Draft summary of the webinar held 2-3 May 2019

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Some background was provided by industry on the history of the 'bioelution concept'. The reflexion started several years ago with the observation that the toxicity of metals-containing materials (e.g. steel alloy) could differ from the toxicity of their ingredients (e.g. nickel or cobalt), related to the bioavailability (release) of metal ions. Main question was how to best reflect this possible difference in properties considering both the duty to ensure a safe handling and communication on the hazard of these materials but also their number and their applications. In the context of the REACH registration, bioelution was increasingly applied to support read-across and grouping (weight of evidence). The use of bioavailability in this context is also referred in the updated OECD Guidance on grouping.

Industry conducted a round robin testing (RRT) in 2010-2011 using a first SOP, based on the ASTM 1517, which was published by Henderson et al. in 2014. Based on the learnings from the RRT a revised SOP was drafted.

Discussions on the concept have taken place in different fora, technical and regulatory ones. For example, REACH text refers to the "properties of the matrix" to be considered for some materials. The CLP includes article 12(b) that allows under certain conditions to consider bioavailability etc. A Bioelution Expert Group, set up on request of Commission, was run by ECHA in 2016-2017 to discuss how to use bioelution results bioelution in the context of article 12(b). It put its activities on hold in summer 2017, waiting for a validated protocol to generate bioelution data.
The documents submitted to ECVAM in 2018 reflect the discussions that have taken place in the different regulatory fora but also with the metal commodities and the labs involved in the RRT and update of the SOP. Industry tried to distil the information in a logical story, in a simple protocol that would give reproducible and conservative results, that would be helpful for the purpose. The two considered applications are read-across/grouping and refinement of CLP for bioavailability.

The summary below reports, the initial noted and actions proposed by industry after the discussion on 2 May (in blue text). Further comments/notes from the call held on 3 May (in green text) and resulting actions for the workplan to be prepared by industry (highlighted in green). The panel's replies and recommendation are noted (in cyan highlights).

1. Why to add NaOH solution to adjust pH? Analytical issues?

Explanation provided by industry: The ASTM protocol on which the SOP is based refers to the addition of 2.55 g of HCl to one litre of water which corresponds to a 0.07N solution of hydrochloric acid solution (calculated pH ~1.2-1.3) and adjusting the pH to 1.5 (see below). The labs indicated that it is difficult therefore to reach the exact pH of 1.5 by dilution with water and that adjustment with a 'drop' of NaOH is needed. The amount of NaOH is estimated insufficient to cause any analytical issue for the ICP. Another option that was considered was to prepare the solution without pH adjustment, but we considered it important to have a precise pH to start with (1.5 +/- 0.1)

7. Reagents

7.1 *Hydrochloric Acid* (0.07 *N*)—Add 2.55 g concentrated hydrochloric acid (HCl) to water and dilute to 1 L with water.

7.2 *Hydrochloric Acid* (0.14 *N*)—Add 5.10 g concentrated hydrochloric acid (HCl) to water and dilute to 1 L with water.

7.3 *Hydrochloric Acid* (2.0 *N*)—Add 72.9 g concentrated hydrochloric acid (HCl) to water and dilute to 1 L with water.

7.4 *Hydrochloric Acid* (6.0 *N*)—Add 218.8 g concentrated hydrochloric acid (HCl) to water and dilute to 1 L with water.

7.5 *Water*, of at least Grade 3 purity in accordance with ISO 3696.

The panel commented that it may be easier to start from a more concentrated (e.g. 0.03N, expected pH 1.52) solution as less NaOH will have to be added.

Actions proposed by industry on 2 May: check with labs and improve/correct text SOP

Reply Panel: Titration – usually in the laboratory, we have 0.1000M (N) HCl – very simple dilution will ensure exact 1.5 pH

Discussion 3 May: the panel explained that using a solution 0.1 N would be more straightforward, allowing to reach the pH of 1.5 more easily by simple dilution. This would simplify the protocol as no adjustment would be needed.

