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Derivation and Application of Effect-Based Trigger Values for Persistent and Non-persistent Aryl Hydrocarbon Receptor-Mediated Activities in Coastal Sediment

Abstract

This study establishes sediment effect-based trigger (EBT) values for aryl hydrocarbon receptor (AhR)-mediated activity using the H4IIE-luc assay, distinguishing between non-persistent and persistent agonists. Sediment-specific EBTs were derived for AhR-mediated activity by translating sediment quality guidelines (SQGs) into bioanalytical equivalent concentrations using relative effect potencies (REPs) determined in the H4IIE-luc assay. Compound-specific bioanalytical equivalents (BEQs) were calculated using SQGs and REPs. The BEQs for persistent and non-persistent AhR activators showed log-normal distributions, and their 50th percentiles were assigned as EBT-BEQchem. The EBT-BEQbio values were derived from EBT-BEQchem by multiplying with mixture factor (MF) derived from potency balance analysis to account for bioassay responses unexplained by quantified target compounds. The resulting EBT-BEQbio for non-persistent AhR-mediated activity dominated by polycyclic aromatic hydrocarbons (PAHs) was 470 ngBaP gdm−1 [expressed as benzo[a]pyrene equivalents (BaP-EQ)]. The EBT-BEQbio for persistent AhR-mediated activity was 7.4 pgTCDD gdm−1 [expressed as 2,3,7,8-tetrachlorodibenzo-p-dioxin equivalents (TCDD-EQ)], by considering polychlorinated dibenzo-p-dioxins and dibenzofurans, coplanar polychlorinated biphenyls, polybrominated diphenyl ethers, and PAHs. Comparisons between bioassay-derived bioanalytical equivalents (BEQbio) for Busan Bay sediments and previously published datasets revealed that BaP-EQbio and TCDD-EQbio frequently exceeded the derived EBTs for non-persistent and persistent AhR-mediated mixtures. The EBT-BEQchem values were exceeded in all cases by the predicted mixture effects of quantified chemicals BEQchem. Benchmarking BEQbio values from previous case studies against EBT-BEQbio showed that sediments from industrialized coastal and riverine regions frequently exceeded EBT-BEQbio thresholds, whereas remote or open-water sites generally remained below them. These findings demonstrate that sediment-specific AhR EBTs incorporating mixture correction provide environmentally relevant benchmarks for sediment quality assessment. The proposed framework enhances the interpretation of bioassay data and supports an integrated sediment quality assessment combining bioanalytical tools with chemical analysis.

Keywords: Effect-Based Monitoring; H4IIE-luc Bioassay; Aryl Hydrocarbon Receptor; Polycyclic Aromatic Hydrocarbons; Sediment.

1. INTRODUCTION

Various chemical substances are released into aquatic environments from point and non-point sources and accumulate in sediments through biogeochemical processes (Neale et al., 2023). Sediment assessments have traditionally relied on chemical analysis combined with sediment quality guidelines (SQGs) or environmental quality standards, which set concentration-based thresholds for individual compounds (Zhao et al., 2024). However, chemical monitoring often fails to detect chemicals present at low concentrations and does not capture mixture effects, transformation products, or unknown compounds, potentially leading to an underestimation of ecological risks (Zhao et al., 2024). To address these limitations, effect-based monitoring (EBM) was introduced to evaluate the biological effects of environmental samples, providing a more holistic view of environmental risk (Escher et al., 2018; Neale et al., 2023). EBM employs in vitro and in vivo bioassays to detect and quantify effects of both known and unknown toxicants, but does not resolve the contributions of individual mixture components (Escher and Neale, 2021). Within EBM, in vitro cell-based assays are rapid, cost-effective, and suitable for targeting specific pathways, making them practical tools for monitoring (Escher and Neale, 2021). Effect-based trigger (EBT) values are used to distinguish between acceptable and unacceptable biological responses (Hong et al., 2023). Although EBTs have been developed for wastewater and surface water, EBTs for sediments remain limited, despite the importance of sediments as sinks for persistent hydrophobic pollutants and as exposure sources for benthic organisms (Neale et al., 2023).

The aryl hydrocarbon receptor (AhR) mediates diverse toxic effects, including carcinogenicity and developmental toxicity, and is activated by ligands such as polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs), coplanar polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), and polycyclic aromatic hydrocarbons (PAHs) (Eichbaum et al., 2014; Hong et al., 2016). Unlike endocrine disruptors, AhR-mediated activity is typically driven by complex mixtures, and measured chemical concentrations rarely explain the observed activity, making bioassay-based evaluation essential (Cha et al., 2019; Escher et al., 2018; Gwak et al., 2022; Kim et al., 2019; Lee et al., 2020). In this study, we differentiated between non-persistent and persistent AhR agonists based on their biotransformation kinetics in the H4IIE-luc assay. PAHs were considered non-persistent because they undergo rapid CYP1A-mediated metabolism and exhibit early-peak AhR activation (typically within 4–6 h of exposure), whereas PCDD/Fs, coplanar PCBs, and PBDEs were classified as persistent due to their resistance to oxidative metabolism and sustained activation at longer exposure durations (typically 24–72 h) (Bock and Köhle, 2006; Larsson et al., 2014). This distinction reflects differences in assay-based persistence of AhR activation rather than environmental degradation and enables discrimination between short- and long-term AhR-mediated responses in sediments, thereby enhancing the ecological relevance of sediment-specific AhR EBTs (Eichbaum et al., 2014; Kim et al., 2019). Non-persistent AhR activity is generally associated with rapidly metabolized compounds such as PAHs, which may reflect relatively recent or ongoing contamination inputs (Otte et al., 2013). In contrast, persistent AhR activity is more commonly associated with highly hydrophobic and

