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Bioelution-Based Approaches for Metals

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Bioelution-Based Approaches for Metals

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Outline

- **Link between bioavailability and toxicity: definitions and general bioelution principles**
- **Use of bioavailability in the regulatory framework**
  - Precedents for use of bioaccessibility data as surrogate for bioavailability in regulations
  - Grouping and read-across:
    - Antimony (Sb)
    - Nickel (Ni)
  - Classification of substances and mixtures
- **Advantages and limitations of bioelution-based approaches**
- **Conclusions**

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Link Between Bioavailability and Toxicity: Definitions and General Bioelution Principles

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Definitions (1)

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/site**

Since the ***toxicity*** of metals and their inorganic substances is primarily associated with the release of ***soluble metal ions***, their bioavailability is defined as the extent to which the soluble metal ion is available at the target organ/site

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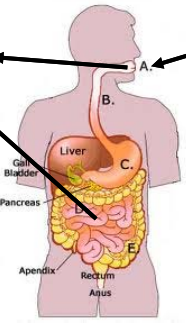
Bioavailability and Toxicity: Oral Route

Systemic Absorption

Bioavailable | Metal ion

Systemic Toxicity Effects
(acute oral toxicity, reproductive toxicity, neurotoxicity, *etc*)



Metal-containing substance

Metal ion Bioavailability → **Toxicity**

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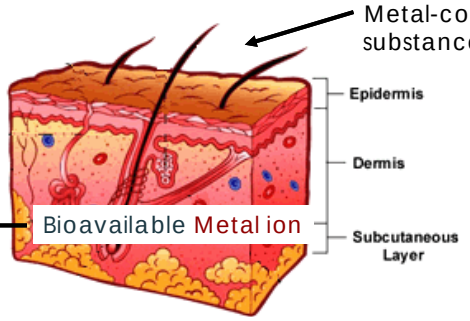
Bioavailability and Toxicity: Dermal Route



Metal-containing substance

Local Toxicity Effects
(sensitization, *etc*)

Metal ion Bioavailability → **Toxicity**

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Definitions (2)

- ***Bioaccessibility*** **is defined as the fraction of a substance that dissolves under *surrogate physiological conditions* (e.g., soluble metal ion) and therefore is "potentially available" for absorption into systemic circulation or for interaction at local sites**

Bioelution refers to the ***in vitro extraction methods*** used to measure the degree to which a substance/metal ion is released into artificial biological fluids, i.e., substance's bioaccessibility

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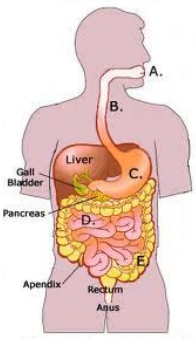
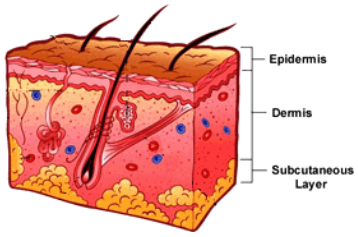
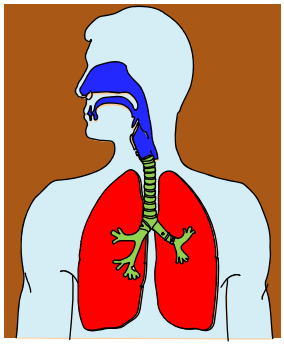
Bioaccessibility as Predictor of Bioavailability: Route of Exposure







Important to consider the ***route of exposure*** as bioaccessibility, absorption and bioavailability are specific to each route of exposure

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Can bioaccessibility be used as a predictor of bioavailability and toxicity in hazard assessment?

- In some cases YES ! In other cases NO !
- In yet other cases, bioaccessibility may be a contributor to predicted toxicity when additional factors are considered

Validation—Verification is needed for each substance and each route of exposure !

