Industry guide note: How to use bioelution data in the context of grouping and read-across of metals and metal compounds?

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1. Aim of this note:

"Grouping and read-across" has extensively been used by the sector as an alternative to animal testing to fulfil hazard information requirements in metal and metal compounds (i.e. metal substances) registrations under REACH (2010, 2013, and 2018).

Read-across involves the "use of relevant information from analogous substance(s) (the 'source'[1] information) to predict properties for the 'target' substance(s) under consideration." The conditions under which 'read-across and grouping' can be used to adapt the standard testing regime are listed in Annex XI 1.5 to the REACH Regulation.

For metal substances, these approaches have generally been based on the occurrence and **"bioavailability" of a common metal ion (cation or anion) and reading-across within a group**. *In vitro* metal ion bioaccessibility, measured in bioelution tests, has been used as an estimate of bioavailability to support the read-across of toxicity information from data-rich (source or reference) substances to the data-poor (target) substances.

In view of the widespread use of read-across in the REACH registrations and its potential impacts on the quality of the REACH data, ECHA has developed several guidance documents and a framework (the Read-Across Assessment Framework or RAAF) to ensure that the approaches used by the registrants fall within the conditions for using grouping and read-across approaches set out in Annex XI 1.5 of the REACH Regulation (i.e. are compliant). Over the last years, read-across has become one of the key aspects looked at in dossiers evaluations.

Read-across has thus, unsurprisingly, been identified by both ECHA and industry as one of the key priorities to tackle under the Metals and Inorganics Sectorial Approach (MISA) and the topic has been discussed at the two first MISA workshops (on human health and environmental information requirements, respectively). The main objective of this MISA priority is to ensure that the read-across justifications used by metal substances' registrants are consistent and robust, fulfil the legal requirements, are understandable by the assessor and explain the metal substances specificities where relevant.

One question that came out from the discussions at the MISA 1 workshop (report available on MISA blog) was how to best integrate bioavailability/bioelution results when performing read-across and grouping. Although it was clearly stated, both during a workshop with ECHA and Member States held on metal

[1] Please note that the terms reference and source substance are used here interchangeably

substances read-across in 2012 and in the OECD Guidance on Grouping of Chemicals (2014, 2017), that "bioavailability results" should be used in a "weight-of-evidence manner", i.e. using all available data sources, there seems to be some lack of clarity on exactly how to best do so. It was thus proposed at the end of the MISA 1 workshop to draft a note to explain the dos and don'ts on the reference to bioavailability and use of bioelution results for grouping and read-across.

It should also be added that the 'bioelution context' has been changing since the first metal REACH registrations in 2010. Industry has made significant efforts, supported by authorities, to validate/standardise a protocol for bioelution testing and hence facilitate its acceptance for use in read-across/grouping and for the classification of alloys. The protocol for the 'gastric test' has been reviewed by ECVAM and has been now submitted as "project" for a possible OECD guidance note to the OECD Testing Guidelines Working Group (decision to be taken in April 2020).

The need to: a) consolidate the robustness of the read-across justifications in the REACH dossiers under MISA and b) work further on the acceptance and coherence of the use of bioavailability for metal substances are the main drivers for this note.

The content focuses primarily on '**the use of bioavailability and bioelution' in the context of grouping and read-across** and is based on the following sources of information:

- ECHA's Read-Across Assessment Framework (https://echa.europa.eu/documents/10162/13628/raaf_en.pdf)
- OECD guidance on Grouping of Chemicals 2014, 2017 (https://www.oecd.org/publications/guidance-on-grouping-of-chemicals-second-edition-9789264274679-en.htm)
- The information submitted on the bioelution gastric test submitted to ECVAM in 2018
- The latest SOP for the gastric bioelution test (November 2019)
- The presentations made at the MISA 1 workshop, i.e. the cobalt case presented by ECHA and the vanadium case presented by industry
- The presentation made by NiPERA at the Eurometaux Science Forum meetings in March 2019

Please note that other elements of metal substances read-across, like the role of the counter ion, are addressed in other documents (MISA workshop report, counter ion note). Please note as well that the use of bioelution for the specific grouping and read-across of nanoparticles is out of the scope of this note[2].

