시험관 생물검사를 활용한 먹는물의 영향기반 수질 평가 및 정량한계 기준 제안

Effect-Based Water Quality Assessment of Drinking Water Using *In Vitro* Bioassays and Proposal of limit of quantification Criteria

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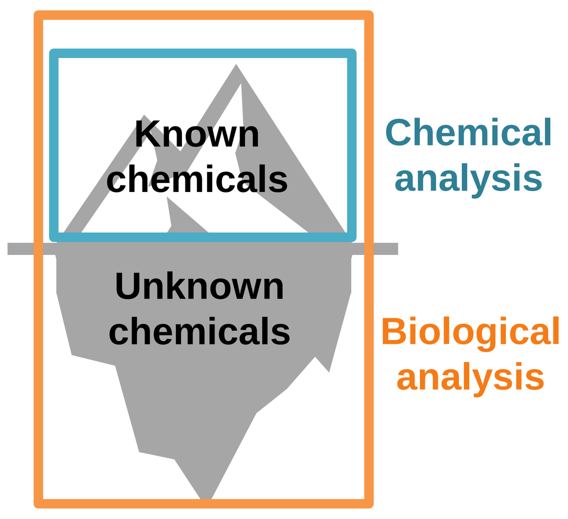
Introduction

In vitro bioassay



Quantification in *in vitro* bioassay

- ❖ Chemical analysis uses direct and standardized linear calibration for quantification.
- ❖ In contrast, in vitro bioassay responses are measured as % effect—indicating the response relative to a maximal effect—or as induction ratio, which reflects the fold change compared to a negative control.
- ❖ These responses are then converted into bioanalytical equivalent concentrations (BEQs) by referencing the dose–response curve of a known reference compound.
- ❖ This quantification is indirect and assay-dependent, influenced by factors such as assay condition.

Quality assurance & quality control (QA/QC) of *in vitro* bioassay

Problem

Reproducibility crisis of *in vitro* bioassay
- Indirect and assay-dependent quantification
- Excessive sample enrichment may cause cytotoxicity, complicating interpretation.
- Lack of reproducibility, reliability and standardization across different laboratories

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***In vitro* bioassay for drinking water**
- Drinking water contains trace-level contaminants, which often result in subtle biological responses.
- These low-level signals can be misinterpreted as false positives in bioassay-based monitoring.

Solution

Addressing through QA/QC
- Accuracy: closeness to true value
- Precision: consistency in results
- Matrix interference: impact of sample composition
- **Sensitivity:** ability to detect low-level response (limit of detection; LOD, limit of quantification; LOQ)

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Research Focus: Analytical sensitivity (LOQ)
- Among QA/QC elements, LOQ is essential for interpreting low-level effects in drinking water.
- A clearly defined LOQ helps prevent overestimation of weak responses and improves the reliability of bioassay results.

Research Objectives

❖To develop a reliable strategy for setting appropriate LOQs in *in vitro* bioassays to ensure accurate interpretation of low-level biological effects in drinking water.

Methods

Method for determining LOQ

Analytically derived values

Bioassay's LOQ
- Assay's detection threshold.
- A range of literature-reported methods was applied to calculate the bioassay's LOQ.

⬇ **Dividing by REF value**

Sample-specific LOQ
- Calculated as bioassay's LOQ / Relative Enrichment Factor (REF; 100 for this study)
- To reflect the sample enrichment

Measured threshold

Laboratory blank
- BEQ values of ultrapure (UP) water
- Representing a practical threshold under laboratory condition.