Resulting actions:

- ***Industry to discuss with the labs the proposal made by the panel and adapt the SOP***

2. Why not to add other ingredients such as pepsine, glycine to better mimicking gastric fluid? Better justification, additional references.

Explanation provided by industry: The ASTM refers to a simple HCl solution and there was also a willingness from industry to go for the simplest medium possible. Still, the question whether we should not use a medium mimicking better the gastric fluid was posed and considered. This was one of the aspects addressed by Yvette Lowney in her work (TST, Attachment 18 in February 2018 submission). She presented and compared the composition of the media (gastric fluids) and metals dissolution results from a series of studies (figure 7, attachment 18 to TST). The simple gastric fluid gave the highest release for As and Pb. The addition of pepsine, glycine is not expected to increase releases in the gastric tests while adding variability to the assay. Important to note here as well is

that we do not aim at generating/predicting an absolute bioaccessibility value in vivo but a relative value.

Reply panel: We agree to have a simple method for the applications proposed. Addition of organic ingredients will create matrix interferences for ICP-OES/ICP-MS measurements. However, depending on the metal, the release in gastric fluid may increase when additional ingredients are added (Whitacre et al, 2017). Therefore, it is important to clarify the applicability domain of the method.

Discussion 3 May: The panel agrees to have a simple method for the applications proposed. Addition of organic ingredients will create matrix interferences for ICP-OES/ICP-MS measurements. However, depending on the metal, the release in gastric fluid may increase when additional ingredients are such as ascorbic acid added (Whitacre et al, 2017). Therefore, it is important to clarify the applicability domain of the method.

Post call, we (industry) checked the publication of Whitacre et al. 2017. This paper compared the metalloid (As) release in two gastric fluids both containing 0.1N NaCl and 1% pepsin, but differing pHs and acids (HCL in OSU-IVG and ascorbic acid in CAB). More As was released in the pH 1.5-ascorbic acid fluid (CAB) than in the pH 1.8- HCl one (OSU-IVG). This is consistent with the current SOP using pH 1.5 as worst case; yet we do not know the effect of ascorbic acid itself on other metals-metalloids besides As. In figure 7 of the Lowney review (attachment 18 to TST) different gastric phase composition fluids are compared regarding their release of As, including OSU-IVG. Simple HCL solution gave higher release than the OSU-IVG.

Resulting actions:

- ***It is important to clarify the applicability domain of the method SOP and Identify those metals for which other ingredients in gastric fluid could enhance metal release***

3. Particle size 100 micron is proposed while 150 micron seems to be used for ingestion. 250 micron is even more common in current literature. Could you confirm that the purpose is to cover oral route of exposure?

Explanation provided by industry: This refers back to the discussions held with the Bioelution Expert Group, where it was requested, if we were to test/classify alloys, to capture their lifecycle in a conservative way. If we were concerned only about ingestion, we could go to 250 micron. However, to address representativeness of samples through the lifecycle, and to be conservative, the proposed approach is to test a smallest size that can also be relevant to the inhalation route (e.g. particles of lead that would be inhaled but brought back to the GI tract by the mucociliary clearance. The 100 micron stands thus as a worst case for oral + reflects particles inhaled and swallowed.

Reply Panel: We agree with the approach proposed.

4. How were the two loadings selected?

Explanation provided by industry: Those are not exact numbers. We started from the ASTM that actually foresees a loading of 20 g/L but -and this was also discussed by Stopford later- in case of high release recommends lowering the loading. The proposed loadings were chosen based on data available for metals to prevent underreporting for samples that release a lot and to assure accurate detection for samples that release too little. We recommend to always test the two loadings. This provides some standardization of future results with this assay to same loadings and allows also to have information on kinetics when comparing by mass. Once you have done all tests/loadings you look at the different releases/mass. Depending on application the worst-case results can be used.

