Direct correlation of the effect biomarker with combined exposure to multiple chemicals and potentially with adverse outcomes. • Considers inter-individual variability in the response to chemicals, e.g., metabolic differences, affecting chemical absorption, distribution, metabolism, and excretion, and its toxicodynamic leading to variability in target organ exposure and associated effects • Accounts for aggregated exposure. • Accounts for known and unknown mixtures • Integrate exposures over time, offering a more comprehensive exposure assessment. • Considers the effectiveness of Personal Protective Equipment (PPE). • Minimal worker involvement in sampling process reduces the likelihood of errors. • A good epidemiological indicator for potential health risks within a group, workplace or industry that can guide further investigation. • Guiding principles and guidelines are in preparation (EFSA and OECD).	• Fewer reference values available for interpretation. • Knowledge on the biological relevance of the effect biomarker is necessary. • Lag time between exposure and measured effect biomarker may be days / weeks / months / years and requires toxicokinetic and toxicodynamic information for interpretation. • Potential high individual variability due to genetics and lifestyle habits. • Difficult to establish contribution of non-occupational exposures. • Requires specialized expertise in analytical laboratories.

2.1. Advantages and disadvantages of using effect biomarkers for mixture risk assessment

Assessment of combined exposure to chemicals in workplaces is a challenge due to the vast number of possible mixtures, leading to potential health effects.(La Rocca and Sarazin 2022; Rim 2023). Additionally, occupational exposures to chemicals are known to vary over time (e.g. during the day, between working days) and between workers carrying out the same or similar tasks (Burdorf 2005; Rappaport et al. 1993). Moreover, there may be significant variation in individual responses to chemical exposures, both qualitatively and quantitatively, as has been shown in toxicological studies. For instance, individual differences in physiological parameters, such as age, weight, and liver function, may affect the distribution of a chemical, and genetic polymorphisms of metabolic enzymes can alter the concentration and pattern of metabolites (DeBord et al. 2022; Ladeira and Viegas 2016).

The need to consider a greater range of factors contributing to potential health effects of combined exposures makes the risk assessment process more complex compared to the assessment of single chemicals. For example, an increased understanding and knowledge about the individual agents, uptake, metabolism, excretion and mechanisms/modes of action in different tissues and cells as well as temporal factors are needed for evaluating health risks of combined and mixed exposures (Silins and Högberg 2011). The unspecific nature of effect biomarkers suggests they have greater potential to reflect complex exposures and can include aggregated and sequential exposures over time. Additionally, using effect biomarkers in studies of complex exposures could help identify both the active components of mixtures/combined exposures and the consequences of specific mixture exposures (Silins and Högberg 2011).

Another advantage of effect biomarkers lies in their interpretation within the context of adverse outcome pathways (AOPs) that are of regulatory significance (DeBord et al. 2015). An effect biomarker should be based on the mechanism of action of chemicals to reflect changes in key steps of AOPs (Zare Jeddi et al. 2021). Therefore, integrating effect biomarker assessments into other monitoring programs would improve occupational mixture risk assessments.

Biological effect monitoring enables occupational health practitioners to detect early health impacts in workers deemed at risk for chemical mixture exposures.

An ideal effect biomarker should have a demonstrated association with exposure to specific chemicals and be predictive of adverse outcomes. It should also be sensitive, robust, and ideally, non-invasive. Several effect biomarkers have been validated for both general population and occupational biomonitoring research studies (Zare Jeddi et al. 2021). However, only a few effect biomarkers have been validated and recommended for use in routine human biomonitoring in occupational health for assessing the risk of exposure to chemical mixtures. Effect biomarkers offer the possibility of identifying early effects from exposure to multiple chemicals that can be reversed or mitigated by ceasing or reducing exposure.

