

Oct 11, 2019 Update on the list of actions agreed during call 25 September and WG discussions on 26 September

1. SOP

	Comment/Discussion	Actions taken
page 6, 2. Applicability of the test method	<p>The applicability domain of the method should be clearly defined. 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.</p> <ul style="list-style-type: none"> • Para on metals/metal compounds in scope needs to become more precise. Metals can be out of scope for technical reasons (e.g. following precipitation) or for producing unreliable results (lower metal releases at low pH). It could also happen that some chemical forms of a metal may be within the applicability domain and others may not. Metal-containing substances outside the applicability domain of the method should simply not be tested. These metal-containing substances should be clearly identified in the SOP. • Metal-containing substances that can be tested and are within the applicability domain of the method should also be identified in the SOP. In the case of metallic forms (M^0), this should also be reflected Annex 1 of the SOP which should be enlarged to contain an edited version of Annex 3 RCOM (see below). Thus, all metal elements described in the SOP as being within the grouping and read across applicability domain of the method and for which a metallic form carries a hazard classification relevant to the oral route (alloy classification applicability domain) should have a M^0 reference material (for alloy classification) identified in Annex 1. This does not preclude the possibility that more reference metals could be added in the future if more metals are classified and provided that it is technically feasible to measure them. 	<ul style="list-style-type: none"> • We contacted the commodities/consortia and clarified the technical applicability of the gastric fluid protocol. Based on existing data, Ag, Hg and some Sb compounds are out of scope. • We clarified for which metals, gastric fluid releases are not a worst case and their consideration under application 1 (grouping and read across) requires additional information. These include some chemical forms of Fe, Mo, Si, and W. • We identified the metals in scope of application 2 (alloy classification) based on their hazard associated with the oral route (and/or classification), and for which reference materials (M^0) will be selected for the sample repository, i.e. Be, Cd, As, Pb, Ni, Co and Se. • Include references where relevant • Annex 1 with table specifying which metals/metalloids are in/out of scope was added.
page 6	Delete part of the sentence: 'under consideration by ECVAM'	Sentence was deleted.

2. Applicability of the test method		
page 6 2. Applicability of the test method And remaining SOP	In the context of alloy classification (application 2), the term relative bioaccessible concentration (RBC%) should be used instead of bioaccessible concentration (BC%).	Accepted the changes made by ESAC WG.
page 7 3. Test materials	Delete word 'additionally' and include 'where possible' It was explained that the intention of this sentence was to address materials that are very hard to grind, e.g. more than 200 µm. Such coarser materials should be covered too, still powders	ESAC's proposed rewording was implemented.
page 7 3. Test materials	Replace word 'desired' by 'representative'	Suggested edits were made.
page 7 3. Test materials	Add sentence: 'The representative particle size(s) to be tested should always be defined and potentially prepared by the applicant/study sponsor	Suggested edits were made.
Page 9 Massive material-epoxy embedded sample	The WG is ok with the procedure but notes that only two epoxy embedded materials in massive form were tested and described in the submission. Just for information. This will be included in the final Working Group Report, but in principle no action needed	No action taken.
page 12 Test medium	Start from a 0.1 M titrated HCl solution and calculate amount of water necessary to reach a pH of 1.5. Titrated HCl solution can be purchased or prepared. In the first case the exact amount of water to be added can be written in the SOP. In the second case this will need to be calculated based on the exact concentration of the in-house titrated solution.	Changed text to recommend as indicated in Table 1 and section 4.2.
page 17 6.3 Observations, sampling and measurements at the end of the test	Clarify text: add words 'analytical sample, from each test material etc.	Text was edited.
page 19 6.5 Data analysis	The SOP should better explain the calculations that are performed using the measurements obtained from the 6 analytical samples and how these are used to check against the acceptance criteria. SDs and CVs obtained from	Text was edited.

	N=3 (averages of technical replicates) or N=6 values (technical and biological replicates together).	
page 25 7. Data application	<p>To simplify the procedure, the same approach of mass/mass should always be used, even when the same exact conditions (e.g., loadings and times) are used for both reference and target samples (i.e., never use. metal releases per volume of solution, e.g. µg metal/L).</p> <p>It should be better explained when it is possible to mix loadings in the read across scenario. Examples of use of different loadings should also be provided in an extra table.</p>	<p>This was clarified in the text as follows: <i>If the target and reference samples have been tested at both of the recommended same two loadings (2 g/L and 0.2 g/L), then the results obtained at the same (high or low) loading should be compared for the purpose of grouping and read across. In most cases, the read across conclusions using data from either loading will be the same. If this is not the case, it is recommended to use the data from the loading that yields the more reproducible data (lowest SD). A deviation from this approach can be justified if for one of the samples, quantitative measurements are only obtained at one loading (e.g., when the low loading yields values < LOQ, the use of the high loading data only would be justified).</i></p>
page 24 7. Data application	Check reference Henderson et al. 2012	This was corrected.
page 25 7. Data application	What is meant by 'most reliable'	Text was edited. Changed to <i>most reproducible based on SD.</i>
page 26 7. Data application (same comment in Annex 1, page 3)	<p>What is the scientific rationale for taking the highest value for the alloy and the lowest for the pure metal, even if coming from different loadings, just to obtain the highest possible RBC%? Is this acceptable? The matrix effect should be evaluated at the same loading, not at different loadings</p>	<p>Text was edited to be consistent with ESAC's advice: <i>To assess matrix effects and calculate the RBC%, it is recommended to consider the data collected at the low and high loading, separately, using Equation 1 above. The most conservative estimate (worst case) of the relative bioaccessible concentration of the</i></p>