recalcitrant compounds such as PCDD/Fs, coplanar PCBs, and PBDEs, which can remain in sediments for extended periods and contribute to long-term biological effects (Qiu and Davis, 2004). Distinguishing between these activity types may improve the interpretation of contamination sources and support the prioritization of sites with greater potential for long-term ecological impacts. Based on this mechanistic distinction, the EBT values developed in this study represent mode-of-action-specific thresholds for AhR-mediated activity, reflecting only the biological responses measured by the H4IIE-luc assay. These AhR-specific EBTs are not intended to indicate overall organic contamination pressure but rather provide criteria for interpreting AhR-mediated activity in complex sediment mixtures.

This study aimed to derive sediment-specific EBTs for both non-persistent and persistent AhR-mediated activity. In previous studies, sediment EBTs were often extrapolated directly from water-based EBTs using the organic carbon (OC)-water partition coefficient of reference compounds (Escher et al., 2025). However, this approach may not fully account for sediment-specific characteristics, such as differences in contaminant bioavailability and the combined effects of complex chemical mixtures. To address these limitations, we applied a bioanalytical equivalent (BEQ)-based framework in which BEQ values were derived individually for known chemicals with available sediment quality guidelines (SQGs) and relative effect potencies (REPs) derived from the H4IIE-luc bioassay. Specifically, this study (i) calculated BEQSQG for non-persistent and persistent AhR agonists using SQGs and REPs, (ii) established EBT for chemical analysis-based equivalent values (BEQchem) and EBT for bioassay-based equivalent concentrations (BEQbio) considering a mixture factor (MF), (iii) compared sediment-derived EBTs with previously proposed water-based EBTs after partitioning correction, and (iv) evaluated their applicability and environmental relevance using sediment extracts from Busan Bay and published case studies across diverse environmental regions.

2. METHODOLOGY

2.1. Derivation of compound-specific BEQs from SQGs

In this study, BEQSQG, i values were calculated by multiplying REPi by the corresponding SQGi for each chemical i with an SQG. The SQGs applied in this study include threshold effect levels (TELs), representing concentrations below which adverse biological effects are rarely expected, and probable effect levels (PELs), above which adverse effects are frequently observed (Eq. 1 and Table 1) (Ingersoll et al., 1996; CCME, 1999).

$$BEQSQG, i = SQGi \times REPi \quad \text{(Eq. 1)}$$

TELs of six AhR agonists were used as SQG proxies for non-persistent AhR-mediated activity, whereas PELs of 23 AhR agonists were applied as benchmark concentrations for persistent AhR-mediated activity within the EBT derivation framework (CCME, 1999) (Table 1). SQGs for PCDD/Fs are not defined at the individual congener level but are instead expressed as toxicity-equivalent (TEQ)-based thresholds and PELs that integrate the relative toxicities of all 2,3,7,8-substituted congeners. REPs were compiled exclusively from studies employing the H4IIE-luc bioassay, using benzo[a]pyrene (BaP) as the reference compound for non-persistent AhR-mediated activity after 4 h of exposure and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) as the reference compound for persistent activity after 72 h of exposure (Eq. 2).

$$REP_i = \frac{EC_{50,\,ref}}{EC_{50,\,i}} \quad \text{(Eq. 2)}$$

2.2. Derivation of AhR EBTs in sediments

Previous studies have proposed eight options for deriving EBTs from bioanalytical data (Escher et al., 2018). These frameworks were largely developed and demonstrated for relatively well-characterized endpoints in surface waters, particularly estrogen receptor-mediated activity, where biological responses are often dominated by a limited number of potent and well-identified compounds (e.g., 17β-estradiol and synthetic estrogens). Under such conditions, chemical analysis can explain a substantial proportion of the observed bioassay response, and EBT derivation can reasonably rely on quantified target compounds. In contrast, AhR-mediated activity in sediments is typically driven by complex mixtures of diverse AhR agonists. Because many contributing compounds remain unidentified or are not routinely monitored, chemical analysis often fails to account for the total observed bioassay response (Escher and Neale, 2021).

Accordingly, in this study, EBT-BEQchem and EBT-BEQbio values were derived to distinguish between thresholds based on chemical analysis-based equivalent values (BEQchem) and thresholds reflecting bioassay-based equivalent values (BEQbio). First, EBT-BEQchem was calculated by plotting the distributions of logBEQSQG for individual compounds and assessing their log-normality. Once log-normality of the distribution was confirmed, the 50th percentile of the log-normal distribution was used as EBT-BEQchem. For simplicity, one can also use the mean of the logBEQ (Eq. 3). This threshold assumes that multiple AhR agonists contribute additively to the overall mixture effect. For example, even if the concentration of one AhR agonist decreases, the overall AhR activity may remain similar if another AhR agonist is present at a higher concentration. Thus, the EBT reflects the combined activity of the chemical mixture rather than the effect of a single compound.

$$\text{EBT-BEQchem} = 10^{\left(\sum_{i=1}^{n} \frac{\log BEQ_{SQG,\,i}}{n}\right)} \quad \text{(Eq. 3)}$$