[2] The current SOP for the gastric bioelution test as submitted to ECVAM and OECD in autumn 2019 states that it **does not apply to nanomaterials** as defined by the EU Commission in 2011 (e.g. a natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50% or more of the particles in the number size distribution, one or more external dimanesions is in the size range 1nm-100 nm). For nanomaterials, the SOP in its current form may not allow for a complete separation of released metal ions from undissolved nanoparticles.

2. Main hypothesis and limitations

The main assumption underlying the grouping of inorganic metal substances is that toxicological and ecotoxicological properties are likely to be similar or follow a similar pattern as a result of the presence of a **common metal ion** (or ion complex including a hydrated metal ion). It is the **bioavailability** of that metal ion (or a redox form of this ion) at target sites that, besides the toxicity potency of that specific ion, will determine the occurrence and severity of the effects to be assessed (OECD 2017). It should be noted that **"(bio) transformation to common compound**(s)" is also reflected in several scenarios of the RAAF (either for an analogue or a category approach) (ECHA, 2017).

The OECD Guidance notes that this is a reasonable assumption for the majority of inorganic compounds and some organic compounds (e.g. metal salts of some organic acids), in the absence of demonstrated relative differences in bioavailability. However, the selection of the substances for which a grouping approach is relevant and reading-across from members of the group to others in that group for which data-filling is required should be done with care.

The main reason for this warning is that several factors may alter the assumption of "commonality" (that metal ion is released inorganic metals) and should therefore be assessed. In particular, one should assess the differences that the metal substances may have regarding the bioavailability and uptake of metal ion, as those will influence toxicity but also how much of the non-common substance is present at the target site. The following elements are identified in the OECD Guidance on Grouping Chemicals (2014, 2017) as affecting bioavailability and toxic potency:

- **Particle size and surface properties**: Particle size can influence the extent of solubility in aqueous and biological media, based on different rates of dissolution and/or corrosion. The particle size of a substance also influences the deposition behaviour in the respiratory tract. All of these could result in significant changes to the toxicological profile of the metal substance.
- **Chemical speciation and valence**: For some metal substances, the chemical speciation and in particular the different valences may result in differences in mechanism of action and a variation in toxicological properties. A classic example is the difference in hazards seen with Cr 3+ and Cr 6+ compounds, and this has also been reported for metals like Sb or V. In some cases, chemical species may be interconvertible, in other cases there is little interconversion between the species. In addition, extrapolating from metal compounds to the metal (valence 0) may require a case-by-case approach.
- **The crystalline structure of insoluble metal compounds** could influence the hazard profile and should be taken into account in the evaluation.
- **Counter ions and other metal ions:** The assumption that the metal ion is responsible for the common property or effect implies that the toxicity of the counter ion or of other metal substances present in the compound will be largely irrelevant in producing the effects to be assessed. The counter ion should be evaluated for its own toxicity potential to confirm that it does not add to toxicity and does not change the *in vivo* bioavailability.

3. How to generate and integrate data on bioavailability in grouping/ read-across of metal substances?

In the absence of actual bioavailability data, bioelution test results can be used to provide a basis for estimation of *in vivo* bioavailability using *in vitro* bioaccessibility data. Since bioaccessibility is based on route of exposure, it also provides an estimate of the amount available for uptake and systemic availability or available at local sites. Bioelution test results indicate the extent of metal ion release *in vitro* in fluids mimicking relevant physiological fluids. Bioelution data can be used to group substances of a given metal and assess if they will have similar or different bioaccessibility in the same fluids as a predictor of bioavailability.

