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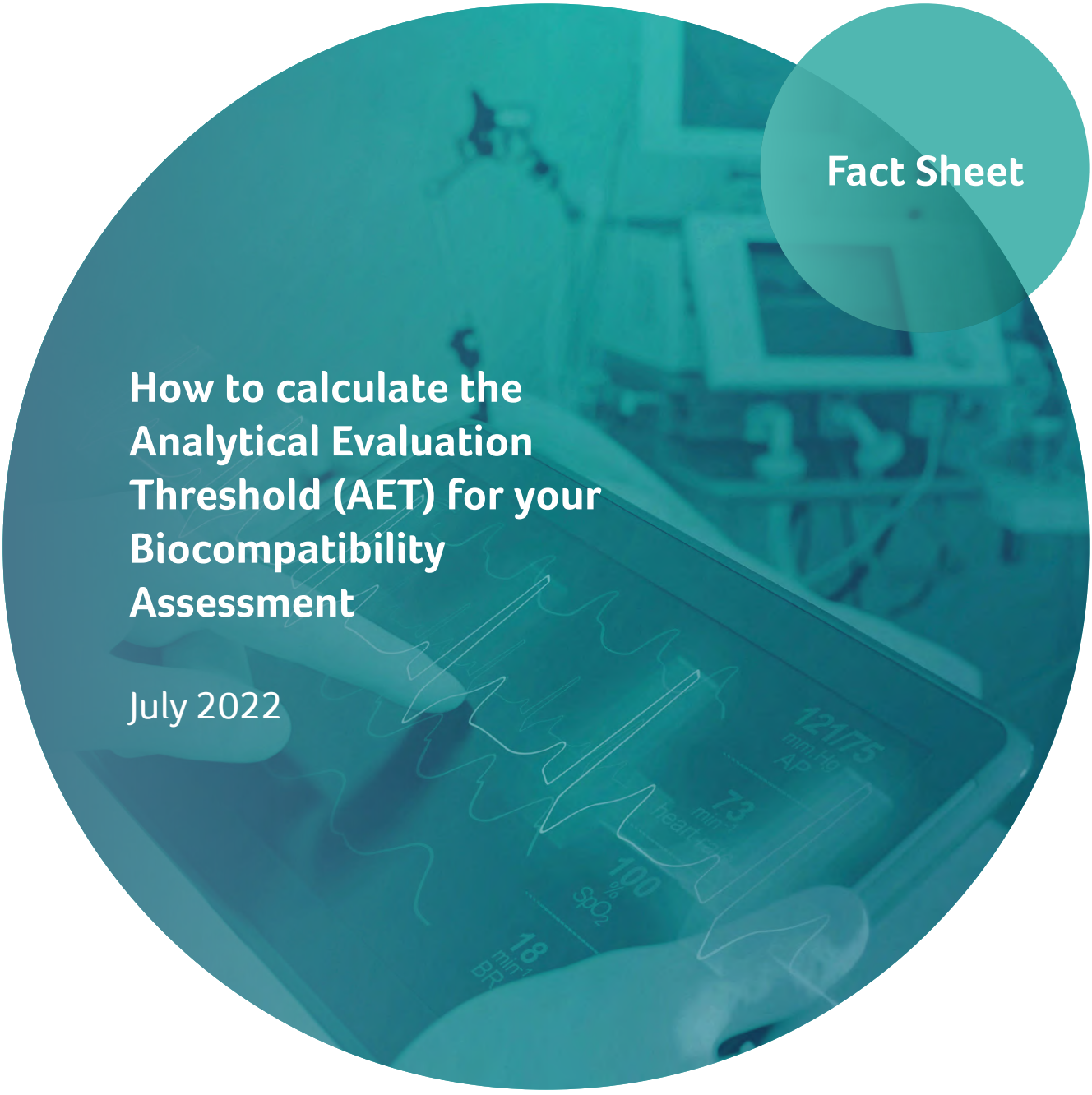

Fact Sheet

How to calculate the Analytical Evaluation Threshold (AET) for your Biocompatibility Assessment