Metal ion Bioaccessibility	→	Metal ion Bioavailability	→	Toxicity
Bioelution: • Saliva • Gastric • Intestinal • Sweat • Alveolar • Lysosomal		**Toxicokinetics:** • Absorption • Excretion • Blood levels • Organ levels		***In vivo* studies:** • Lethality • 2-generation reproductive • *Etc.*

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Use of Bioavailability in the Regulatory Framework

Precedents for use of bioaccessibility data as surrogate for bioavailability in regulations

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Precedents for Use of Bioelution in Regulatory Frameworks: Environment

Classification for acute and chronic aquatic toxicity

- Bioaccessible metal ion is measured in the Transformation-Dissolution Protocol (TD/P).
- **Absolute** metal release at different mass loadings are compared to metal-specific **toxicity reference values** (ERVs) (derived from the 100% bioaccessible form) to determine acute and chronic aquatic toxicity **classification** of metal compounds and different physical forms of metals, alloys, etc.

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Precedents for Use of Bioaccessibility Data for Human Health in EU Regulatory Frameworks

- **EN 71.3 & ASTM F-963**
  - Standard and method for determining toy safety; specifies limits for the migration of metals from toy materials
- **REACH Restriction of Nickel & EN 1811**
  - Considers allowable nickel release from consumer articles intended for prolonged and direct skin contact and provides method to assess Ni ion release
- **CLP & EN 1811**
  - Addresses the classification of alloys as dermal sensitizers based on nickel ion release rate
- **REACH Restriction of Lead in consumer articles** (http://echa.europa.eu; adopted opinions on restriction **proposals):**
  - Considers allowable lead release from consumer articles that can be mouthed by children (Urrestarazu et al., 2014)

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Precedents for Use of Bioelution in GHS

GHS Classification of hazardous substances and mixtures

- "The effect of a substance or mixture on biological and environmental systems is influenced, among other factors, by the physico-chemical properties of the substance or mixture and/or ingredients of the mixture and *the way in which ingredient substances are biologically available*. Some groups of substances may present special problems in this respect, for example, some polymers and metals.
- A substance or mixture *need not be classified when it can be shown by conclusive experimental data from internationally acceptable test methods that the substance or mixture is not biologically available.* **Similarly, *bioavailability data* on ingredients of a mixture should be used where appropriate in conjunction with the harmonized classification criteria when classifying mixtures**." (section 1.3.2.4.5.1)

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Use of Bioavailability in the Regulatory Framework

Grouping and read-across (REACH):

Antimony (Sb)

Nickel (Ni)

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Grouping and Read-across: Antimony (Sb)

- **Naturally occurring element:**
  - Ores: mainly as sulfide (e.g., stibnite Sb_2S_3)
- **Commercially used as Sb metal and Sb compounds**
  - Most important compound antimony trioxide (ATO; Sb_2O_3)
    - Flame retardant synergist (>85%)
    - PET catalyst (5%)
    - Pigments/paints/ceramics (5%)
- **Forms compounds in valence state (-3, +3, +5)**

Source: Courtesy of J. Mertens- International Antimony Association

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Grouping and Read-across: Antimony (Sb)

- **Differential toxicity of Sb(III) versus Sb(V)**
  - Sb (V) used in treatment of leishmaniosis
    - Affects 2 million people and kills 70 000 per year
    - Caused by Leishmania species (family Trypanosomatidae)
  - Sb(V) is prodrug—reduced intracellulary in parasites by glutathione (GSH) to the active Sb(III) species
  - Sb(III) assumed to be more toxic than Sb(V)
- **8 main Sb substances needed assessment; only one data rich (ATO)**
  - Theoretically: 4 antimony read-across groups possible

highly soluble Sb(III)	poorly soluble Sb(III)
highly soluble Sb(V)	poorly soluble Sb(V)

ATO

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Grouping and Read-across: Antimony (Sb)

