Detailed protocol for the determination of relative metal/metalloid release using a simple simulated gastric fluid (0.032 M HCl)

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Note: this document was initially made available on 26/02/2020 after its evaluation and review by EURL ECVAM and its Scientific Advisory Committee (ESAC). In follow-up, the protocol was submitted to the OECD Testing Guidelines Working Group (WNT) to draft an internationally agreed protocol (OECD Test Guideline). While the OECD project was withdrawn in 2024, the discussions with the OECD Expert Group resulted in useful clarifications and improvements to this protocol. This 2025 protocol version is here made publicly available.

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1. Purpose of the method

The systemic toxicity of most metals and metalloids (hereafter referred to as 'metals') is associated with the release of metal ions ('moiety') and their uptake by the body and/or interaction at their target organ sites (i.e., their bioavailability) since the metal ion is the toxic entity (Goyer and Clarkson, 1996; Tokar et al. 2013). The simple presence of a metal or inorganic metal compound does not necessarily impart to the substance or mixtures the biological properties of the released metal/inorganic species; it is the **bioavailability** of the released metal at the site of action in the organism that is the most important factor determining toxicity for metals and minerals.

Information on bioavailability can be derived from *in vivo* sources, such as toxicokinetic or toxicological test data, or predicted using different models. When the bioavailability of a metal or mineral substance is not known or it is not feasible to determine *in vivo*, the amount of released metal "available for absorption" may be measured using *in vitro* methods[1]. The term **bioelution** refers to these *in vitro* methods, which are used to measure the degree to which a substance is released (e.g. as metal ions) into simulated biological fluids. The purpose of such physico-chemical tests is thus to determine the release of metal ions from various metal-containing materials in test media simulating physiological conditions associated with different routes of human exposure.

This protocol describes how to obtain material-specific metal ion release data from materials such as metals, inorganic metal compounds and other inorganic complex metal-containing materials (e.g., alloys; pigments; Unknown or Variable composition, Complex reaction products and Biological materials (UVCBs)) in a simple simulated gastric fluid composed of 0.032M HCl, pH 1.5 ± 0.1. This gastric fluid represents the oral route of human exposure.

The method compares metal ion releases between two or more materials containing the same metal, i.e. a test and reference material (i.e., Relative Metal release). The applications of metal release data are described in section 2. Applicability of the method, and sections 6.5. Data analysis and 6.8 Interpretation of results. Namely, Application 1 is used for grouping and read-across of compounds of a given metal, comparing the releases of the metal ion in simulated gastric fluid from a reference and a test material. Application 2 is for the calculation of the Relative Metal release-based Concentration (RMC) of metal ingredients in complex metal-containing materials based on comparison of releases of metal ions from the complex material (e.g. alloy) and reference materials (e.g. metal ingredient or constituent).

The test conditions described in this protocol are chosen to approximate metal ion release in gastric fluid after oral ingestion in humans. It is important to stress that it is not intended to address the entire gastrointestinal tract, completely mimic conditions in the stomach, or estimate absolute or relative oral bioavailability. Instead, the protocol is a simple, reproducible approach based on stomach composition intended to measure the *in vitro* relative release of metal ions for the majority of tested materials included in Annex 1.

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[1] This can be referred to as 'in vitro bioaccessibility' or IVBA, by e.g. the US EPA (2024)

This protocol builds upon the experience and knowledge gained from a round-robin study examining the method's performance when applied to various metal-containing materials (Henderson et al., 2014). The protocol used in the round-robin included one loading, and the simulated gastric solution, 0.07 M HCl, was prepared from a 12 M HCl solution and adjusted to 1.5 ± 0.1 with NaOH before the materials were added. The composition of this fluid (HCl) was similar to the BS EN 71-3 (2013) method used to measure metal release from toys and to the ASTM D5517 protocol (ASTM 2014) developed to measure metal release from Arts Materials. This fluid has the same pH (1.5) as in the US EPA 1340 glycine buffer method to measure As and Pb release from soils (US EPA 2024). However, there are also differences from these existing protocols, reported in Table 1 below:

Table 1: differences between this protocol and ASTM D5517, US EPA 1340

	ASTM D5517	**US EPA 1340**	**This protocol**
Objective	Measure absolute metal releases as estimates of metal bioavailability after ingestion of art materials; data used in risk assessment to refine exposure	Measure absolute release of Pb or As to estimate metal bioavailability after ingestion of soils; data used in risk assessment to refine exposure. Method specifically developed and validated to assess in vivo bioavailability of Pb from soils based on in vitro releases	Measure relative metal releases from metals, inorganic metal compounds, and inorganic complex metal -containing materials. Relative values used in hazard identification applications (e.g., ranking, grouping & read-across, presence of matrix effects in case of alloys or other complex materials)
Start Solution	12M (37%) HCl	0.4M glycine adjusted to pH 1.5 ± 0.05	0.1M HCl
End solution before adding sample	0.07M HCl (pH ~ 1.15)	0.4M glycine adjusted to pH 1.5 ± 0.05	0.032M HCl at pH 1.5
Method	Mix with sample and adjust to pH ≤1.5 w/HCl if needed (t=0h)	Mix with sample.	Mix with sample, measure pH in 4th vessel and adjust to pH 1.5 in triple vessels if needed (t=0h)
pH	• pH at t=0 (after sample added): ≤1.5 (not same for all samples) • pH not adjusted during 2 hours • pH at t=2h: Not measured	• pH at t=0 (after sample added): ~ 1.5 but not measured • pH not adjusted during 1 hour • pH at t=1h: Measured & must be 1.5± 0.5	• pH at t=0 after sample added, is adjusted if needed (based on 4th vessel) to 1.5±0.1 • pH not adjusted during 2 hours • pH at t=2h: measured & must be 1.5± 0.1
Loading	20 g/L (<250 ppm release) and 1g/L (>250 ppm)	10 g/L; dilute if Pb release > 500 mg/L	2g/L and 0.2 g/L
Sample	powder, liquid, comminuted, ground art material	Intact or ground soils (sieved to ≤150 µm)	Massive and powder (sieved to ≤100 µm)* forms of metals and metalloids, inorganic metal compounds and other inorganic

			complex metal/metalloid-containing materials
Separation	Centrifugation and filtration 0.45µm filter	Filtration 0.45µm filter	Filtration 0.2µm filter
Controls	Negative	blank & Lab Control samples, control soil	negative & positive; proficiency materials; reference metals for alloys
Data Acceptance Criteria & Reporting	not well defined	well-defined & described	well-defined & described

**: For more details see section 3*

A glossary of the terms and acronyms used in this protocol is available here (Section 8).

2. Applicability of the method

The metal release method applies to test materials such as most metals, inorganic metal compounds, and complex metal- containing materials (e.g. alloys, UVBCs, pigments) in massive form (≥ 1mm) or powder form (≥ 0.1µm - <1mm).

Importantly, this protocol **<u>does not apply to</u>** nanomaterials as defined by the EU Commission in 2022[2]. For nanomaterials, the metal release method (in its current form) may not allow for a complete separation of released metal ions from the undissolved nanoparticles to be achieved.

For some metals, such as mercury (Hg) and silver (Ag), or specific chemical forms of elements, such as antimony (Sb) trichloride ($SbCl_3$) and antimony pentachloride ($SbCl_5$), the results in simulated gastric fluid of low pH (e.g., 0.032 M HCl, pH 1.5 ± 0.1) are unreliable due to precipitation. In the case of Sb, this was observed with two chemical forms ($SbCl_3$ and $SbCl_5$) that are corrosive but not with other chemical forms of antimony (e.g., Sb metal, Sb oxide). Precipitation in 0.032 M HCl will be rare, with the exceptions mentioned here. Any chemical forms of a metal showing clear (naked eye) precipitation in 0.032 M HCl are outside the technical applicability domain of this protocol, as precipitates could either count as undissolved or pass through the filter and be counted with the dissolved fraction.

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[2] 'Nanomaterial' means a natural, incidental or manufactured material consisting of solid particles that are present, either on their own or as identifiable constituent particles in aggregates or agglomerates, and where 50 % or more of these particles in the number-based size distribution fulfil at least one of the following conditions: (a) one or more external dimensions of the particle are in the size range 1 nm to 100 nm; (b) the particle has an elongated shape, such as a rod, fibre or tube, where two external dimensions are smaller than 1 nm and the other dimension is larger than 100 nm; (c) the particle has a plate-like shape, where one external dimension is smaller than 1 nm and the other dimensions are larger than 100 nm. In the determination of the particle number-based size distribution, particles with at least two orthogonal external dimensions larger than 100 µm need not be considered. However, a material with a specific surface area by volume of < 6 m2 /cm^3 shall not be considered a nanomaterial (EU Commission, 2022)

2.1 Simulated gastric fluid technical applicability

The following metals and their compounds or complex materials have demonstrated that releases in simulated gastric fluid can be measured without technical limitations and hence are within the technical applicability domain of this protocol: arsenic (As), gold (Au), boron (B), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), germanium (Ge), indium (In), manganese (Mn), molybdenum (Mo), nickel (Ni), lead (Pb), palladium (Pd), platinum (Pt), rhenium (Re), rhodium (Rh), ruthenium (Ru), antimony (Sb) (non-corrosive compounds), selenium (Se), silicon (Si), titanium (Ti), vanadium (V), tungsten (W), zinc (Zn) and zirconium (Zr) (See Annex 1).

For most metals and their chemical forms in the technical applicability domain, fluids with pH~1.5 may lead to higher metal releases compared to fluids of neutral pH. However, for some of these metals, releases in 0.032 M HCl (simulated gastric fluid) may be lower than releases in other fluids with higher pH that simulate other parts of the oral exposure pathway, such as intestinal fluid. It is important to be aware of the known cases where metal ion release, as described in this protocol, may not be the highest release for the oral route. In the cases of Mo, Fe, Si, W and Sb, the ion releases from some of their compounds and complex metal-containing materials have demonstrated not to be the highest in 0.032 M HCl (e.g., Mo from MoO_3 and some Mo alloys; Fe from FeMo alloy, Si from FeSi alloy and Al silicate). For these elements (in all chemical forms), data on relative water solubility and/or relative metal release tests in a second fluid of neutral pH should also be collected/generated to provide information on whether simulated gastric fluid could be an acceptable fluid to generate relative releases for the intended application (Mörsdorf et al. 2015; Herting et al. 2014; Stefaniak et al. 2010; IITRI 2010; Hedberg et al. 2010).