July 2022

Smart consulting,
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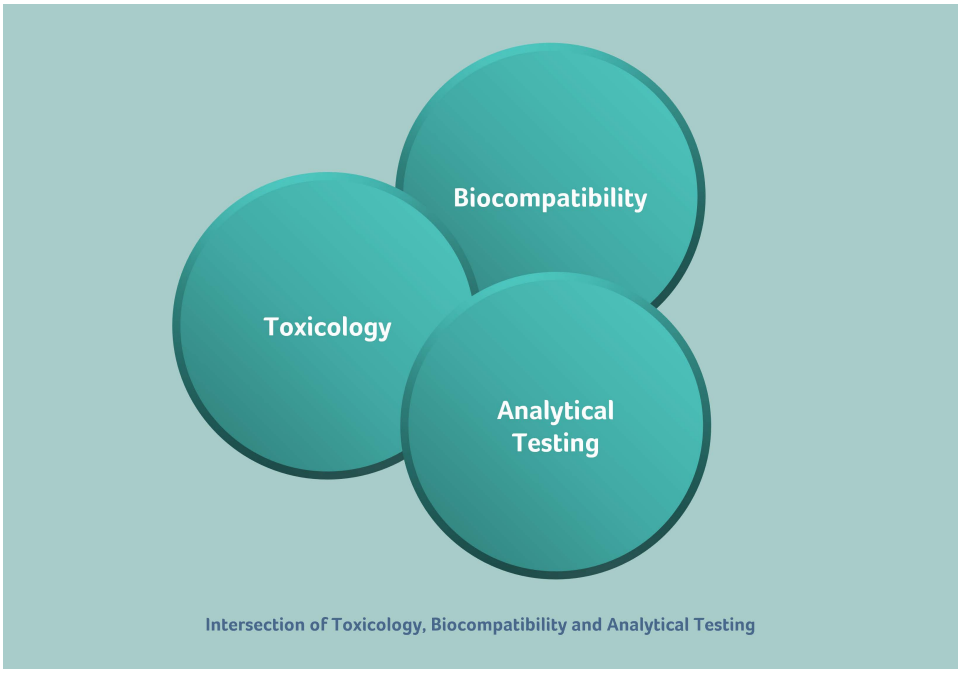
How to calculate the Analytical Evaluation Threshold (AET) for your Biocompatibility Assessment

What will you find in this fact sheet?

The **Analytical Evaluation Threshold (AET)** is the crucial parameter for demonstrating that your analytical results sufficiently assess the biological safety of your medical device, and is subsequently the intersection between Toxicology, Biocompatibility and Analytical Testing.



Intersection of Toxicology, Biocompatibility and Analytical Testing

This easy-to-read fact sheet includes:

- **An explanation of the importance of the AET including its connection with the threshold of toxicological concern (TTC) limits**
- **How to calculate your AET, including the applicability of ISO/TS 21726:2019, ISO 10993-17, and ISO 10993-18**
- **Tips & considerations for your next AET calculation**

Here's a short reference glossary for the key terms used in this fact sheet:

- **AET** | Analytical Evaluation Threshold
- **TTC** | Threshold of Toxicological Concern
- **LOD** | Limit of Detection
- **LOQ** | Limit of Quantification
- **TRA** | Toxicological Risk Assessment
- **CoC** | Cohort of Concern

The importance of the Analytical Evaluation Threshold (AET)

Biocompatibility testing applies a tiered approach:

1. Chemical characterisation & cytotoxicity testing
2. If necessary, *in-vitro* testing such as genotoxicity
3. If necessary, *in-vivo* testing (animal testing)

The AET is particularly important for the first tier – chemical characterisation.

The importance for implants

When undergoing the biocompatibility assessment for implants, the AET is the most critical factor in the chemical characterisation, because it connects the testing with the toxicological parameters - i.e., the threshold of toxicological concern (TTC) limits outlined in the standard **ISO/TS 21726:2019**.

Thereby, your AET must demonstrate that the analytical methods performed are sufficient to assess the toxicological risks for your respective device.

The AET is the key means to demonstrating that your analytical results (detected compounds) do not fly under the radar, and that you detect what you need.

What about instruments?

For medical devices classed as instruments the AET is of lesser significance: the requirements can be met relatively easily compared with implants because of the higher applicable TTC and lesser sensitivity of the analytical methods required.