Given that AhR-mediated activity in sediments is frequently underexplained by chemical analysis, an MF was introduced to account for bioassay responses not captured by quantified target compounds. The MF was derived from comparisons between BEQbio, i and BEQchem, i across individual compounds with available SQGs. Specifically, the median fraction of mixture effect explained by the quantified chemicals, i.e., the ratio of BEQchem,i to BEQbio,i was used (Eqs. 4 and 5), thereby accounting for mixture effects attributable to both quantified and unquantified AhR agonists. Finally, the EBT-BEQbio was derived by multiplying the EBT-BEQchem by the MF (Eq. 6).

$$\text{Median contribution} = \text{median}\left(\frac{BEQ_{chem,\,i}}{BEQ_{bio,\,i}}\right) \quad \text{(Eq. 4)}$$

$$MF = \frac{1}{\text{Median contribution}} \quad \text{(Eq. 5)}$$

$$\text{EBT-BEQ}_{bio} = MF \times 10^{\left(\sum_{i=1}^{n} \frac{\log BEQ_{SQG,\,i}}{n}\right)} \quad \text{(Eq. 6)}$$

Intuitively, one would divide by the MF if we think of a risk cup approach (Backhaus et al., 2025), where incrementally adding more chemicals would eventually lead to an overflow of the risk cup. This would mean that any of the known chemicals could be replaced by equi-effective quantity of unknown chemicals. However, we can also argue that the unknown chemicals may be natural ligands or very short-lived ligands and that we accept the risk cup to overflow by the mixture of unknown chemicals if that does not exceed the ratio between the mixture effects of known and unknown chemicals. The previous use of a mixture factor was in the same direction, only the MF was not derived from iceberg results but rather from expert judgment (Escher et al., 2018). An MF value of 1 would indicate that analytically identified compounds fully explain the observed AhR-mediated bioassay response, whereas MF values greater than 1 indicate that acceptable bioassay responses exceed those predicted from quantified chemicals alone. Higher MF values reflect a greater contribution from unidentified or unquantified AhR agonists. The MF is a multiplicative correction factor defined as the inverse of the fraction explained, and therefore, it is not constrained by an upper limit. The final sediment-specific AhR EBT-BEQbio values were obtained by applying

the MF to the EBT-BEQchem, separately for non-persistent and persistent AhR-mediated activities.

2.3. Conversion of WQG-based EBTs to sediment-equivalent concentrations

To enable a quantitative comparison between the AhR EBTs derived in the present study and previously proposed EBTs for surface waters, water quality guidelines (WQG)-based EBTs reported in earlier studies were converted to sediment-equivalent concentrations (EBT-EQsediment) using OC-water partition coefficients (KOC) (Eq. 7). This conversion accounts for the preferential partitioning of hydrophobic AhR agonists to sediment organic matter and allows comparison of EBTs across environmental matrices.

$$\text{EBT-EQsediment} = \text{WQG-based EBT} \times \text{KOC} \times \text{fOC} \quad \text{(Eq. 7)}$$

BaP and TCDD were used as reference compounds for non-persistent and persistent AhR-mediated activities, respectively, to ensure consistency with the bioassay calibration and REP framework applied in this study. Partitioning parameters for BaP (log KOC = 4.8) and TCDD (log KOC = 7.0), which are estimated values obtained from the ChemSpider database (https://www.chemspider.com), were used for the conversion of previously reported water-based EBTs (Escher et al., 2015, 2018; Escher and Neale, 2021; van der Oost et al., 2017). The fraction of organic carbon (fOC) value was assumed to be 0.01 (1% OC in sediment). The converted sediment-equivalent EBTs were used solely for comparative purposes and were subsequently compared with the AhR EBTs derived in this study.

2.4. Application of AhR EBTs to sediments from Busan Bay

2.4.1. Sample collection and extraction

In 2022, forty-one surface sediment samples were collected from North Harbor (N1–20, n = 20), South Harbor (S1–12, n = 12), and Gamcheon Harbor (G1–9, n = 9) in Busan Bay, South Korea (Figure S1). Samples were stored in glass bottles, kept on ice during transport, and stored at –20 °C until analysis. The sediments were freeze-dried, sieved through a 1-mm mesh, and homogenized. Twenty grams of sediment were extracted with dichloromethane (DCM; Honeywell, Charlotte, NC) using an accelerated solvent extractor (ASE 350, Dionex, Thermo Scientific, Salt Lake, UT). The extraction procedure was based on previously validated methods for sediment-associated organic contaminants and AhR agonists, with reported extraction efficiencies ranging from approximately 70–100% for representative AhR agonists (Cha et al., 2019; Hong et al., 2016; Lee

et al., 2020). Elemental sulfur was removed from the raw extracts (REs) by adding activated copper and allowing the mixture to react for approximately 1 h. REs were then concentrated to 2 mL using a rotary evaporator, followed by nitrogen blowdown. The REs were split: 1.0 mL for chemical analysis and 1.0 mL reserved for bioassays. For bioassays, REs were solvent-exchanged to dimethyl sulfoxide (DMSO; Sigma-Aldrich, St. Louis, MO).