How? For systemic toxicity endpoints, it is proposed to use the SOP for the gastric test that is currently being validated by ECVAM (Eurometaux, 2019) and to compare the results of a given metal ion released from different substances of the same metal. The measured values of the metal ion (expressed as µg metal/L[3]) of the target substance can be compared to those from one or more reference (= source) substances[4] (when using same loading for target and reference substances); i.e. by calculating the relative bioaccessibility:

$$\text{Relative bioaccessibility (\%)} = \frac{\text{metal release from the target substance (average µg metal/L ±SD) X 100}}{\text{metal release from reference substance (average µg metal/L ±SD)}}$$

The resulting relative bioaccessibility is used to group metal substances that release similar amounts of metal ion and to use this information in a weight-of-evidence approach (i.e. considering all data sources available in the conclusion that is drawn) to determine if target and reference substances can be predicted to have similar or different oral systemic effects.

Example of use of relative bioaccessibility for grouping (results shown for the same loading)

Samples	**Results (average µg metal/L ± S.D.)[a]**	**Relative bioaccessibility to reference 1 (%)**
Reference substance 1	1000 ± 90	100
Reference substance 2	10 ± 0.5	1

[3] Units: one can also use calculated values expressed as µg metal/g sample, µg metal/cm² sample (for metals, not metal compounds), or as percent of metal content

[4] The selection of the reference material(s) will depend on each metal(metalloid) of interest and what the purpose of the read across is (e.g. assess if one compound is similar enough in bioavailability to another one to warrant the same classification; assess how the bioaccessibility of many different compounds of a metal compare to a few reference metal compounds for which toxicity data already exists). The exact materials used as reference/source material will depend on the exact question that is being addressed. Comparability and reliability is assured by always testing the reference materials in parallel to the target samples. The rationale behind the choice of reference material should be clearly documented

Target substance 1	900 ± 100	90
Target substance 2	800 ± 75	80
Target substance 3	100 ± 9	10
Target substance 4	9 ± 1	0.9
Target substance 5	7.5 ± 1	0.75

a After subtracting negative control values.

The information presented in the table could be used as follows, to support and increase the confidence on your read-across adaptation, together with further information:

- Target substances 1 and 2 have metal release properties comparable to reference substance 1 and could be grouped with this substance.
- Target substances 4 and 5 have similar metal release to reference substance 2 and could be grouped with this substance.
- For target substance 3, relative bioaccessibility data cannot be used in isolation but needs to be complemented with other existing information (e.g. physico-chemical properties, toxicokinetics, and/or toxicity data in a weight of evidence approach) before deciding whether reference substance 1 or 2 should be used for read-across.

Important notes:

- Loading: when source and target samples are tested at equal loadings and times in the same solutions, metal releases per volume of solution can be directly compared and no further modification of the data is needed (see example above). In some cases, when very high or very low releases are expected, different loadings may be applied to the reference (=source) and target samples. In this case, each loading can be considered to express the results as mass of released metal ions / mass of sample. Metal ion release per mass of sample from reference and target substances and mixtures can then be compared.
- Information on sample properties (purity, composition, particle characteristics) should be available to assess representativeness but also comparability of the reference and target substances.

4. Limitations and way forward:

As actual bioavailability data is rather scarce and requires animal testing, industry is using bioelution to estimate *in vivo* bioavailability and hence to estimate uptake and systemic toxicity. This is also compatible with the 3Rs (Replacement, Reduction and Refinement of animal testing). ECHA supports the approach but considers that estimations of internal systemic exposure are subject to some uncertainties if based **solely** on an *in vitro* bioelution. Bioelution is a starting point.