One approach for assessing exposure to chemicals is to divide the population into Similar Exposure Groups (SEGs), and design biomonitoring programs that minimize both cost and effort (Irving et al. 2008; Mulhausen et al. 2006). The SEG approach is used in testing compliance with occupational exposure limit values (Technical Committee CEN 137, 2020). SEGs are defined as “group of workers having the same general exposure profile for the agent(s) being studied because of the similarity and frequency of the tasks performed, the materials and processes with which they work, and the similarity of the way they perform the tasks” (Derby 2011; Mulhausen et al. 2006). A lognormal distribution of exposure can be assigned for workers in a SEG, including those who have not been sampled. This lognormal distribution of exposure can be used to assess compliance of the SEG with the occupational biomonitoring exposure limit. SEG collective assessment is recommended, if possible, for air and/or personal exposure biomonitoring measurements. There are circumstances where the random variability is so great within a SEG group that the use of SEG approach would not be possible. SEGs may be inappropriate when within-group exposure variability exceeds a 2- to 3-fold range or when environmental and process-related factors lead to significant individual differences, suggesting the need for more refined or individual-level assessments (Rappaport et al. 1993).

However, the application of effect biomarkers is accompanied by several limitations that must be carefully considered. Firstly, there is a limited availability of established reference values, which complicates the interpretation of measured biomarker levels. Accurate assessment requires a thorough understanding of the biological relevance and mechanistic role of the selected biomarker. Additionally, the temporal relationship between exposure and the manifestation of a measurable biological effect can vary widely—from days to several years—necessitating detailed toxicokinetic and toxicodynamic data for meaningful interpretation. Moreover, it is often difficult to distinguish the contribution of occupational exposures from non-occupational sources, particularly in complex exposure scenarios. Potential interlaboratory variability should be considered, as for other exposure monitoring approaches.

3 Effect biomonitoring sampling strategies using SEGs approach

Whether for single substances or combined exposure to multiple chemicals, it is recommended to classify workers into SEGs according to EN689:2018 (CEN 2018) when assessing workplace exposure to chemicals using biological effect monitoring. EN 689:2018 specifies a strategy using SEGs to perform representative measurements of exposure by inhalation to chemical agents in order to demonstrate the compliance with occupational exposure limits (OELs) (D'Errico et al. 2022). This strategy is needed because interpreting the effects of workplace exposure on an individual basis might not be feasible, as individual variability (e.g. genetics and personal habits) may affect the results. The purpose of SEGs is to group workers with similar exposure profiles for more accurate risk assessments and exposure monitoring. Using a SEG approach is not intended to replace individual biological effect biomonitoring as a component of medical surveillance practices depending on the country's occupational laws and regulations.

3.1. Investigating the influence of specific factors within SEGs

The most important step in defining SEGs involves evaluating work processes to identify all exposure determinants-- factors that influence the levels (intensity), frequency, and duration of exposure to chemical agents in the workplace with the help of industrial hygienists/occupational health professionals. It is necessary to gather exposure information beyond the scope of typical human biomonitoring of exposure biomarkers or air sampling programs to ensure the relevance and high quality of exposure data. Individual factors may affect the results (e.g. pre-existing diseases, medications, alcohol consumption, physical activities) this should be addressed by questionnaires for all involved workers and in the control group. It is recommended to follow OECD guidance developed for occupational exposure biomonitoring (OECD 2022), since biomonitoring of effect biomarkers also encompasses all routes of exposure, similar to biomonitoring of exposure biomarkers.

The information gathered is then used to define exposure profiles and SEGs as well as make initial judgments on exposures (Bullock et al. 2006). Workers are attributed or classified into specific SEGs based on their exposure profiles. The SEG approach can utilize the same statistical analyses as those applied in identifying SEGs for exposure monitoring (OECD 2022). In the SEG approach, certain exposure-related parameters such as the type of chemical handled, the duration of exposure, and the use of personal protective equipment are kept constant within a SEG. By controlling these variables, it helps to ensure that the exposure level is similar for all members of the group, thereby reducing the complexity and cost of monitoring each individual.