2.4.2. Target chemical analysis

Target analysis was conducted in this study for 7 legacy PAHs and 14 emerging PAHs using gas chromatography (GC; Agilent 7890B, Agilent Technologies, Santa Clara, CA) coupled with a 5977B mass selective detector (MSD; Agilent Technologies). Instrumental conditions for the GC-MSD are provided in Table S1. Concentrations of PCDD/Fs, PCBs, and PBDEs in sediments were obtained from the National Marine Environment Monitoring Program of Korea (https://www.meis.go.kr/portal/main.do).

2.4.3. H4IIE-luc in vitro bioassay

AhR activation induced by sediment organic extracts was measured using H4IIE-luc recombinant cells (Table S2). Cells were detached using trypsin and seeded into 96-well plates at a density of 7.0 × 104 cells mL−1 (250 µL per well). After incubation at 37 °C with 5% CO2 for 24 h, cells were exposed to controls and REs. BaP was used as the positive control for 4-h exposure, and TCDD was used for 72-h exposure. Negative controls consisted of 0.1% DMSO in culture medium. Maximum concentrations of BaP and TCDD were set at 50 nM (= 100% BaPmax) and 320 pM (= 100% TCDDmax), respectively. Test compounds were serially diluted (1:3) to yield six concentrations. REs were tested in the bioassay using six serial dilutions (1:3), with the highest test concentration corresponding to 10 g sediment mL–1. After exposure, the culture medium was removed, and 75 µL of Dulbecco's phosphate-buffered saline and 75 µL of Steady-Glo® Luciferase Assay Reagent were added to each well, followed by incubation for 15 min. Luciferase activity was measured using a Victor X3 multilabel plate reader (PerkinElmer, Waltham, MA), and bioassay responses were expressed as percentages of the maximum BaP or TCDD response. In this study, sediment extracts and positive controls were exposed to H4IIE-luc cells for 4 h to assess non-persistent AhR-mediated activity and for 72 h to assess persistent AhR-mediated activity.

2.4.4. Potency balance analysis

Potency balance analysis was conducted by comparing bioanalytical equivalent concentrations derived from chemical analysis (BEQchem) and measured BEQbio for the same samples to assess

the contribution of quantified AhR agonists to total AhR-mediated activity. BEQchem was calculated as the sum of analytically measured concentrations of individual AhR agonists in the same sediment samples and their corresponding REPs (see Eq. 8). The REPs for persistent and non-persistent target AhR agonists were compiled from previous studies and are listed in Tables S3 and S4 (Behnisch et al. 2003; Cha et al., 2019; Gwak et al., 2022; Kim et al., 2019; Lee et al., 2015, 2022; Takigami et al., 2005). Bioassay-derived BEQbio (expressed as ngBaP gdm−1 for non-persistent activity and pgTCDD gdm−1 for persistent activity) was calculated from dose-response relationships using the corresponding reference compound. Similarly, BEQchem (ngBaP gdm−1 and pgTCDD gdm−1) values were calculated using the effect concentration (EC50) of the reference compound and the sediment extracts (Eq. 9).

$$BEQ_{chem} = \sum_{i=1}^{n} C_i \times REP_i \quad \text{(Eq. 8)}$$

$$BEQ_{bio} = \frac{EC_{50,\ ref}}{EC_{50,\ sediment\ extract}} \quad \text{(Eq. 9)}$$

2.5. Compilation of AhR activation data from sediment case studies

Case studies that applied bioassays to sediment extracts were collected from the peer-reviewed literature. BEQbio and BEQchem values from 10 previous studies (54 BaP-EQbio and 36 BaP-EQchem) were compiled for non-persistent AhR-mediated activity (Cha et al., 2019; David et al., 2010; Gwak et al., 2022, 2024a, 2024b; Kinani et al., 2010; Kim et al., 2019; Louiz et al., 2008; Lee et al., 2020; Vondráček et al., 2001) (Table S5). In addition, data from 14 studies (190 paired TCDD-EQbio and TCDD-EQchem values) were collected for persistent AhR-mediated activity (Hollert et al., 2002; Hong et al., 2012; Khim et al., 2001; Koh et al., 2004, 2005, 2006; Lee et al., 2017, 2022; Luo et al., 2009; Nieuwoudt et al., 2009; Qiao et al., 2006; Song et al., 2006; Suares Rocha et al., 2002; Takigami et al., 2005) (Table S6).

3. RESULTS AND DISCUSSION

3.1. Derivation of EBT for non-persistent AhR agonists in sediments

An EBT for non-persistent AhR agonists in sediments was derived using SQG-based TELs of individual compounds. The probit plot of log-transformed BEQSQG values showed an approximately linear relationship (R2 = 0.9862) (Figure 1A), supporting the assumption of a log-normal distribution. The distribution is unimodal and moderately spread, indicating that most

individual compounds yield BEQSQG values within a comparable range when expressed as BaP-EQ. The 50th percentile of the logBEQSQG distribution was at BEQSQG of 12 ngBaP gdm−1 (EBT-BaP-EQchem).

In order to derive the MF, datasets linking chemical analysis and bioassay responses were examined (Cha et al., 2019; David et al., 2010; Gwak et al., 2022, 2024a, 2024b; Kinani et al., 2010; Kim et al., 2019; Louiz et al., 2008; Lee et al., 2020; Vondráček et al., 2001). The six PAHs for which SQGs were available accounted for a median of only 2.6% (n = 76) of the non-persistent AhR-mediated activity measured after 4 h of exposure (Figure 1B). This low explanatory power indicates that most of the observed bioassay responses cannot be attributed solely to the known AhR agonists. Unexplained activity arises from additional low-potency AhR agonists, unidentified or nontarget compounds, transformation products, and potential mixture effects that are not captured by chemical analysis.

