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

(25-26 September 2019)

Minutes

Attendees:

Core ESAC Members

Chantra Eskes (Chair)

Emanuela Corsini

Ad-hoc ESAC Members

Valerie Hanley

Irina Karadjova

Mina Suh

JRC (Directorate F)

João Barroso

Pilar Prieto

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 proposed context of use of the method is to assess the relative bioaccessibility (metal release from the test material compared to metal release from a reference material) for classification purposes (classification of alloys and use for grouping and read across). It is not to be used for predicting relative bioavailability for application in a risk assessment context.
- The method is representative of the gastric compartment. 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, these additional components have varying bioelution effects for the different metals and add complexity to the method that may lead to extra variability. Therefore, the ESAC WG supports keeping the method as simple as possible and acknowledges that the simple gastric solution is fit-for-purpose considering the proposed context of use.
- 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 metals tested (i.e. Co, Cu, Fe, Ni Pb, Zn tested in the round robin as published by Henderson et al., 2014), the ESAC WG agrees that the within- and between-laboratory reproducibility were acceptable. In concept, the ESAC WG agrees that this should work for other metals; however, data have not been provided to support this assumption.
- This method is not to be used for risk assessment. As such, the absence of *in vivo* relative bioavailability data to make a direct comparison to relative bioaccessibility data does not compromise the assessment of the scientific validity of the method. The ESAC WG supports this method for grouping and alloy classification but not for risk assessment.
- The ESAC WG agrees that comparing measurements of metal release between pure metal and alloy to calculate the relative bioaccessible concentration and assess if a matrix effect occurs, is appropriate. In this context, 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 metal mixture) and the total content of that metal in the material were appropriate.
- The ESAC WG acknowledges the fact that the submitters modified the protocol to indicate that reference materials will always need to be run in parallel to test materials as recommended by the WG.

- The SOP is considered consolidated with the revisions made to address the ESAC WG comments from May 2019 and with the addition of comments made during the WG meeting of September 2019.

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

- To provide clarifications and revisions to the SOP:
    - preparation of gastric fluid (*ongoing*)
    - use of reference materials (*ongoing*)
    - applicability domain (*ongoing*)
    - homogeneity of the test sample (*addressed*)
    - qualification of sample loading (*addressed*)
    - data interpretation procedures (*addressed*)
    - confidence intervals of RBC% (*addressed*);
- In the context of alloy classification (application 2), the term relative bioaccessible concentration (RBC%) should be used instead of BC% (*WG revisions made on the SOP for consideration by the submitters*).
- The submitters should perform additional testing to aid proof-of-concept of the revised SOP (*ongoing*): test (i) leaded brass, lead metal, cobalt metal and a cobalt-based alloy using a 0.1 M titrated HCl and (ii) cobalt metal and a cobalt-based alloy using the 2018 protocol (NaOH pH adjustment). Due to the nature of the method being the calculation of relative bioaccessibility values, test materials should always be tested concurrently to reference materials in both applications (grouping/read across and alloy classification). This is further supported by the variability of metal release values observed in the round robin trial (Henderson et al. 2014). On this basis, the data that were generated with the Co powder with the 2018 protocol needs to be regenerated concurrently to the alloy. Having this full dataset will allow calculating and comparing RBC% and CIs for the two approaches and investigating if/how potential differences observed in the absolute releases observed between tests and/or HCl procedures affect the RBC% and CI values. It is noted that the increase in metal release values of 10-27% obtained with concentrated HCl appear to be in the range of the variabilities observed in the round robin trial.
- The applicability domain of the method should be clearly defined (*ongoing*). This should consider the known technical/mechanistic and predictive limitations. The SOP should clearly describe the metals that fall outside the applicability domain (e.g. those that have high releases in neutral pH or precipitate with chloride), as well as those that fall inside.
- Linked to the previous point, additional work is needed to identify and characterise the reference materials for alloy classification for all those metals that fall within the applicability domain of the method (*ongoing*). At the moment, there seems to be a

disconnect between the reference materials described in Annex 3 RCOM (As, Pb, Ni, Co, Cd and Be metal) and those tested in the round robin trial (Co, Cu, Fe, Ni Pb, Zn; Henderson et al., 2014). All metals within the applicability domain should have a defined reference material in the SOP. Metals that could be within the applicability domain but that will never need to be tested, can be left out of the SOP (neither mentioned as being within nor out of the applicability domain) and would not need a reference material being defined.

- An edited version of Annex 3 RCOM should be added to Annex 1 of the SOP, including the criteria for selecting reference materials (*ongoing*).
- Data on the selected proficiency and reference materials should be generated once the final SOP is agreed and acceptance criteria should then be defined on the basis of those data (*to be done before OECD Test Guideline adoption*).

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.