Water solubility of main chemical forms of Sb

Substance	Molecular Formula	Valence	Water Solubility [mg/L]
Antimony	Sb	0	18.2
Diantimony trioxide (ATO)	Sb_2O_3	+III	25.6
Diantimony trisulfide	Sb_2S_3	+III	43.5
Diantimony tris(ethylene glycolate)	$Sb_2[OCH_2CH_2O]_3$	+III	0.4–1.2 µg/L
Antimony trichloride	$SbCl_3$	+III	Hydrolyses
Diantimony pentoxide (APO)	Sb_2O_5	+V	453.0
Sodium antimonate	$NaSbO_3$	+V	225.0
Sodium hexahydroxoantimonate (SHHA)	$NaSb(OH)_6$	+V	594.0

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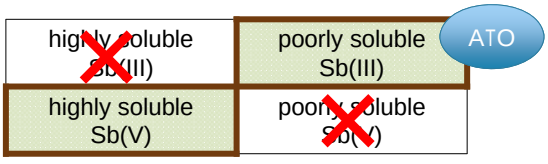
Grouping and Read-across: Antimony (Sb)

- **Bioaccessibility of key Sb substances: 0.1 g/L loading, 2 and 24 h exposures**
- **Dissolution kinetics expressed as dissolved mass per surface and time [mg/cm²/h] for 24 h exposure period**

	Blood	Inhalation		Oral	Dermal
Test item	PBS pH 7.4	GMB pH 7.4	ALF pH 4.5	GST pH 1.6	ASW pH 6.5
Sb	4.1	5.6	8.1	2.0	6.0
Sb_2O_3 (ATO)	0.1	0.2	6.2	0.3	
Sb_2S_3	0.6	0.3	0.4	0.3	0.3
$NaSb(OH)_6$ (SHHA)	2.5	4.7	5.1	8.0	5.1

Sb(III) substances

Sb(V) substances



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Grouping and Read-across: Antimony (Sb)

- **8 Sb substances needed assessment—two data rich reference compounds were determined to be sufficient for read-across based on water solubility/bioaccessibility/valence**

Substance	Molecular Formula	Valence
Antimony	Sb	0
Diantimony trioxide (ATO)	Sb_2O_3	+III
Diantimony trisulfide	Sb_2S_3	+III
Diantimony tris(ethylene glycolate)	$Sb_2[OCH_2CH_2O]_3$	+III
Antimony trichloride	$SbCl_3$	+III
Diantimony pentoxide (APO)	Sb_2O_5	+V
Sodium antimonate	$NaSbO_3$	+V
Sodium hexahydroxoantimonate (SHHA)	$NaSb(OH)_6$	+V

Sb(III)-group
Poorly soluble
Read-across from ATO

Sb(V)-group
Highly soluble
Read-across from SHHA & APO data

Thanks J. Mertens- International Antimony Association

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Grouping and Read-Across: Nickel (Ni) Compounds

- **Naturally occurring transition metal** (lateritic and sulfidic ores)
- **Main commercial applications:** stainless steels, metal finishing, batteries, catalyst, electronics, etc
- **Essential for bacteria, plants** and some mammals
- **Ni (II) ion is considered to be the toxic moiety** for systemic effects and a significant contributor to local toxicity effects
  - Not very redox active unless it is bound to other ligands (e.g., proteins)
  - Exposure via oral, inhalation, and dermal routes

- **Three main chemical forms of nickel compounds**
  - Water soluble, sulfidic, and oxidic

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Grouping and Read-across: Nickel (Ni) Compounds—Oral Route

Robust datasets-evaluations available for Ni sulfate, Ni oxide, and Ni subsulfide; 10 nickel compounds needed to be assessed!

Reference or source nickel compounds	Example: Oral route-systemic effects
Ni sulfate	Acute Tox 4; H302; Repr. 1B; H360D
Ni subsulfide	No classification Acute Tox No classification Repr.
Ni oxide	No classification Acute Tox No classification Repr.

