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)

ESAC Request 2019-01

EURL ECVAM Scientific Advisory Committee

(ESAC)

EURL ECVAM REQUEST FOR ESAC ADVICE

on the

Bioaccessibility testing (Bioelution) of metals, inorganic metals compounds and metals-containing materials in a simulated gastric fluid

Title page information	
Abbreviated title of ESAC request	Bioelution validation
ESAC REQUEST Nr.	2019-01
Template used for preparing request	EP 3.02
Date of finalising request	13/03/2019
Date of submitting request to ESAC	13/03/2019
Request discussed through	Written procedure previous to ESAC 45
Opinion expected at (date)	ESAC 45 (June 2019)
File name of this request	**ER2019-01_ESAC_REQUEST_Bioelution.doc**

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1. TYPE OF REQUEST

Request Type	Identify request ("YES")
R1 ESAC Peer Review **of a Validation Study**	**YES, external validation study** **(i.e. not coordinated by EURL ECVAM)**
If R1)applies please specify further:	
►Prospective Validation Study	**YES** **The submission includes the outcome of an inter-laboratory study (five laboratories) with 6 test items as well as retrospective data on *in vivo* bioavailability and *in vitro* bioaccessibility data of metals from soil.**
►Retrospective Validation Study	**YES (see above)**
►Validation Study based on Performance Standards	**NO**
R2 Scientific Advice on a test method submitted to EURL ECVAM for validation **(e.g. the test method's biological relevance etc.)**	**NO**
R3 Other Scientific Advice **(e.g. on test methods, their use; on technical issues such as cell culturing, stem cells, definition of performance standards etc.)**	**NO**

2. TITLE OF STUDY OR PROJECT FOR WHICH SCIENTIFIC ADVICE OF THE ESAC IS REQUESTED

External validation of the Bioaccessibility testing (Bioelution) of metals, inorganic metals compounds and metals-containing materials using a simulated gastric fluid

3. BRIEF DESCRIPTION OF THE STUDY OR PROJECT

The Bioelution test method is an *in vitro* extraction method used to measure metal releases in artificial gastric fluid (pH 1.5). It does not address a biological effect. It provides *in vitro* estimates of oral bioaccessibility, which is the first step that leads to oral absorption. In this way oral bioaccessibility is related to systemic bioavailability of metal ions.

The claim made is that the systemic effects of metal containing materials are related to the bioavailability of the metal ion after systemic absorption. In this way, the proposed protocol is relevant for systemic health effects of metals and metal compounds.

The Bioelution test method is proposed to generate relative bioaccessible data by comparing the *in vitro* metal ion releases from target and reference material for the oral route of exposure. The relative metal ion release is then used to make predictions on how the test material will behave compared to the reference material, based on relative metal ion releases.

The *in vitro* relative bioaccessibility data generated are proposed for two regulatory applications, called 1-Grouping and 2-Alloy classification. Under Grouping the *in vitro* relative bioaccessibility data are used, in a weight of evidence approach, to support grouping and read across of metal-containing substances and mixtures (bridging). Under Alloy classification, bioaccessibility data are used to calculate the bioaccessible concentration of a metal ingredient in an alloy, which is then compared to its bulk concentration in order to assess the presence of a matrix effect. This would then warrant the use of the bioaccessible concentration for the purpose of alloy classification for systemic health effects according to the EU CLP[1].

The issue on how to classify alloys under CLP for human health endpoints was triggered at the REACH Committee in relation to the inclusion of a new harmonised classification of lead in Annex VI of the CLP Regulation. Member States raised concerns and questions around the applicability and interpretation of Article 12(b) [specific cases requiring further evaluation] in the case of mixtures/alloys. An Expert Group established by the European Chemicals Agency (ECHA) in September 2016 further debated the regulatory application of the Bioelution test method. This group limited the discussions to alloys and their systemic effects after oral exposure in the context of hazard assessment related to CLP. EURL ECVAM actively participated in these meetings.