Actions proposed by industry on 2 May: Incorporate more clarity into SOP on how to select the release data from high or low loading for each application. see also question **9.**

Reply panel: We agree with the revision of the SOP accordingly.

Resulting action:

- ***Incorporate more clarity into SOP on how to select the release data from high or low loading for each application. see also question 9***

4b. The issue of reference material: when, which one, how many?

Explanation provided by industry: this will depend on the application.

- For read-across/grouping, you will have more than one reference material. The reference material(s) will always be run in parallel with the test material. Examples of reference (source) materials can be the soluble compound, an oxide etc. This will depend on each metal and what they are looking for: e.g. check if 2 soils are similar enough or register 5 cobalt compounds under REACH. What you will use as reference/source material will depend on the problem.
- For classification of alloys: we have a huge number of alloys families and the risk is that they may all use a different reference material (e.g. different pure Ni metal samples). This should be avoided. The idea was at some point to have a repository of metal (M^{o}) samples (massive and powder) that would then be used as references by anybody testing alloys containing that metal. This should be feasible, is not specifically articulated in the SOP yet as we were waiting for a validated protocol to test them (10 measurements/material, open access etc.), and to have guidance from regulators on how to choose them. For the alloys, their classification is based on the classification of the metal they contain. Sometimes this classification has been the result from a worst-case read-across from the soluble form but still the classification of the metal itself is what determines the classification of the alloy.

The panel confirmed that it is obligatory to have a clear database of reference materials, with well characterised samples as the whole concept relies on relative bioaccessibility.

Actions proposed by industry on 2 May: define/describe more clearly in the SOP how we choose the reference (material and include the justification for the selection in the bioelution report for both applications.

Reply Panel: The above statement is not completely correct. The panel is of the opinion that a list of references materials should be established for each application. Furthermore, the panel believes that it is critical to always run the appropriate reference materials in parallel to the test materials. All this needs to be clearly described in the SOP, i.e. the list of reference materials and the strategy to select that (in section 6.2).

Discussion 3 May: the panel explained that there are two elements to be addressed in this context:

a) The fact that reference materials shall be run in parallel to the test materials every single time for both applications (read-across/grouping, classification of alloys)
b) Clarify what is meant by repository, database etc.

The panel had discussed the reference material issue to a detailed extent before the call. They would like to have more clarity on criterions for reference material selection. They gave the following example for read-across/grouping: assuming that the cobalt sector would have to perform read-across, it would be helpful for them to be able to use a reference (highly or low soluble cobalt compounds) included in the repository. This would allow more coherence.
Industry agreed to have the reference and test samples run in parallel but asked to be able to keep some flexibility when it comes to the selection of the reference material for the read-across/grouping application. The choice of the source substance will depend on what you want to do, and the list of reference materials may change depending on the purpose, the availability of toxicological data and time. If the protocol is too narrow or the list of reference materials is too rigid, the list will be quickly outdated and useless. Criterion to define adequately the reference substance (e.g. phys-chem, tox data etc.) and to instruct in the SOP how to choose references shall be explained in the report.

The panel agreed that **criteria to select the reference material (or source substance) in the context of read-across and grouping** would be useful. Tox data, classification profile and solubility information seem to be straightforward criteria. The panel suggested also to check the RAAF recommendations to identify the criteria they use for the selection of the source substance when performing read-across/grouping and to include a reference to the ECHA RAAF in the documents.

For the alloys, classification application, it would be different: **the reference would be the metal-metalloid ingredients.** More discussion on this topic is reported under the next question.

Actions proposed by industry on 2 May: commit to set up repository of reference materials once we have clarity from regulatory authorities on criteria for choosing the reference samples.

Discussion 3 May: industry explained that a repository of samples would be set up. Typical samples would be the metals used as reference materials for the alloys' classification application. Two labs would run the revised SOP on the samples that in parallel would also be very well characterised, so

as to have a database of results that could be used by the lab performing an alloy bioelution test as internal control. Access to the samples would be for free (costs for shipping?), they would be stored under nitrogen.