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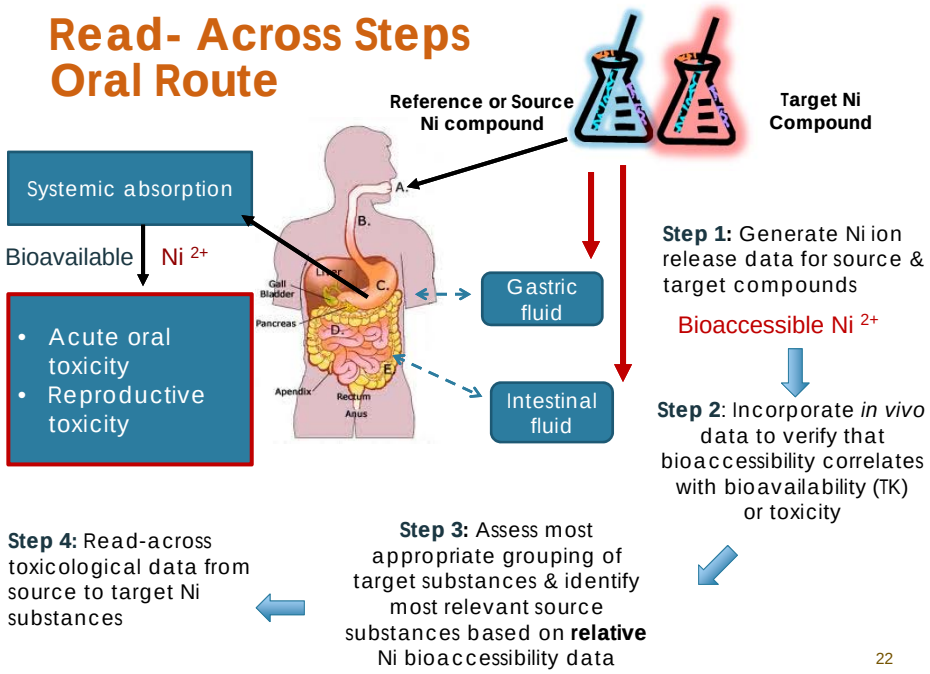
Read- Across Steps Oral Route



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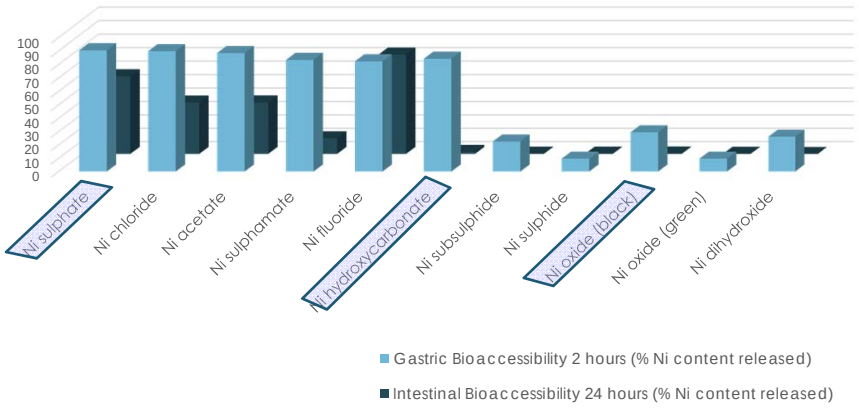
Bioaccessibility -> Bioavailability -> Toxicity

- **Step 1: Generate bioaccessibility data**



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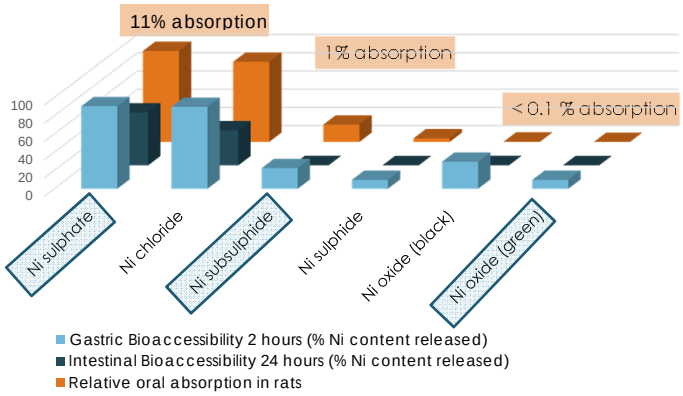
Bioaccessibility -> **Bioavailability** -> Toxicity