Despite controlling for known variables, there will always be uncontrolled factors that contribute to the variance in exposure levels within a SEG. These uncontrolled factors might include environmental conditions (e.g. temperature or humidity), slight variations in task executions, or differences in individual behavior (e.g. compliance with work procedures). Uncontrolled factors must be accounted for in the risk assessment process. The SEG approach allows for targeted investigations regarding how factors, such as different types of Personal Protective Equipment (PPE), affect exposure levels within similar groups.

By comparing SEGs that differ only in the type of PPE used, researchers can isolate the impact of this variable on worker exposure. In the context of SEGs, variance in exposure levels does not need to be categorized as inter-individual (between persons) and intra-individual (within the same person over time). Instead, as proposed in evaluation schemes such as OECD Guide #370 (Table 14), the total variance observed in the exposure data can be considered as a composite measure that includes effects from multiple sources.

Random variability refers to the unpredictable fluctuations in exposure levels that occur even under controlled conditions. When workers are classified into SEGs based on similar job roles, tasks, and exposure conditions, individual exposures can vary due to factors that are not easily quantifiable or controlled. For example, minor differences in the way tasks are performed, fluctuations in environmental conditions (e.g. ventilation rates, ambient temperature), variations in the amount of substance handled, differences in adherence to work protocols can contribute to variable exposures among members of the same SEG. This variability in exposure levels is treated as part of the natural distribution of exposures within the SEG.

Occupational health professionals should design sampling strategies to account for variability in exposure levels among members of the group, e.g. sampling at different times and under different conditions within the SEG.

3.2. Stepwise workplace exposure assessments using effect biomarkers

Following the characterisation of workers' exposure and the identification of SEGs, sampling and analysis of the SEG approach comprises the following seven steps (Figure 1).