If metal releases are significantly lower in the gastric simulated fluid than in the neutral pH solution or water, it is recommended to rule out the possibility that precipitation may have occurred (at lower pH). Of note, a relative comparison of metal releases in 0.032 M HCl can still be useful even if the acidic conditions do not always represent a maximum release compared to neutral pH solutions.

Note 1: This method determines the metal's release as the element's concentration in solution. However, the applied analytical method does not determine the species in which the metal is released. For many elements, a single oxidation state will be present at pH 1.5 0.032M HCl. For some metals, for which different speciation (oxidation states) may be suspected depending on the exact material (e.g., chromium III/VI, antimony III/V and vanadium IV/V) and where such difference in speciation is expected to impact the toxicity, additional information is required to understand whether the metal form(s) released from the test material is/are the same as the form(s) released from the reference material. Examples of analytical methods that can be used for speciating the ions include Ion Chromatography-Inductively Coupled Plasma Mass Spectrometry (IC-ICP-MS), High-Performance Liquid Chromatography coupled to Inductively Coupled Plasma – Mass Spectrometry (HPLC-ICP-MS), and electrochemical tools such as stripping voltammetry and ion selective electrodes.

Note 2: Additional metal substances may be tested in the future. If results become available and demonstrate that measurements in this simulated gastric fluid for these metals are technically feasible, they will be added to Annex 1.

2.2. Applications of the results

Comparing metal ion releases between test and reference materials can be used to **rank** different materials containing the same metal (e.g. for grouping and read-across).

For alloys or other complex metal-containing materials like UVCBs, pigments, the relative releases from test and reference materials can detect the presence of **matrix effects**, which can affect the predicted ion releases in 0.032 M HCl.

Application 1: To support grouping and read-across

Relative metal ion release data can be used to group substances of a given metal or complex materials containing the metal based on comparable metal release in the simple gastric fluid used in this protocol. Such data can be used to support read-across for systemic effects via the oral route in a weight-of-evidence approach, to, e.g., fulfil human health information data requirements and/or hazard classification under chemical management programmes. In the context of this application, test and reference materials are also referred to as target and source substance(s), respectively, in guidance documents on grouping and read-across (e.g. in the ECHA RAAF (2017) and the OECD Guidance on the Grouping of Chemicals, under final revision).

In practice, the releases of the metal ion from two or more materials of the same metal (“source and target”) are measured in parallel under the same testing conditions, and the values are compared. Therefore, the relative metal release (%) (Equation 1) is the ratio of the measured value of the metal ion (e.g. expressed as µg metal released/g sample, or % of metal content) of the target material compared to those from one or more source materials containing the same metal.

Eq 1:

$$Relative\ metal/metalloid\ release(\%) = \frac{mg\ metal\ ion\ released\ \frac{measured\ in\ extract}{g}\ target\ sample}{mg\ metal\ ion\ released\ \frac{measured\ in\ extract}{g}\ source\ sample}\ x\ 100$$

This relative release can be used with other data as one line of evidence to group metal substances that release similar amounts of metal ions to determine if they can be predicted to have similar types and levels of effects. Further guidance and examples can be found in the OECD Guidance 194 on the Grouping of Chemicals, section 6.8 and the ECHA RAAF (2017).

Application 2: To establish the presence or absence of a matrix effect in alloys or other complex metal-containing materials like alloys, UVCBs, pigments based on the %RMC (Relative Metal release-based Concentration%)

Relative metal releases between alloys and their pure metal ingredients can be used to assess the presence of matrix effects in alloys and other complex metal-containing materials, as matrix effects can affect the metal release and, therefore, toxicity, which would not be the same as that predicted based on the release from the pure metal ingredient. The ratio of metal release value from the pure ingredients and the alloy is then used to calculate the %RMC of each metal ingredient in the alloy (or the complex metal-containing material).

To minimise variability in the release from pure metal ingredients, it would be desirable to have the same sample of a metal classified for human health systemic effects tested consistently alongside the alloys. These samples could be kept in a repository and made available to the laboratories. In the future, it is possible that other metals may be classified for systemic effects and could fall within the scope of this application. If the measurements for these metals in simulated gastric fluid are technically feasible, then reference materials will be added to that repository. The selection of reference materials is further discussed below and in section 6.6.

3. Test materials

3.1. Types of test material

Test materials are intended to be metals in elemental form, inorganic metal compounds, or complex metal-containing materials such as alloys, UVCBs and pigments.

The selection of the test materials will depend on the application for which the release data will be used. The selection process should follow a stepwise approach that considers physical form, purity, and relevance. Test materials can be in powder or massive form. When selecting test materials, particular attention should be paid to the composition and form that best represents the materials as they are placed on the market or would reasonably be used[3]. Depending on the type of test material and physical form, specific sample preparation and test execution apply. Generally, test and reference materials should be tested in the same physical form. For hazard classification of alloys, however, if the reference sample in the physical form that led to the hazard classification of the metal is available, this reference sample should be selected,.

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[3] For example, Article 5 of the EU CLP states: *The information shall relate to the forms or physical states in which the substance is placed on the market and in which it can reasonably be expected to be used*. Article 8(6) states that *Tests that are carried out for the purposes of this Regulation shall be carried out on the substance or on the mixture in the form(s) or physical state(s) in which the substance or mixture is placed on the market and in which it can reasonably be expected to be used.*

When metals and complex metal-containing materials do not fit the definition of massive (≥ 1 mm, A9.7.5.4.3, UN GHS 2023), they are referred to as **powders** (they contain particles < 1 mm diameter but above 0.1 µm diameter). For these powders, the subfraction of particles ≤100 µm in diameter should be tested. While particles smaller than 150 or 250 µm are often used for measuring metal release after oral intake (US EPA 2000), testing a subfraction enriched in finer particles (≤ 100 µm) for the oral route also covers the inhaled particles that are deposited in the mid to upper regions of the respiratory tract and are then swallowed and passed into the gastrointestinal tract, providing a conservative estimate due to increased release with smaller particle size.

Note 3: For alloy powders for which the lower limit of the particle size distribution is > 100 µm but below 1mm, testing of the powder without further treatment is recommended. For alloy powders with diameters below 100 µm, testing them as they are marketed without further sieving is recommended. If a %RMC for a hazardous metal present in this material falls close to a regulatory classification cut-off (e.g., general or specific concentration limit), testing a smaller diameter subfraction that more closely matches the size of the reference metal powder may be recommended, depending on sieving options and amount of material available to achieve the similar size fraction.

Note 4: It is important to note that particles < 0.2 µm may pass through the filter recommended in this protocol and will be reported as soluble ions. Therefore, the sample's particle size distribution (PSD) should be examined to determine whether the possible contribution of < 0.2 µm particles to the overall metal release would be likely and to what degree that would affect the release results. In a volume-based PSD, the d0.1 (10% lower end of PSD) should not be lower than 0.2 µm for the relative metal release method to be applicable.

If the test material on the market or its expected use is only in the massive form (particles ≥ 1 mm), this form should be tested.

The massive form of the test material can be tested as it is or tested as an epoxy-embedded sample. Embedding samples in epoxy resin may facilitate sample handling and provide a standardised exposed surface. The surfaces of the reference and the test materials are to be treated in the same way to maximise comparability of results. Annex 2 provides a description of the epoxy embedding process and the advantages and disadvantages that it confers to the massive sample. If the massive sample needs to be cut to a smaller size for testing, care must be taken since grinding and polishing operations during sample preparation may cause the exposed surface not to be representative of the as received material anymore (this may be overcome by allowing the polished surface to be oxidised for a specified period of time). In addition, care must be taken to not introduce crevices that promote corrosion/metal release.

If particles are expected to be generated from the massive forms during normal handling and use, then particles (<1 mm diameter) representative of the activity of interest (e.g., grinding) should be collected for testing. To obtain the representative particle size, sieving of particles generated during the process of interest (e.g., grinding, sanding) is the preferred method, as this method does not impart any physical or chemical changes to the sample (e.g., possible surface oxidation/passivation). If the process of interest is not well defined or particles cannot be collected, other potential methodologies for sample preparation include crushing, grinding, and/or ball milling, all followed by sieving. Under these circumstances, it will be important to test the metal ion release from the material both as initially received and after being crushed/ground/milled to establish whether the crushing/grinding/milling operation impacts the test material's release properties. If the metal release is significantly different between the massive and the crushed/ground/milled forms, tests such as Electron Spectroscopy for Chemical Analysis [ESCA, also known as X-ray photoelectron spectroscopy (XPS)] or Auger Electron Spectroscopy (AES) complemented by Scanning Electron Microscopy (SEM), can be conducted on the samples before and after the crushing/grinding/milling operation, to assess any changes on the surface of the materials that can explain the differences in metal release. Any such change in surface properties should be recorded and reported.

3.2 Test sample identification and characterization

For traceability, safe handling and storage of the materials, a test sample has to be accompanied by documentation (e.g. a certificate of analysis or equivalent and Material Safety Data Sheets (MSDS)), as well as the batch number and source of the sample. All of the information needed is described under section 6.10. Reporting and record keeping.

3.3 Test sample storage

Samples should be supplied to the testing laboratory in a condition ready to be tested. The supplier of the sample is requested to determine the dry matter or moisture content. If the sample contains moisture, it preferably should be dried and packed airtight for shipping. This will stop the oxidation and conversion reactions as much as possible and the characteristics of the sample will be stable over a more extended period, increasing the reproducibility of the results. .

It is important to perform a visual inspection of the test samples to assess their homogeneity by the naked eye and to follow any storage instructions provided by the manufacturer or supplier (e.g., stored under a nitrogen or an inert atmosphere, stored in a sealed container, stored in a desiccator, etc.). In the absence of storage instructions, the test material should be stored at room temperature (15-25°C) in an appropriate container, in a dry place (≤ 70% room humidity) and in dark conditions to protect it from moisture and further surface oxidation and to preserve the integrity of photosensitive materials.

Note 5: If the supplier can only provide a moist sample representative of the sample on the market, the supplier should indicate how the sample should be stored in the testing laboratory and how the metal release values should be adjusted based on the water content of the sample. Moisture content needs to be provided by the manufacturer.

