
**Water quality — Calculation of
biological equivalence (BEQ)
concentrations**

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Contents

Page

Foreword.....	iv
1 Scope.....	1
2 Normative references.....	1
3 Terms and definitions.....	1
4 Principle.....	2
5 Procedure.....	5
5.1 General.....	5
5.2 Procedure for the calculation of biological equivalence (BEQ) concentrations.....	5
5.2.1 Assessment of the suitability of the experimental data for the calculation of a biological equivalence (BEQ) concentration.....	5
5.2.2 Fitting of concentration-effect data for the reference compound.....	8
5.2.3 Calculation of quality measures for the fit.....	9
5.2.4 Normalization of data from the reference compound and samples.....	10
5.2.5 Calculation of the x %-effect level of the reference concentration-effect relationship and the respective RC_x -value.....	11
5.2.6 Assessment of the validity of the experimental data for the calculation of a biological equivalence (BEQ) concentration.....	12
5.2.7 Calculation of the concentration factor of the sample at x %-effect level by linear interpolation.....	12
5.2.8 Calculation of the biological equivalence (BEQ) concentration.....	13
6 Validity criteria.....	14
7 Test report.....	14
Annex A (informative) Illustration of χ^2 statistics using data from Table 1.....	15
Bibliography.....	18

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

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

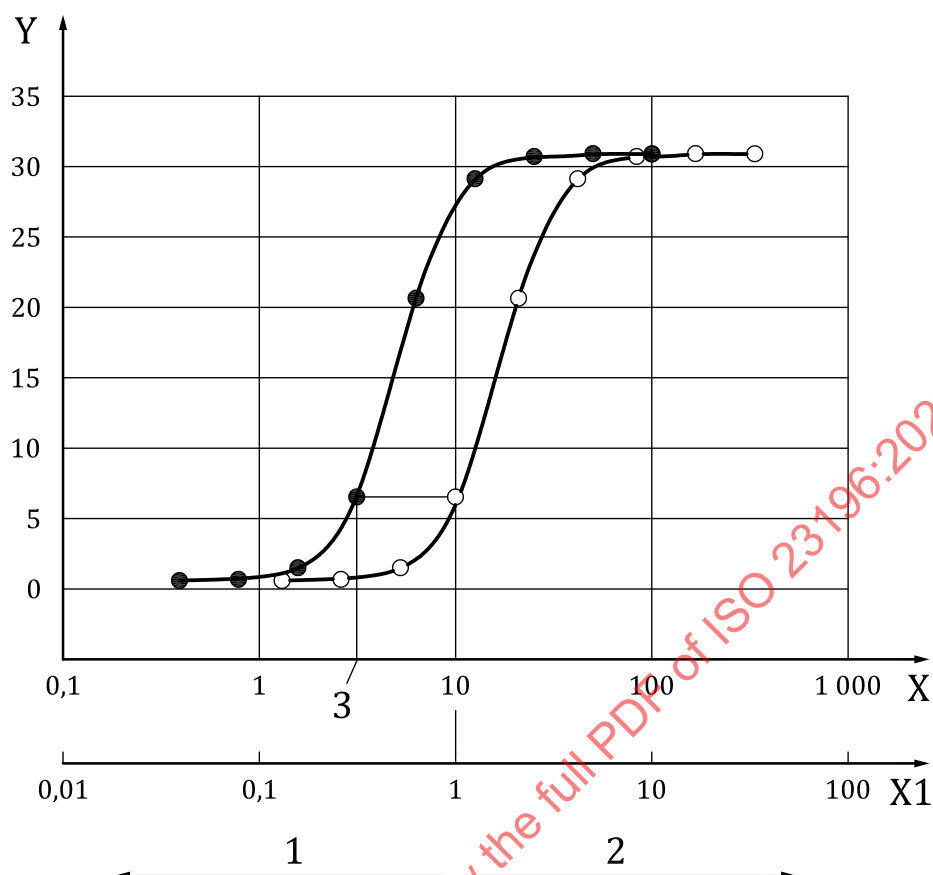
[SOURCE: ISO 15952:2018, 3.6]

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.

