INTERNATIONAL STANDARD

ISO 23196

First edition
2022-02

Water quality — Calculation of biological equivalence (BEQ) concentrations

Reference number
ISO 23196:2022(E)

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Published in Switzerland

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Foreword

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This document was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 5, *Biological methods*.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Water quality — Calculation of biological equivalence (BEQ) concentrations

1 Scope

This document specifies the derivation of biological equivalence (BEQ) concentrations for results of in vitro bioassays which are based on measuring effects on a biological process such as enzyme induction or cellular growth. The concept described here can be used for any biological assay after the proof of its applicability.

To derive BEQ concentrations, the effect on a biological process caused by a sample – i.e. the activity of the sample – is expressed in terms of a concentration of a reference compound which results in an equivalent effect on the process. The term "sample" used in this document addresses environmental samples as well as defined mixtures and pure compounds used as test item in a bioassay. BEQ concentrations can be derived for environmental water samples, extracts of environmental water samples including tap water or solutions of pure chemicals or mixtures of chemicals.

2 Normative references

There are no normative references in this document.

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

3.1
biological equivalence concentration
BEQ concentration
concentration of a *reference compound* (3.6) that causes the same effect as the effect measured in a sample, a sample dilution or a solution containing one or more chemicals

3.2
concentration-effect relationship
response to a concentration gradient of an environmental sample or a known substance or mixture of substances which is described by pre-determined diagnostic indicators

[SOURCE: ISO 6107:2021, 3.127, modified — the term "environmental sample" added; Note 1 to entry has been deleted.]

3.3
concentration factor
CF
ratio of the actual concentration of the sample compared to the original sample taking all enrichment and dilution steps of the sample into account

3.4
limit of quantification
LOQ
lowest value of a determinant that can be determined with an acceptable level of accuracy and precision

[SOURCE: ISO 15839:2003, 3.18]

3.5
negative control material
well characterized material and/or substance that, when evaluated by a specific test method, demonstrates the suitability of the test system to yield a reproducible, appropriately negative, non-reactive or minimal response in the test system

Note 1 to entry: In practice, negative controls include blanks, vehicles/solvents and reference materials.

[SOURCE: ISO 7405:2018, 3.4, modified — Note 1 to entry has been deleted.]

3.6
reference compound
RC
compound with one or more property values that are sufficiently reproducible and well established to enable the calibration of the measurement method

Note 1 to entry: For the purpose of this document, a reference compound is any well characterized material and/or substance that, when tested by the procedure described, demonstrates the suitability of the procedure to yield a reproducible, predictable positive response.

[SOURCE: ISO 7405:2018, 3.5, modified — "compound" replaces "material"; "the calibration of the measurement method" replaces "use of the material or substance for the calibration of an apparatus, the assessment of a measurement method or for the assignment of values to materials". Note 1 was modified to cover only a reference compound resulting in a positive response – otherwise the proposed concept is not applicable.]

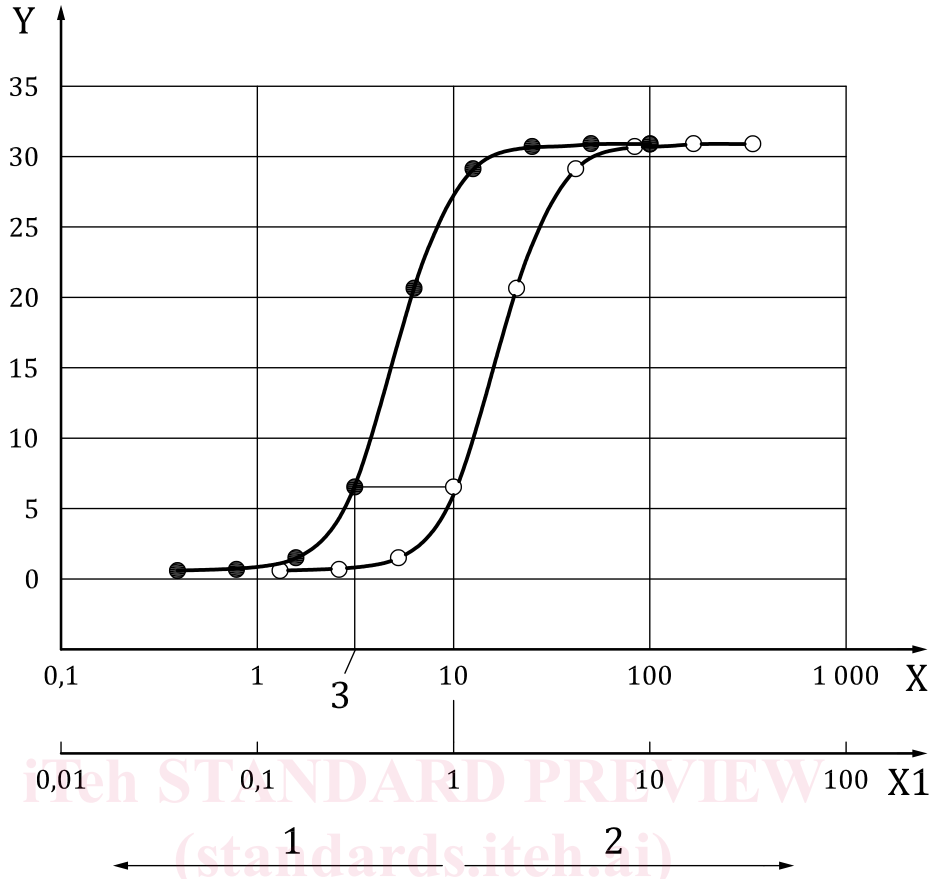
3.7
***x* %-effect concentration**
EC_x
concentration at which a specific effect is detected; *x* is the percentage (e.g. 10, 25, 50) of this effect, e.g. growth inhibition