- **Step 2A: Compare to toxicokinetic data**



Source: Ishimatsu et al. (1997)

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Bioaccessibility -> Bioavailability -> **Toxicity**

- **Step 2B: Compare to acute toxicity data**

Sample	Ni content (%)	Bioaccessibility Gastric Fluid (% Ni content released)	*In vivo* Acute Toxicity (oral LD_{50}, mg/kg b.w.)
Ni Oxide-Green (N112)	81	0.00	11000
Ni Subsulfide	70	22.65	11000
Ni Sulfate	23	90.55	362
Ni Oxide-Green (N9/N46)	77	0.33	11000
Ni Oxide-Black (N10)	75	3.60	11000
Ni Dihydroxide	54	26.30	5000
Ni Oxide-Black (N105)	75	29.60	9990
Ni Flouride	32	82.35	310
Ni Sulfamate	18	83.40	1098
Ni Hydroxycarbonate	49	84.30	2000
Ni Acetate	24	88.50	550
Ni Chloride	25	89.85	500

Source-reference compounds
Source: Henderson et al., 2012a,b

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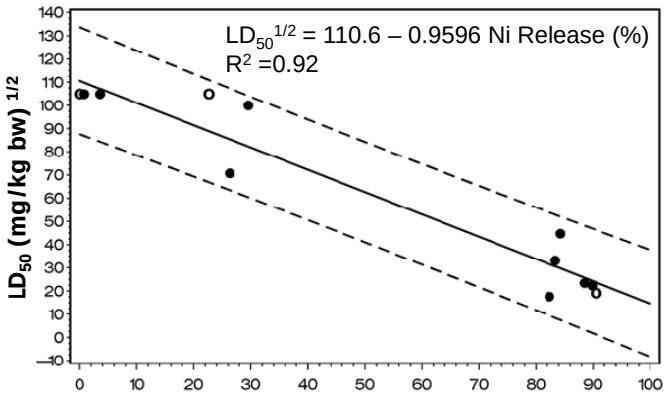
Bioaccessibility -> Bioavailability -> **Toxicity**



Step 2B

Source: Henderson et al., 2012b.

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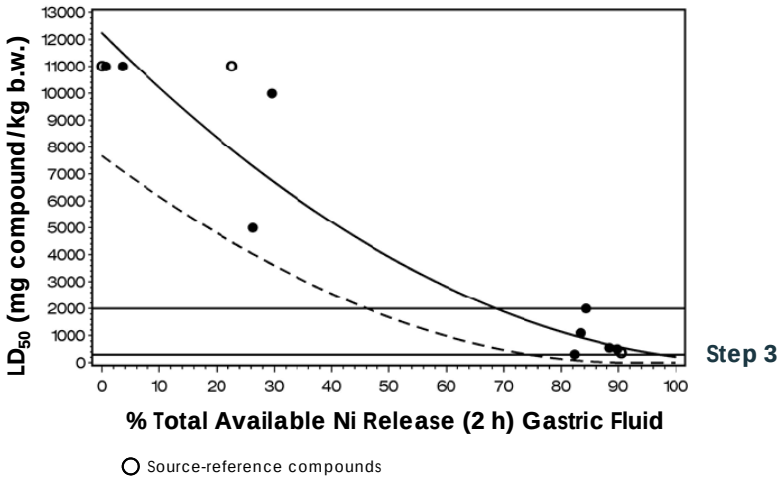
Grouping and Read-across: Nickel (Ni) Compounds—Oral Route