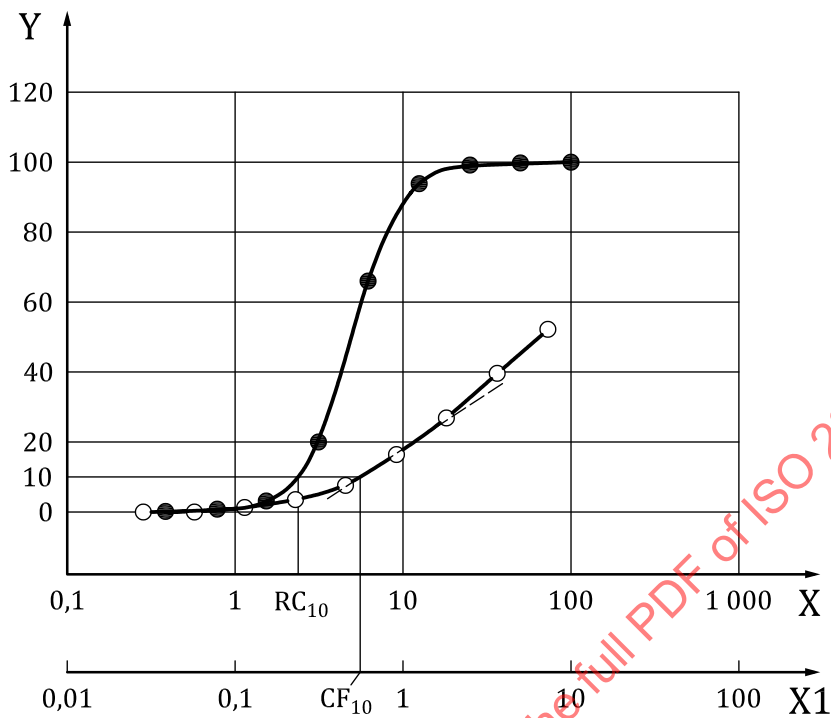
Figure 1 — Basic principle of the derivation of a biological equivalence (BEQ) concentration

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:

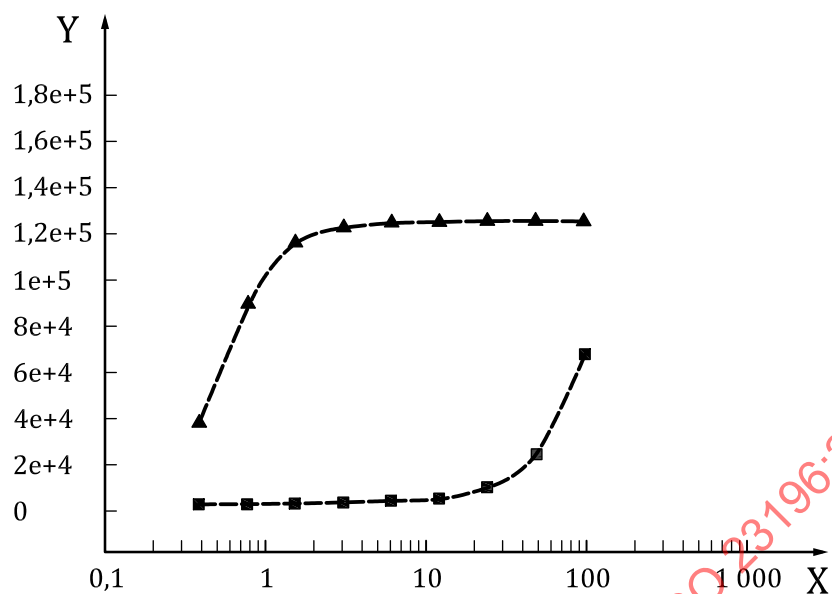
- a) at least two more data points than the number of parameters describing the logistic function of the curve are required for the concentration-effect relationship of the reference compound, i.e. in case of the five-parametric logistic function described in [5.2.2](#) seven data points are required;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