3.4 Test sample handling

Powder particles shall be weighed in dry, acid-cleaned, chemical-resistant glass or polymeric test vessels using cleaned metal-free spatulas or spoons. For massive forms of the test material that are not epoxy embedded (e.g.in the form of discs, sheets, etc.), the weight, geometry, surface finish and geometric surface area of the sample should be recorded. Further information on the handling of massive samples is available in Annex 2.

4. Test system

The test system consists of the chosen test vessel (usually a 250 mL Erlenmeyer flask) and the appropriate volume of test medium (a minimum of 50 mL).

4.1. Test vessels

Inert, chemical-resistant vessels with flat bottoms such as Erlenmeyer flasks (e.g. Polyethylene Terephthalate Glycol (PETG) or borosilicate glass) of 250 mL capacity or larger vessels in case of testing massives should be used for testing (up to two-litre flasks). The vessels shall be properly covered (e.g. metal free screw caps, silicon or rubber stoppers) to avoid (cross)contamination or evaporation of the test medium.

Care should be taken to ensure that the test vessels do not interact with the test material by causing abrasion or releasing metal ions to the test medium. Abrasion has been observed with some materials like slags in borosilicate glass. Borosilicate can also contribute Si and B to the test medium and should be avoided when Si or B are elements of interest (e.g., silicates). In cases where gas release can be expected (e.g., carbonate salts brought in an acidic environment), care needs to be taken so that no pressure builds up in the vessel (avoid too tight-fitting caps). All test vessels, whether new or used, should be cleaned appropriately to reduce possible background levels of the elements of interest before use and to ensure reproducibility when working with metals at trace levels.

An example of a simple and appropriate cleaning procedure for new and re-used test vessels: acid soak for 24h in 10% HNO_3, then rinse at least 4x in ultrapure water (e.g. MilliQ water 18.2MΩ·cm) and dry (by air at room temperature or in low temperature, < 50 ºC oven).

For the handling of powders, prior to each use, metal-free spatulas or spoons shall, as a minimum, be carefully cleaned in ultrapure water and isopropyl alcohol or, preferably, acid-cleaned in the manner described above. Do not use the same spatula or spoon on different test samples unless it has undergone cleaning after the previous use.

4.2. Test medium.

The test medium is 0.032 M HCl solution at 37 ± 1 °C and pH 1.5 ± 0.1. The chemical composition is shown in Table 2. It simulates a simple gastric fluid. The solution of 0.032M HCl must be prepared freshly every day. Once the temperature of the solution is stable at 37 ± 1 °C, the pH of the final solution must be confirmed that it is 1.5 ± 0.1. If the resulting pH is not 1.5 ± 0.1, then it must be adjusted using 0.1 M HCl or Ultrapure water as needed until this correct pH is reached.

Table 2. Chemical composition of simple simulated gastric fluid (0.032 M HCl).

Chemical	**Amount**
HCl solution (analytical grade or better)	316 mL of a purchased 0.1 M HCl solution of verified concentration at 37 ± 1 °C
Ultrapure water (18.2 MΩ cm)	Ultrapure water at 37 ± 1 °C.
The temperature of the simulated gastric fluid should be maintained at 37 ± 1 °C (e.g., water bath)	

5. Equipment

5.1 Agitation equipment

A thermostated linear (horizontal) or orbital shaker (37 ±1°C, 100 rpm; stroke length=1 inch) can be used to provide agitation. Gentle agitation at 100 rpm is used to maintain the flow of the aqueous medium over the test materials while maintaining the integrity of the surface of the test materials and any solid reaction product coatings formed during the test. The abrasion of particles or sample surfaces will be minimised in this way.

5.2 Thermometer

Calibrated thermometer, readable to 0.1 °C.

5.3 pH meter

A calibrated pH meter readable to 0.01 pH units.

5.4 Balance

A calibrated microbalance at least readable to 0.01 mg; controlled with standard weights before and after each use. The weight of two decimal numbers should be reported.

5.5 Filtration equipment

Disposable 0.2 µm membrane filters, e.g., Whatman UNIFLO syringe filters (Polytetrafluoroethylene (PTFE) membrane), Pall Acrodisc syringe filters (Polyethersulfone (PES) membrane), or equivalent filter systems should be used, as these filters do not adsorb or release metals in any significant amount. The use of filtration to separate undissolved from dissolved metal ions is consistent with ASTM D5517. Appropriate single-use latex- and oil-free (e.g., disposable polypropylene) syringes (volume depending on the amount required for the analysis technique) should be used. Appropriate high-density polypropylene sample tubes known not to release or adsorb significant amounts of the metals of interest should be used.

Note 6: The filter's effectiveness in separating dissolved from undissolved metal for a particular test material can be confirmed by visual inspection and/or light scattering or equivalent method. Any particles <0.2 µm can, in theory, pass the filter and will be counted as soluble ions (see Note 2).

6. Experimental Procedures

6.1 Sample loadings

Two loadings (0.2 and 2 g /L) are tested for each material so that the ones that release low amounts of ions can be reliably assessed at the high loading and the ones that release high amounts do not reach saturation at the low loading. The physical form of the test material (i.e. powders, non-epoxy-embedded massive samples and epoxy-embedded massive samples) determines how loading is defined (see 6.2 Test setup below). The loading of epoxy-embedded massive samples can be expressed as surface area equivalent.

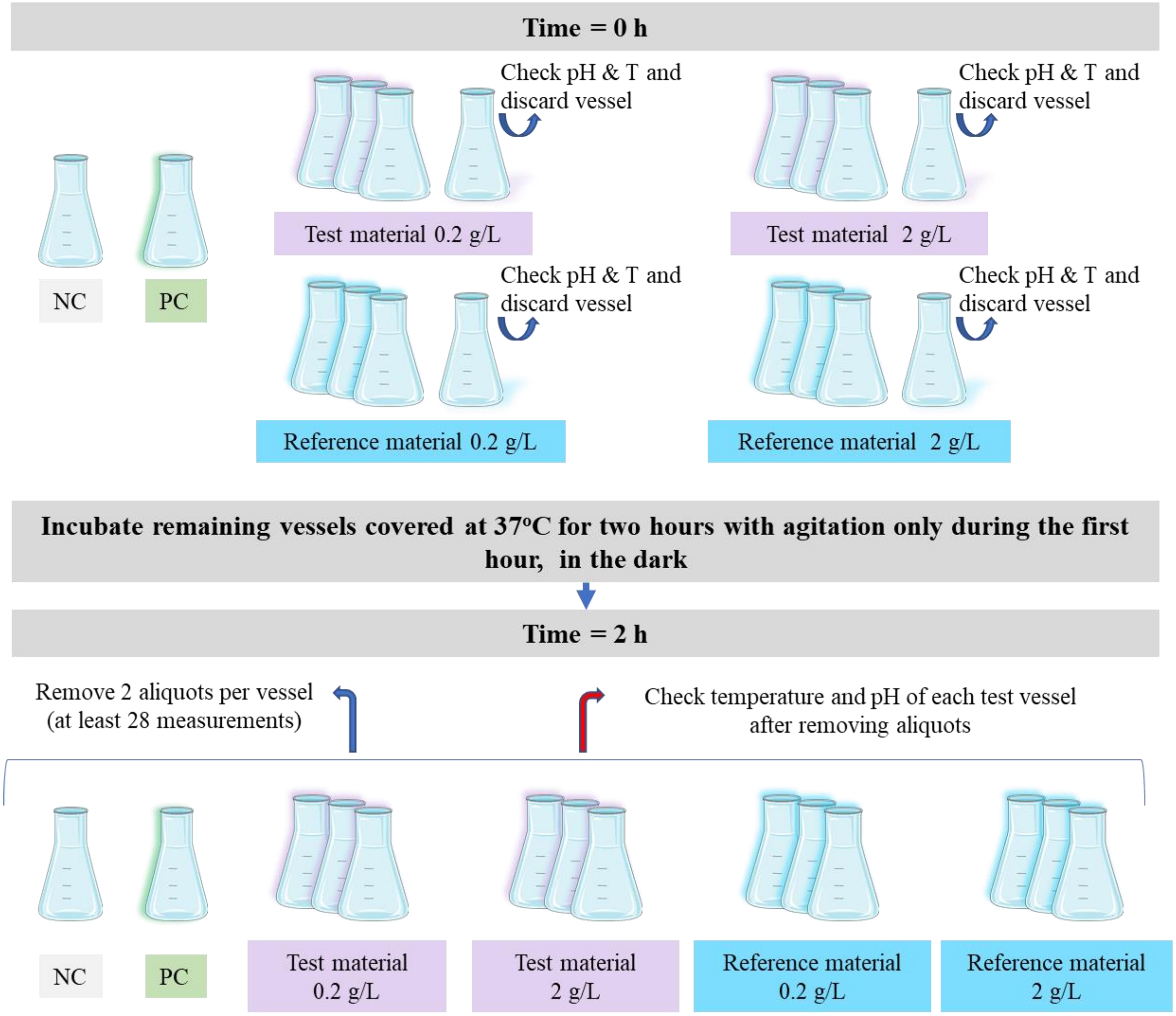
6.2 Test setup

6.2.1 Set up of the vessels

In short, at least eighteen pre-heated vessels should be set up for each comparison of release from materials (i.e., test and reference materials), with eight vessels for each material (i.e., three vessels for analysis in triplicates plus one vessel for pH and temperature (T) measurement at time=0, for each of the two loadings) and positive and negative control vessels (at least 1 each) (see Figure 1).

The size of the vessels and the amount of sample and volume of solution added to these vessels will be determined for each of the two loadings based on the physical form, sample characteristics, and analytical needs. A minimum of 50 mL of simple simulated gastric fluid is needed with a liquid volume to headspace volume ratio of approximately 1:4. This flexibility is necessary to make sure that enough fluid is available to cover all the surfaces of massive samples, which come in different shapes (whether epoxy embedded or not).

Figure 1. Test setup: Flow chart for one run with two materials. Minimum number of vessels needed, pH and temperature (T) measurements and aliquots taken for analysis (NC: negative control, PC: positive control)



More details are given below, depending on the physical form of the samples to be tested.