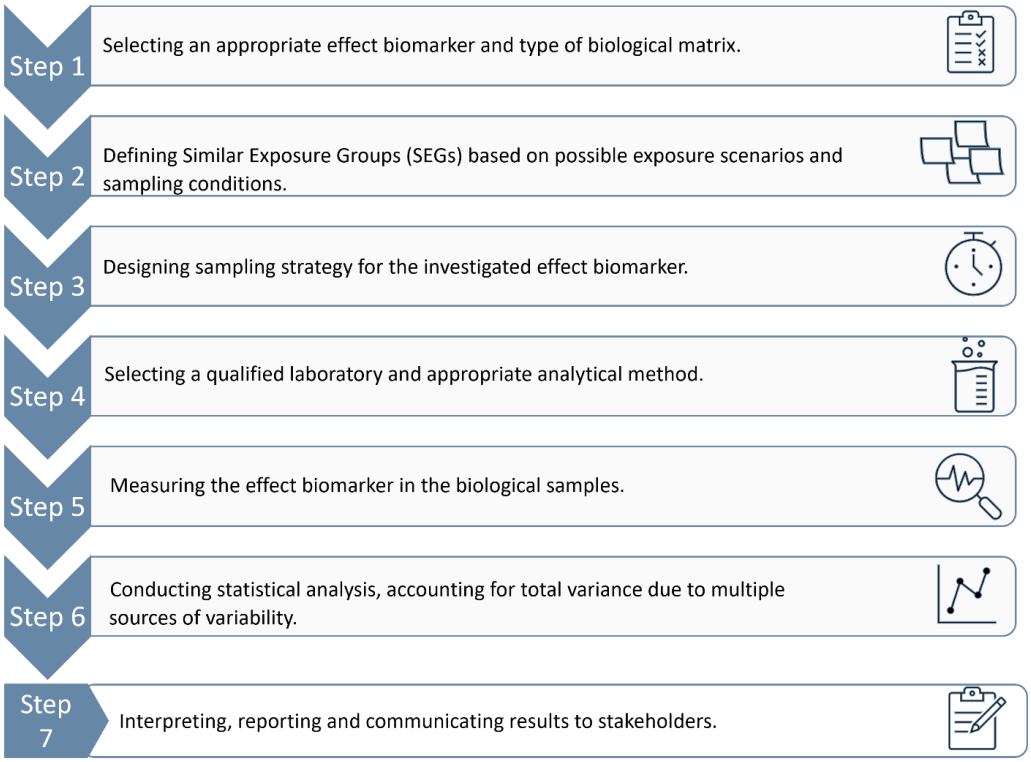

Figure 1. Stepwise approach for bringing effect biomarkers into workplace assessments

The seven steps include:

Step1: A suitable biomarker is one that aligns with the rationale of the specific exposure scenario, reflecting the relevant biological processes and timing of exposure(See chapter 1 of Guiding principles for mixture threshold derivation from effect biomarkers and Zare Jeddi et al. (2021)).

Step 2: Defining the (mathematical) set of most likely exposure scenarios and sampling conditions that could occur within the SEG. This set encompasses various work shifts, tasks, and exposure durations to represent the full range of variability within the SEG. Although workers perform similar tasks, day-to-day differences in work pace, environmental conditions, or use of protective equipment can lead to fluctuations in exposure. The goal is to mathematically define the most likely exposure scenario by capturing this variability, ensuring that biomonitoring data accurately reflect real-world conditions.

Box 3.1. Example: Calculating Potential Annual Exposure Days for a Similar Exposure Group (SEG)

Imagine a SEG containing 25 workers, all performing the same task under similar conditions. The task is carried out for 12 working weeks (5 days/week) per year. This results in 60 potential exposure days annually per worker (12 weeks × 5 days/week = 60 days). Exposure day(s) refers to day(s) on which a worker performs a task that may result in exposure. Since there are 25 workers in the SEG, the total number of individual exposure-days across the group is:

25 workers × 60 days = 1500 worker-exposure days per year.

It is important to note that this number (1500) represents the total number of potential exposure events, not necessarily the number of actual measurements taken. In occupational hygiene, it is typically not feasible or necessary to take a measurement for every exposure event. Instead, a representative sampling strategy is used. The number of samples depends on the variability of exposure and the purpose of the assessment (e.g., compliance vs. risk assessment).

Step 3a: *Sampling time*: Effect biomarkers often have a specific window of presence. The lag time between exposure and the appearance of a measurable effect biomarker can range from short to very long durations after exposure, with their levels influenced not only by the intensity and duration of exposure but also by individual differences in biological repair and clearance mechanisms. Effect biomarkers can vary not only with exposure intensity but also with biological rhythms, co-exposures, and individual susceptibility. Accurate interpretation of this lag time requires both basic toxicokinetic and toxicodynamic (AOPs) information from expected mixtures (DeBord et al. 2022; Reale et al. 2024). If the interpretation of an effect biomarker depends on cumulative exposure over consecutive days, sampling should reflect typical exposures of the SEG and adequately captures the variability across days.

Step 3b: *Stratified* r*andom sampling***:** Once all possible exposure scenarios within a SEG have been identified, the next step is to strategically and randomly select a subset of these scenarios for sampling. Stratified random sampling ensure that all relevant exposure scenarios are proportionally represented; sampling avoids systematic bias (e.g., only sampling during low-exposure periods); the dataset reflects temporal and task-related variability.

A representative subset must be chosen because it is impractical, costly, and logistically challenging to sample every exposure day (e.g., 1500 exposure days per year in the previous example). It is crucial to ensure that the samples are representative of the entire SEG, thereby reducing bias and improving the accuracy of the exposure assessment. This subset should capture the variability in exposure

conditions across the SEG, including different work shifts (e.g., day vs. night); tasks performed; exposure durations; environmental conditions (e.g., temperature, ventilation), and worker-specific factors (e.g., metabolism, health status, years of work experience, PPE use). Variability in effect biomarkers with very long half-lives is greater among workers who have experienced prior occupational exposures, as residual biomarker levels from previous jobs can persist and influence current measurements, unlike in colleagues without such histories.