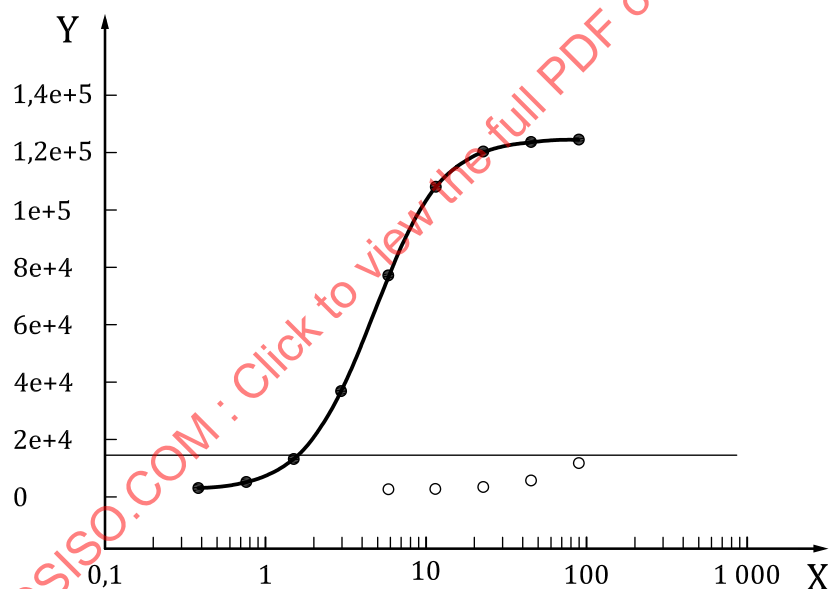
[Figure 3](#) a) shows two concentration-effect relationships of the reference compound that are not suitable for the calculation of biological equivalence (BEQ) concentrations. In case of the upper curve (black triangles, high response curve), the bottom of the concentration-effect relationship is not 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).

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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

5.2.2 Fitting of concentration-effect data for the reference compound

Use the following five-parametric logistic function given by [Formula \(1\)](#) to fit the measured concentration-effect relationship to the model.

$$f(x_i) = y_i = d + \frac{a-d}{\left(1 + \left(\frac{x_i}{w}\right)^b\right)^f} \quad (1)$$

where

y_i is the effect measure at concentration x_i ;

x_i is the compound concentration which activates the test system to the effect y_i ;

d is the calculated parameter for the bottom curve plateau of the reference compound;

a is the calculated parameter for the top curve plateau of the reference compound (the sum of d and a describes the maximum curve plateau);

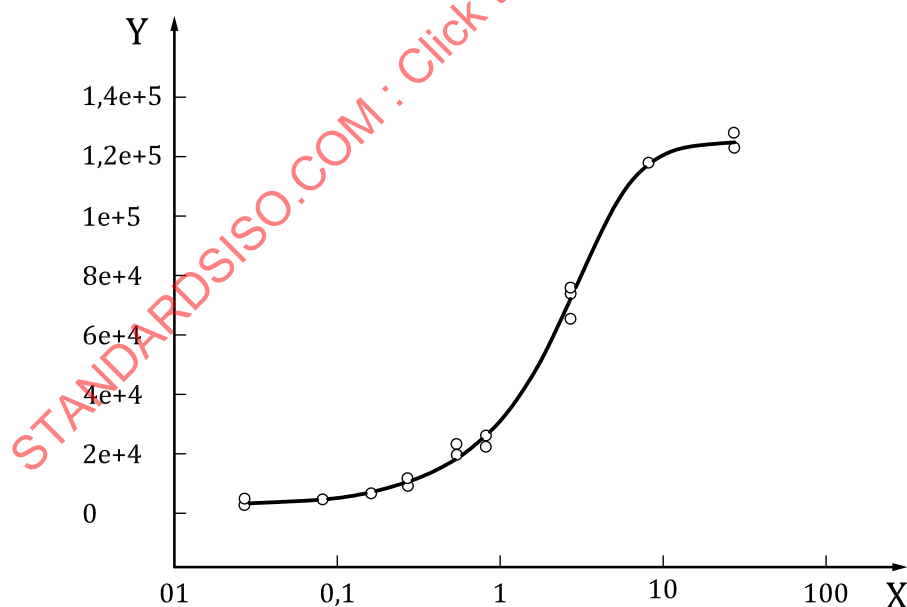
w is the curve point of inflection;

b is the slope factor or hill slope which describes the steepness of the curve;

f is the symmetry factor of the sigmoidal curve.

NOTE If $f = 1$, the curve is symmetrical.

[Figure 4](#) shows an example of a concentration-effect relationship with the reference compound 17 β -estradiol, which was modelled using [Formula \(1\)](#) (line). The calculated parameters for the function are summarized in [Table 1](#).