The Bioleution test method underwent an external inter laboratory comparison (initiated in 2010) to investigate the within-lab repeatability and between-lab reproducibility of bioaccessibility testing in gastric fluid. Five laboratories measured metal release from six metal-containing materials following one defined protocol The outcome of the study was published in 2014 by Henderson et al.

[1] https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX%3A32008R1272

In July 2016 EURL ECVAM received a pre-submission of the Bioelution test method and in November launched a consultation within the PARERE network with the aim of getting their views with regard to 1) the interpretation of the CLP legal text, 2) the regulatory applicability of the Bioelution test method, 3) its limitations and scientific relevance, 4) the potential further validation of the test method, and 5) the impact on animal testing. On the basis of the evaluation of the pre-submission and the feedback received from PARERE members, EURL ECVAM invited the test submitter to submit a full submission and to provide clarifications and more detailed information on a number of questions that emerged from the assessment.

Full submission was received in February 2018 together with clarifications on the questions that were raised. The submission also included a review of the available information to understand the relationship between *in vivo* bioavailability and *in vitro* bioaccessibility for metals. Following evaluation, in November 2018 EURL ECVAM received a revised full submission together with responses to the comments raised by EURL ECVAM in June 2018.

EURL ECVAM assessed this revised submission, and the assessment report is made available to ESAC in the accompanying documents of the current request.

4. OBJECTIVES, QUESTIONS, TIMELINES

4.1 OBJECTIVE

Objective *Why does EURL ECVAM require advice on the current issue?*	The simple presence of a metal in a material does not necessarily impart to that substance the biological properties associated with the fully water-soluble metal ion or the metal by itself. It is the bioavailable metal ions that drive the systemic toxicity of metal-containing materials after oral ingestion of micron size powders or massive forms. Therefore, when the bioavailability of the released metals (e.g. ions) at relevant exposure levels differs, their toxic effect differs as well. The *in vitro* relative bioaccessibility data generated by the Bioelution test method are proposed for two regulatory applications, called 1-Grouping and 2-Alloy classification. The opinion of ESAC should, therefore, provide expert advice to EURL ECVAM on the scientific validity of the relative bioaccessibility data obtained with the Bioelution test method in view of the two proposed regulatory applications.

4.2 QUESTION(S) TO BE ADDRESSED

Questions *What are the questions and issues that should be addressed in view of achieving the objective of the advice?*	The ESAC should review the available evidence as provided with the Submission, and provide an opinion on the following issues: 1) The number and quality of the test items used in view of the study objectives. 2) The reliability (reproducibility within and between laboratories) of the results obtained *in vitro*. 3) The biological relevance with regard to the *in vitro* system and experimental conditions used as being: • representative of the gastric compartment, • worst case scenario for the oral route of exposure, • relevant in relation to the dermal and inhalation routes of exposure

	4) Appropriateness of identification of applicability and limitations of the Bioelution test method. 5) The relationship between *in vitro* bioaccessibility in oral fluids and relative *in vivo* bioavailability (e.g. from soil of metal compound studies) and its relevance in the context of the two regulatory applications proposed (1-Grouping and 2-Alloy classification). 6) Appropriateness of the relative measurements of bioaccessibility between pure metal and alloy used to calculate the bioaccessible concentration to show metal releases affected by matrix effects. 7) Appropriateness of 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. 8) Clarity and completeness of the protocol and prediction model. 9) The readiness of the method for regulatory use.

4.3 TIMELINES

Timelines concerning this request *When does EURL ECVAM require the advice?*	**Timeline**	**Indication**
	Finalised ESAC Opinion required by:	**End of May 2019**
	Request to be presented to ESAC by written procedure (e.g. due to urgency) prior to the next ESAC	**YES**
	Request to be presented to ESAC at ESAC plenary meeting	**NO**