Panel indicated that the SOP method need clarification on what is meant by "repository": 1) How it will be established? 2) What will be included? 3) How will this be used? 4) How it will be made available to the end users on a long-term basis?

Resulting actions:

- ***For the read-across/grouping application:***
  - ***Propose criteria for the selection of the reference material(s) & check the criteria used in the context of the RAAF to define the source substance and add a citation***
  - ***Include the instruction in the SOP that the choice of the reference substance should be explained in the report***
- ***For the alloy classification application:***
  - ***Propose criterion to select metal ingredients as reference substance and set up repository (see below).***
- ***Repository. Describe: 1) How the repository will be established, 2) What will be included, 3) How will this be used, 4) How it will be made available to the end users on a long term basis?***
- ***Set up repository: explain which metals will be included, how they will be characterised, test results that will be available and clarify modalities of access etc.***

5. Massive material: when to use epoxy embedded material?

Explanation provided by industry: Using epoxy embedded materials facilitates comparison among samples, same surface area, same finishing (polishing). It has initially been used in the context of the TDp for metals for which there was some abrasion. For some metals like nickel, results from tests done side by side using or not an epoxy mounting were compared and did not yield significant differences in terms of releases.

Actions proposed by industry on 2 May: give more context or guidance as to when an epoxy embedded sample can/should be used

Reply panel: We agree that further guidance needs to be provided.

Resulting actions:

- ***give more context or guidance as to when an epoxy embedded sample can/should be used***

6. How to present the final result, mass to mass or mass to surface area?

Explanation provided by industry: This depends on the application. The original results (=raw data) are mass per volume and then from there we can report the results as mass/mass or mass/surface area.

- Classification of alloys: the CLP refers for the Ni dermal sensitisation to an absolute release rate in sweat expressed as µg/cm²/week. By contrast for classification for other endpoints, a bulk concentration % is used (mass/mass) but you could also calculate a rate if of interest. The various ways to report data are included in the protocol but there is no prescription. For alloys, surface area plays a role (corrosion). So, results/surface area can be used if one wanted to e.g. compare releases from massive and powders for life cycle investigation. Still for now, the classification of alloys under CLP is based on % mass/mass, so it is important to have results expressed as e.g. mg/g
- For grouping/read-across: this relates to compounds! Surface area should not be used to correct the metal releases here

Actions proposed by industry on 2 May**:** give more direction on what metrics to use under each application. see also question 7

Reply panel: We agree with the approach proposed.

Resulting action:

- ***Give more direction on what metrics to use under each application. see also question 7***

7. Prediction model (DBALM protocol): Can you clarify how the data is used to make a prediction?

The panel explained that the prediction model in the DBALM should be replaced by a clear guidance on what to do/how to use the results for each application. It is proposed to use Annex 1 to the SOP for this and to focus on the SOP rather than the DBALM.

Actions proposed by industry on 2 May: include guidance in Annex 1 in the main body of the SOP, clarifying also the use of the different units (question 6)

Reply panel: We agree with the approach proposed. However, this should be described as a procedure and not as an example. In addition, it is possible that relative bioaccessibility of the alloy (test material) is greater than 100%. The SOP should discuss that the absolute bioaccessibility of the alloy (test material) can be higher than that of the pure metal (reference material).

Discussion 3 May: The panel stressed that guidance on the use of the results should be part of the procedure. On the BC>100%, industry explained that in the context of the Bioelution Expert Group, approaches/flowcharts were proposed to consider cases where bioaccessible concentration would be >100% (safety net). However, the ways to use the results and the use of a safety net had not been approved by ECHA's Expert Group when its activities were put on hold. We may need to be a bit cautious. The panel clarified that what is important is to stress in the SOP that one may have a BC>100% and that this does not necessarily correspond to an error. The SOP should include the

calculation of the BC and explain that indeed depending on the matrix effect, BC may be higher or lower. This can be relatively brief but is important for a self-standing SOP.