Box 3.2. Example Sampling Strategy

In the example of step 2, the SEG has 1500 potential worker-exposure days per year. Let's assume that due to resource constraints, only 10% of the 1500 worker-exposure days per year can be sampled—i.e., 150 worker-exposure days per year. A computer-generated random selection process can be used to select these 150 days from the full set of 1500. This ensures that each exposure day has an equal probability of being selected, thus reducing selection bias. Stratified random sampling is often preferred over simple random sampling to improve robustness. For example, divide the 1500 worker-exposure days per year into strata based on shift type, task category, or season. Then randomly select a proportional number of days from each stratum.

This approach ensures that key sources of variability are captured, which is especially important when interpreting effect biomarkers, as these may be influenced by both exposure and non-exposure-related factors.

Using a stratified random sampling approach, 150 sampling events are distributed across different shifts, tasks, and environmental conditions to ensure representativeness. Each sampling event involves collecting a biological sample (e.g., urine, blood) from a worker at a time point relevant to the biomarker's response window.

Step 3c: *Determine an appropriate sample size*: The appropriate number of samples should balance statistical reliability and logistical feasibility. When applying for ethical approval for effect biomarker studies, it is essential to perform a priori power calculations to justify the proposed sample size. These calculations help ensure that the study is capable of detecting meaningful effects while minimizing unnecessary participant burden. The ideal sample size depends on several factors:

i. Statistical Power**:** A larger sample size increases the power of the study, reducing the likelihood of Type II errors (failing to detect an effect when one exists); Power calculations should be based on expected effect sizes, variability of the biomarker, and desired confidence levels (typically 80–90% power at a 5% significance level).

ii. Practical Constraints: Time, budget, and resource availability may limit the feasible number of samples; These constraints must be balanced against the need for scientific validity.

iii. Expected Exposure Variability Within the SEG: Greater heterogeneity in exposure scenarios (e.g., tasks, shifts, environmental conditions) requires a larger sample size to adequately capture the range of exposures.;

iv. Nuisance Parameters (Confounding Factors): If confounders (e.g., diet, smoking, temperature) are well-controlled or determined to have a minimal impact, a smaller sample size may suffice. If such factors are uncontrolled or unknown, a larger sample size is needed to maintain statistical robustness.

v. Effect Biomarkers Variability: Some effect biomarkers (e.g., markers of oxidative stress or inflammation) have high intra- and inter-individual variability. Sample size should be (if possible) adjusted accordingly, ideally based on pilot data or literature estimates of biomarker variability.

vi. *Control group Requirements*: For effect biomarker lacking established population reference values (e.g., genotoxicity biomarkers), a **matched** control group is essential. Controls should be matched for age, sex, **lifestyle factors,** and other relevant confounding variables to ensure valid comparison.

vii. *Exclusion Criteria*: Certain medications, medical conditions **or lifestyle factors** (e.g., recent infections, chronic diseases, medical regimen) can significantly influence effect biomarker levels. Therefore, individuals affected by these factors should be excluded based on predefined criteria to avoid data distortion.

Step 4: The selected laboratory must operate under recognized quality standards to ensure the reliability and comparability of biomonitoring data. This can be demonstrated by accreditation of analytical methods, participation in external quality assessment (EQA) schemes or inter-laboratory comparisons, demonstrated accuracy in analysing certified reference materials (CRMs), when available, or use of validated methods for the specific effect biomarkers being analysed (OECD 2022).

Step 5: Measurement of samples involves the actual analysis of biological samples (e.g., blood, urine) using the selected analytical methods with appropriate detection limits. Key considerations include chain of custody documentation to ensure sample traceability, blinding of samples to reduce analytical bias and inclusion of quality control samples (e.g., blanks, duplicates, spiked samples) in each batch.