6.2.1.1 Test and reference materials in powder form

The (at least) 18 pre-heated vessels should be set up as follows (see Figure 1):

- At least 1 negative control vessel containing only the test medium 0.032 M HCl at pH 1.5 ± 0.1 at 37 ± 1 °C.
- At least 1 positive control vessel containing a solution of the dissolved salt of the element(s) of interest in 0.032 M HCl, (preferably) at a concentration within the range that can be expected to be released from the test material (see also section 6.6). A positive control stock should be diluted in 0.032 M HCl pH 1.5 ± 0.1, pre-heated to 37 ± 1 °C, to the desired final concentration.
- Eight test material vessels at 0.2 g/L loading (4 vessels for each test and reference material, respectively): approximately 10 mg (± 0.05 mg) test material should be weighed into each of the 3 replica test material vessels and 1 extra test material vessel for pH measurement at the start of the test. The exact weight should be noted to two decimal places.
- Eight material vessels at 2 g/L loading (4 vessels for each test and reference material, respectively): approximately 100 mg (± 0.5 mg) test material should be weighed into each of the 3 replica test material vessels and 1 extra test material vessel for pH measurement at the start of the test. The exact weight should be noted to two decimal places.

Swirling is not recommended for powders, as they may adhere to the vessel walls.

6.2.1.2 Test and reference materials in massive non-epoxy- embedded form

These materials include materials placed on the market, e.g. non-embedded coupons, discs, sheets, coarse pellets, and granules (fitting the definition of massive forms, see section 3.1).

At least 18 pre-heated vessels should be set up as follows (see Figure 1):

- At least 1 negative control vessel containing only the test medium 0.032 M HCl pH 1.5 ± 0.1 at 37 ± 1 °C.
- At least 1 positive control vessel containing a solution of the element(s) of interest in 0.032 M HCl, (preferably) at a concentration within the range that can be expected to be released from the test material (see also section 6.6). A stock of the positive control should be diluted in 0.032 M HCl pH 1.5 ± 0.1, pre-heated to 37 ± 1 °C, to the desired final concentration.
- Eight material vessels at 0.2 g/L loading (4 vessels for each test and reference material, respectively): one or more pieces of test/reference material should be weighed into each of the 3 replica test/reference material vessels and 1 extra vessel (for pH measurement at the start of the test). The exact weight should be noted.
- Eight material vessels at 2 g/L loading (4 vessels for each test and reference material, respectively): one or more pieces of test/reference material should be weighed into each of the 3 replica test/reference material vessels and 1 extra vessel (for pH measurement at the start of the test). The exact weight should be noted.

Note 7: For non-epoxy-embedded massive samples (e.g., sheets, ≥ 1 mm diameter pellets or granules), the weight of the sample can be used to modify the fluid volume and comply with the desired loading. [A surface equivalent loading can also be calculated for metal and alloy samples using the geometric exposed surface area of the sample].

Vessels containing massive non-epoxy embedded forms will need to be gently swirled and visually inspected to ensure that the sample is covered by fluid and/or the coarse pellets or granules are dispersed in the solution.

6.2.1.3 Test and reference materials for massive epoxy-embedded forms (surface area loading)

At least 18 pre-heated, suitable-sized vessels should be set up as follows (see Figure 1):

- At least 1 negative control vessel containing a blank piece of epoxy resin similar in size to that used in the test and reference samples and the test medium.
- At least 1 positive control vessel containing a blank piece of epoxy resin + a solution of the element(s) of interest in 0.032 M HCl, (preferably) at a concentration that is within the range that can be expected to be released from the test material (see also section 6.6). A stock of the positive control should be diluted in 0.032 M HCl pH 1.5 ± 0.1, pre-heated to 37 ± 1 °C, to the desired final concentration.
- Eight material vessels at 0.2 g/L surface equivalent loading (4 vessels for each test and reference material, respectively): pieces of epoxy resin with test/reference material with a known exposed metal surface should be introduced into each of the 3 test/reference material vessels and 1 extra vessel (for pH measurement at the start of the test). The exact weight should be noted.
- Eight material vessels at 2 g/L surface equivalent loading (4 vessels for each test and reference material, respectively): pieces of epoxy resin with test/reference material with a known exposed metal surface should be introduced into each of the 3 test/reference material vessels and 1 extra vessel (for pH measurement at the start of the test). The exact weight should be noted.

Note 8: The loading of epoxy-embedded massive samples (2 g/L or 0.2 g/L) can be expressed as surface area equivalent. The surface equivalent loading calculation is described in Annex 2.

Vessels with epoxy-embedded massive samples should be gently swirled and visually inspected to ensure that the sample is covered by fluid.

6.2.2 Common steps

Before adding the medium to the triplicate vessels containing the test and reference materials the effect of medium addition to the sample in the 4th vessel should be considered.

Some samples may create a ‘pH drift’ when the medium is added. To ensure the pH remains at pH of 1.5 ± 0.1, the following step is needed before adding the medium to all the test and reference vessels (which corresponds to the launch of the test or t=0):

- Add an initial volume of 0.032 M HCl consistent with the desired loading (e.g., 50 mL in the examples mentioned above) at pH of 1.5 ± 0.1, pre-heated to 37 ± 1 °C to the 4th extra test/reference material vessel (one for each loading, for the test and reference material) and measure the pH. If the pH is not 1.5 ± 0.1, then it should be adjusted using HCl or NaOH (analytical grade or better, at concentrations chosen to avoid dilution of the test medium) as needed until this value is reached. The amounts of HCl or NaOH added should be carefully recorded. The total volume of medium corresponds to the sum of the initial volume and the volume needed for the adjustment of pH. Once this total volume has been defined, the extra test/reference material vessels and their content can be discarded.

At t=0 hours value:

- Add the total volume of medium defined above to the negative control vessel and to each of the remaining test/reference material vessels containing the pre-weighted material.
- After adding the pre-heated test medium to the vessels with the materials as described above, the vessels should be covered as soon as possible and placed in a thermostatic shaker (37 ± 1 °C) for 2 hours: 1 hour at an agitation rate of 100 rpm and 1 hour without agitation. The test should be performed in the dark to preserve the integrity of photosensitive materials and to reflect the dark environment of the stomach.

6.3 Observations, sampling and measurements at the end of the test

Any visual observations of, e.g. precipitation, colouration, or discolouration that differ between replicate test vessels or are consistent for all three replicates should be recorded as they can help explain the metal release results.

The following sampling procedure should be used to collect and preserve the samples for elemental analysis.

- At the end of the 2 hours incubation period, swirling is recommended before removing two aliquots (e.g., 15 mL, dependent upon the analytical setup of each laboratory) from each remaining vessel (at least one negative control, at least one positive control, and 3 replicates of 2 materials at each of the 2 loadings) at a depth of 2/3 up from the bottom of the liquid (at least 28 analytical samples[4]). These aliquots should be filtered through a 0.2 µm filter (e.g., syringe), and transferred to uniquely labelled (e.g., 15 mL polypropylene) sample tubes for analysis. The filter should be pre-wetted with 1 mL of the test medium before filtration of the test material to avoid metal adsorption to the filter.
- The samples should be preserved by adding 100 µL HNO_3 concentrated per 10 mL of sample volume to keep the pH of the samples < 2 to maintain the released ions in the solution.

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[4] Analytical sample is the sample that gets measured for metal concentration in Section 6.4 Sample Analysis

- The samples should be covered (to avoid evaporation and increased concentration) and stored at room temperature in the dark until analysis, up to 1 month after sampling.
- After sampling, the temperature and pH of the test medium left inside all vessels, including the positive and negative control(s), should be measured and recorded. The pH should be measured after sampling to avoid contamination of the samples.
- Sampling procedures are expected to be conducted promptly (no longer than 10 minutes per test/reference material at the end of the incubation period and in the order in which the medium was added) to keep variation between replica vessels as low as possible.

6.4 Sample analysis

The concentrations of released metal ions are measured with an appropriate (validated) measurement method. To guarantee the basic quality (use of a validated measuring method, involvement of appropriately trained staff, traceability of the measurements, data processing and archiving,…), the sample analyses are preferably performed in a laboratory that works according to a standardised quality system (e.g., GLP, GMP, ISO17025).

Examples of universal and commonly used methods for measuring dissolved element concentrations (and thus recommended) are listed below. However, other validated analytical methods can also be applied (such as graphite furnace or flame atomic absorption spectrophotometry, AAS).

- ICP-MS (inductively coupled plasma-mass spectrometry); well documented in ISO 17294-1:2004 Water quality - Application of inductively coupled plasma mass spectrometry (ICP-MS) - Part 1: General guidelines and ISO 17294-2:2016 Water quality - Application of inductively coupled plasma mass spectrometry (ICP-MS) - Part 2: Determination of selected elements including uranium isotopes.
- ICP-AES (inductively coupled plasma-atomic emission spectrometry); well documented in ISO 11885:2007 Water quality - Determination of selected elements by inductively coupled plasma optical emission spectrometry (ICP-OES)

Only 1 measurement result is reported for each analytical sample (i.e. 2 analytical samples from each test vessel) and metal (in µg/L or mg/L) for at least 28 analytical samples.

In addition to the measurement results of the test/reference material samples, an experimentally derived reporting limit must be provided for each metal of interest (e.g., limit of quantification (LOQ), limit of detection (LOD), see glossary for definitions, or other reporting limits derived in the laboratories according to standard use). Other analytical performance characteristics of the analysis (e.g. measuring range, repeatability, reproducibility, accuracy, measurement uncertainty) should also be reported.

6.5 Data analysis

Results should first be reported as measured metal release (µg or mg metal release per L simulated gastric fluid). The releases need to be corrected by subtracting the negative control values when they exceeded the quantification limit (LOQ). All data should be corrected based on the actual loading for each test run.

Results should also be presented as:

- release expressed as µg or mg (released) metal ion / g test or reference material. This applies to powders of e.g., metals, metal compounds, UVCBs, etc.
- release as a percentage of the total amount of metal contained in the test/reference material (%) that is released when the metal concentration in the test sample is known
- release per unit surface area (mg/m²) in the case of metals (zero valence) and alloys, in powder and massive (non-epoxy-embedded) forms
- release per unit exposed surface area (mg/m²) in the case of epoxy-embedded massive forms.