Applicability of the threshold of toxicological concern (TTC)

It is important to note, that the threshold of toxicological concern (TTC) concept is only applicable to specific biological endpoints:

Applicability of TTC	
The TTC values in ISO/TS 21726:2019 CAN be used:	**The TTC values in ISO/TS 21726:2019 CANNOT be used:**
For comparing to a maximum concentration of an identified or unidentified constituent in an extract	For constituents with adequate toxicity data for deriving a tolerable intake (TI) value
For supporting toxicological equivalence	For other biological endpoints assessed as part of the biological evaluation of a medical device, per ISO 10993-1, for example: • cytotoxicity • irritation • sensitisation • hemocompatibility • material mediated pyrogenicity • local effects that occur in tissues at the site of contact between a medical device and the body (e.g. the observations from implantation studies).
For comparing to a maximum exposure dose estimate of an identified constituent	For potential exposure via gas pathways of medical devices
As protective values for carcinogens, systemic toxicants, and reproductive toxicants	For the safety assessment of constituents belonging to the cohort of concern

Calculating your AET

The applicability of ISO/TS 21726:2019, ISO 10993-17 & -18

The threshold of toxicological concern (TTC) is defined in the standard **ISO/TS 21726:2019**, which refers to standards **ISO 10993-17** and **ISO 10993-18**.

The table below shows the recommended TTC values based on medical device contact categories as outlined in ISO 10993:

Medical device contact category	Limited (< 24 h)	Prolonged (24 h to 30 d)	Long-term[a] (> 30 d)		
Duration of body contact	≤ 1 month		> 1 month to 12 months	> 1 year to 10 years	> 10 years to lifetime
Daily intake (µg/d) of any one constituent	120		20	10	1,5[b]

[a] Long-term includes devices commonly described as permanent contacting (see ISO 10993-1).
[b] The 1,5 µg/d value is based on 10^{-5} cancer risk and 60kg (adult) body weight.

Recommended ICH M7(R1) (2017) TTC values based on ISO 10993-1 medical device contact category

According to Annex E of ISO 10993-18, to calculate the AET, the following parameters are typically considered:

- Product properties (intended patient group e.g. children/adults, maximum amount of products to be implanted, size, exposure duration)
- Extraction conditions
- Uncertainty factor of the analytical method

Using the calculation equation as outlined in **Annex E of ISO 10993-18** along with the typical parameters outlined above, you can convert the dose-base threshold (e.g. the TTC) into a concentration-based threshold (AET).

Calculating your AET (continued)

Utilisation of LOD & LOQ for comparison

To determine whether the AET for your tests is sufficient, you can compare it with the parameters of the applied analytical methods below:

- **LOD: Limit of Detection**
- **LOQ: Limit of Quantification**

If your AET is above your LOD and LOQ, the test method provides sufficient means for detection.

If your AET is below your LOD and LOQ, the test method is not capable of detecting compounds in a certain range, leaving a potential gap in your results. In this case, the method should either not be applied, or be supported with further information such as:

- a justification as to why the results are still valuable
- other analytical methods

Special Case

If your AET is above your LOD, but below your LOQ, this means that your method can detect the compounds within the specified range, but the quantification doesn't meet the necessary level of precision. As such, further investigation would be required.