[SOURCE: ISO 15952:2018, 3.6]

3.8
***x* %-effect concentration of a reference compound**
RC_x
concentration of a *reference compound* (3.6) at which a specific effect is detected; *x* is the percentage (e.g. 10, 25, 50) of this effect, e.g. growth inhibition

4 Principle

The general idea for the derivation of BEQ concentrations, assessed by the test method, is shown in Figure 1. The biological activity of a sample is expressed in terms of a concentration of a reference compound which affects a biological process to the same extent. By this means, BEQ concentrations allow an indirect quantification of results and a comparison of results obtained by different laboratories. Furthermore, a robust way to calculate BEQ concentrations is a necessary requirement for a possible use of effect-based trigger (EBT) values in regulations[5],[6],[7],[8].



Key

X	concentration of the reference compound, e.g. in ng/l
X1	concentration factor of the sample compared to the original water sample
Y	effect in arbitrary units
1	sample diluted
2	sample concentrated
3	biological equivalence (BEQ) concentration
—●—	reference compound
—○—	sample

NOTE The measured effect of a sample or a sample dilution is extrapolated to a concentration of a reference compound which induces the bioassay to the same extend as the sample or sample dilution to derive a biological equivalence (BEQ) concentration, here an example of an agonistic action is shown.

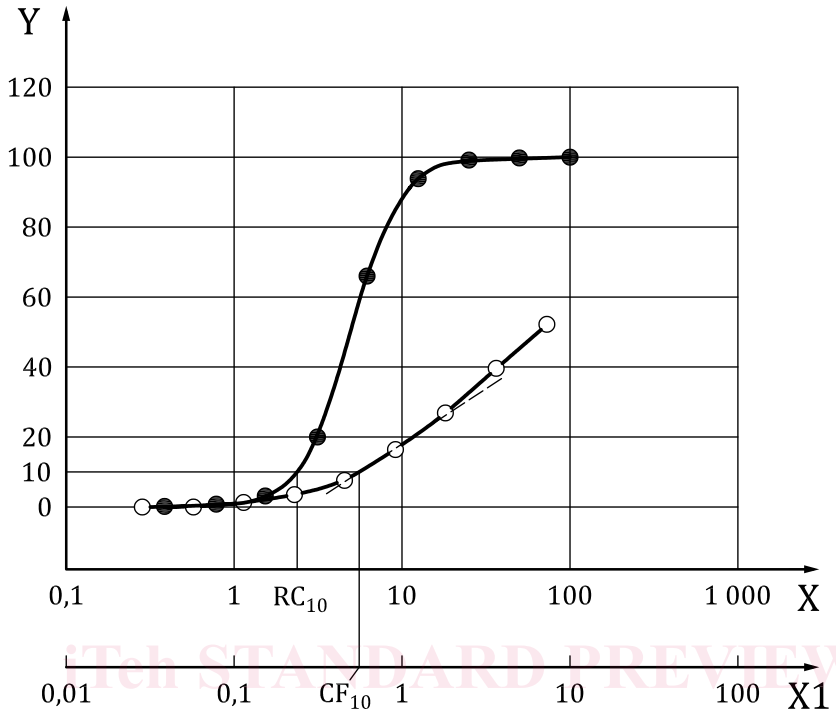
Figure 1 — Basic principle of the derivation of a biological equivalence (BEQ) concentration

Several different methods for the derivation of BEQ concentrations have been published, but not all methods are applicable to all kinds of concentration-effect relationships. For a detailed discussion about advantages and disadvantages of the various mathematical approaches, see References [1],[3],[5],[6]. The method described in this document, termed RC_x-approach, is reported to be a robust method for the derivation of BEQ concentrations[11],[12] and outlined as well in OECD 455[13].