Resulting actions:

- ***include procedure on how to use the results in the main body of the SOP, clarifying also the use of the different units***
- ***Include the calculation of the BC in the SOP and explain it can be <, = or > 100%***

8. Protocol, section 6.1, note: Last sentence is not clear, "If there is a small amount of sample, the test material should be mixed in between weighing the three samples".

Explanation provided by industry: this was a suggestion made by one of the labs/sponsors of the test. Aim is to increase representativity of samples tested by mixing, taking a sample, mixing, taking a sample etc, when you have small amounts.
The panel did not like this text and agreed to replace it with a sentence referring to the fact that it is important to have a good sample homogeneity in order to have a good representativity. This is more about homogenisation when there is heterogeneity

Actions proposed by industry on 2 May**:** edit sentence and mention the need for having a homogeneous sample prior to start the testing.

Reply panel: We agree with the approach. However, the SOP should clarify who will be responsible for sample representativeness and homogeneity. Usually, this is a kind of agreement between laboratory and client. Sample homogeneity is a source of variability, and the whole test could be discarded based on this reason. It is evident but should be better clarified in SOP

Discussion 3 May: it should be stressed in the SOP that the sample shall be representative. We may also specify that a sufficient amount should be provided to the lab to avoid that they have too small quantities. The panel added that a word of caution should be included to prevent the sequence sampling, mixing, sampling, mixing as the continuous shaking will change the material and thus affect the representativity/comparability of samples.

Resulting actions:

- ***Edit SOP to indicate that a sufficient amount of a homogeneous sample (check minimum amount with lab) should be delivered by the sponsor to the lab and include clarifications about mixing between samples in the SOP***

9. What happens if the sample changes the pH at the end of the 2 h exposure? It is outside of applicability domain or will the experiment be repeated?

Explanation provided by industry: at the start we wanted to measure the pH initially, then once the sample was added to correct the pH and again two hours later. However, this was increasing the number of manipulations/possible sources of errors. Therefore, the suggestion was to include an

additional sample to measure the initial pH and to also measure it at the end of the 2 hours period. Also, this was a good argument to have the two loadings as the buffering capacity will be different (higher capacity at lower loading). This should allow us to get some information at the end of the test. The use of triplicates should also allow some 'control': if one of the three samples has a pH shift, then on can consider there is an artefact, if all triplicates are consistent, then this seems to be inherent to the sample. How to proceed is explained on page 19 of the current SOP. Still the question remains posed: if you have a pH of 5 in the lower loading, do you invalidate the results? Can this be overcome?
The panel made several comments: if you have a continuous increase in pH, why discarding results? The fear is that the in vivo stomach would keep pumping protons and would keep pH low while we cannot do it in vitro. Should we repeat the test at a loading < 0.2 g/L? It should be clarified when results should be repeated or discarded. Results from the loading that has pH closer to 1.5 should take precedent. Industry explained that we do not have a lot of experience with pH drifts in the metal results accumulated until now.
Also, if you have significantly different results (mass/mass) between two loadings: which result should you use? We should use the more conservative as the idea is to have a worst-case scenario. E.g. when calculating the concentration of a metal in an alloy based on bioaccessible ions, the worst case is to use the loading that results in higher releases for the alloy and the loading that results in lower releases for the pure metal; that combination results in the highest (most conservative) concentration. This should be clarified in the SOP.

Actions proposed by industry on 2 May**:**

- clarify what to do when there is a pH drift (when to discard, when to repeat)
- clarify which loading results to use for each application (worst-case)

Reply panel: We agree with the approach proposed.