Key

X concentration of the reference compound 17 β -estradiol [ng/l]

Y effect in arbitrary units

Figure 4 — Example of effect data of the reference compound 17 β -estradiol (circles depict triplicate data)

Table 1 — Calculated parameters for the function of reference compound data

<i>d</i>	2 275,95
<i>a</i>	125 034,65
<i>w</i>	4,63
<i>b</i>	-2,81
<i>f</i>	0,34

5.2.3 Calculation of quality measures for the fit

The statistical background for this approach – Chi square Goodness-of-fit test – is described in Chapter 11 of Reference [16]. In short, the statistic χ^2 Formula (2) characterizes for polynomials the variance s^2 of the fit.

$$\chi^2 = \sum \frac{(y_i - f_i)^2}{\sigma_i^2} \quad (2)$$

where

y_i is the effect measure at concentration i ;

f_i is the effect measure predicted by the model at concentration i ;

σ_i is the standard deviation of the effect measure at concentration i .

χ^2 is the characteristic of the spread of the data given by σ^2 and the accuracy of the fit. The definition of χ^2 , as the ratio of the estimated variance s^2 to the parent variance σ^2 times the number of degrees of freedom ϑ , makes it a convenient measure of the goodness of fit. If the fitting function is a good approximation to the parent function, then the estimated variance s^2 should agree well with the parent variance σ^2 and the value of the reduced chi-square should be approximately unity, $\chi_{red}^2 = \frac{\chi^2}{\vartheta} = 1$ [12].

To estimate the quality of the fitted function use Formula (3) to calculate the reduced chi-square also termed as mean square weighted deviation (MSWD).

$$\chi_{red}^2 = \sum_i \frac{(y_i - f_i)^2}{\sigma_i^2} \times \frac{1}{\vartheta} \quad (3)$$

where

y_i is the effect measure at concentration i ;

f_i is the effect measure predicted by the model at concentration i ;

σ_i is the standard deviation of the effect measure at concentration i ;

ϑ is the degrees of freedom given by number of data points minus number of fit-parameters.

This calculation is exemplified in Table 2 by using the data presented in Figure 4. A value of $\chi_{red}^2 \gg 1$ would indicate that the fitting function is not appropriate for describing the data. The observed value for χ_{red}^2 will differ between experiments because of the uncertainty in the determination of s^2 . Upper boundaries for acceptable values of χ^2 can be defined based on a 5 % threshold by making use of the distribution of χ^2 that is a function of the degrees of freedom ϑ only.

The integral of the χ^2 -distribution between the calculated value of χ^2 and ∞ "describes the probability, that a set of n data points, drawn from the parent distribution would yield a value of χ^2 equal or greater than the calculated value" [16]. In other words, in case of a low probability it is highly unlikely that the

measured data deviate randomly from the fitted function indicating a systematic discrepancy between the data and the fit.

Table 2 — Example for the calculation of χ^2 and χ_{red}^2

c(E2) ng/l	Signal replica 1	Signal replica 2	Signal replica 3	Rounded mean y_i	Rounded standard deviation σ_i	Variance σ_i^2	Fit (f_i)	$(y_i - f_i)^2 / \sigma_i^2$
0,027	2 776	4 999	3 144	3 600	1 200	1 440 000	3 232	0,00
0,082	4 041	4 938	4 680	4 550	460	211 600	4 977	0,05
0,16	6 842	7 186	6 559	6 860	310	96 100	7 478	0,00
0,27	9 139	10 613	12 001	10 600	1 400	1 960 000	10 707	0,91
0,55	19 567	20 009	23 191	21 000	2 000	4 000 000	18 501	1,50
0,82	24 358	22 269	26 053	24 000	1 900	3 610 000	16 039	0,01
2,7	76 217	73 982	65 543	72 000	5 600	31 360 000	71 730	3,84
8,2	117 182	117 440	118 054	117 560	450	202 500	117 658	0,84
27,2	123 336	122 930	128 175	124 800	2 900	8 410 000	124 753	0,12
							Sum	7,27
							(χ^2)	
							ϑ	4
							χ_{red}^2	1,82