NOTE In the literature, other terminologies might be found for RC_x, such as PC_{10}. PC_{10} describes the concentration of the positive control that induces a 10 % effect-level. In this document, the term “reference compound” is used instead of “positive control”. Therefore, RC is used instead of PC.

The approach is illustrated in Figure 2. As a first step of the RC_x-approach, the concentration-effect relationship of the reference compound is modelled to determine the maximum effect of the assay for the reference compound. Next, the effect levels are normalized to a percentage scale whereby the effect

level of the negative control material is defined as 0 % and the maximum effect level of the reference compound is defined as 100 %. Then, the concentration of the reference compound that affects the assay to the *x* %-effect level is calculated (RC_x).



Key

X	concentration of the reference compound, e.g. in ng/l
X1	concentration factor of the sample compared to the original water sample
Y	normalized effect in %
RC_{10}	concentration of the reference compound at 10 % effect level
CF_{10}	concentration factor of the sample at 10 % effect level with respect to the reference compound
—●—	reference compound
—○—	sample

NOTE Following data normalization, where 0 % equals the effect in the negative control material and 100 % is the modelled maximum effect of the reference. The sample concentration factor (CF) and reference compound concentration (RC) required to reach an effect level of *x* % (here 10 %) are interpolated from the data using linear interpolation (dashed line). The BEQ concentration is derived by dividing RC_x by CF_x.

Figure 2 — Illustration of the RC_x-approach for the derivation of a biological equivalence (BEQ) concentration

In a further step, the concentration factor of the sample is determined at which the sample affects the assay to the selected *x* %-effect level (CF_x). As defined in 3.3, the concentration factor describes the ratio of the actual concentration of the sample compared to the original water sample taking all enrichment and dilution steps of the sample into account. If a sample is, under consideration of a quantitative process, enriched 1 000-fold by solid phase extraction, the final concentration factor of the sample after a 100-fold dilution with, for example, growth medium is 10. If this sample is tested in a series of six 1→2 dilutions, the resulting rounded concentration factors are 10, 5, 2,5, 1,25, 0,62, 0,31 and 0,15. The biological equivalence (BEQ) concentration of the sample is finally given by the ratio RC_x/CF_x.

5 Procedure

5.1 General

Usually, concentration-effect relationships of reference compounds and samples (see Clause 1) in reporter gene assays and proliferation assays are sigmoidal. Depending on the individual shape of the concentration-effect relationship, a suitable mathematical model has to be selected for fitting. In general, a five parametric logistic model[14],[15] can be applied as shown in 5.2.

The overall procedure for the calculation of BEQ concentrations consists of the following steps that are described in detail in 5.2:

— assessment of the suitability of the experimental data for the calculation of a biological equivalence concentration (see 5.2.1);

— fitting of concentration-effect data for the reference compound (see 5.2.2);

— calculation of quality measures for the fit (see 5.2.3);

— normalization of data from the reference compound and samples (see 5.2.4);

— calculation of the x %-effect level of the reference compound and the respective RC_x-value (see 5.2.5);

— assessment of the validity of the experimental data for the calculation of a biological equivalence concentration (see 5.2.6);

— calculation of the concentration factor of the sample at the x %-effect level by linear interpolation (see 5.2.7);

— calculation of the biological equivalence (BEQ) concentration (see 5.2.8).

5.2 Procedure for the calculation of biological equivalence (BEQ) concentrations

5.2.1 Assessment of the suitability of the experimental data for the calculation of a biological equivalence (BEQ) concentration

To assess the validity of the experimental data for the calculation of biological equivalence (BEQ) concentrations (see 5.2.6) by the procedure described in this document, some calculations should be performed. The experimental data should be assessed prior to this procedure as described below to evaluate its general suitability. Use only experimental data which fulfil the validity criteria of the respective standard or guideline for the calculation of biological equivalence (BEQ) concentrations.