Method for determining bioassay's LOQ

Table 1. Literature methods for determining bioassay's LOQ determination

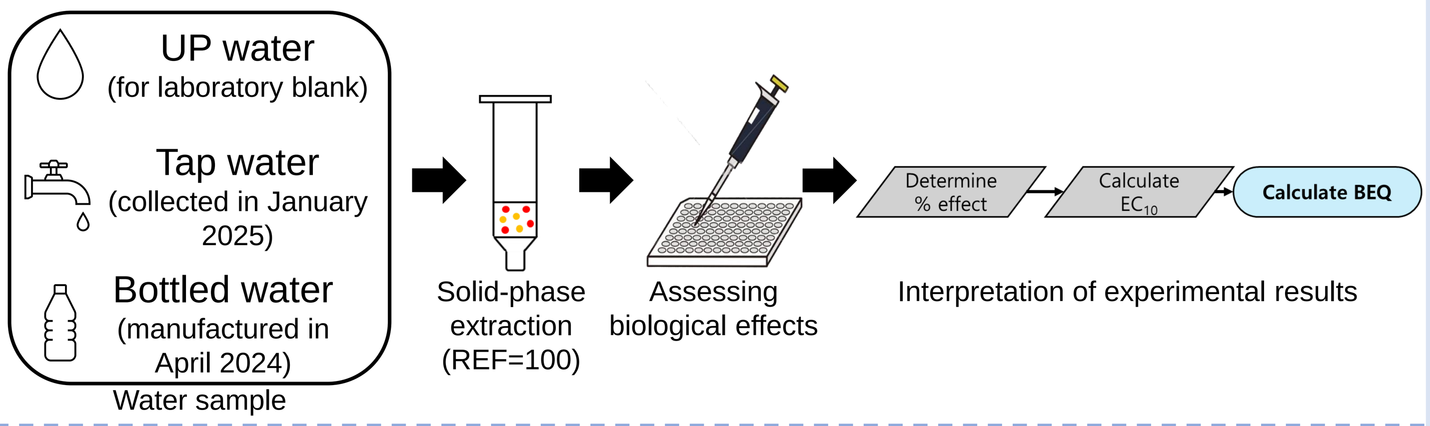
Method for determining bioassay's LOQ	Classification
The lowest reference compound concentration with a coefficient of variation (CV) below 30%.	CV-based
Reference compound concentration causing 10% effect (EC_{10})	EC_{10}-based
LOQ = Average of the blank or solvent control +10×standard deviation (SD) of solvent control	SD-based
LOQ = Average of the solvent control + 3×SD of solvent control	

CALUX used in this research

Table 2. CALUX assays, endpoints, and reference compounds used in this study

CALUX	End Point	Reference compound
ERα	Estrogenicity	17β-estradiol (E2)
PAH	Endocrine system disruption	Benzo[a]pyrene (B[a]P)
PXR	Liver metabolism	Nicardipine (Nic)
p53	Genotoxicity	Actinomycin D (Act)
Nrf2	Oxidative stress response	Curcumin (Cur)

Samples and experimental scheme



Data analysis

- ❖ BEQs express the effect of a sample as the concentration of a reference compound producing the same response.
- ❖ BEQ calculation requires the effect concentration (EC), indicating the concentration that triggers a specific biological effect. The reference compound is a known substance with established biological activity.

For ERα, PAH, PXR CALUX

$$BEQ = \frac{EC_{10}(\text{reference compound})}{EC_{10}(\text{Sample})}$$

For p53, Nrf2 CALUX

$$BEQ = \frac{EC_{IR1.5}(\text{reference compound})}{EC_{IR1.5}(\text{Sample})}$$

Results & Discussion

Determining bioassay's LOQ

Table 3. Bioassay's LOQ values calculated according to methods reported in the literature. Parentheses indicate the corresponding % effect or induction ratio.

Classification	Method for determining bioassay's LOQ	Bioassay's LOQ ERα (ng/L)	PAH (ng/L)	PXR (μg/L)	p53 (μg/L)	Nrf2 (μg/L)
CV-based	Lowest tested dose showing CV under 30%	0.17 (6.3%)	N/A	140 (13%)	N/A	368 (1.04)
EC_{10}-based	Concentration causing 10% effect (EC_{10})	0.43 (10%)	960 (10%)	91 (10%)	N/A	
SD-based	LOQ = Average of the solvent control +10×SD	0.050 (1.4%)	300 (3.3%)	180 (18%)	698 (2.14)	2120 (1.43)
	LOQ = Average of the solvent control + 3×SD	0.010 (0.43%)	100 (0.99%)	130 (12%)	195 (1.34)	1000 (1.13)