		<i>metal in an alloy can then be identified and selected.</i>
page 27 7. Data application	One should not talk about matrix effect when different loadings are combined for the calculation of "worst case RBC%". This will however be ok if the worst case scenario is defined as proposed in the comment/reply above.	Text was edited.
page 27 7. Data application (same comment in Annex 1, page 3)	<p>The free QuickCalcs software does not provide sufficient significant digits and is not considered appropriate to calculate Confidence Intervals in this situation. Please revise the way Confidence Intervals are calculated using something like Excel, R or SPSS. The formula should also be provided in the SOP.</p> <p>Reply from submitters: The Quickcalcs was included in Annex 1, in response to a question posed by ESAC WG in May to calculate 95% CI. ESAC WG asked why only the numerator value (alloy) is multiplied by 100. Without multiplying by 100, the values of the 90, 95, 99% CI calculated by the software fall in a range (less precise): 0.06-0.07. The question was posed how precise we need to be (number of digits). It may be sufficient in some cases to have a 0.1 % value for example and in other cases (lower releases) we may need to have more digits. It was also proposed to rely on more common software for the calculations of the CI, like R, SPSS, or even Excel or to NOT push for a specific tool, limit the instructions to the equation and that the CI should be calculated checking the level of precision that is needed</p>	The reference to QuickCalcs was deleted. Text was edited to include only the reference to Fieller, 1954. It will be up to the user to decide how they want to implement it (Excel, R or SPSS). We now note that the CI should be calculated checking the level of precision that is needed
page 33 Annex 1	Avoid making reference to the TST in the SOP	Text was corrected.
page 33 Annex 1	This Annex should be extended to provide the information given on annex 3 RCOM describing the physical repository that will be established (as if it is already established). Both Proficiency and Reference Materials should be described. The criteria for selecting reference materials should be included	Annex was expanded.

2. Annex 1 of Response to ESAC Comments

Additional tests were agreed to be conducted (see below) and results will be reported when finalized.

3. Others

	Comment/Discussions	Actions
Reports ECTX	Clarify reported values	We clarified with ECTX that 'blue results' (x average, S between, CV between are the ones needed. Included in SOP only what is needed
Repository	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.	Timeline and milestones will be forwarded <ul style="list-style-type: none">• November 2019: contacts with 3 possible candidate labs (Germany, Sweden, Belgium) for setting up repository and comparison of conditions/modalities. November 2019: discussion within sector on funding/maintenance mechanisms of repository• November 2019: contacts with companies to define/ collect sufficient amounts of samples of reference metals. Organise characterisation and shipping• December 2019 January 2020: selection of the lab in charge + second lab to test the reference samples• January-February 2020: identification, collection of proficiency materials

	<p>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 of alloy classification 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 Annex 3 and would not need a reference material being defined.</p> <p>Before OECD Test Guideline adoption, 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</p>	<ul style="list-style-type: none"> • Tests on reference samples by two labs and set up Shewart charts database to begin when OECD gives green light to the SOP <p>There is a disconnect because the purposes of the studies were different. The selection of samples for RR included examples of different types of materials: alloys, compounds and M^o. For alloy classification only M^o of classified metals/metalloids need to be included as reference materials.</p> <p>Understood. This will depend on discussions at OECD WTN group; please see timeline above.</p>
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The table below lists the samples to be tested in October 2018⁹ indicating under which conditions they are proposed to be tested (X) and under which conditions they have already been tested.

Samples	2018 HCl method (pH adjustment with NaOH)	2019 HCl method (from 0.1 M HCl)	Comments
Leaded brass	In RR & in annex 1	X (2 loadings)	Will allow comparison with results from RR and recent ones reported in annex 1.
Pb powder	In annex 1	X (2 loadings)	Will allow comparison with 2018 HCl method, HCl from concentrate, and 2019 method (HCl from 0.1 M solution). Will allow calculation of RBC and comparison with RBC calculated using 2018 HCl method
Co powder	X (2g/L loading)	X (2g/L loading)	Will allow calculation of RBC for Co in alloy at high loading using 2018 and 2019 methods to prepare HCl pH 1.5.
Co alloy	X (2g/L loading)	X (2g/L loading)	