The release per g test/reference material (µg/g or mg/g) is calculated considering the actual weighed amounts of test/reference item per test vessel.
For epoxy-embedded massive materials not all the mass added to the vessel is available for release but the release per g test/reference material can be calculated based on equivalent loading (Annex 2).

The percent of total metal ions released (%) is calculated as the ratio of the average measured amount of metal ions released in the test solution to the total amount of metal in the test material initially added to the test solution as in **Equation 2**:

Eq 2

$$Metal\ ion\ released\ (\%) = \frac{average\ metal\ ion\ measured\ in\ extract\ (mg)}{metal\ in\ material\ sample\ (mg)}\ x\ 100$$

where *average metal ion measured in extract (mg) = average concentration of metal ion in extract (mg/L) x vol extract (L)*; and *metal in test material sample (mg) = metal concentration in sample (mg/g) x mass sample (g).*

In the case of metal (zero valence) or alloy powders, the element released per unit surface area (mg/m^2) is also calculated as the measured amount of metal ion in the test solution per exposed surface area (usually based on the BET measurement).

An average element release is calculated first from the two analytical sample measurements from each replicate test material sample and checked against the data acceptance criteria. After subtracting the negative control value (if applicable), the average element release, the SD and the coefficient of variation (CV) based on all three replicates of a test sample are calculated for each loading. The between-vessel CV is then used to check against the data acceptance criteria.

6.6 Negative and Positive Controls and Reference Materials

Negative control: A test vessel with test medium only. For embedded massive samples, it would also include test medium with a clear resin. A negative control run in parallel helps to detect background values of the elements of interest in the test fluid and helps rule out contamination. The measured values of the elements of interest in the negative control should generally be < 20 x standard deviation (SD) of negative controls (based on 10 or more replicates[5]). Higher values would indicate possible contaminations of the test system, glassware, etc. (see also Note 10).

Positive control: A test vessel with a test medium containing a specific amount of a highly soluble form of the element(s) of interest. For example, the positive control can easily be prepared by spiking certain amounts of the Certified Standard Metal solution into the test medium. A concentration in the range that can be expected to be released from the test material at one of the 2 loadings should be added to the test medium at time = 0 hours and run through the protocol. When there is no information about the possible release behaviour of the test material, a concentration that leads to values that can be accurately measured (within calibration curve) should be chosen. Recovery of 100 ± 10% is acceptable, based on the mean value of 10 or more replicate measurements of the positive control obtained in the laboratory. The purpose of the positive control is to assure that the relative metal release method produces repeatable results over time and to account for any precipitation of the metal ion in 0.032 M HCl. The positive control provides evidence that the test system is responsive under the actual conditions of the assay and is run in parallel with the test materials of that element. The positive control can also assess the recovery of the metal ions of interest through the test (mixing with test medium, incubation, filtering, sampling, etc.) and show any interaction with the test medium.

Reference materials should always be run in parallel with the test materials to ensure comparability and reliability. The reference material(s) may span a range of water solubilities. Examples of reference materials can be the water-soluble chemical form of the metal, the metal in its elemental state (zero valence), a representative metal oxide, etc.

The selection of the reference material(s) will depend on each metal of interest, the test material's physical state and the purpose of the test. As indicated in section 3 for test materials, the selection of the reference material (i.e., size, form and surface treatments) should also follow a stepwise approach that considers physical form, purity, and relevance.

> For Application 1- Grouping and read-across: It is not possible to predetermine what reference material(s) should be selected for assessing how the metal release of different materials of the same metal compared to reference materials. In this case, the sponsor of the study should select (an) appropriate reference material(s) based on the same physical form and the existence of toxicological

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[5] A minimum of 10 negative control measurements with the test medium need to be obtained when the method is first set up in the laboratory. As the laboratory conducts more and more tests with gastric fluid, the number of negative control values (negative test medium) will increase and the acceptance criterion for the negative concurrent control will be adapted accordingly.

information and/or oral reference values.

For Application 2 Assessment of the presence of matrix effects by of %RMC of a metal in complex metal-containing material (e.g., alloys): the pure metal ingredients of the complex metal-containing material are used as reference materials and are run in parallel with the test materials. To assess matrix effects, the reference material(s) and the test material should have the same physical form (powder or massive). If the reference sample in the physical form that led to the hazard classification of the metal is available, this reference sample should be selected for the calculation of %RMC. Otherwise, an effort should be made to match physical form.

The rationale behind the choice of reference material should be documented, and information on particle size distribution and specific surface area should be provided.

6.7 Data Acceptance Criteria

A test using this protocol can be assumed **valid** (i.e., satisfies the quality criteria of the test system) if all of the following criteria are met:

- The values of pH and temperature of the negative and positive control at the end of the test fall within the set boundary conditions, i.e., pH 1.5 ± 0.1 and temperature at 37 ± 1 °C.
- The average pH values of the three test/reference material sample replicates at the end of the test fall within the set boundary conditions, i.e., pH 1.5 ± 0.1, for at least one of the two loadings (see note 10).

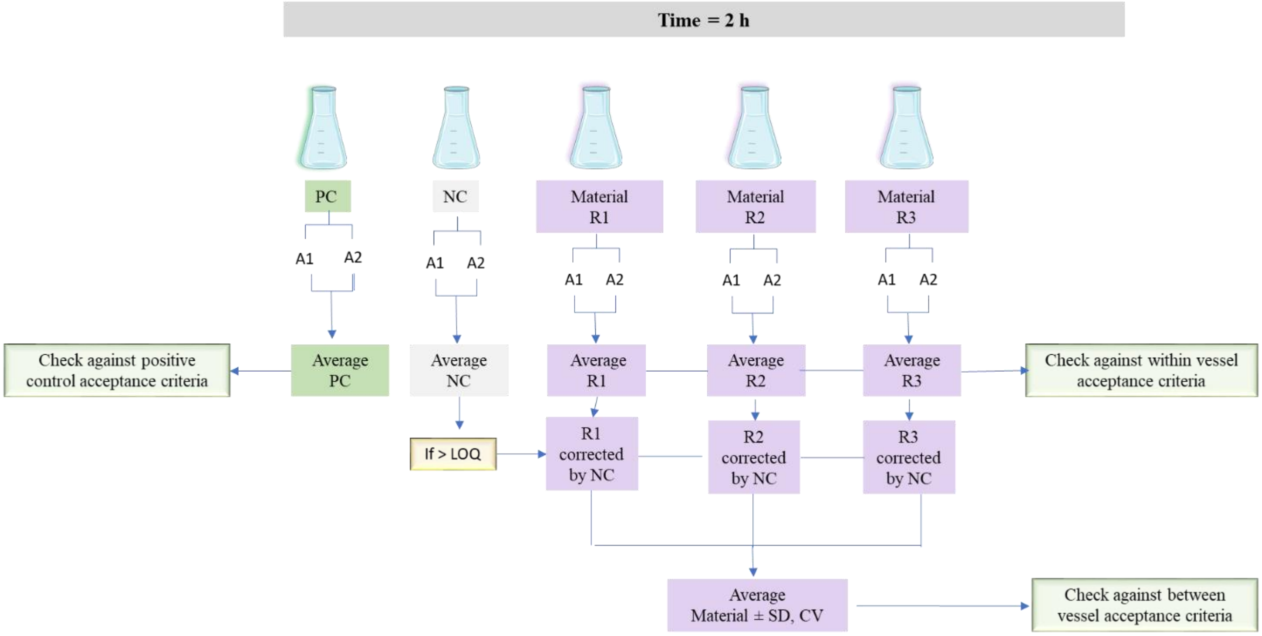
Note 9: While the above criterion applies to homogeneous materials, for some complex materials (e.g. UVCBs), the heterogeneity of the test item may explain the differences in pH among the sample replicates (hence the importance of the visual inspection in section 3.3 Test sample storage). This will need to be documented. If the difference can be explained by heterogeneity, repeating the test may not improve the results. In this case, it may be acceptable to report the metal release results even if the acceptance criterion for pH is not met.

Note 10: At the end of the test, the average pH of the triplicate test material vessels may be outside the acceptable range of 1.4-1.6, depending on the nature of the test material; this deviation should be recorded. Where the pH values of the triplicate test material samples are lower than 1.4 or higher than 1.6, it is recommended to compare the changes in pH between the high and low loadings. If one of the two loadings has an average pH at the end of the test within the acceptable range of 1.4-1.6, the data from that loading should be used to calculate %RMC. For example, an increase in pH can happen when the metal ion released has alkaline properties and/or if the sample has alkaline properties. At the low loading, the buffering capacity of the test medium will be less exhausted than at the high loading, and it is likely to yield pH values closer to 1.5. If neither the low nor the high loadings data meet the pH acceptance criterion, the test is considered invalid, and the data cannot be used.

The test results are considered acceptable if all the following criteria are met:

- A LOQ or other reporting limit is available for each element of interest.
- Results for the two analytical samples from each test material vessel (within-vessel) do not differ by more than 20% for measurements in the range from LOQ to 10x LOQ (LOQ ≤ range ≤ 10x LOQ), or by more than 10% for measurements > 10x LOQ.
- The between test material vessels' Coefficient of Variation (CV) is ≤ 40% for each element of interest in the range from LOQ to 10x LOQ (LOQ ≤ range≤ 10x LOQ), or CV ≤ 20% for measurements > 10x LOQ.
- Accurate element measurements (± 10% of reported values for Certified Metal Solution selected as positive control) are obtained from a positive control certified solution that contains the element of interest.

Figure 2. Flow chart for calculations. Average metal releases and application of acceptance criteria. NC: negative control, PC: positive control, A: analytical sample, Material: test material or reference material, R: replicate, LOQ: limit of quantification.



Note 11: When the CV values of test materials exceed the recommended values (leading to data rejection), a thorough review of the sample characteristics should be conducted before the study is repeated; in some instances, the large variability in the metal release could be linked to the intrinsic nature of the sample. Some materials (e.g., UVCBs) are very complex and heterogeneous and can, despite being from the same batch, have a wide composition and particle size range, which could result in substantial variations within a vessel and between vessels. Significant variations will therefore provide crucial information on the test material and therefore a range of release values may be reported. Still, this information needs to be combined with proper physical-chemical characterisation of the test material, e.g. Scanning Electron

Microscopic-Energy Dispersive X-Ray Spectroscopy (SEM-EDS), XPS, infrared spectroscopy (IR), BET, size distribution, ICP-MS, ICP-AES, etc.