Use the following guidance to assess a general suitability of the data:

a) at least two more data points than the number of parameters describing the logistic function of the curve are required for the concentration-effect relationship of the reference compound, i.e. in case of the five-parametric logistic function described in 5.2.2 seven data points are required;

b) the upper curve plateau of the sigmoidal concentration-effect relationship of the reference compound is indicated by the data, i.e. might be estimated by the human eye;

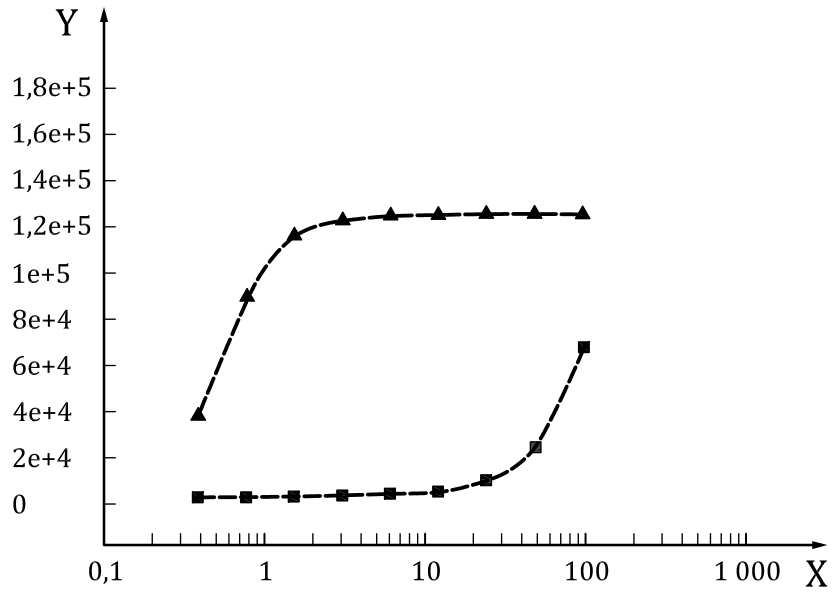
c) the lower curve plateau of the sigmoidal concentration-effect relationship of the reference compound is indicated by the data, i.e. might be estimated by the human eye;

d) the effect measures of the tested sample concentrations are likely to cross the chosen x %-effect level.

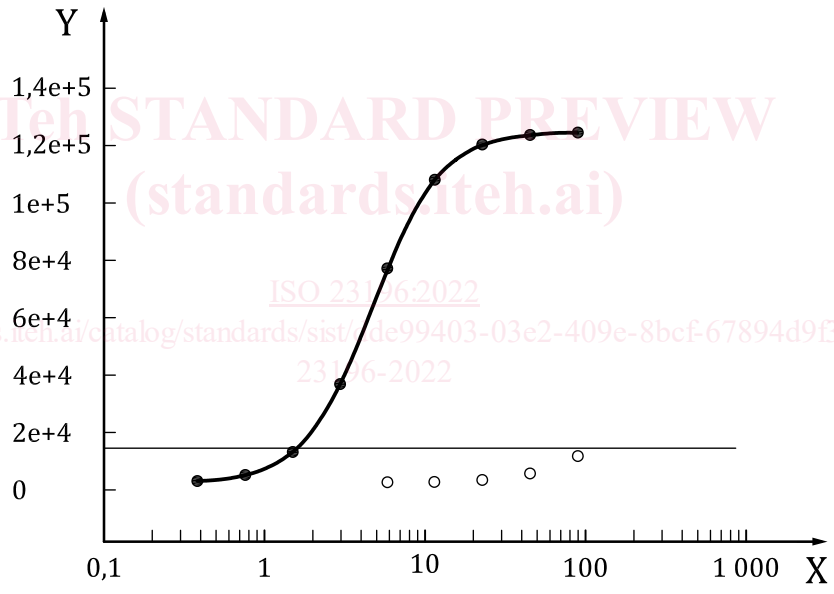
Figure 3 shows two examples to guide the assessment of the suitability of the experimental data for the calculation of an equivalence concentration.

Figure 3 a) shows two concentration-effect relationships of the reference compound that are not suitable for the calculation of biological equivalence (BEQ) concentrations. In case of the upper curve (black triangles, high response curve), the bottom of the concentration-effect relationship is not defined; in case of the lower curve (black squares, low response curve), the top (maximum to infinite effect range) is not defined. In such cases, adjust the concentration range for the reference compound to generate a complete concentration-effect relationship.

Figure 3 b) shows a suitable concentration-effect relationship for the reference compound but the effect response of the sample is too low to reach or exceed an equivalent of the 10 % effect threshold of the reference compound (RC_{10}-value, 10 % is indicated by a line).



a)



b)

Key

X concentration of a reference compound and a sample
Y effect in arbitrary units
reference compound without defined top
reference compound without defined bottom
reference compound
○ sample

Figure 3 — Examples for guidance on assessment of the suitability of experimental data