Additional Panel Comment May 3:
The SOP should require a measure of variability like 95%CIs to be calculated for %BC

Discussion 3 May: this was agreed. It is important considering possible later applications, e.g. comparison with a classification cut-off

Resulting actions:

- ***clarify what to do when there is a pH drift (when to discard, when to repeat)***
- ***clarify which loading results to use for each application (worst-case)***
- ***Request that 95% CI be provided with each calculated BC%***

10. How did you determine the number of samples (6) in the RRT?

Explanation provided by industry: the story of the RRT was briefly detailed: participating labs= existing labs with experience with TDp and bioelution testing in 2010 + ECTX who later joined the exercise. Selected samples covered a range of different materials (powder, alloy, ore and

concentrate, soluble compound) and a number of metals from these materials (matrix of measured metals).

Reply panel: For the six metals tested, we agree that the reproducibility (between) and repeatability (within) were acceptable. In concept, we agree that this should work for other metals; however, data have not been demonstrated for the proposed protocol.

11. Where the samples tested blind?

Explanation provided by industry: samples were not tested blindly (labs knew what they were testing) but the statistician did analyse the results 'blindly'

Reply panel: It is likely that the laboratories did not know what the results will be. In addition, the statistician did the analyses in a blind fashion. Therefore, we found this to be an appropriate approach.

12. Was the revised SOP ever checked in an independent laboratory?

Explanation provided by industry: no new RRT was launched (waiting for the protocol and feedback from ESAC-ECVAM to know which parameters to further improve/correct) but the protocol was tested by a lab in the US in the context of nanos (so changing the separation step) but without a possible comparison with earlier results. The SOP is used by labs like ECTX and a lab in Spain but not on the exact same materials tested in the RRT.

Actions proposed by industry on 2 May: Consider a new RRT with recommended protocol at some stage

Reply panel: We believe that the RRT is not necessarily needed. However, we 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.

Discussion 3 May: it is proposed to rather focus on the reference materials for alloy classification and to test those with the revised SOP. Idea would be to use 2 labs, do a number N of measurements. During the discussions, it was mentioned that it would be interesting to include one of the materials of the 2014 RRT to compare and give some confidence that the revision of the protocol did not generate different results (refined and better SOP, not different). We could also include a couple of metals with low bioavailability like sodium arsenate, etc.

Additional Comment from panel: Please discuss and provide an assessment of %BC variability.

Discussion 3 May: it is important to realise that it is the method that is examined, not only the protocol but also how results are used. Referring already to the use of the data will facilitate further regulatory use. The variability of the BC% is important to mention in this context (CI?)

Resulting actions:

- ***Draft a list of potential reference materials that could be tested and submit proposal to panel***
- ***Include the variability of the BC% in the SOP***

13. What evidence you have that the method is conservative enough?

Explanation provided by industry: reference was made to the work done by Yvette Lowney and the correlation curves.
The panel stressed that because the applications compare metal releases, it is very important that the reference materials are run in parallel. Industry agreed to further work on the aspect of reference material but mentioned as well that this part will also be further discussed by regulators who will come with recommendations on reference material selection. Using the gastric fluid is a worst case for oral bioavailability and because relative bioaccessibility is used, if there is any shift, it will also affect the relative bioaccessibility.

Actions proposed by industry on 2 May: further clarify when to use which reference material for which application (see question 4b).

Reply panel: See our response to Point 12.

Resulting action:

- ***Further clarify when to use which reference material for which application (see question 4b).***

14. Did you ever consider testing some chemicals in a blind manner having in vivo data as a proof of concept?

The panel explained that the suggestion would be to take some materials for which we have some bioavailability data and to run them through a gastric test. This would help to feel more comfortable that the method is conservative in its predictions.
Industry agreed this could be done

Actions proposed by industry on 2 May: consider running gastric tests for samples of materials (soils?) for which bioavailability data are available. Suggestions from ESAC?

Reply panel: We would rather recommend having a proof of concept testing with well-characterized reference materials.