5. EURL ECVAM PROPOSALS ON HOW TO ADDRESS THE REQUEST WITHIN ESAC

5.1 EURL ECVAM PROPOSAL REGARDING REQUEST-RELATED STRUCTURES REQUIRED

Specific structures required within ESAC to address the request *Does the advice require an ESAC working group, an ESAC rapporteur etc.?*	**Structure(s) required**	**Required according to EURL ECVAM? (YES/NO)**
	S1 ESAC Rapporteur	**NO**
	S2 ESAC Working Group	**ESAC core members** - Chantra Eskes (Chair) - Emanuela Corsini - Annete Kopp-Schneider **ESAC ad-hoc members** - Valerie Hanley - Irina Karadjova - Mina Suh
	S3 Invited Experts	**NO**
	Ad S3: If yes – list names and affiliations of suggested experts to be invited and specify whether these are member of the EEP	
	If other than above (S1-S3):	**NO**

5.2 DELIVERABLES AS PROPOSED BY EURL ECVAM

Deliverables *What deliverables (other than the ESAC opinion) are required for addressing the request?*	**Title of deliverable other than ESAC opinion**	**Required? (YES/NO)**
	D1 ESAC Rapporteur Report and draft opinion	**NO**
	D2 ESAC Peer Review Report and draft opinion	**YES**
	If other than above (D1-D2):	**NO**

6. LIST OF DOCUMENTS TO BE MADE AVAILABLE TO THE ESAC

Count	Description of document	Already available? (YES/NO)	File name
1	Bioelution test submission (TST)	YES	181109TSTrev clean.docx
2	EURL ECVAM Assessment Report on the Bioelution test submission	YES	Assessment of Full Submission_TM2016-02_Bioelution_100119.docx
3	Protocol of the Bioelution test method	YES	181109TSTAttachment1arev clean.docx
4	Bioelution test method DB-ALM protocol	YES	181109TSTAttachment1blongrev clean.docx
5	Bioelution - List of test items used to develop and optimise the test method, including their CAS number	YES	181109TSTAttachment2rev.docx
6	Bioelution - List of test items used to assess WLR and BLR, including their CAS number and relevant properties	YES	180216TSTAttachment3.pdf
7	Bioelution - Data used for WLR and BLR assessment (Tables and/or Figures)	YES	181109TSTAttachment4rev.docx
8	List of test items used to assess PC, including their CAS number and relevant properties	YES	180216TSTAttachment18.pdf
9	Data used for PC assessment (Tables and/or Figures)	YES	180216TSTAttachment18.pdf
10	Inter-laboratory study - Project plan(s)	YES	181109TSTAttachment13rev clean.docx
11	Bioavailability of Metals from Soil: Review of In Vitro Methods to Predict RBA (Alloy LLC)	YES	180216TSTAttachment18.pdf
12	List of possible proficiency materials	YES	181109TSTAttachment19rev.docx
13	Summary of studies conducted by ECTX for Eurometaux at ECVAM's request	YES	180216TSTAttachment20.pdf
14	Report-ECTX study X02a-081 (2 hours Bio-elution Study on Stainless Steel 316L Massive (Epoxy resin embedded) at a 2 g/L loading (surface equivalent) in a simulated gastric fluid), June, 18, 2017	YES	FR X02a-081-SS epoxy massive.pdf
15	Report-ECTX study X02a-130 (2 hours Bioelution Study on Fe-Cr-Ni Powder Mixture (68.6/16.4/10.7 mass fraction) at a 0.2, 1 and 2 g/L loading in a simulated gastric fluid), December 19, 2017	YES	FR X02a-130.pdf
16	Report Amendment no.1-ECTX study	YES	FR X02a-144 Correction.pdf