Note 12: The average negative control measurements that exceed the LOQ should be subtracted from test sample values. Contamination is suspected when negative control values are > 20 x SD of the mean negative control for the laboratory (which is based on 10 or more replicate measurements). In the latter case, a thorough review of all the test data should be conducted (including ratio of test sample value to negative control value) before deciding whether the study should be repeated.

6.8 Interpretation of results (to be done by the Sponsor of the study)

The metal releases in the simple simulated gastric fluid from different materials containing this metal (test material and reference material in the same physical form, but not necessarily the same particle size) can be compared to produce relative metal release values. Relative metal/release values can be used to compare two materials for grouping or read-across (Application 1) or to assess the presence of a matrix effect in an alloy (Application 2).

The results obtained with the test and reference materials at each of the two loadings (2 g/L and 0.2 g/L), should be reported. The initially measured release values are reported as µg metal/L or mg metal/L. The calculated values (expressed as mg metal/g sample, mg metal/cm^2 sample, or as percent of metal content) can be compared to those from reference materials.

Application 1: To support grouping and read-across

The relative metal release (%) should be calculated as in **Equation 3:**

Eq 3

$$Relative\ metal\ release(\%) = \frac{mg\ metal\ ion\ released\ (measured\ in\ extract)/g\ Test\ sample}{mg\ metal\ ion\ released\ (measured\ in\ extract)/g\ Reference\ sample}\ \boldsymbol{x\ 100}$$

The relative metal release values using data from either loading are likely to be comparable. If this is not the case, it is recommended to use the data from the loading that yields the highest reliable metal ion release for each individual material (e.g., to avoid using data from the high loading where saturation could have led to lower releases/g sample, or from the low loading where low releases/g sample could lead to higher variability). This means that in certain situations, the relative metal release may be calculated by combining data from the two different loadings. If, for one of the samples, quantitative measurements are only obtained at one loading (e.g., when the low loading yields values < LOQ), the data from that loading should be used as long as they fulfil the acceptance criteria described above. In the case of massive metal samples (e.g., alloys) and solid inorganic UVCBs, a correction by surface area needs to be applied (mg metal/cm^2 sample) to compare releases from samples that differ in exposed surface area.

Although this can also be applied to metal powders to assess if/how surface area may influences the release between powder samples, the release by mass of sample should be used to calculate the relative metal release since surface area is difficult to measure accurately for powders and is not the only thing that affects release from metal powders. It is not recommended to apply correction by surface area when comparing different chemical forms of metals that are not in the elemental (valence of zero) form. E.g., for metal compounds, differences in the chemical nature of the metal bonds (rather than just surface area) will be the main driver for the observed differences in metal release. Correction by surface area can be applied to compare results for different particle sizes of the same material.

Application 2: To establish the presence or absence of a matrix effect in alloys or other complex metal-containing materials like alloys, UVCBs, pigments

Metal release data generated from a test material alloy (in powder or massive form) and from a reference material can also be compared as mentioned above to establish the existence of a matrix effect. The reference material will be the alloy ingredient on its own at high and low loadings. In this case, the ratio of metal release values multiplied by 100 corresponds to the Relative Metal release-based Concentration (%RMC) of the metal in the alloy and can be calculated using the following **Equation 4:**

Eq 4

$$RMC\ (\%) = \frac{mg\ metal\ ion\ released\ (measured\ in\ extract)/g\ alloy\ sample\ tested}{mg\ metal\ ion\ released\ (measured\ in\ extract)/g\ pure\ metal\ sample\ tested}\ x\ 100$$

While the releases corrected by surface area are reported to give an indication if/how surface area influences the release between reference and test samples, the metal releases per mass of sample is used to calculate the %RMC.

Equation 4 above should be used to assess matrix effects and calculate the %RMC.

%RMC results from both loadings are likely to give similar information on the presence or absence of a matrix effect when compared to the nominal concentrations of the metals in the alloy. If this is not the case, it is recommended to use the data from the loading that yields the highest reliable metal ion release per mass of sample for each individual material (e.g., to avoid using data from the high loading for the reference material where saturation could have led to lower releases/g sample, or from the low loading for the alloy where low releases/g sample could lead to higher variability) to calculate the %RMC. This means that in certain situations, the %RMC may be calculated by combining data from the two different loadings, provided that both loadings meet the acceptance criteria described in section 6.7 (Data acceptance criteria).

The calculated %RMC of a metal in an alloy may be equal, higher or lower than the %bulk concentration of that metal in the alloy, and in some cases, it may exceed 100%, which can be explained by the following possibilities.

- The %RMC will equal the %bulk concentration when there is no matrix effect in the alloy; in other words, the alloy behaves as a simple mixture.
- The %RMC will be lower than the %bulk concentration when there is a matrix effect in the alloy that lowers the effective release of that metal; in this case, the alloy behaves as if it had a lower %bulk concentration of the metal.
- The %RMC will be higher than the %bulk concentration when there is a matrix effect in the alloy that enhances or promotes the effective release of the metal; in this case, the alloy behaves as if it has a higher %bulk concentration of the metal.

Once the relative metal release or the %RMC are calculated, the 95%-confidence interval (CI) can be calculated. The approach described by Fieller (1954) for obtaining the approximate 95% CI around the ratio of metal release values (i.e., %RMC) can be applied. The formula involves calculation of the standard error of the ratio (quotient) by error propagation, and then multiplying this by the appropriate quantile and add/subtract to/from the ratio of the means, as per **Equation 5**:

Eq 5

$$\mathrm{SE_Q} = \mathrm{Q}\sqrt{\frac{\mathrm{SEM_A^2}}{\mathrm{A^2}} + \frac{\mathrm{SEM_B^2}}{\mathrm{B^2}}}$$

$$95\%\ \mathrm{CI{:}}\ \mathrm{Q} - \mathrm{t^*} \cdot \mathrm{SE_Q}\ \mathit{to}\ \mathrm{Q} + \mathrm{t^*} \cdot \mathrm{SE_Q}$$

Where SEQ: Standard error of the quotient
Q: quotient of A/B
t*: 0.975-quantile of the t-distribution with n-2 degrees of freedom, where n is the number of replicates for A plus the number of replicates for B
A: release of metal from alloy
B: release of metal from the pure element
SEM: standard error of the mean of A or B

There are several software tools that facilitate calculating the CI. A free online calculator for Fieller's approach for n < 20 can be found at https://www.graphpad.com/quickcalcs/ErrorProp1.cfm. The numerator metal release value needs to be multiplied by a suitable factor in order to get the required number of significant digits. The number of significant digits needed may vary depending on the value of the relative metal release or the %RMC, and the level of precision needed.

6.9. Proficiency material

Proficiency materials are well characterised, and defined materials (e.g. regarding elemental composition and particle size) that are tested to ensure the consistency of the protocol results. These materials may or may not include the metal of interest but need to match the physical form of the test materials (e.g. samples of powder metals or metal compounds, embedded and non-embedded massive forms of alloys). These materials are tested initially by the laboratory when establishing the protocol (to demonstrate technical proficiency prior to routine use of the method) and are run periodically afterwards (at least once a year). Results corresponding to ± 20% of reported values are deemed acceptable. Proficiency materials are independent of the application of the test data.

Such proficiency materials should:
- Be easily (and foreseeably) available in the same physical form as the test material
- Have consistent quality and quantity from a reliable source that is expected to continue to provide the same materials with the same characteristics at a relatively low cost
- Have well characterised metal release data
- Be stable under ambient conditions
- Not be extremely toxic so no special measures need to be taken other than the usual protective clothing, dust mask, glasses and gloves.

In powder form, the proficiency materials should be characterised in terms of particle size distribution and range, and specific surface area (m^2/g) should be measured using BET analysis or an equivalent technique.

A list of possible proficiency materials is provided in Annex 3.

6.10. Reporting and record-keeping

A test report is prepared for each test material and shall include (but is not limited to) the following information:

- **Information on the study and test laboratory**
  - Description of the study
  - Identification of the sponsor(s), the test facility, and the study director
- **Description of the test and reference materials**:
  - Name of the test material, including identifiers such as CAS/EINECS numbers when relevant
  - Batch number and/or sampling date
  - Expiration date when applicable
  - Details of supplier(s)/manufacturer/importer (e.g. address, contact information)
  - Information available on composition (e.g. Certificate of Analysis), at least all metals/metalloids > 0.1 wt% to assess the purity of the sample and nature of impurities (% or ppm). To note: sometimes a 100% CoA is not available as elements like Fe, Si, …can be ignored even if present in high concentrations if the customer has asked for specific elements.
  - Information on the method of manufacture (particularly for powder forms), if available, indicating if atomisation (water or air), or grinding method was used.
  - Information on processing, preparation, surface finish, transport and storage, if available (e.g. heat treatment/fabrication history; metallurgical microstructure, particularly for massive forms)

  - A justification of sample preparation if a representative powder sample is generated from a massive form (not recommended due to possible changes in properties that would affect release)
  - Information on agglomeration, surface charge (particles), surface composition, surface reactivity, and other surface properties (e.g. surface oxidation for both massive and powder forms; surface finish particularly for massive forms), if available. If surface charge information is available, include the method and medium used to measure the surface charge (e.g. composition, ionic strength, pH).
  - Particle size distribution (powders only) using parameters such as $d_{0.1}$, $d_{0.5}$, and $d_{0.9}$. Report the assessment method and if values are based on volume or number distribution and in which media (e.g. composition, ionic strength, pH) the size distribution was measured (e.g. air, water). *For example, size distribution may be determined by sieve analysis or laser diffraction.*
  - Information on morphology (shape of the particles) and metallurgical structure, if available. For example, *the morphology of particles may be determined by examination using a Scanning Electron Microscope (SEM).*
  - A specific surface area in m^2/g for powders of metals (zero valence) is required. This information may also be available for some metal compounds. *For example, the BET analysis (absorption of nitrogen or krypton at cryogenic conditions) can determine particle surface area.* The geometric exposed area of massive materials and embedded test samples can be measured with a Vernier calliper or other suitable graduated measuring device, e.g., a measuring projector.
  - If the sample (powder) was sieved, the type of sieve (and material of which it is made) and information about the original sample should also be provided.