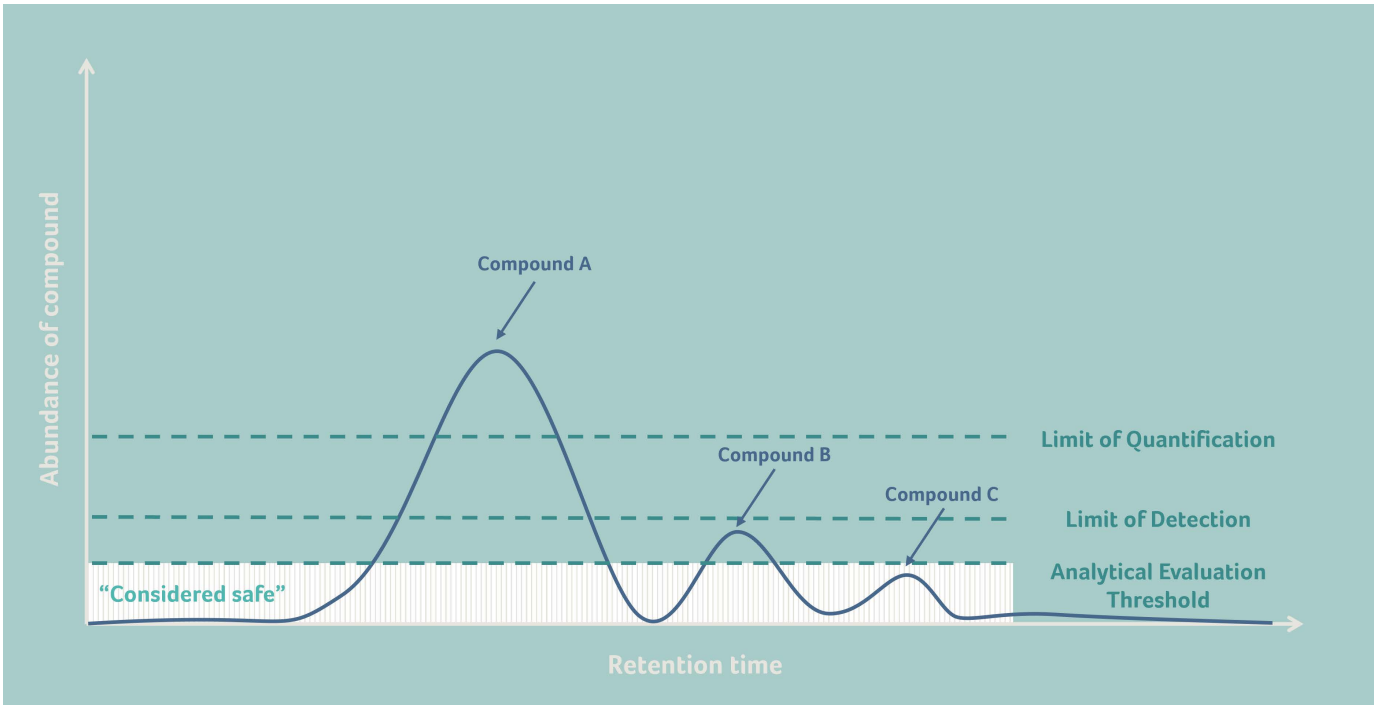
A common approach is to assume that the detected compound is present in an amount that equals the LOQ - but this assumption must be justified.

Calculating your AET (continued)

Using a chromatographic method as an example, the figure below is a **graphical representation of the method's Analytical Evaluation Threshold compared with the Limit of Detection and Limit of Quantification**.

The curve highlights the principle, including compounds that would not be detected as their amount is below the LOD. The representation only considers the presence of compounds for which the principle is fully applicable, in particular regarding the "considered safe" area. The AET in this example lies below both the LOD and LOQ, highlighting the potential challenge regarding the detected compounds:

- **Compound A is detected** and can be toxicologically assessed
- **Compound B is not detected but present** in an amount that might be toxicologically relevant (i.e. could represent a potential risk to the patient)
- **Compound C is not detected** but since present below the AET, it is also not toxicologically relevant