Based on the median contribution (2.6% or 0.026), an MF of 38 was derived (Figure 1B). Thus, EBT-BaP-EQbio for non-persistent AhR agonists in sediments was 470 ngBaP gdm−1. For comparison, substantially higher MF values have been applied in water-based assessments. In surface waters, numerous low-potency AhR agonists typically account for less than 1% of total AhR-mediated activity, necessitating a larger extrapolation factor when deriving water-based EBTs (Escher et al., 2018). Accordingly, MF values of up to 100 have previously been applied to water matrices. The lower MF derived for sediments in the present study reflects the generally higher contribution of quantified hydrophobic AhR agonists in this matrix. Nevertheless, MF values are not fixed and may vary across environmental scenarios. Such variability is comparable to other sources of uncertainty in effect-based assessments, including incomplete coverage of target compounds, the presence of transformation products, variability in REP values, and differences in bioavailability across environmental matrices (Escher et al., 2018).

To further contextualize the derived sediment-based EBT, comparisons were made with previously proposed WQG-based EBTs for non-persistent AhR agonists. A WQG-based EBT range of 62–1800 ngBaP L−1 reported in earlier studies was converted to sediment-equivalent concentrations using the KOC of BaP and an assumed fOC of 0.01 (Escher et al., 2018; Escher and Neale, 2021; van der Oost et al., 2017). This conversion yielded values ranging from 42 to 1200 ngBaP gdm−1. These values align well with the proposed independent derivation from SQG of 486 ngBaP gdm−1, indicating general consistency between sediment-based and water-based EBTs after accounting for sediment-water partitioning.

3.2. Derivation of EBT for persistent AhR agonists in sediments

An EBT for persistent AhR agonists in sediments was derived using the same general approach as for non-persistent AhR agonists, with adjustments reflecting differences in potency, persistence,

and bioassay response characteristics. Persistent AhR-mediated activity was evaluated using REPs measured in the H4IIE-luc assay after 72 h of exposure, which better capture the sustained activation typical of highly potent and persistent AhR agonists, such as dioxin-like compounds.

Compound-specific BEQSQG values for persistent AhR agonists were calculated based on SQG concentrations and corresponding REPs and expressed as TCDD-equivalent concentrations (Table 1). The distribution of the resulting BEQSQG values is shown in Figure 1C. The probit plot of log-transformed BEQSQG values showed an approximately linear relationship ($R^2 = 0.9346$) (Figure 1C), indicating that the BEQSQG are log-normally distributed. The 50th percentile of the distribution of logBEQSQG was at EBT-TCDD-EQchem of 0.72 pgTCDD gdm^{-1}.

To assess the contribution of target compounds, chemical and bioanalytical datasets were examined. Quantified persistent AhR agonists explained a median of 9.7% of the total AhR-mediated activity (n = 194) following 72 h of exposure (Hollert et al., 2002; Hong et al., 2012; Khim et al., 2001; Koh et al., 2004, 2005, 2006; Lee et al., 2017, 2022; Luo et al., 2009; Nieuwoudt et al., 2009; Qiao et al., 2006; Song et al., 2006; Suares Rocha et al., 2002; Takigami et al., 2005) (Figure 1D). Although this contribution is substantially higher than that observed for non-persistent AhR agonists, it still indicates that a considerable fraction of the bioassay response cannot be explained by the quantified target AhR agonists alone. Sediments contain complex mixtures of known and unidentified dioxin-like compounds, and AhR-mediated responses are generally assumed to follow concentration addition. Therefore, unidentified dioxin-like compounds may substantially contribute to the unexplained biological activity observed in sediments. To account for the fraction of biological activity not explained by the target compounds, an MF of 10 was applied. Application of the MF to the 50th percentile of logBEQSQG value resulted in a corrected EBT-TCDD-EQbio of 7.4 pgTCDD gdm^{-1}, which provides a more realistic estimate of mixture-driven persistent AhR-mediated activity in sediments.

The sediment-derived EBT was compared with previously reported WQG-based EBTs for persistent AhR agonists. A WQG-based EBT of 50 pgTCDD L^{-1} reported in previous studies was converted to a sediment-equivalent concentration using the KOC of TCDD and an assumed fOC of 0.01, resulting in a corresponding value of 32 pgTCDD gdm^{-1}. The converted value was within a factor of 4 of the sediment-specific EBT derived in the present study (7.4 pgTCDD gdm^{-1}), supporting the applicability of the proposed EBT framework across environmental matrices and the robustness of EBT derivation despite limited input data.

3.3. Application of AhR EBTs to sediments of Busan Bay

The EBTs for non-persistent and persistent AhR-mediated activity were compared with BEQbio and BEQchem values from 41 sediment samples collected in Busan Bay (Tables S7 and S8). This comparison provides insight into the extent to which chemically quantified AhR agonists explain

observed bioassay responses and evaluates the adequacy of conventional chemical monitoring for assessing AhR-related risks in complex sediment matrices.