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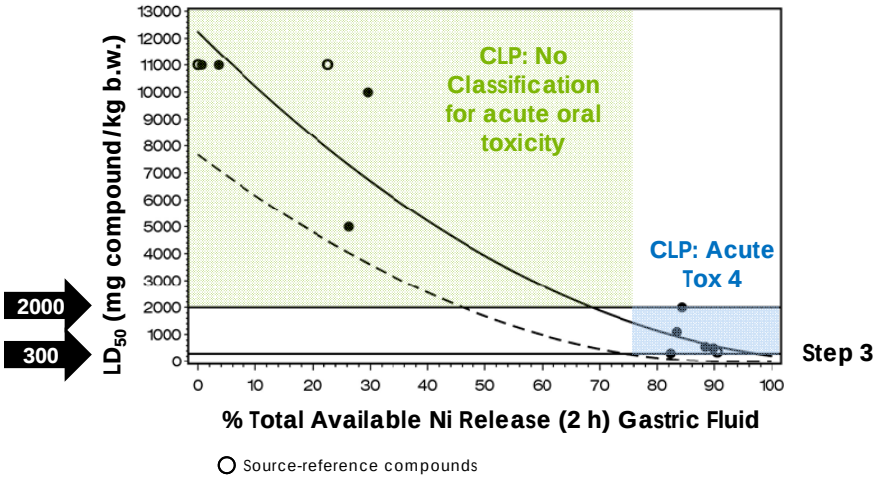
Grouping and Read-across: Nickel (Ni) Compounds—Oral Route



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Use of Bioavailability in the Regulatory Framework

Classification of substances and mixtures

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Grouping and Read-across: Nickel (Ni) Compounds—Oral Route

- Read-across among Ni compounds based on Ni bioaccessibility data for the **oral** route of exposure was verified for acute *and repeated exposure* health endpoints (systemic effects)using acute oral toxicity and/or toxicokinetics data
  - **More and less stringent classifications of Ni compounds were warranted** *(Step 4)*

Approach can be applicable to other metal substances and routes of exposure. Verification for each metal substance and each route of exposure needed

Case Study:
Using bioaccessibility data for grouping metal compounds

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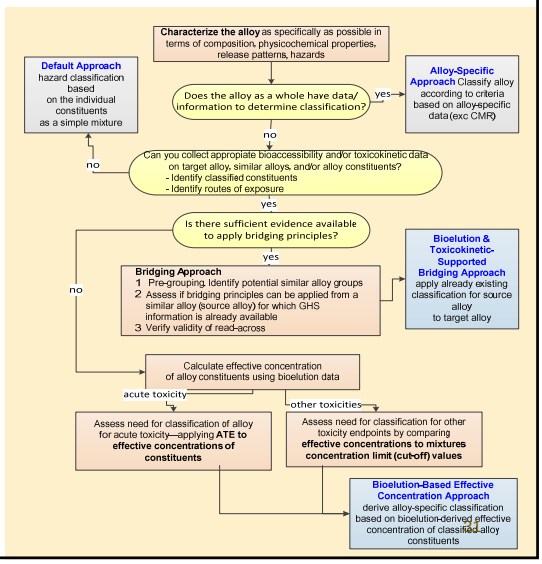
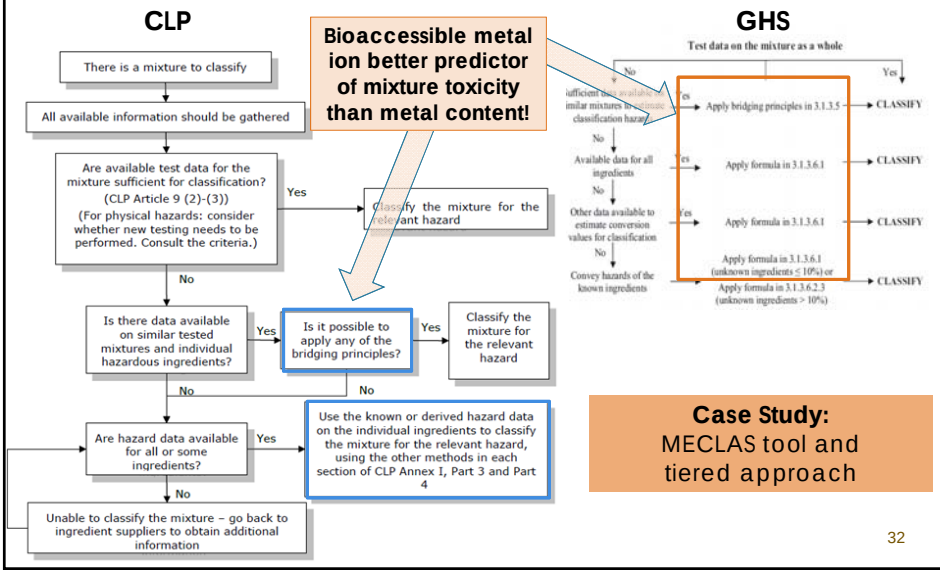
Classification of Complex Mixtures: Proposed Framework Incorporates Bioaccessibility Data