More specifically, bioavailability (uptake) in vivo is a dynamic process and a limitation of the bioelution test is that is does not predict the quantitative in vivo bioavailability. Indeed, in biological systems, the dynamics are different from the static conditions encountered in the in vitro bioelution test. Bioelution

methods currently cannot mimic all key components of in vivo bioavailability like competitive inhibition of uptake, transport mechanisms, absorption or interactions.
It is generally assumed that not all the released metal predicted based on bioelution tests gets absorbed. A literature review of 38 soil studies examining the relationship between in vitro bioaccessibility and in vivo bioavailability concluded that bioelution data can provide a conservative estimate of absolute bioavailability (Alloy Inc 2017, Dutton et al. 2019).
Therefore, bioelution data is useful when different cations are compared, when trend analysis is performed and when read-across is substantiated with weight- of- evidence information. Bioelution data is thus a supportive element in the justification of a read-across or category, but not sufficient on its own. The read-across adaptations based on 'bioavailability' results shall therefore ensure that these uncertainties or possible over/underestimations are analysed, minimised, and taken into account. Bioaccessibility studies should be used in a weight-of-evidence approach.

How?

- By using **supportive data:**
    - Acute studies, sub-acute studies or reproductive toxicity screening studies ("bridging studies") can help bringing confidence in a proposed 'category'. The hypothesis and the prediction will be strengthened in case the bioaccessibility data and bridging studies are relatively consistent in regard of the trend within the category. In case of inconsistency, the trend or grouping or the prime hypothesis needs to be reconsidered. See also the examples below.
    - Supporting information can also include information on a number of different factors (e.g. physicochemical properties such as water solubility, degree of dissociation of the metal – containing substance, particle size and structure, or toxicokinetics).
    - If there is evidence that the surface properties and particle size influence the bioavailability significantly and therefore impact the severity of the effects of the substance to be assessed, this should be taken into account.
    - The influence of the counter ion should be checked for each endpoint. If there is reason to believe that the counter ion (such as cyanates, oxalates) or other metal ions present in the substance significantly influence the effects and bioavailability of the substance to be assessed and alter the assumption of commonality, this should be taken into account in the evaluation[5].

All the available data can be included in a **matrix**

Category member[6]	**Metal substance 1**	**Metal substance 2**	**Metal substance 3**	**Metal substance 4**

[5] Please note that a document summarising the data on a number of typical metal counterions and in particular the information of toxicity and possible impacts on the ion bioavailability is currently prepared by EBRC and Eurometaux and can be attached to the read-across justification where relevant

[6] This 'matrix' is geared towards the category approach, but is also applicable to the analogue approach in the consideration of comparing one-to-one or one-to few substances

Bioaccessibility (e.g. in gastric test)				
E.g. Water solubility				
E.g. Particle size or surface area when appropriate				
Toxicity counter ion (effect on bioavailability/toxicity?)				
Other in vitro tests (e.g., toxicity)				
Toxicokinetic data				
Mode/mechanism of action				
E.g. Acute oral toxicity (LD50…)				
E.g. Repeated dose toxicity (NOAEL,…)				
E.g. Reproductive toxicity (NOAEL)				

The matrix will show the consistency and inconsistencies among the category members and for example whether the bioelution/bioaccessibility data matches with the toxicity studies.

- By following **the stepwise approach proposed by the OECD Guidance on Grouping of Chemicals:**
    - Step 0: "Determine if the metal substance is already a member of a group or existing category." Some authorities and/or industry groups have indeed already applied the principles of grouping for use in assessment of health and environmental hazard properties and this may be of help/provide useful information, facilitating grouping. Information sources on existing categories include for example: eChemportal (www.echemportal.org), Canada (https://www.canada.ca/en/health-canada/services/chemical-substances/substance-groupings-initiative.html), EPA/OPPT (Office of Pollution Prevention and Toxics) chemical categories programme

    - Step 1: Generate metal release data in an appropriate bioaccessibility testing set up (using appropriate fluids related to the route of exposure considered) for source and target substances. A preliminary grouping can be done based on these data.