For non-persistent AhR-mediated activity, BaP-EQchem in sediments exceeded EBT-BaP-EQchem (12 ngBaP gdm−1) at all sites. The BaP-EQbio exceeded EBT-BaP-EQbio (470 ngBaP gdm−1) at only 37 of the 41 sites (Figure 2A). Notably, at four of 41 sites, BaP-EQchem exceeded the threshold, while BaP-EQbio did not, demonstrating a discrepancy between chemical analysis-based predictions and actual bioassay responses (Figure 2B).

Across all 41 sediment samples, quantified non-persistent AhR agonists explained 1.2–>100% of the measured BaP-EQbio (Figure 2B). This low explained fraction suggests that a substantial portion of the observed AhR activity is attributable to unidentified or unquantified AhR agonists not included in current target analytical lists. These findings highlight the limitations of target chemical analysis for assessing AhR-mediated activity in complex environmental matrices such as sediments. Contribution analysis of individual compounds revealed that benzo[b]anthracene (BbA) was the major contributor to non-persistent AhR-mediated BaP-EQchem, accounting for a mean of 41% of the activity explained by quantified agonists (Figure 2C). This dominance reflects both the relatively elevated concentrations of BbA in Busan Bay sediments and its higher REP (11) compared with BaP under short-term exposure conditions (4 h) in the H4IIE-luc assay.

A similar trend was observed for persistent AhR-mediated activity as for non-persistent AhR activity. TCDD-EQchem in sediments exceeded EBT-TCDD-EQchem (0.72 pgTCDD gdm−1) at all sites. Among the 41 sediment samples, only four showed more than 50% of the TCDDmax response, allowing EC50 values to be calculated. TCDD-EQbio exceeded EBT-TCDD-EQbio (7.4 pgTCDD gdm−1) at three of these four sites (Figure 2D). These results again demonstrate the discrepancy between chemical analysis-based and bioassay-based assessments. At these sites, quantified AhR agonists explained 41–>100% of the TCDD-EQbio (Figure 2E). BbA also showed the highest contribution to persistent AhR-mediated activity, accounting for a mean of 59% of the explained TCDD-EQbio (Figure 2F). However, the role of BbA is not straightforward. While BbA exhibits higher AhR potency than BaP under short-term exposure conditions, its REP decreases markedly under prolonged exposure (72 h of exposure), reaching 5.1 × 10–6 relative to TCDD. This substantial decline is consistent with the rapid metabolic degradation of BbA in H4IIE-luc cells (Larsson et al., 2014). Nevertheless, BbA retains intrinsic AhR-binding affinity and, when present at elevated concentrations in sediments, may still contribute to AhR-mediated activity under conditions of sustained or repeated exposure. This finding indicates that compounds typically categorized as non-persistent may still influence longer-term AhR activation when environmental concentrations are sufficiently high.

Overall, non-persistent and persistent AhR-mediated activities observed in Busan Bay sediments were only partially explained by quantified target AhR agonists. The frequent exceedance of EBTs based on BEQbio, coupled with the low contribution of BEQchem, reinforces the value of

bioassays as integrative tools for assessing toxicological potential in complex sediment environments. When BEQbio exceeds the EBT, complementary approaches such as effect-directed analysis (EDA) and nontarget screening (NTS) are warranted to identify the primary drivers of AhR activity. Such an integrated strategy will improve the precision of AhR-related risk assessment and management in complex, multi-source coastal systems such as Busan Bay.

3.4. Comparison of derived EBTs with published sediment AhR activation data

Application of the derived EBT to various regions and environmental conditions revealed distinct differences in the spatial distribution of AhR-mediated activity (Figure 3 and Tables S5 and S6). For non-persistent AhR-mediated activity, BaP-EQchem exceeded EBT-BaP-EQchem (12 ngBaP gdm−1) in 87% of all sites, and BaP-EQbio exceeded EBT-BaP-EQbio (470 ngBaP gdm−1) in 77% of the sites. For persistent AhR-mediated activity (Cha et al., 2019; David et al., 2010; Gwak et al., 2022, 2024a, 2024b; Kinani et al., 2010; Kim et al., 2019; Louiz et al., 2008; Lee et al., 2020; Vondráček et al., 2001), TCDD-EQchem exceeded EBT-TCDD-EQchem (0.72 pgTCDD gdm−1) in 95% of the samples, and TCDD-EQbio exceeded EBT-TCDD-EQbio (7.4 pgTCDD gdm−1) in 92% of the samples (Hollert et al., 2002; Hong et al., 2012; Khim et al., 2001; Koh et al., 2004, 2005, 2006; Lee et al., 2017, 2022; Luo et al., 2009; Nieuwoudt et al., 2009; Qiao et al., 2006; Song et al., 2006; Suares Rocha et al., 2002; Takigami et al., 2005). These results indicate that chemical analysis-based predictions generally have higher exceedance rates than bioassay-based assessments, and suggest that chemical assessments based on simple concentration addition may, in some cases, overestimate actual biological responses. For both non-persistent and persistent AhR-mediated activity, EBT exceedances were primarily observed in sediments located near coastal and industrial areas with dense industrial complexes. This pattern suggests that pollutants discharged from petrochemical industries, shipbuilding facilities, and other heavy industrial sources, as well as urban runoff, contribute collectively to the accumulation of AhR agonists in sediments. These findings indicate that mixture-driven AhR-mediated activity remains an important environmental concern in industrialized regions.