	X02a-144 (2 hours Bio-elution Study on Stainless Steel 316L Powder at a 2 g/L loading in a simulated gastric fluid), April 21, 2018		
17	Report-ECTX study X02a-145 (4 hours Bio-elution Kinetics Study on Stainless Steel 316L Powder at a 2 g/L loading in a simulated gastric fluid), April 21, 2018	YES	FR X02a-145.pdf
18	Report-ECTX study X02a-146 (4 hours Bio-elution Kinetics Study on Z45 (massive) at a 2 g/L loading (surface equivalent) in a simulated gastric fluid) April 21, 2018	YES	FR X02a-146.pdf
19	Proposed responses to questions raised in the Test Pre-Submission Assessment Report	YES	181109Response to PARERE questionsrev clean.docx
20	Absolute in vivo bioavailability of metal compounds	YES	180216PARERE Annex2.pdf
21	Gastrointestinal Absorption of Microparticles and Nanoparticles after Oral Administration	YES	181109PARERE Annex3rev clean.docx
22	Perkin-Elmer guidance	YES	180216PARERE Annex4.pdf
23	Key references	YES	References.zip
24	Clarifications provided on full submission package	YES	181109RCOM ECVAM.docx

7. TERMS OF REFERENCE OF THE ESAC WORKING GROUP

7.1 ESTABLISHMENT OF THE ESAC WORKING GROUP

The ESAC unanimously agreed on the composition of a new ESAC Working Group for the review on the scientific validity of the Bioelution test method on the 4th of the December during the ESAC44 plenary meeting.

7.2 TITLE OF THE ESAC WORKING GROUP

Full title:
ESAC Working Group on the Bioelution Test Method

Abbreviated title:
ESAC WG Bioelution

7.3 MANDATE OF THE ESAC WORKING GROUP

The ESAC WG is requested to conduct a review of the scientific validity of the Bioaccessibility testing (Bioelution) of metals, inorganic metals compounds and metals-containing materials using a simulated gastric fluid. The review needs to address the questions put forward to ESAC by EURL ECVAM under section 4.2 of the current request.

7.4 DELIVERABLES OF THE ESAC WORKING GROUP

The ESAC WG is requested to deliver to the chair of the ESAC and the ESAC Coordinator a detailed **ESAC Working Group Report** outlining its analyses and conclusions and a **draft ESAC Opinion.** A template intended to facilitate the drafting of the WG report is available and will be provided by the ESAC Coordinator (see section 7.7).

The conclusions drawn in the report should be based preferably on consensus. If no consensus can be achieved, the report should clearly outline the differences in the appraisals and provide appropriate scientific justifications.

7.5 PROPOSED TIMELINES OF THE ESAC WORKING GROUP

Item	Proposed date/time	Action	Deliverable
1	2-3 May 2019	ESAC Working Group meeting	Draft ESAC WG report and draft ESAC opinion
2	End of May 2019	Circulation of final WG Report and draft ESAC Opinion to ESAC	Final draft ESAC WG Report and draft ESAC Opinion
3	3-5 June 2019	Endorsement of WG Report and ESAC Opinion at ESAC45 meeting	Final ESAC WG Report and ESAC Opinion

7.6 QUESTIONS WHICH SHOULD BE ADDRESSED BY THE ESAC WORKING GROUP

The review should address the **questions put forward to ESAC by EURL ECVAM** (see section 4.2) and the information requirements of the ESAC Working Group Template, where applicable. The ESAC Coordinator will provide guidance if needed.

When preparing the final ESAC WG Report to address these questions, the ESAC WG is requested to use a pre-defined reporting template. This template (see section 7.7) follows EURL ECVAM's modular approach and addresses to which extent the standard information requirements have been addressed by the study. The template allows moreover for addressing the issues specific to this study as outlined in section 4.2. The ESAC Coordinator will provide guidance if necessary.

7.7 REPORTING TEMPLATE

The ESAC WG template suggests a structure that is in close agreement with the EURL ECVAM information requirements ("modules") for scientific review following validation and allows at the same time for the description of the analysis and conclusions concerning more specific questions.

The template can be used for various types of validation studies (e.g. prospective full studies, retrospective studies and performance-based studies). Depending on the study type and the objective of the study, not all sections may be applicable.

However, for reasons of consistency and to clearly identify which information requirements have not been sufficiently addressed by a specific study, this template is uniformly used for the evaluation of validation studies.

The current template is

TEMPLATE_ESAC-WG_REPORT-20190424.doc