- Step 2: Consider the bioaccessibility data in a weight-of-evidence approach with data on physical-chemical properties (e.g. water solubility, degree of dissociation of the metal-containing substance/mineral, particle size and structure), knowledge on mode and if possible mechanism of action (in particular for local effects), and factors like presence of counter ions. Incorporate existing *in vivo* data and as appropriate targeted (lower tier) *in vivo* toxicity and/or toxicokinetic testing[7] in order to verify that the bioaccessibility data correlate with the toxicity endpoint(s) considered and to generate a reference range (here again a matrix can be used). In relation to establishment of a chemical category: Pay particular attention to the need to robustly define **the borders of the category** (the most comprehensive test data are required for the substances at each end of the category).
  Note: grouping is necessary for the category approach, and also for the analogue approach even if not as comprehensive. The analogue approach also utilises this concept to determine similarity between the source and target substances with support from the bioelution test data.

- Step 3: Assess the most appropriate grouping of substances and identify the source substance for each target substance based on the weight-of-evidence approach described above.

- Step 4: Use the new paradigm to read-across toxicological data from source substances to target substances based on the weight-of-evidence approach described above.

- Important: the boundaries of the **category** to which the read across is applied to should be clear and transparent. Both *in vivo* data and bioaccessibility data play a role in this perspective. In case only few substances have adequate data, it will indeed be difficult to confirm the category hypothesis and predict the toxicity of the target substances. On the other hand, with more data (scenario 2), one may consider creating subcategories in such cases to avoid the application of a worst-case (scenario 1). At the MISA workshop the following examples were given:

  - Scenario 1: one category

Substance 1	Substance 2	Substance 3	Substance 4	Substance 5	Substance 6

Lowest solubility……Highest solubility

Lowest bioaccessibility……Highest bioaccessibility

In vivo data is available for the most soluble substance: substance 6

Substance 1	Substance 2	Substance 3	Substance 4	Substance 5	Substance 6

Read-across

In this scenario, the read-across is applied from substance 6 to substances 1 to 5, based on a worst-case prediction. This is done by endpoint.

[7] Targeted test on a few substances to address a larger group of substances

- Scenario 2: subgroups in the category based e.g. on bioavailability

Substance 1	Substance 2	Substance 3	Substance 4	Substance 5	Substance 6

Lowest solubility……Highest solubility

Lowest bioaccessibility……Highest bioaccessibility

In vivo data available for TWO members of the category: substances 3 and 6

Substance 1	Substance 2	Substance 3	Substance 4	Substance 5	Substance 6

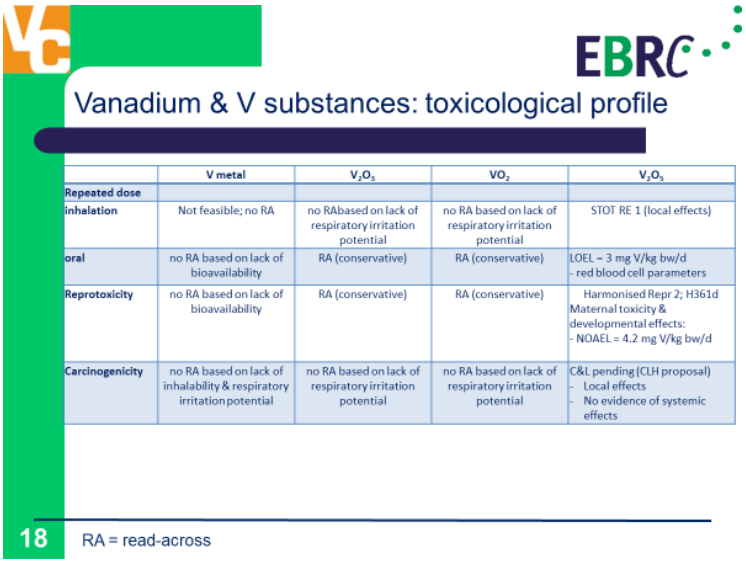
Read-across subgroup1 Read-across subgroup 2

5. Reporting/examples

The following examples are available for consultation:

- **The cobalt testing proposal example**, as analysed and presented by ECHA on 2 October 2018 referred for example to the following elements (please see the full example available on the MISA blog or on request to Dokou@eurometaux.be):
    - "To support the proposed read-across hypothesis, the Registrant has provided experimental data on water solubility, cobalt speciation in the environment, *in vitro* bioaccessibility studies (of all cobalt substances in the proposed category) in various artificial physiological fluids, and four sub-acute toxicity studies (28-day). The results from the *in vitro* bioaccessibility studies demonstrate that the predicted bioaccessibility (gastric release) is high for most substances within the category regardless of water solubility."
    - "In order to verify the assumption that the systemic toxicity of the cobalt substances is correlated with the *in vitro* bioaccessibility in artificial gastric fluid, the bioaccessibility data was put into relation to the effect levels of oral repeated dose toxicity studies, by plotting the NOAEL values against the bioelution data. The data cover the full spectrum from readily to poorly bioaccessible cobalt substances." (ECHA, 2018)

- The **vanadium example** as analysed and presented by the V consortium on 2 October 2018. The elaboration of the read-across concept for the 18 substances (vanadium metal & interstitial carbides, tri-, tetra-, pentavalent vanadium salts & oxides) is based on the combination of water solubility, *in vitro* bioaccessibility in different physiological fluids, *in vivo* toxicokinetics and physico-chemistry (oxidising properties / reactivity/acidity). The vanadium substances tested in bioelution tests were selected based on water solubility screening results, oxidation state and commercial relevance. Poor water solubility was generally indicative of low bioaccessibility. A redox speciation analysis was included (as valence is toxicologically relevant) and showed that the speciation in body fluids depended mostly on the bioelution medium and less on the vanadium substance tested. Vanadium substances transform mostly in soluble pentavalent species (except in the artificial lysosomal fluid) and tetravalent/pentavalent forms occur simultaneously depending on the physiological conditions. For systemic toxicity, unlimited read-across was applied from the pentavalent substances to the soluble vanadium substances. Low bioavailability was considered for the V metal



	V metal	V_2O_3	VO_2	V_2O_5
Repeated dose inhalation	Not feasible; no RA	no RAbased on lack of respiratory irritation potential	no RA based on lack of respiratory irritation potential	STOT RE 1 (local effects)
oral	no RA based on lack of bioavailability	RA (conservative)	RA (conservative)	LOEL = 3 mg V/kg bw/d - red blood cell parameters
Reprotoxicity	no RA based on lack of bioavailability	RA (conservative)	RA (conservative)	Harmonised Repr 2; H361d Maternal toxicity & developmental effects: - NOAEL = 4.2 mg V/kg bw/d
Carcinogenicity	no RA based on lack of inhalability & respiratory irritation potential	no RA based on lack of respiratory irritation potential	no RA based on lack of respiratory irritation potential	C&L pending (CLH proposal) - Local effects - No evidence of systemic effects

- The nickel example of an analogue read-across approach structured along the RAAF tables and presented during the Science Forum meetings on 25 March 2019 is shown below.
  This example provides justification for the read across of reprotoxicity data from Ni sulphate to Ni acetate. All Ni compounds, whether inhaled or ingested, are considered to transform (to different extents) upon inhalation or ingestion into the common soluble Ni ion. Based on structural similarity of the toxic moiety (Ni ion), an analogue approach was selected for read-across, where properties (such as water solubility, *in vitro* bioaccessibility data, *in vivo* bioavailability data, and toxicological data) from the source substance are used to predict similar effects for the target substance. The RAAF table below presents a concise version of the read-across approach for Ni substances. However, more in-depth details are provided in the dossier as read-across background documents (CSR appendices) that are specific for the inhalation and oral routes of exposure.