A probability around 50 %, i.e. an area of 0,5, is an optimal value. A probability below 5 % is not acceptable. This is illustrated in [Annex A](#) using the data from the example with $\vartheta = 4$ and $\chi^2 = 7,27$ resulting in a $P_\chi = 0,122\ 3$, i.e. with a probability of 12,23 % higher values of χ^2 can be expected. [Figure A.1](#) shows the situation for $\chi^2 = 9,487\ 73$ and $\vartheta = 4$ resulting in an unacceptable low probability ($\leq 5\ %$) of a higher value of χ^2 . In turn an overfitting of the data would be indicated by a probability above 95 %. Therefore, the value for χ^2 for the fit has to be above the value for $P_{\chi(\chi^2; \vartheta)} = 0,95$ and below the value for $P_{\chi(\chi^2; \vartheta)} = 0,05$. The respective χ^2 -values for ϑ from 1 to 10 are given in [Table A.1](#) and can be found in statistical text books^[17].

5.2.4 Normalization of data from the reference compound and samples

Normalize the measured effect data for the reference compound and the sample to 100 % using [Formula \(4\)](#):

$$y_i (\%) = \frac{y_i - d}{a} \times 100 \quad (4)$$

where

$y_i(\%)$ is the normalized effect measure at concentration i ;

y_i is the effect measure at concentration i ;

d is the calculated parameter for the bottom curve plateau of the reference compound;

a is the calculated parameter for the top curve plateau of the reference compound, i.e. the maximal activation of the test system by the reference compound.

5.2.5 Calculation of the x %-effect level of the reference concentration-effect relationship and the respective RC_x -value

Calculate the RC_x value of the reference compound by applying the parameters for the reference compound determined in 5.2.2 using Formula (5):

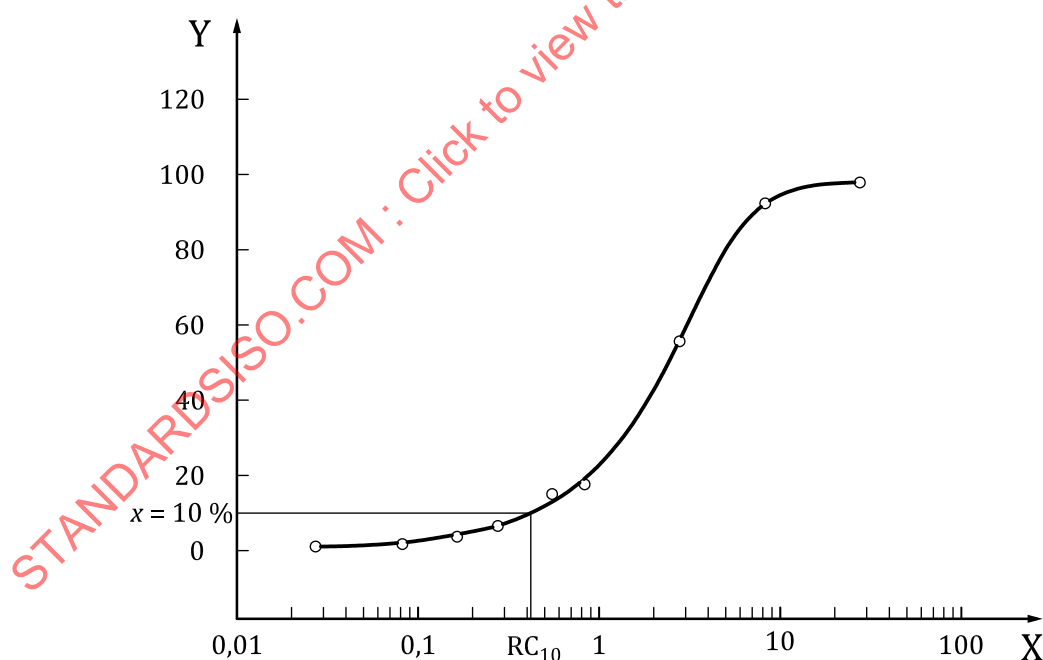
$$RC_x = \left(\left(\frac{100}{x} \right)^{\frac{1}{f}} - 1 \right)^{\frac{1}{b}} \cdot w \quad (5)$$

where

- x is the chosen effect level in % at which the equivalent concentration is to be calculated;
- RC_x is the x %-effect concentration (EC_x) of the reference compound causing an x %-effect level;
- w is the curve point of inflection;
- b is inverse proportional to the slope of the function;
- f describes the asymmetry of the sigmoidal curve.