- → LOQ methods were selected based on the corresponding % effect or induction ratio, excluding those yielding N/A values.
- → To align with BEQ calculations (EC_{10} or $ECIR_{1.5}$), methods below these thresholds were prioritized.
  - For % effect endpoints: average of the solvent control + 10×SD
  - For induction ratio endpoints: average of the solvent control + 3×SD
- → These approaches were also the most reported in the literature.
- → These bioassay's LOQs were divided by 100 (REF value) to derive sample-specific LOQ

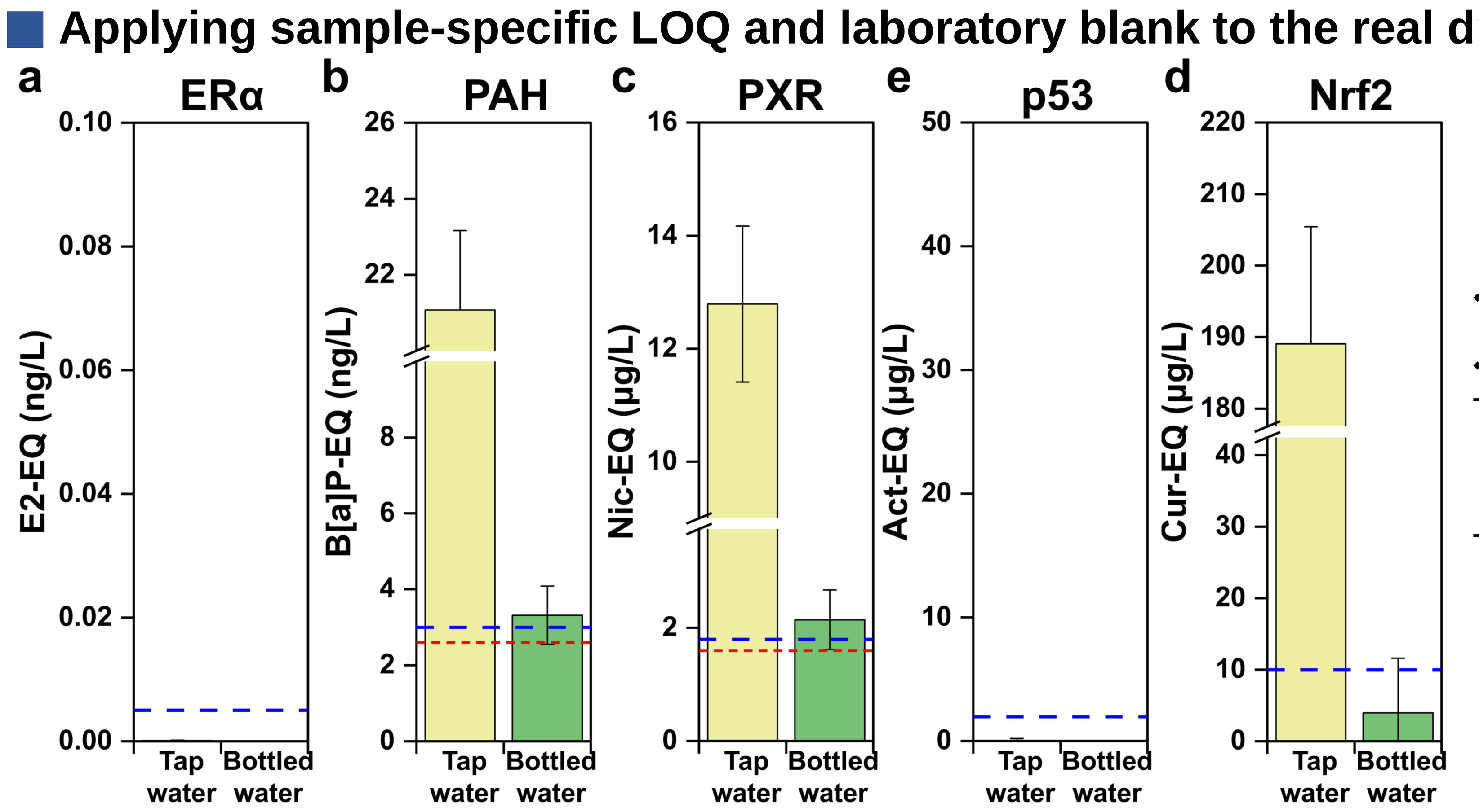
Applicable LOQs of each endpoints

Table 4. LOQ values for each endpoint calculated by using three different criteria.