Read-across framework: reproductive toxicity studies with nickel sulphate read-across to nickel acetate

Assess-ment Elements	Assessment Element Type	Justification
A.1	Characterisation of source substance	Ni sulphate. CAS 10101-97-0, EC 232-104-9. Mono-constituent substance; main constituent is present at >98.0%. No impurities identified
A.2	Link of structural similarity and differences with proposed prediction	Both substances are inorganic, contain the same cation (nickel ion) and a different anion (counter-ion). Read-across is based on the bioavailability of the nickel ion (Ni^{2+}) from the source (nickel sulphate) and target (nickel acetate) substances using a weight of evidence approach. All Ni-containing compounds have been shown to release soluble Ni (II) ions to different extents, depending on the compound and the medium (route of exposure-specific fluids or cell culture) tested (Henderson et al., 2014; Fletcher et al., 1994). **The absorbed soluble Ni ion is the toxic moiety responsible for the reproductive effects of nickel compounds**. All Ni compounds transform (to different extents) into a common soluble Ni (II) ion.

Assess-ment Elements	Assessment Element Type	Justification
A.2	 Link of structural similarity and differences with proposed prediction	The potential for most nickel-containing compounds to cause adverse systemic effects has been shown to be dependent upon **water solubility, toxicokinetics, bioaccessibility, and systemic bioavailability of the Ni^{2+} ion at target sites** (Fletcher et al., 1994; Ishimatsu et al, 1995; KMHC, 2010; FDRL, 1983; CSR Appendices B1 and B2). These properties enable grouping of target Ni compounds for classification purposes according to bioaccessibility in vitro. Gastric fluid yields the highest estimates of bioaccessibility and can be used to differentiate between nickel salts that yield 90-100% bioaccessible Ni ion (e.g., Ni acetate, Ni sulphate, Ni chloride) and Ni compounds with much lower bioaccessibility (Ni oxides and sulphides) (Henderson et al., 2012a). Bioaccessibility results show similar release rates of nickel sulphate and nickel acetate in both gastric and intestinal fluids. **Toxicokinetic studies have shown that oral absorption of Ni correlates well with the bioaccessible fraction of Ni ion from Ni compounds in gastric fluid**, meaning that higher systemically absorbed fractions are typically observed with soluble nickel substances compared to less soluble nickel substances (Ishimatsu et al., 1995). A strong correlation is also observed between **bioaccessibility and acute toxicity for oral exposure** (Henderson et al, 2012a). A weight of evidence assessment based on water solubility, oral bioavailability, bioaccessibility, toxicokinetics, and acute toxicity suggest that the systemic effects such as reproductive toxicity of nickel acetate should be read-across from nickel sulphate (CSR Appendix B1).
A.3	Reliability and adequacy of source studies	The source studies are one and two-generation reproductive toxicology studies in rats with Ni sulphate (SLI, 2000a,b) conducted according to GLP. At least K2 for reliability for these endpoints.

Read-across framework: reproductive toxicity studies with nickel sulphate read-across to nickel acetate

Assess-ment Elements	Assessment Element Type	Justification
1.1	Formation of common(identical) compound	Both source (Ni sulphate) and target (Ni acetate) samples release **similar relative amounts of soluble Ni (II) ions** in gastric and lung fluids (Appendices B1 and B2).
1.2	The biological targets for common compound	The common compound for nickel sulphate and nickel acetate is the Ni^{2+} ion. Thus, since the **common compound is the same, the biological targets and resulting effects would be the same as well.**
1.3	Exposure of biological target to common compounds	Reprotoxic effects are considered to be the result of systemic exposure to the common compound, Ni^{2+}. Oral exposure to soluble nickel compounds will lead to similar nickel ion absorption, as shown quantitatively in a toxicokinetic study (Ishimatsu et al., 1995). Since the solubility of nickel acetate in gastric fluid is the same as for those compounds, the common compound (Ni^{2+}) will have **similar biological targets, internal exposures, & biological effects**.