…developed based on adaptation of the mixture classification guidelines identified in GHS and EU CLP, the concepts of special preparations (alloys) under EU REACH, grouping, and read-across formulated as part of EU REACH/OECD and other programmes, and available scientific data on metal-containing complex mixtures and alloys



Current CLP-GHS Classification Frameworks for Mixtures



Case Study: MECLAS tool and tiered approach

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Advantages and Limitations of Bioelution-Based Approaches

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Advantages and Limitations of Bioelution-Based Approaches

- **Advantages**
  - Reduction in animal testing
  - Reproducibility of relative bioaccessibility data
  - Inexpensive, rapid, conservative
  - Can be tailored to exposure pathways
- **Limitations**
  - Know your chemistry (example silver)
  - No internationally agreed test method to date (OECD validation of guidance note or protocol being sought)
  - Bioelution outcomes should not be used in isolation to predict local effects !

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Bioelution Testing and Bioaccessibility Data for Metals: Conclusions (1)

- Bioleution testing is not "new"— several **precedents**
- Bioaccessibility data **(relative metal ion releases)** provides a useful tool to predict relative bioavailability for inorganic substances of a particular metal
- Bioelution testing/dissolution is a **kinetic** process – consider time-dependance and influence of surface area
- Relevant **route of exposure** must be considered

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Bioelution Testing and Bioaccessibility Data for Metals: Conclusions (2)

- **Validation** by *in-vivo* toxicokinetic and/or toxicity data needed
- Bioaccessibility data is part of a **weight-of-evidence** approach
- Bioleution tests have been used successfully for **screening/grouping, read-across** and **classification** of metal substances and are proposed as refinement tools for the clasification of metal-containing complex substances and **alloys**

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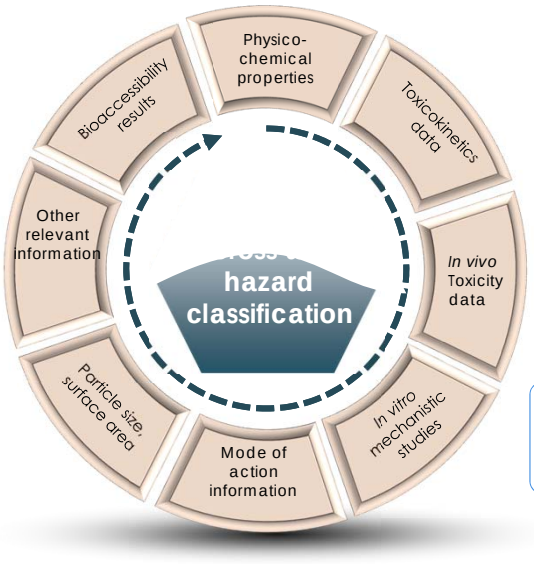
Thanks for Your Attention!



Appreciation of contributions by V Verougstraete, J Mertens, and other metal associations colleagues

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References Cited

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