Meanwhile, for non-persistent AhR-mediated activity, no EBT exceedances were observed at any site in the Mediterranean Sea (Louiz et al., 2008). This suggests that AhR-mediated activity remains relatively low in open marine environments distant from direct industrial emission sources. These results indicate that the proposed AhR EBT can distinguish between impacted and relatively uncontaminated environments, reflecting differences in contamination intensity and mixture complexity. Interestingly, in sediments from the Svalbard region of the Arctic, BaP-EQbio of 2800 and 5300 ngBaP gdm−1, exceeded the non-persistent AhR EBT-BaP-EQbio (Gwak et al., 2024a). Although the Arctic is generally considered a remote region with limited direct industrial emissions, accumulation of AhR-active compounds through long-range atmospheric transport has been documented. In addition, some locations have a history of coal mining activity, indicating

potential contributions from local sources. These findings suggest that AhR-mediated activity may be elevated even in remote environments due to global transport of pollutants or legacy contamination. Therefore, bioassay-based assessments provide a useful tool for evaluating sediment quality not only in densely industrialized regions but also in remote environments.

The EBT derived in this study was established based on coastal sediment SQG, but its applicability was also evaluated using river sediment datasets. For non-persistent AhR-mediated activity, the BaP-EQbio exceeded EBT-BaP-EQbio in 12 of 24 river sediment samples (Cha et al., 2019; David et al., 2010; Vondráček et al., 2001), with exceedances primarily observed near industrial or urban areas, indicating that these sources contribute significantly to AhR agonist inputs in freshwater environments. The Morava River in the Czech Republic provides a representative example of the potential management utility of the EBT (Vondráček et al., 2001). AhR-mediated activity exceeded the EBT at all sites before pollutant removal, whereas no exceedances were observed after remediation, demonstrating that the proposed EBT is responsive to changes in contamination levels and may serve as a useful indicator of sediment remediation effectiveness. For persistent AhR-mediated activity, the TCDD-EQbio exceeded EBT-TCDD-EQbio at 67 of 71 river sediment sites, most of which were located near industrial areas (Hollert et al., 2002; Khim et al., 2001; Koh et al., 2005, 2006; Lee et al., 2017; Luo et al., 2009; Nieuwoudt et al., 2009; Suares Rocha et al., 2002). However, because the EBTs presented in this study were derived from coastal sediment SQGs, freshwater-specific thresholds may be required to account for differences in sediment characteristics and contaminant behavior. Freshwater and marine sediments differ in salinity, organic matter composition, particle size distribution, and pollutant sources, which influence chemical partitioning and bioavailability (Knezovich et al., 1987). Developing freshwater-specific EBTs would improve the accuracy and applicability of sediment risk assessments across environmental settings.

For practical application, it should be noted that the EBT derived in this study was established based on an assumed 1% OC content in sediments. A default fOC value of 0.01 was applied in this study, which falls within the typical range reported for coastal sediments (generally 0.5–5% OC) and is commonly used as a conservative assumption in sediment quality assessments (Graversen et al., 2025). Because hydrophobic AhR agonists preferentially partition to organic matter, the OC content in sediments directly influences the relationship between measured BEQbio values and ecotoxicological thresholds. For example, for sediments with a 2% OC content, the fOC should be adjusted to 0.02 for comparison. This normalization process ensures consistency in comparisons between sites with different sediment compositions and enhances the reliability of bioassay interpretations under diverse environmental conditions.

3.5. Implications for sediment quality assessment and management

This study derived EBT values for both non-persistent and persistent AhR-mediated activity in

sediments and differentiated between EBT for mixtures of known chemicals (EBT-BEQchem) and mixtures of known and unknown chemicals directly quantified using bioassays (EBT-BEQbio). The proposed EBTs should be interpreted primarily as screening and prioritization benchmarks rather than hazard- or risk-based thresholds directly linked to ecological effects. Because EBT-BEQchem was derived from SQG-based concentration distributions translated using REP values, these thresholds are intended to support interpretation of bioassay responses and identification of sediments requiring further evaluation. Future development of tiered trigger frameworks may further improve applicability for screening and management purposes. Even when SQGs are not exceeded, complex chemical mixtures in sediments, including uncharacterized contaminants, may act additively and pose ecological risks that are not captured by single-compound-based assessments (Hong et al., 2023). Accordingly, sediments that remain below SQGs but exceed EBTs may require further evaluation, including potency balance analysis, to better characterize mixture-driven biological effects.

Integration of bioassay-based screening with source-tracking and complementary analytical approaches may further improve identification of contamination sources and support sediment remediation and management strategies. In addition, incorporation of contaminant bioavailability, desorption potential, leaching behavior, and trophic transfer assessments may improve interpretation of bioassay responses in relation to ecological exposure and exposure-based sediment risk management. The framework proposed in this study may also be expanded to incorporate additional toxicological endpoints relevant to sediment-associated contaminants, such as estrogen receptor-, androgen receptor-, and peroxisome proliferator-activated receptor-mediated activities, thereby broadening its applicability across a wider range of environmental risk assessments. Because bioassay-based approaches can capture mixture-driven biological effects without requiring comprehensive identification of all contaminants, this framework may be particularly valuable in regions where conventional chemical monitoring and source-control capacity remain limited. Standardization of effect-based sediment assessment through international monitoring frameworks could improve comparability across regions and support broader application of bioanalytical tools in global sediment quality assessment and environmental monitoring programs.