NOTE If $f = 1$ the curve is symmetrical.

Using average data shown in Figure 4 the RC_x at the chosen effect level of $x = 10$ % is calculated as $RC_{10\%} = 0,41$ ng/l (see Figure 5; the use of x to denote the effect level along the y-axis is based on historic nomenclature.).



Key

- X concentration of the reference compound 17 β -estradiol [ng/l]
- Y normalized effect in %
- RC_{10} concentration of the reference compound 17 β -estradiol at 10 % effect level = 0,41 ng/l

Figure 5 — Calculation of the concentration of the reference compound (17 β -estradiol) exhibiting x % of the maximal effect (RC_x)

5.2.6 Assessment of the validity of the experimental data for the calculation of a biological equivalence (BEQ) concentration

The following criteria shall be met for the valid calculation of an equivalence concentration:

- the maximal effect measured for one or more of the tested reference compound concentrations is >90 %;
- the minimal effect measured for one or more of the tested reference compound concentrations is below the chosen x %-effect level;
- the LOQ for the reference compound, as defined by the respective standard of the test method applied, is $< RC_x$ (determined according to 5.2.5);
- the effect measures of the tested sample concentrations are crossing $y_{x\%}$ determined in 5.2.5 based on the chosen x %-effect level.

5.2.7 Calculation of the concentration factor of the sample at x %-effect level by linear interpolation

In case of samples showing only a weak induction (or activation) of the assay, a fitting of the data by a sigmoidal function is not appropriate because the data do not provide sufficient information. To allow a robust calculation of the concentration factor of the sample at x %-effect level even for samples with low activity, a linear interpolation is applied, using the data points adjacent to the selected effect level.

Identify the data point in the normalized concentration-effect relationship of the sample that is directly below the selected effect level of, for example, 10 %. Identify the data point in the normalized concentration-effect relationship of the sample that is directly above the selected effect level of, for example, 10 %.

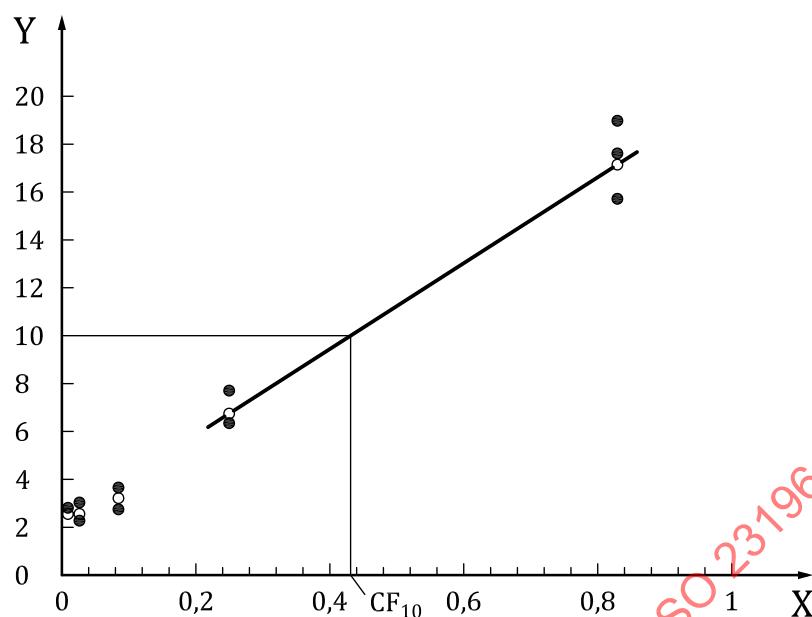
Calculate the concentration factor of the sample at the x %-effect level (CF_x) based on the normalized sample data using [Formula \(6\)](#):

$$CF_x = (x - y_{low}) \cdot \left(\frac{x_{high} - x_{low}}{y_{high} - y_{low}} \right) + x_{low} \quad (6)$$

where

- x is the chosen effect level in % at which the equivalent concentration is to be calculated;
- x_{low} is the concentration factor of the sample at the effect level y_{low} ;
- x_{high} is the concentration factor of the sample at the effect level y_{high} ;
- y_{low} is the %-effect level below the chosen effect level x ;
- y_{high} is the %-effect level above the chosen effect level x .