Method for LOQ	ERα (ng/L)	PAH (ng/L)	PXR (μg/L)	p53 (μg/L)	Nrf2 (μg/L)
Sample-specific LOQ	0.0005	3	1.8	1.95	10
Laboratory blank	0.000008	2.6	1.6	N/A	0.015

- → For ERα, PAH, and PXR CALUX assays, the sample-specific LOQs were comparable to the laboratory blank, with no statistically significant differences observed (p > 0.05).
- → In contrast, the sample-specific LOQs for p53 and Nrf2 CALUX assays were much higher than their laboratory blank.

Applying sample-specific LOQ and laboratory blank to the real drinking water samples



- ❖ Blue dashed line: sample-specific LOQ
- ❖ Red dashed line: laboratory blank
- → Laboratory blanks for ERα and Nrf2 CALUX assays are not shown in the graph, as their values were below the visible range.
- → The laboratory blank for p53 CALUX is also not shown, as its value was not available.

Figure. CALUX assay results for each sample across five endpoints, expressed as BEQs (n = 3). Error bars represent SD. The blue dashed line indicates the sample-specific LOQ. The red dashed line indicates the laboratory blank. (a) ERα CALUX, (b) PAH CALUX, (c) PXR CALUX, (d) p53 CALUX, and (e) Nrf2 CALUX. Notably, significant BEQs were observed only in tap water for PXR, PAH and Nrf2 endpoints.

Hormone activity

❖ **ERα**

→ All samples: BEQ < LOQ, no significant biological activity

Xenobiotic metabolism

❖ **PAH**

- → Tap water: highest BEQ (190 μg/L), activity only observed in tap water
- → BEQ of tap water exceeded effect-based trigger (EBT) value (6.2 ng/L) by ~4 times
- → Bottled water: no significant difference with laboratory blank (p > 0.05)

❖ **PXR**

- → Tap water: highest BEQ (13 μg/L), but lower than EBT value (154 μg/L)
- → In contrast, bottled water showed similar BEQ to laboratory blank (p>0.05) → no significant biological activity

Adaptive stress response

❖ **p53**

→ All samples: BEQ < LOQ, no significant biological activity

❖ **Nrf2**

- → Significant BEQ observed in tap water (190 μg/L)
- → This suggests the potential presence of oxidative stress-inducing compounds formed during drinking water treatment
- → BEQ of tap water exceeded EBT value (3.2 μg/L) by ~60 times
- → The BEQ of bottled water (4 μg/L) exceeded the laboratory blank (0.015 μg/L) but was below the sample-specific LOQ (10 μg/L), indicating that the activity is unlikely to be biologically meaningful.

Sample-Specific LOQ: Recommended LOQ approach for bioassays

- → Offers a conservative threshold to prevent overestimation
- → In some endpoints, values aligned with laboratory blanks and BEQs of bottled water—practical and realistic in actual sample conditions

Conclusion

- ❖ This study proposed three methods for determining the limit of quantification (LOQ) to ensure the reliable application of in vitro bioassays and evaluated their validity.
- ❖ In particular, the bioassay's LOQ—calculated as the average of the solvent control plus ten times the standard deviation—and the sample-specific LOQ—obtained by dividing the bioassay's LOQ by a relative enrichment factor (REF) of 100—showed values like the laboratory blank of some endpoints. Sample-specific LOQ provides more conservative and practical threshold.
- ❖ In addition, PAH, PXR, and Nrf2 activities in tap water exceeded the LOQ, suggesting potential biological risks. These findings indicate that in vitro bioassays can be effectively used to evaluate drinking water treatment processes.

Acknowledgement

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