Metals Read-across Workshop
Helsinki, October 1, 2012

Short draft report
Revised version

The workshop opened with a short *tour de table*. Participants were mainly ECHA and EU Member States scientists and the case study presenters were scientists from the Co, Mo, Ni, Sb and V consortia, plus Eurometaux. The final attendance list is attached as Annex 1. Short bios and contact details of the speakers are provided in Annex 2. The presentations are attached as Annex 3.

Discussion outline:

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<td>14.15-14.45</td>
<td>Introductory presentation:</td>
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<td>• When is read-across used in the context of REACH?</td>
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<td>14.45-17.15</td>
<td>Presentation of examples, illustrating the weight of evidence approach &amp; questions</td>
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*Eurometaux* made an introductory presentation highlighting the afternoon’s objectives, i.e. an open discussion on how read-across has been used in metal dossiers in the context of REACH. Metals and metal compounds present a number of specificities that need to be considered when assessing hazard and/or making hazard predictions using read-across and grouping, including speciation, valence (oxidation state), bioavailability, crystalline structure and particle size, and inclusion in complex materials. In preparation for the 2010 deadline, the metals sector -mainly handling high tonnages- realized that only very few compounds had reliable data for most of the human health endpoints and significant testing requirements would be necessary. Read-across and grouping were used as alternatives to testing to address REACH Annexes 9 and 10 data requirements and to fill in data gaps, to draft testing proposals, to derive DNELs, based on metal content, and to classify.

Metals read-across uses a weight of evidence, case-by-case approach. It relies on data and considerations that come from several areas and that are interlinked: physico-chemical properties...
(including water solubility), bioavailability, speciation and valence, toxicokinetics/toxicodynamics, mechanism of action, structure and particle size.

It is also key to assess the plausibility of the read-across, to check whether its rationale is supported, and to estimate residual uncertainty. All this will be reported and explained in a transparent way. It was acknowledged that the burden of the registration work in 2010 may have impacted upon the quality of some of the explanations included in some metal dossiers, and that the sector is willing to work on the basis of continuous improvement in subsequent dossier updates.

A number of cases were subsequently presented, aimed at illustrating the following concepts/considerations in metals read-across:

- **Antimony: speciation, valence, grouping.** Based on the combination: water solubility/bioaccessibility/valence. Two groups of Sb compounds could be formed: highly soluble Sb(V) compounds, and poorly soluble Sb(III) compounds. Whereas read-across from a complete data set was possible from diantimony trioxide, two major data gaps (90 days oral toxicity and developmental toxicity) were identified, for which approval was given by ECHA in the meantime.

- **Molybdenum: speciation, substance sameness for read-across purposes.** The rationale of the Mo read-across is that the physiologically and environmentally significant species is the \([\text{MoO}_4]^2-\) ion. The x-ray crystal structures of certain molybdate transporter proteins show that the simple \([\text{MoO}_4]^2-\) ion is the form which is bound and taken into biology. At the pHs and Mo concentrations of relevance for REACH, Mo compounds transform into \([\text{MoO}_4]^2-\) and enter the organism as such. Water solubility with UV spectra for speciation analysis of aqueous solutions of molybdates and powder X-ray diffraction for solids characterization, with the example of a molybdenum disulfide catalyst, were presented.

- **Nickel presentation: bioaccessibility, grouping, verification with acute toxicity data and DNEL adjustment.** Ni(II) ion is considered to be the toxic moiety for systemic effects and a significant contributor to local toxicity effects. Three source compounds were used to carry out read-across in order to cover information requirements, for classification and for deriving DNELs. Suggested changes in classification (towards a less severe or more severe classification) further to bio-elution based read-across combined with in vivo verification were summarized in a “substances x health endpoints” matrix.

- **Cobalt presentation: water solubility-bioaccessibility- verification and grouping.** Here, too, the assumption is that the metal ion common to all Co compounds is the driving toxic entity and that it is the bioavailability of the ion that matters. Water solubility was chosen as the initial screening parameter, varying for cobalt compounds over nine orders of magnitude. Bioaccessibility data indicated arguments in favour of defining two groups within inorganic Co compounds (highly accessible, poorly accessible compounds). Two reference substances were chosen. Grouping was also explained: a line drawn one order of magnitude to the left and right of the mean release rate of CoCl₂ defined the boundaries. The results will be further refined with the results of testing proposal. Cobalt has 28-day repeated oral exposure studies for various compounds (Co₃O₄, CoS, Co).
- **Vanadium presentation: water solubility, bioaccessibility and speciation.** Based on water solubility, seven groups of V compounds can be anticipated. Information on electrochemistry / thermodynamics, speciation, bioaccessibility, in-vivo bioavailability enabled the number of groups to be reduced to two. Toxicokinetic data showed that V(IV) + (V) occur simultaneously under physiological conditions. Read-across was performed between V substances for selected human health endpoints (chronic toxicity, mutagenicity & skin sensitisation).

The main focus of the case presentations was on systemic effects.

