EUROPEAN COMMISSION
DIRECTORATE-GENERAL
JOINT RESEARCH CENTRE
Directorate F - Health, Consumers and Reference Materials
European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM)

Meeting of the ESAC Working group on Bioelution

(2-3 May 2019)

Minutes

Attendees:

Core ESAC Members

Chantra Eskes (Chair)

Emanuela Corsini

Annette Kopp-Schneider (joined by WebEx on the second day of the meeting)

Ad-hoc ESAC Members

Valerie Hanley

Irina Karadjova

Mina Suh

JRC (Directorate F)

João Barroso

Pilar Prieto

No conflicts of interest were declared by the members of the ESAC Working Group (ESAC WG).

Mina Suh agreed to act as rapporteur and to present the outcome of the ESAC WG (i.e. scientific advice) to ESAC at the ESAC plenary meeting in December 2019.

Chantra Eskes chaired the two days meeting.

Pilar Prieto briefly presented the Bioelution test method submission file. As a follow up, members of the group shared the individual viewpoints and controversial issues and a number of points were collected and discussed with the test method submitters by WebEx.

The statistician of the group, Annette Kopp-Schneider, could not attend in person but on request joined the discussions by WebEx and addressed a number of issues that were raised by group (i.e. appropriateness of within and between reproducibility assessment and the statistics used by test submitter, need to show variability of relative measurements, and reply provided by test submitter with regard to the uncertainty associated with available *in vitro*/*in vivo* correlations).

The main outcome of the meeting is summarised as follows:

- There is an agreement by the ESAC WG that this is an atypical method that ESAC evaluates (borderline between ISO and OECD TG);
- There is an agreement by the ESAC WG that this is an useful approach;
- The method is representative of the gastric compartment
- The objectives of the test are to measure relative bioaccessibility for grouping and classification of alloys. It is not to be used for predicting relative bioavailability. Publications in the peer-reviewed literature show that additional components (e.g., ascorbic acid, pepsin, and glycine) could be added to the gastric solution to increase solubility of certain metals (e.g., Whitacre et al. 2017). However, for the use of this method, the worst case scenario for the oral route of exposure may not be necessary due to the relative nature of the test, i.e., metal release of the test material (alloy) compared to metal release in the reference material (pure metal).
- The test is to be used for the oral route of exposure only. However, use of the 100 micron size was used to reflect particles inhaled and swallowed.
- For the six metal tested (i.e. round robin as published by Henderson et al., 2014), the ESAC WG agrees that the reproducibility (between) and repeatability (within) were acceptable. In concept, the ESAC WG agrees that this should work for other metals; however, data have not been demonstrated for the proposed protocol.
- In the peer-reviewed literature, there is a clear relationship observed between *in vitro* relative bioaccessibility and *in vivo* relative bioavailability. The objectives of the test are to measure relative bioaccessibility for grouping and classification of alloys. It is not to be

used for predicting relative bioavailability. Because of this, variability of *in vivo* data is not relevant for this application (grouping and classification of alloys). ESAC WG supports this approach for grouping and alloy classification but not for risk assessment.

- ESAC WG agrees that the relative measurements of bioaccessibility between pure metal and alloy used to calculate the bioaccessible concentration to show metal releases affected by matrix effects, is appropriate. However, it should be clearly defined as relative bioaccessible concentration and not %BC.
- ESAC WG agrees that the experiments carried out to demonstrate that there is a linear relationship between the fraction of metal ion release in the absence of a matrix effect (simple mixture) and the total content of that metal in the material were appropriate.

The main recommendations of the ESAC WG to test submitters were the following:

- To provide clarifications and revisions to the SOP (i.e. preparation of gastric fluid, homogeneity of the test sample, use of reference materials, qualification of sample loading, data interpretation procedures, confidence intervals of %BC, and applicability domain);
- To perform additional testing to aid proof-of-concept of the revised SOP, in particular the reference materials.
- Additional work to identify and characterise the reference materials. The ESAC WG would like to see an evaluation of the recommended reference materials that are to be added to the revised SOP. This is a critical component to demonstrate applicability of the method across various metals, i.e., alloy classification
- There are bits and pieces of information spread over different documents and it would be useful to have those clearly described in the SOP. These should include technical/mechanistic and the predictive limitations. The SOP should clearly describe the metals (e.g. those that have high releases in neutral pH or precipitate with chloride) that fall inside and outside the applicability domain.
- ESAC WG recommended further clarification to the protocol and the prediction model, as described above.

Until the issues raised above are addressed, the ESAC WG cannot make a final recommendation on the readiness of the method for regulatory use.

The test submitter agreed to address all points raised by the ESAC WG and to send a proposal to the group with regard to the additional work the group has recommended.

The next teleconference of the ESAC WG was scheduled for 21 May at 4 p.m. to discuss the proposal that the test submitter will eventually provide and to prepare for the ESAC plenary meeting in June 2019.