[Figure 6](#) exemplifies the calculation of the concentration factor of the sample at the 10 %-effect level (CF_{10}) based on the normalized sample data using [Formula \(6\)](#).

**Key**

X concentration factor of the sample

Y normalized effect in %

CF₁₀ concentration factor (no dimension) of the sample at 10 % effect level = 0,43

● raw data

○ mean values

Figure 6 — Example for the calculation of the concentration factor of a sample at x %-effect level by linear interpolation

The CF at the chosen effect level of $x = 10\%$ is calculated as $CF_{10\%} = 0,43$ (see Figure 6). Thus, the sample at a concentration level of 43 % (original sample concentration = 100 %) induces the assay to a similar extend as 0,41 ng/l of the reference compound 17 β -estradiol (E2; see Figure 5).

5.2.8 Calculation of the biological equivalence (BEQ) concentration

Calculate the biological equivalence (BEQ) concentration of the sample as given in Formula (7):

$$y_{\text{BEQ}} = \frac{RC_x}{CF_x} \quad (7)$$

where

y_{BEQ} is the biological equivalence (BEQ) concentration of the sample;

RC_x is the concentration of the reference compound causing the x %-effect level (see 5.2.5);

CF_x is the concentration factor (CF) of the sample at x %-effect level (see 5.2.7).

The biological equivalence (BEQ) concentration of the sample shown in Figure 6 is 0,95 ng/l 17 β -estradiol-equivalence.

6 Validity criteria

Consider the derivation of a biological equivalent (BEQ) concentration valid if the following conditions are met:

- a) the bioassay meets validity criteria of the experimental method used;
- b) the bioassay involves a concentration-effect relationship of a reference compound;
- c) number of tested reference compound concentrations exceeds fitted parameters by two or more;
- d) Chi square Goodness-of-fit test is met;
- e) the LOQ for the reference compound is $< RC_x$ (x determined according to 5.2.5);
- f) the lowest measured effect caused by the reference compound is less than x % (x determined according to 5.2.5) the highest measured effect > 90 %;
- g) the effect measures of the tested sample concentrations are crossing $y_{x\%}$ determined in 5.2.5 based on the chosen x %-effect level.

7 Test report

The test report shall include the information described below. Concerning minimal information requirements for in vitro bioassays see Reference [9]:

- a) a reference to this document, i.e. ISO 23196:2022;
- b) statement that the validity criteria defined in Clause 6 were met;
- c) all data required for identification of the test sample;
- d) the sample enrichment and maximal concentrations tested and all dilutions of pure compound or concentrations of compounds in a mixture or concentration factors of the samples tested in the bioassay, for example, if the sample is enriched 1 000 times, then diluted 1:100, the maximal concentration factor is 10;
- e) compound used as reference including compound name and CAS number;
- f) chosen effect level x used to calculate the equivalence concentration;
- g) limit of quantification for the reference compound;
- h) model used for the fit of the concentration-effect relationship;
- i) goodness of fit given by χ^2_{red} and χ^2 ;
- j) number of degrees of freedom for the χ^2 statistics and the threshold value of χ^2 for $P_{\chi(\chi^2, \nu)} = 0,95$ and χ^2 for $P_{\chi(\chi^2, \nu)} = 0,05$ (see Table A.1 or Reference [17]);
- k) value of the equivalence concentration.

Annex A (informative)

Illustration of χ^2 statistics using data from [Table 1](#)

A.1 General

This annex illustrates the χ^2 statistics using data from [Table 1](#).

A.2 Assessment of the quality of fit using data from [Table 2](#)

The [Figure A.1](#) shows two χ^2 -distributions. In [Figure A.1](#) a) the calculated χ^2 of 7,27 is indicated. The integral from $\chi^2 = 7,27$ to ∞ is 0,122 3. Therefore, with a probability of 12,23 % higher values of χ^2 can be expected indicating a sufficient quality of the fit. The [Figure A.1](#) b) illustrates the situation for a probability of 5 % to obtain higher values of χ^2 if a set of n data points would be drawn from the parent distribution. The respective value for χ^2 is 9,487 73. In other words, a value for χ^2 above 9,487 73 indicates an insufficient quality of the fit.