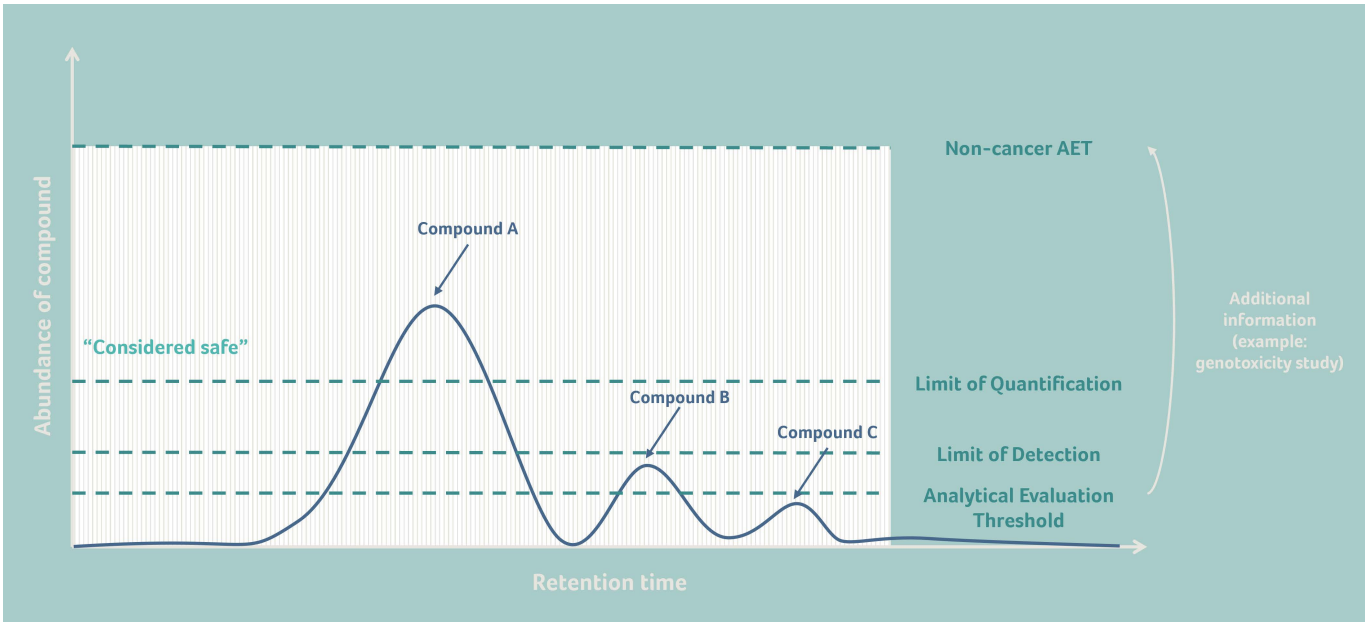
How to "shift" your AET

TTC/AET can be “shifted” by conducting further *in-vitro* tests such as genotoxicity in addition to the chemical characterisation.

The TTC values listed in the table on page 4 were developed to be **protective for cancer-based and non-cancer effects**. The threshold for cancer-based effects is lower than for non-cancer effects. As such, by performing testing that detects potential cancer-inducing effects (like genotoxicity), you can justify the use of the non-cancer threshold instead of the cancer-base threshold – of course only if the testing passes the criteria and demonstrates absence of carcinogens etc.

The figure below is a graphical representation of the Analytical Evaluation Threshold compared to the Limit of Detection and Limit of Quantification - this time with inclusion of a non-cancer AET. **This exemplifies how additional information can affect your biological assessment.**

If the presence of potential carcinogens can be ruled out, for example by providing additional genotoxicity testing, the TTC for non-cancer effects can be included in your AET calculation. In this example, you’ll subsequently see that the amount of all three compounds is below the respective AET and therefore, would not be considered toxicologically relevant.



Tips & considerations for your next AET calculation

Choose the right evaluation strategy

For your overall evaluation strategy there are two options according to the tiered approach mentioned earlier:

1. **Sequential approach (standard approach):** Perform the chemical characterisation first, and if the results are above the AET and toxicological risk assessment is not possible (for example because of a poor identification of the compound) perform a subsequent *in-vitro* test. This strategy can result in a longer total evaluation time, but potentially saves time and resources if a subsequent *in-vitro* test is not required.
2. **Parallel approach:** This strategy involves performing the chemical characterisation and an *in-vitro* test (such as a genotoxicity test) in parallel. Whilst overall time is initially saved with a parallel approach, the results of the chemical characterisation may indicate that an *in-vitro* test was not required, and as such budget and resources could have been allocated elsewhere.

Talk to your friendly local contract lab

Collaboration with your preferred lab is highly recommended when calculating and checking your AET. Their knowledge of the analytical methods which they provide should place them in a strong position to offer an **expert opinion**.

Don't forget the TRA

The **Toxicological Risk Assessment (TRA)** is a key element of a comprehensive biological assessment. It involves a toxicologist who uses the detected compounds and available data on those compounds (such as existing literature, tox databases etc.) to calculate potential patient exposure and the associated margin of safety.

Only consider *in-vivo* testing as a last resort

Should chemical characterisation and *in-vitro* testing not be sufficient, *in-vivo* testing is an option. However, the need to utilise this option is fortunately very rare – and **very much a last resort**. This position is supported by ISO 10993-2 which instructs the avoidance of animal testing as far as possible.

Prepare for "unknowns"

The possibility of being confronted by "unknowns" and "poorly identified compounds" is an important consideration in practice. If their amount is below the AET, you could argue that your device is still safe (but you better have your rationale ready as to why CoC compounds are not expected).

If their levels are above the AET, they are toxicologically relevant – and so without being able to identify the compounds, further investigation would likely be required. This investigation could take the form of genotoxicity tests, the provision of more information on potential residues to improve the identification of results, or follow-up analysis using alternative methods.

Consequently, you should **factor in some budget and time contingency** to allow for these potential hurdles.

Should you have a biocompatibility challenge, our Operations team is ready and happy to help. Simply get in touch to start the conversation.

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