- **Description of the test system and loading:** the following information should be provided
  - Test/reference material weight, final loading and final volume

  - pH and temperature at the start and the end of the test. If pH adjustment for a vessel with test material is necessary, describe the initial and final pH and how the adjustment was done.

  - Description of how the positive control sample was prepared.

  - Detailed descriptions of the test apparatus and procedure, including analytical instruments used

  - Surface area to volume ratio for massive materials

- **Results from the analyses of metal concentrations:**
  - Date of test

  - LOQ (or at a minimum, the LOD)

- Metal release (µg/L or mg/L) (measured element concentrations in the samples using one of the methods described in Section 6.2, including negative and positive controls, test material samples and reference materials).
  - Mean metal release per g material (calculated mg/g)
  - Mean percent of total element released (calculated %)
  - Mean element release per unit surface area for metals and alloys (calculated mg/m²)
  - Relative Metal release-based Concentration or %RMC

- **Description of the quality assurance program applied**
- **Deviations from this protocol, if any and reason(s) for such**
- **Any circumstances that may have affected the results (i.e., description of other effects observed, e.g., precipitation)**
- **Reference to the records and all raw data necessary to reconstruct the study**

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8. Glossary

- Bioavailability: *In vivo* bioavailability is defined as the extent to which a substance is taken up by an organism and is available for metabolism and interaction at target organ/sites (e.g. kidney, skin).
- Bioaccessibility: can be defined as the fraction of a substance that dissolves under surrogate physiological conditions and is potentially available for absorption into systemic circulation.
- Relative Metal release-based Concentration (RMC): for complex materials like alloys, UVCBs, pigments, the metal release-based concentration is calculated according to the formula below and compared to the bulk concentration to assess the presence or absence of a matrix effect.

$$RMC\% = \frac{\mu g\ metal\ ion\ released\ (measured\ in\ extract)/g\ alloy\ sample\ tested}{\mu g\ metal\ ion\ released\ (measured\ in\ extract)/g\ pure\ metal\ sample\ tested}\ x\ 100$$

- Bioelution: *In vitro* physico-chemical test that measures metal releases in artificial physiological fluids (e.g. artificial gastric juice)
- BET: Brunauer, Emmett and Teller's method to measure the specific surface area of a powder (Brunauer, 1938)
- CAS number: Chemical Abstracts Service number
- Certified Standard Metal solution: Standard solutions containing the metal of interest used for analytical calibration and may be used to prepare the positive controls.
- EINECS number: European Inventory of Existing Commercial Chemical Substances number
- GLP: Good Laboratory Practices
- GMP: Good Measurement Practices
- Grouping: a general approach for considering more than one chemical at the same time. It can include the formation of a chemical category or identification of (a) chemical analogue(s) to fill data gaps as appropriate
- ICP-AES: Inductively Coupled Plasma-Atomic Emission Spectrometry
- ICP-MS: Inductively Coupled Plasma-Mass Spectrometry
- IR: Infrared
- LOD (Limit of Detection): The smallest amount or concentration of an analyte in the test sample that can be reliably distinguished from zero (Thompson et al. 2002; ECHA 2016). The LOD for each metal can be calculated as three times the standard deviation of the mean measured concentration of a negative control test medium (e.g. average of 10 measurements).
- LOQ (Limit of Quantification): Concentration below which the analytical method cannot operate with acceptable precision for the given matrix (Thompson et al. 2002). The LOQ for each metal can also be calculated as six times the standard deviation of the mean measured concentration from the negative control test medium (e.g. average of 10 measurements).
- MSDS: Material Safety Data Sheets
- Positive control: The positive control is a solution of known concentration of the highly soluble salt of the metal of interest at a concentration estimated to be similar to the release of the metal being tested to test the accuracy and repeatability of the protocol for measuring that metal ion.

- Proficiency materials: Well-characterised and defined materials (e.g. regarding elemental composition and particle size) that are tested to ensure the consistency of the protocol results. These may or may not include the metal of interest but need to match the physical form of the test materials (e.g. samples of powder metals or metal compounds, embedded form and non-embedded massive forms of alloys). When establishing the protocol, these materials are tested initially by the lab and run periodically afterwards (at least once a year). Proficiency materials are independent of the application of the test data.
- Read-across: Extrapolation of known data from one reference material (also named “source” in the ECHA RAAF and OECD Guidance on the Grouping of Chemicals) to a similar test material (also named “target material”) based on the assumption that the two substances will cause similar biological responses. This is used to fill data gaps for substances without additional testing.
- Reference materials: Materials containing the same metal as the test material. The selection of the exact reference material to use in each case depends on the application (Application 1 or 2) and the characteristics of the test materials. See also Section 6.6.
- SiC: silicon carbide
- Target material: materials (e.g. substances) lacking toxicological data for one or more health endpoints but that have sufficient physico-chemical data. Their bioaccessibility is usually compared to the bioaccessibility of a reference (source) material (substance) (see also relative bioaccessibility and read-across)
- Test material: Test materials are metals, inorganic metal compounds, or complex metal-containing materials e.g. alloys, UVCBs, pigments. Test materials may be called target materials for Application 1 (read-across and grouping).
- Test medium: simulated gastric fluid (0.032M HCl solution at 37±1°C and pH 1.5±0.1).
- Test system: Test medium and test vessels.
- UVCB: Substance of Unknown or Variable composition, Complex reaction products or Biological materials
- XPS: X-ray photoelectron spectroscopy

Annex 1. Metals (and metalloids) within and out of the scope of this protocol

This table will be expanded with increasing experience with metals/metalloids (availability of results, assessment of existence of technical limitations)

Metal/metalloid	Simulated gastric fluid technical applicability[a]	Examples of useful references
Ag	NA	Unpublished PMC data
Ag compounds	NA	Unpublished PMC data
As	√	REACH Consortium Arsenic and Arsenic compounds 2012
As compounds	√	CIMM 2010: Copper and Copper compounds in gastric mimetic fluids. Report to the European Copper Institute (unpublished) REACH Consortium Arsenic and Arsenic compounds 2012
Au	√	Unpublished PMC data
B	√	Unpublished Boron Consortium data
B compounds	√	Unpublished Boron Consortium data
Cd	√	Unpublished IZA data 2018
Cd compounds	√	Unpublished IZA data 2013
Co	√	Stopford et al. 2003, Henderson et al. 2014, ECTX reports e.g. XO2a-218, XO2a-231, Danzeisen et al. 2020, Heim et al. 2020, Verougstraete et al. 2022
Co compounds	√	Stopford et al. 2003, Danzeisen et al. 2020, Verougstraete et al. 2022
Cr	√	Hedberg et al. 2010, 2016,
Cr compounds	√	Jiang et al. 2012
Cu	√	CIMM 2010: Copper and Copper compounds in gastric mimetic fluids. Report to the European Copper Institute (unpublished), ECI unpublished data 2010, Delbeke et al. 2020
Cu compounds	√	CIMM 2010: Copper and Copper compounds in gastric mimetic fluids. Report to the European Copper Institute (unpublished), Hnderson et al. 2014, ECI unpublished data 2010
Fe	√	Hedberg et al. 2016, Mörsdorf et al. 2015
Fe compounds	√	Unpublished Iron Platform Services LTd/Iron Oxides REACH Registration Consortium data 2018
Ge	√	Unpublished IZA data 2014
Ge compounds	√	Unpublished IZA data 2015
Hg	NA	
Hg compounds	NA	
In	√	Unpublished IZA data 2014, Lombaert et al. 2018
In compounds	√	Unpublished IZA data 2014, Lombaert et al. 2018
Mn	√	Hillwalker and Anderson, 2014
Mn compounds	√	Hillwalker and Anderson, 2014
Mo	√	Mörsdorf et al., 2015
Mo compounds	√	Mörsdorf et al., 2015
Ni	√	Henderson et al. 2014, Hedberg et al. 2016, Heim et al. 2020

Ni compounds	√	Henderson et al. 2012
Pb	√	CIMM 2015:Bioelution studies in gastric mimetic fluid of copper alloys and lead in massive form. Report to the European Copper Institute (unpublished), Unpublished IZA data 2017, ECTX studies e.g. XO2a-058, XO2a-217
Pb compounds	√	CIMM 2010: Copper and Copper compounds in gastric mimetic fluids. Report to the European Copper Institute (unpublished), CIMM 2015:Bioelution studies in gastric mimetic fluid of copper alloys and lead in massive form. Report to the European Copper Institute (unpublished)
Pd	√	Unpublished PMC data
Pd compounds	√	Unpublished PMC data
Pt	√	Unpublished PMC data
Re	√	Unpublished PMC data
Rh	√	Unpublished PMC data
Rh compounds	√	Unpublished PMC data
Ru	√	Unpublished PMC data
Sb	√	Unpublished i2a data 2010
Sb compounds	√	Unpublished i2a data 2009, Unpublished IPC data
Se compounds	√	Sadiq and Beauchemin 2017
Si	√	Herting et al. 2014
Si compounds	√	Herting et al. 2014
Ti	√	Unpublished IPC data 2018
Ti compounds	√	Hedberg et al. 2012, Unpublished TDMA/TDIC data 2015
V	√	Unpublished Vanadium Consortium data 2010
V compounds	√	Unpublished Vanadium Consortium data 2010
W	√	Stefaniak et al. 2010
W compounds	√	Stefaniak et al. 2010
Zn	√	Henderson et al. 2014, Unpublished IZA data 2014
Zn compounds	√	CIMM 2010: Copper and Copper compounds in gastric mimetic fluids. Report to the European Copper Institute (unpublished),
Zr	√	Hedberg et al. 2012

[a] A check mark means that the element can be measured in simulated gastric fluid without technical limitations (e.g. no precipitation, no complexation).

NA: not applicable

Annex 2. Handling of massive samples

Testing of Massive Samples

Massive forms of the test material can be tested as present on the market or as epoxy-embedded samples. For massive forms such as discs, rods, sheets, balls, pellets, etc., the weight, geometry, surface finish and geometric surface area should be recorded. Massive test material samples should, generally, be tested as close as possible to their as-received condition (i.e., in the form and with the surface properties in the original state or reflecting conditions of foreseeable use).