4. CONCLUSION

This study established sediment-specific EBT-BEQbio of 470 ngBaP gdm−1 for non-persistent and 7.4 pgTCDD gdm−1 for persistent AhR-mediated activity by integrating SQG-based benchmarks with REP values and MFs. The application of mixture factors improved the environmental relevance of the derived EBTs by accounting for bioassay responses that quantified target compounds could not explain. For the first time, EBT-BEQchem is defined, with mixture effects calculated for detected chemicals. This concept provides a practical benchmark for linking

chemically derived potency estimates with bioassay responses and for identifying sediments where unidentified AhR agonists or mixture-driven effects may substantially contribute to observed biological activity. Application to Busan Bay sediments demonstrated frequent exceedance of EBTs based on BEQbio. Discrepancies between BEQbio and BEQchem highlighted the limitations of conventional target chemical monitoring in complex sediment matrices. Comparisons with previously published datasets further showed that EBT exceedances were predominantly observed in industrialized coastal and riverine regions. At the same time, remote or open marine environments generally remained below the proposed thresholds. Overall, the derived EBTs provide a framework that is mixture-sensitive and scientifically supported for sediment quality assessment. They support integrative monitoring strategies that combine bioassays, potency balance analysis, and advanced chemical identification tools, and provide practical utility for environmental risk assessment, remediation evaluation, and policy decision-making.

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672 Table

673 Table 1. Relative effect potency (REP) values for non-persistent and persistent AhR agonists
674 determined in the H4IIE-luc assay following 4-h and 72-h exposures, respectively, together with
675 the corresponding threshold effect levels (TELs), probable effect levels (PELs), and derived
676 BEQSQG values.

Non-persistent AhR agonists	REPsa	TELb (ng gdm−1)	BEQSQG (ngBaP gdm−1)	References
BaA	3.2 × 10-1	16	5.0	Kim et al. (2019)
Chr	8.5 × 10-1	27	23	Kim et al. (2019)
BbF	5.0 × 10-1	27	14	Kim et al. (2019)
BaP	1.0	32	32	Kim et al. (2019)
IcdP	5.8 × 10-1	17	9.9	Kim et al. (2019)
DbahA	6.6 × 10-1	10	6.6	Kim et al. (2019)
Persistent AhR agonists	REPs	PELc (pg gdm−1)	BEQSQG (pgTCDD gdm−1)	References
PCDD/Fs	1.0	21.5	21.5	Lee et al. (2015)
CB105	7.0 × 10−7	189,000	0.13	Lee et al. (2015)
CB118	1.0 × 10−7	189,000	0.019	Lee et al. (2015)
BaA	1.9 × 10−6	693,000	1.3	Lee et al. (2022)
Chr	2.3 × 10−6	846,000	1.9	Lee et al. (2022)
BaP	1.6 × 10−6	763,000	1.2	Lee et al. (2022)
DbahA	4.6 × 10−6	135,000	0.62	Lee et al. (2022)

677 a Assay-specific REP values obtained from previous studies.

678 b TEL: Threshold Effect Level (Ingersoll et al., 1996).

679 c PEL: Probable Effect Level (CCME, 1999).

Figure captions

Figure 1. (A) Probit plots of the logarithms of BEQSQG and visualization of the log-normal distributions of BEQSQG for non-persistent target AhR agonists. Blue dashed lines represent the 50th percentile of logBEQSQG. (B) Boxplots showing the mixture factors (MF) derived from the distribution of the ratio between BEQbio and BEQchem for non-persistent AhR-mediated activity. For non-persistent activity, an MF of 38 was derived from the median BEQbio/BEQchem ratio (n = 76). (C) Probit plots of the logarithms of BEQSQG and visualization of the log-normal distributions of BEQSQG for persistent target AhR agonists. (D) Boxplots showing the MF derived from the distribution of the BEQbio/BEQchem ratios for persistent AhR-mediated activity. For persistent activity, an MF of 10 was derived from the median ratio (n = 194).

Figure 2. (A) Boxplots of non-persistent AhR-mediated activities derived from bioassays (BaP-EQbio) and chemical analysis (BaP-EQchem) in sediments from Busan Harbor, South Korea. (B) Potency balance between BaP-EQbio and BaP-EQchem. (C) Major non-persistent AhR agonists in sediment extracts (top 10 contributors). (D) Boxplots of persistent AhR-mediated activities (TCDD-EQbio and TCDD-EQchem) in sediments from Busan Harbor. (E) Potency balance between TCDD-EQbio and TCDD-EQchem. (F) Major persistent AhR agonists in sediment extracts (top 10 contributors). Red and blue dashed lines indicate the EBT-BEQbio and EBT-BEQchem values derived in this study.

Figure 3. Cumulative distribution of BEQbio and BEQchem for (A) non-persistent AhR agonists (BaP-EQbio and BaP-EQchem) and (B) persistent AhR agonists (TCDD-EQbio and TCDD-EQchem), derived from this study and previously published datasets (Tables S7 and S8). Red dashed lines represent the EBT-BEQbio values for non-persistent (470 ngBaP gdm−1) and persistent (7.4 pgTCDD gdm−1) AhR-mediated activity derived in this study, and blue dashed lines indicate the corresponding EBT-BEQchem values for non-persistent (12 ngBaP gdm−1) and persistent (0.72 pgTCDD gdm−1) activity.