Step 6*:* Statistical analysis is essential to interpret biomarker data and assess exposure distributions. Goodness-of-fit tests can be used to evaluate whether the biomarker data follow a specific distribution. While the plots of biomarker data may follow a variety of distributions, in principle, lognormal distribution is often appropriate for biomarker data due to right-skewed nature, but this must be empirically verified. If the distribution does not fit the data well, consider revisiting the earlier steps including SEG definition, sampling strategy, and data transformation methods. When using a lognormal distribution, the Geometric mean and standard deviation should be calculated. These estimates help in assessing compliance with occupational exposure limits by comparing the calculated values against established thresholds and characterizing exposure variability within the SEG.

Step 7: Interpretation of findings, includes comparison to reference or guidance values, identification of high-exposure subgroups as well as uncertainty and limitations. A detailed report should include study objectives and design, sampling strategy and SEG definitions, analytical methodology, results, and interpretations. These parameters support a transparent description of how data were collected, analyzed, and interpreted. Furthermore, detailed information about sampling methods, analytical data quality (accuracy, precision, reproducibility), and potential for analytical contamination must be documented. Proper data handling methods are essential to prevent errors and ensure the integrity of the findings (OECD 2021). The report should also outline any assumptions made, potential sources of bias or error and the limitations of the analysis.
Communications of results to different stakeholders need to be tailored accordingly. Communication to workers and participants in SEG should be conducted in a clear, non-technical language. Employee and work councils might need detailed technical reports.

4 Ethical and legal aspects of effect biomonitoring

Since effect biomonitoring requires the use of human samples, ethical principles and data protection legislation must be respected. The study design, including the use of SEGs and sampling strategy, should be reviewed and approved by an ethics committee. Considerations of fairness, informed consent, and data protection should be included. Participation in biomonitoring programs should be voluntary, and using non-invasive methods such as urine sampling, which is generally more acceptable than blood, can increase the likelihood of worker participation. Privacy requirements are stipulated in local regulations and will differ according to the sociocultural and national legal context in which they are applied. All workers should, in principle, have the same opportunity to fully benefit from the human biomonitoring program and have their data assessed at both individual and collective (group) levels to provide the most adequate information for further actions. The use of SEGs for collective (group) level assessments is a scientifically valid and resource-efficient approach, assuming that all members of the group share comparable exposure profiles. However, it also introduces limitation as not every worker will have their own biomarker data assessed. To uphold the principle of equal opportunity while maintaining scientific and logistical feasibility, it should be clearly explained to all workers why SEGs are used and how group-level data will inform workplace exposure decisions. Additionally, it should be emphasized that even if not individually sampled, workers benefit from the collective findings. Moreover, optional individual sampling should be offered for workers outside the selected subset, particularly if they have specific concerns or risk factors.

The employer has a duty to protect the workers from chemical hazards, including providing information on the chemical risks to which the workers are exposed, as well as exposure reduction methods and tools. The worker has a right to receive biomonitoring results that have been interpreted by the occupational health professionals. Recommendations based on the exposure assessment results should be elaborated for both the employer and the individual workers. Overall, this guiding principle focuses on SEGs assessment, aggregating individual results.

5 Recommended use of assessment levels

The overall results from the effect biomonitoring program shall be assessed against an established effect biomarker assessment level value. Communications can be based on the threshold levels described in the OECD "Occupational Biomonitoring Guidance Document" (370, Table 4 p. 70). That terminology for Occupational Biomonitoring Level (OBL) has been adjusted for effect biomarkers as occupational Effect Biomonitoring Level (OBEL) and used for seven effect biomarkers in the (Guiding principles for mixture threshold derivation for effect biomarkers 2025). Table 2 shows the four tiers (0-1-2-3) of assessment for human health (TOBEL, ROBEL, POBEL, OBEL) and environmental health (T-EBT, R-EBT, P-EBT, EBT) and their meanings.