However, there are cases when massive samples need to be modified before testing. If the volumes of medium required to achieve the required loadings of a massive sample (2 g/L and 0.2 g/L) are too large to be feasible (maximum volume = 1250 mL for a two-litre flask), two options are possible: 1) cut the sample down to a smaller size and/or 2) reduce its exposed surface by epoxy embedding it.

Option 1: Cutting massive samples to smaller size (with or without subsequent embedding)

When smaller test material samples are prepared from the original -as received- material, cut edges should be wet ground to 1200 grit [Silicon carbide (SiC) paper][6] FEPA "P" grit sizes or equivalent to provide flat surfaces to aid area measurements and to prevent lacerations from sharp edges. All wet grinding should be followed immediately by mild ultrasonic cleaning in ethanol for 10 minutes and subsequently in isopropyl alcohol for 10 minutes. If, however, the as-received surface of the massive material has been damaged by the sampling operation (e.g., scratched), the test surface and cut edges should be prepared for testing by wet grinding with silicon carbide papers from coarse to fine grit until a uniform surface finish with 1200 grit is attained (this will also provide a flat surface to aid with area measurements). Grinding surface finishing should be followed immediately by mild ultrasonic cleaning in ethanol for 10 minutes and subsequently in isopropyl alcohol for 10 minutes, with a final rinse with isopropyl alcohol. Afterwards, the samples should be dried under a stream of cold (not hot) nitrogen gas and stored in a desiccator for a minimum of 168 hours before the start of the test. Cut pieces must be measured to the 0.1 or 0.01 mm scale using the appropriate measuring device. During the testing, care should be taken to avoid the creation of crevices (not usually present in the sample) that may lead to increased corrosion and release of metals. Cutting soft metals and alloys should be carried out carefully to avoid damaging the test surface. However, further special polish techniques may be required to provide a scratch-free surface. For example, polishing with a proprietary polishing fluid on a Selvyt cloth.

Option 2: Epoxy embedding massive samples (with or without cutting them first)

When the sample cannot be cut to a smaller size without altering its surface's physico-chemical properties and possibly affecting the metal release, epoxy embedding (as described below) is an option. Epoxy embedding decreases the effective surface area (i.e., the surface area available for interaction with the medium) and presents some benefits. The surface properties of the embedded sample can reflect the material's original state, or the surface can be treated by polishing when comparing similarly processed

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[6] While there are several scales for grit papers, two of the most commonly used are the ANSI/CAMI in USA and the FEPA in Europe. For example, a FEPA P-grade 1200 has a median diameter of 15.3 µm and is equivalent to an ANSI grit 600 with a median diameter of 14.5 µm. An example of a comparison table can be found at https://www.buehler.com/grinding-and-polishing-guide.php

samples. When the sample is cut to a smaller size before embedding, fresh-cut surfaces can be shielded with the epoxy.

In addition to decreasing the volume of fluid needed for the test, **embedding** has the advantage of avoiding abrasion during testing and yielding a regular shape. Therefore, the exposed surface area can be accurately determined.

Polishing an embedded sample has the advantage of creating a standardised, uniform, and well-defined surface, which may increase reproducibility. The disadvantage is that the polished surface no longer reflects the material's surface in the original state.
Polishing is not recommended for metal or alloy massive samples that:

- Do not have a sufficiently homogeneous microstructure.
- Have a porous structure. Openings may be created and filled with metal dust during polishing.
- Have a loosely grained structure, such that pieces break off during polishing.
- Have a low melting point. Polishing causes the temperature to rise locally despite water cooling.
- React strongly with the solvents (water, isopropanol) that are commonly used during sample preparation.
- Soft metals and alloys require special attention. The polishing needs to be adjusted for soft materials; the abrasive paper may need to be softened with wax or material polishing, and/or a final etching step may be recommended.

Epoxy embedding process

The preparation of epoxy-embedded samples is briefly described below and may occur in up to three steps. The first step is cutting the massive metal when needed. The second step is the epoxy embedding itself. A third step, surface preparation, can be skipped when samples that reflect the material's surface properties in the original state are desired.

a. Cutting (optional): typically, coupons are cut from the massive metal by suitable means (e.g., water-cutting, laser cutting, hacksaw, shears, etc.) depending on the size and shape of the sample (see further details under Option 1 above). It is important that whatever technique is selected, it does not alter the properties of the surface that will be exposed to the medium after embedding. The samples are degreased with ethanol before embedding in epoxy resin.

b. Embedding: To embed the samples, one can use EpoFix Kit from Struers or equivalent. The polymerisation process is done as directed by the resin supplier, e.g., Struers, as follows: to prepare the resin fluid, an amount of hardener is mixed into the correct amount of resin (25 parts of resin / 3 parts of hardener by weight). The mixture is mixed well for approximately 2 minutes without introducing too many air bubbles. The mixture is left to rest for 2 minutes (pot life of this mixture: about 30 minutes). The epoxy is carefully poured over the specimen in an achieved mould. A vacuum system can be used to reduce air bubbles. The embedded samples are left at room temperature for hardening for at least 12 hours.

c. Surface preparation (optional): the surface preparation may depend on the nature and hardness of the test material. This is best done by following the manufacturer's instructions for the polishing machine used (e.g., Struers). Ultimately, a uniform and scratch-free surface should be obtained. The followed polishing step must be well documented and subsequently reported. The following procedure was, for example, well applicable for medium hard materials:

Each epoxy sample is wet ground with 400 grit, 800 grit, 1200 grit and finally, 2000 grit SiC paper in sequence. To avoid maintaining a memory imprint of the grinding scratches deeper into the bulk material matrix; it is important to apply only very gentle pressure during the grinding. Each sample is ground only for approximately one minute on each grit size paper with very gentle pressure, recognising that hard and soft surfaces may require different grinding times. After wet grinding, the samples are rinsed with ethanol and dried with nitrogen gas to avoid corrosion during the sample preparation. Polishing is performed using 3, 1 and 0.25 µm diamond paste (DP-Paste M) in turn with ethanol as a lubricant and with utmost care. To obtain smooth, scratch-free surfaces, the samples are held very gently against the polishing cloth with very little applied pressure. Plenty of ethanol is used as a lubricant to obtain a well-defined surface. Each polishing step with diamond paste takes about 5 to 10 minutes, depending on the type of material. After polishing, the samples are cleaned with ethanol and quickly dried with cold nitrogen gas. To remove any residual particles left on the surface after grinding and polishing, all samples are first cleaned ultrasonically in ethanol for approximately 10 minutes, followed by ultrasonic cleaning in isopropyl alcohol for another 10 minutes, and a final rinsing with isopropyl alcohol. Isopropyl alcohol is used as the last cleaning medium. After the ultrasonic cleaning, all samples are dried quickly again with cold nitrogen gas.

The polished surface is checked using a microscope. The metal surfaces should be free from scratches and spots, otherwise, re-polishing is necessary. Before testing, the samples are stored for a minimum of 168 hours in a desiccator (passivation time). While in most cases, the average free metal surface is about 100 mm², the exact exposed surface should always be measured and reported. After testing, the surface of massive samples should be again looked at under a microscope to assess if the testing conditions triggered surface alterations.

Figure 3. Example of polished surfaces embedded in epoxy resins

Surface Equivalent Loading for embedded materials

Steps involved in the calculation of the surface equivalent loading for test materials in massive epoxy-embedded form:

1. Starting from a selected mass loading (e.g., **2 g/L**) and knowing the test material's density, a surface equivalent loading can be calculated by considering the massive material as a 1 mm diameter sphere. 1 mm is the particle diameter, which is the generally accepted to be the cut-

off between powders and massive (e.g., for aquatic toxicity classification under ECHA CLP Guidance (2024) or in Annexes 9 and 10.7 of the UN GHS rev. 10 (2023).

2. A spherical particle with a diameter of 1 mm has a surface area of 3.14 mm^2 ($4\cdot\pi\cdot r^2$) and a volume of 0.524 mm^3 ($\frac{4}{3}\cdot\pi\cdot r^3$). Using the information on the density of the material (e.g., 8.38 mg/mm^3), it can be calculated that a Ø 1 mm spherical reference particle has a mass (mg) = density (mg/mm^3) x volume (mm^3). In the example: 8.38 mg/mm^3 x 0.524 mm^3 = 4.39 mg per particle.
3. A loading of e.g., 2 g/L means that 2000 mg can be divided by 4.39 mg (the weight of a particle in this example) to calculate the number of particles needed per litre of fluid (i.e., 456 particles/L in the example above).
4. Since each particle has a surface area of 3.14 mm^2, a surface loading (e.g., in mm^2/L) can be calculated. In the example, a 2 g/L loading corresponds to 456 reference spheres and a total free metal surface loading of **1432 mm^2/L** (456 particles x 3.14 mm^2).
5. So, to test a massive embedded test material with a density of 8.38 mg/mm^3, an exposed metal surface of 1432 mm^2/L is required. For a standard test volume of 50 mL this means an exposed metal surface area of 71.65 mm^2 (1433 mm^2/L x 0.05 L = 71.65 mm^2) is needed. The loading for other available surface areas can be adjusted by adding the corresponding amount of test medium (within the vessel volume limits of minimum 50 mL and maximum 1250 mL).
6. If the massive embedded test material sample has, for example, 50 mm^2 exposed surface per piece, 29 pieces per litre would be needed for a 2 g/L loading (1432 mm^2/L ÷ 50 mm^2/piece massive representative sample = 29 pieces/L). Depending on the flask size and considering the volume limits of a minimum 50 mL and a maximum 1250 mL, 2 pieces with 70 mL medium or 35 pieces with 1225 mL medium could be set up for testing.

Annex 3. Proposed List of possible proficiency materials

Proficiency materials are well characterised and defined materials (e.g., regarding elemental composition and particle size) that may or may not include the metal of interest but need to match the physical form of the test materials (e.g., samples of powder metals or metal compounds, embedded form and non-embedded massive forms of alloys). Examples of possible massive and powder proficiency materials are shown below.

- Massive
    - SS316 sheets: 68.1% Fe (balance), 16.2% Cr, 11.2% Ni, 2.27% Mo, 1.10% Mn, 0.65% Si, 0.25% Cu, ≤0.18% Co, 0.12% Al.
    - Brass Cu63/Zn37 discs: (63% copper, 37% zinc)

- Powders
    - Cobalt metal (CAS 7440-48-4)
    - Lead metal powder (CAS 7439-92-1)