Discussion 3 May: The panel explained that it is not so much needed to have a comparison of bioelution data with in vivo data but to demonstrate that the protocol works. As the proposed use of data is not to predict absolute bioavailability in vivo but to do a "relative" exercise (and classify below/above cut-offs or reference metals for example), it is more important to have appropriate, well-tested reference materials. Industry referred to the paper comparing BC with acute toxicity levels for nickel compounds and the 'ranking' between the compounds that came out of it (Henderson et al.

Henderson et al 2012.pdf

2012). A proposal would be to test whether this ranking holds with the new protocol.

Resulting actions:

- ***Tests a couple of materials from previous round robin with updated protocol (summer)***
- ***Consider a couple of Ni compounds referred to in Henderson et al. 2012 with revised protocol and assess if ranking remains the same***

15. Is it possible to compile the metals (e.g. those that have high releases in neutral pH or precipitate with chloride) that fall inside and outside the applicability domain within the SOP?

The panel explained that there are bits and pieces of information spread over different documents and it would be useful to have those in one place.
Industry agreed but stressed that we need to distinguish cases where the gastric test did not generate the highest release compared to other biological fluids from cases where we cannot apply the SOP. In other words, we need to distinguish applicability of the SOP and applicability of the data.
The panel explained that the limitations of a test/SOP include both the technical/mechanistic and the predictive limitations. The SOP should clearly state under applicability domain:

- What is explicitly excluded because of technical issues (e.g. nanos) or predictive issues (e.g. metals that precipitate or metals that release higher amount at neutral pH
- Or make a proposal (e.g. for this metal, check release in fluids with different pHs)

Actions proposed by industry on 2 May: further clarify predictive limitations (the clarification that nanos are excluded is fine)

Reply panel: We agree with the approach proposed.

Resulting action for industry:

- ***further clarify predictive limitations in the SOP***

Timelines:

The panel discussed several possible scenarios:
Overall, the debates seem to indicate that the test is fine, but the protocol is not explicit enough on a number of issues mentioned above. Further work is required on the SOP to optimize it, keeping in mind that what is examined is the 'method', i.e. protocol + interpretation of data.

ESAC could theoretically reach an opinion by end of June or by December, but to be able to provide an opinion before the summer industry would have to deliver on identified actions asap. It was agreed that it may be easier and more efficient to deliver revised SOP and other deliverables by October so as to allow ESAC to reconvene second half of the year and have an opinion in December. The package would be more robust.

This timeline does not prevent to submit a project to the WNT (deadline: 15 November), as in any case the WNT starts looking at the materials after the deadline (Q1 -Q2 next year).
Issue to solve in this context is to identify a sponsor: Commission, an EU Member State (at this stage not a lot of volunteers) or other OECD country.

Industry proposed to re-send the notes of the exchange (amended with notes discussions on 3 May) + a workplan and milestones.
A call with the panel will be held on 21 May to discuss workplan (see annex 1).

Annex 1: proposed workplan: actions to be addressed before October 2019

1. **SOP**

Required work (questions posed by ESAC)	Proposed milestones
• Use a 0.1N solution of HCl that will allow to reach the pH of 1.5 more easily by simple dilution. This will simplify the protocol as no adjustment would be needed (question 1)	• Discuss the issue with labs (May) • Clarify text SOP (June)
• Check publication of Whitacre et al. 2017 (and possible others) and identify metals for which there may be higher release in presence of some organic ingredients. It is important to clarify the applicability domain of the method SOP and Identify those metals for which other ingredients in gastric fluid could enhance metal release (question 2)	• Inspect if the literature search of Y. Lowney has information on metals that may have higher release in presence of pepsin, glycine, ascorbic acid (May-June) • Prepare list of metals that are of concern (summer) • Include the outcomes in the section on the "applicability domain" of the SOP (summer)
• Confirm that the purpose of the method is to cover oral route of exposure and provide a worst case to address the comment that whilst a particle size of 100 µm is proposed, 150 or 250 micron seem to be used /more common for ingestion (question 3)	• Check clarity of the explanation in text SOP (June)
• Incorporate more clarity into SOP on how to select the release data from high or low loading for each application and that it should be worst case (questions 4, 9)	• Clarify text SOP (June)