Read-across framework: reproductive toxicity studies with nickel sulphate read-across to nickel acetate

Assess-ment Elements	Assessment Element Type	Justification
1.5	Formation and impact of non-common compounds	Both source (Ni sulphate) and target (Ni acetate) have different non-common compounds (anions) but these moieties (sulphate, acetate) are not expected to influence reproductive toxicity to any significant extent. No intermediates are formed in the transformation of the source or target compounds.
A.4	Bias that influences the prediction	**A weight of evidence approach was used to determine the most appropriate scenario for read-across**. Water solubility and bioaccessibility data were supported with in vivo toxicity and toxicokinetic data. Soluble nickel compounds have structural similarity as well as similar Ni^{2+} ion release in the appropriate synthetic biological fluids. This overcomes uncertainties in read-across based solely on water solubility. Given that the oral bioavailability of Ni from repeated exposure can be extrapolated from acute Ni bioavailability, the results of the gastric bioaccessibility and acute oral toxicity (LD_{50}) testing can serve as the basis for performing read-across for chronic endpoints such as reproductive and developmental toxicity (see CSR Appendices B1 and B2). .

6. Glossary

- Anion: a negatively charged ion
- Analogue: In an analogue approach, read-across is employed within a group of a very limited number of substances for which trends are not apparent: i.e. the simplest case is read-across from one source substance to one target substance.
- Bioaccessibility: In vivo bioaccessibility is defined as the fraction of a substance that is released in the gastrointestinal/respiratory tract/skin and becomes available for absorption. This can be estimated in vitro by measuring the dissolved quantity of a metal ion released under surrogate physiological conditions (in vitro bioaccessibility) in bioelution tests
- 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).
- Bioelution in vitro physico-chemical test that measures metal releases in artificial physiological fluids (e.g. artificial gastric juice, artificial sweat, artificial lysosomal fluid)
- Bridging studies: toxicological studies that help to support the read-across hypothesis by providing relevant, reliable and adequate information allowing to compare the properties of the target and source substance.
- Category: In a category approach, read-across is used within a group of a number of substances for which trends are apparent.
- Cation: a positively charged ion
- Counter ion: ion that accompanies an ionic species in order to maintain electric neutrality
- CSR: Chemicals Safety Report
- EPA: Environmental Protection Agency
- ECVAM: EU Reference Laboratory for alternatives to animal testing
- Insoluble substances: substances that will not dissolve in water.
- LD50: Lethal Dose for 50% of subjects
- NOAEL: No Observed Adverse Effect Level
- OECD: Organisation for Economic Co-operation and Development
- OPPT: Office of Pollution Prevention and Toxics
- RAAF: Read-Across Assessment Framework
- SOP: Standard Operating Procedure
- Source and target substance: read-across is regarded as a technique for predicting endpoint information for one substance (target substance), by using data from the same endpoint from (an)other substance(s), (source or reference substance(s)).
- Sparingly soluble substances: A sparingly soluble metal compound can be considered as one for which a solubility product can be calculated, and which will yield a small amount of the available form by dissolution.
- Speciation: specific form of an element defined as to isotopic composition, electronic or oxidation state, and/or complex or molecular structure

References

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- Dutton MD, Vasiluk L, Ford F, Bellantino B, Perco S, Taylor R, Lopez K, Bolger GT, Gopalapillai Y, Hal B. 2019. Towards an exposure narrative for metals and arsenic in historically contaminated Ni refinery soils: Relationships between speciation, bioavailability, and bioaccessibility. Science of the Total Environment 686 (2019) 805–818
- ECHA's Read-Across Assessment Framework (https://echa.europa.eu/documents/10162/13628/raaf_en.pdf)
- OECD guidance on Grouping of Chemicals 2014, 2017 (https://www.oecd.org/publications/guidance-on-grouping-of-chemicals-second-edition-9789264274679-en.htm)
- MISA website https://echa.europa.eu/nl/misa https://www.reach-metals.eu/metals-and-inorganics-sectoral-approach-misa