Table 2. Proposed concept of Occupational Biomonitoring Effect Level (OBEL) allowing their interpretation.

Human Health (HH)	Tier	Level	Meaning if exceeded
OBEL=Occupational Biomonitoring Effect Level	**3**	**Refined**	**Health risk indicated**
POBEL	**2**	**Provisional**	**Health risk may be indicated**
ROBEL	**1**	**Reference**	**Exposure above or below a reference level (e.g.>95%, <5%)**
TOBEL	**0**	**Technical**	**Exposure detected or not (e.g. >LOQ or >LOD)**

The information of the SEG distribution can be applied to the tiers in the following way:

- **Tier 3-Quantifying direct health risks:** Similar to the European Standard EN 689:2018 (CEN 2018), we recommend to use an OBEL for investigated effect biomarker, when available.(CEN 2018). OBEL is clearly linked to an adverse outcome pathway. Specifically, the upper bound of the 70 % tolerance interval for the 95th percentile estimate of the SEG distribution should not exceed the OBEL. This allows for occasional exceedances of the OBEL, fitting within this risk management framework.

- **Tier 2-Quantifying direct potential health risks:** In the intermediate scenario, the POBEL is a point of departure (PoD), triggering investigation of modes of action. Depending on the adverse effects connected to the POBEL, the median of the SEG distribution should not exceed the POBEL, applying an average concept approach[1]. This evaluation indicates whether there is a potential health risk. It is important to note that this determination is not based on a single measurement but rather on a series of measurements, providing a more comprehensive assessment of exposure levels. The response of an effect biomarker quantified either as a Bioanalytical Equivalent Concentration (BEQ) for well-known mode of action (MoA) specific reference compounds or directly as an effect-level indicating an increased exposure or potential or direct adverse risk level or classes. This level of effect biomarker response is linked to accepted adverse PoDs in risk assessments. BEQ is an integrative response of an effect biomarker translated into an effect concentration of a reference compound. For example, the combined effect of estrogen receptor-binding substances may be expressed in estradiol equivalents, and the combined effects of dioxin-like acting substances in dioxin-equivalents. In some cases, the POBEL directly corresponds to well-characterized stressors as for genotoxicity (e.g., Chromium, Formaldehyde) and neurotoxicity (e.g. Lead), so a simple translation to risks is possible.
- **Tier 1-Interpreting an occupational exposure:** The ROBEL is derived from the exposure distribution of a reference (non-exposed) population. Analysis involves comparing whether the SEG distribution significantly overlaps with the reference population's distribution. For example, if the ROBEL represents the 95th percentile of the reference population, then the lower limit of the 95% confidence interval of the SEG's 95th percentile should be below the ROBEL.
- **Tier 0-Monitor occupational exposures:** When monitoring occupational exposures, the TOBEL represents Limit of Detection (LOD) and Limit of Quantification (LOQ) required to sensitively monitor exposures and effects. The TOBEL should be set well below the values being monitored (e.g., ROBEL, POBEL, OBEL, or SEG distribution) to ensure sufficiently sensitive detection of occupational exposures and effects.

For each of the assessment levels (OBEL, POBEL, ROBEL, TOBEL), the statistical analysis must be consistent with the definitions and intended outcomes specific to the selected effect biomarker. It recognized that individual responses to effect biomarkers may sometimes deviate significantly from expected ranges. These individual exceedances should be interpreted with caution, as many factors beyond occupational exposures—such as chronic diseases, medications, recreational activities, or the use of herbal remedies—can influence biomarker levels. Nevertheless, such deviations can still serve as early warnings, highlighting potential risks associated with specific exposures or working conditions, even in the absence of group-level assessments.

[1] The median of the lognormal distribution is estimated by the geometic mean. If the upper limit of the 95%-confidence interval of the geometric mean is below the POBEL, 95% confident that half of the SEG population is below the point of departure.

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