Required work (questions posed by ESAC)	Proposed milestones
• Clarify the choice of the reference material: *For the read-across/grouping application:* - Propose criteria for the selection of the reference material(s) & check the criteria used in the context of the RAAF to define the source substance and add a citation - Include the instruction in the SOP that the choice of the reference substance should be explained in the report *For the alloys classification application:* - Propose criterion to select metal ingredients as reference substance and set up repository (see below) (questions 4b, 13)	• Clarify in SOP that reference materials shall be run in parallel to test materials (June) • Check criteria to select/describe the source substance in ECHA RAAF (May) • Clarify in SOP that reference materials shall be run in parallel to test materials • Clarify text SOP on selection/description choice reference material for alloys (June) • Set up repository (see below)

Required work (questions posed by ESAC)	Proposed milestones
• Give more context or guidance as to when an epoxy embedded sample can/should be used (question 5)	• Include text on when an epoxy embedded sample can/should be used (June)
• Include procedure (currently only example is provided in Annex 1) on how to use the results in the main body of the SOP, clarifying also the use of the different units • Include the calculation of the BC in the SOP and explain it can be <, = or > 100% • Request that 95% CI be provided with each calculated BC% (questions 6, 7, 12)	• Clarify text in SOP (include in Annex 1 as 'procedure') (summer)
• Clarify issue of homogeneity of the sample clarifications in the SOP • Edit SOP to indicate that a sufficient amount of a homogeneous sample (check minimum amount with lab) should be delivered by the sponsor to the lab and include clarifications about mixing between samples in the SOP (question 8)	• Explore if there is an agreement existing between labs and client on amount/pre-treatment of the sample and include (June) • Include clarification in the SOP • Include word of caution to prevent the sequence sampling, mixing, sampling, mixing as the continuous shaking will change the material
pH drift • clarify what to do when there is a pH drift (when to discard, when to repeat) • clarify which loading results to use for each application (worst-case) (question 9)	• Check if text in SOP is clear enough (June)
Further clarify predictive limitations in the SOP: The SOP should clearly state under applicability domain: • What is explicitly excluded because of technical issues (e.g. nanos) or predictive issues (e.g. metals that precipitate or metals that release higher amount at neutral pH) • Or make a proposal (e.g. for this metal, check release in fluids with different pHs)	• Clarify text in SOP (summer)

(question 15)	

2. Other actions

Required work (questions posed by ESAC)	Proposed milestones
• Set up physical repository of reference materials for alloys = metals • Describe: 1) How the repository will be established, 2) What will be included, 3) How will this be used, 4) How it will be made available to the end users on a long-term basis? • Explain which metals will be included, how they will be characterised, test results that will be available and clarify modalities of access etc. • Have an evaluation of the recommended reference materials that are to be added to the revised SOP (questions 12, 13)	• Draft a list of potential reference materials that could be tested and submit proposal to panel (< 21 May) • Clarify if some of these reference materials can also be used as proficiency materials for this application of bioelution (May) • Clarify modalities of set up of repository: materials should be well defined and characterized (e.g. regarding elemental composition and particle size), massive and powder physical form of the test materials will be available. Conditions of storage and access should be detailed. These materials would be tested by two labs so as to have a series of results that can be made available using the revised protocol (summer)
• Consistency between updated and initial protocol (question 14)	• Tests a couple of materials from previous round robin with updated protocol (summer) • Consider re-testing a couple of Ni compounds referred to in Henderson et al. 2012 with revised protocol and assess if ranking for bioavailability and toxicity remains the same (summer)