An overview of the participants’ comments to the presentations is given below. The issues and comments that were raised were re-arranged as follows, associated with follow-up actions:

1. **Terminology issues, to be further clarified or specified (both in dossiers and generic approach for metals read-across)**

2. **Generic recommendations/questions that require either further reflection and/or technical work (with an indication of scope of application and timing when possible).**

3. **Metal-specific comments**

### 1. Terminology issues, to be further clarified

- Explain how considerations on metabolism and speciation may differ or are interlinked
- Considerations on “Crystalline structure”, “particle size”: what is their respective importance?
  - It was suggested to include examples and/or the reasoning. For the inhalation route, particle size is used as discriminator, there is a possibility to consider differences between workplace and e.g. animal testing settings. For the oral route: surface area (or as auxiliary parameter: particle size) will make a difference, not influencing absorption itself, but rather overall release and rate of dissolution, and therefore overall bioaccessibility.

### 2. Generic recommendations requiring further reflection and work

- **Particle size:**
  - Particle size (ranges), used for bioaccessibility testing, for example, may vary and have varying impacts depending on the route of exposure: one way of proceeding that has been used is to normalize for surface area. **Proposed action: highlight and explain the normalization for surface area to address variability of particle size**
  - Could the particle size considerations presented also be extrapolated to nanos and coated particles? It was explained that coating can cause a change to the surface;
bioaccessibility can give some information provided it can be absorbed. With regard to nanos, research is still ongoing to determine whether “nanos” as particulates become bioavailable or whether the dissolution of the particle drives its bioavailability/effect. As far as inhalation is concerned, for the time being, the majority of analytical procedures do not allow verification of a particular substance as “nano” — instead, only the number of nanoparticles is measured. With regard to metals, however, in the occupational setting, metals are not anticipated to be associated with ultrafine particulates; instead, metals are predominantly associated with “larger” particles (>> 1 µm). Conversely, nanos may represent diesel exhaust fumes, refractory materials etc. (exception: welding fumes!). Research on exposure is still ongoing. Proposed actions: include a sentence in generic presentations/guidance notes clarifying scope (pending for nanos) and a reference to coated particles

Speciation:

- The graph presented in the Mo case showing the species present at different pHs raised the question of similar information for other metals. Proposed actions: ensure that we have some information on species in presence at relevant pHs

Bioaccessibility:

- This question was raised in the context of the Ni presentation, but is valid for all metals: how validated is the read-across from acute to long-term endpoints? Proposed action: reflect on how to better justify this, particularly for endpoints like reprotoxicity; what data and framework are needed? (e.g. repeated dose toxicokinetic data or repeated dose toxicity data)
- Is gastric bioaccessibility sufficiently representative for the oral route? It is considered as a worst case: material that becomes dissolved in the gastric milieu may theoretically precipitate upon neutralisation in the intestine, but in ultrafine form, for which uptake cannot be completely excluded through a variety of processes. Intestinal absorption data may be warranted in case the pH dependency indicates preferential dissolution under neutral/slightly alkaline condition. This may be extrapolated from bioaccessibility testing in other physiological media, for example. Proposed action: check BARGE data/setting, for example, and further elaborate on this. Check data on the intestinal dissolution of metals
- Dissolution kinetics have been used for Co; how does this fit in with e.g. 2- or 24-hour bioaccessibility testing? It is relevant to take into account the kinetics aspects of Co release during the incubation period in view of the limited residence time of the compounds in the GI tract (most relevant for poorly soluble compounds). Overall, in vivo verification can indicate whether dissolution rates or overall releases of metal ions are better at predicting the observed toxicities. Proposed action: clarify
Other comments to keep in mind:

- Finding a correlation does not imply causality. How many data points are needed for a correlation (e.g. 3)? **Proposed action: better highlight how we move from observations to conclusions**
- Would read-across for a particular endpoint be acceptable if the source substance has no test for that effect but has a harmonized classification, based on relative toxicokinetics data?
- Scientifically, read-across is an iterative process, as it could be refined with newly generated data, but there are limitations associated with this process (regulatory process, but also industry resource constraints). How can we motivate industry to keep collecting better data when their preliminary read-across leads to no classification? Should industry cases based on robust science be used in dialogue seeking to propose corrections of existing EU harmonized classifications?

3. Metal-specific comments:

- **Sb presentation:**
  - The ALF contains citric acid that is intended to mimic protein binding; in the case of antimony, known to form very stable soluble complexes with trivalent organic acids, we cannot exclude that the bioaccessibility is overestimated.
  - There was a clarification question about the difference in loading rate versus solubility for Sb(V); in view of the 100% dissolution of Sb(V), the dissolution rates given were not very informative; it would have been better to give maximum release concentrations side-by-side also
  - Could Sb(III) and Sb(V) compounds co-exist together, based on the thermodynamics of the pentavalent? This was answered as being only the case for a limited extent; because both valence states are known to be stable under physiol. conditions, testing proposals for Sb(V) had to be submitted.
  - The graph on the first and last slide (data gaps) does not enable a clear distinction to be made between waiving and read-across; in fact, the colour codes suggested to the participants that the read-across from Sb(III) was extended to Sb(V). The scope of the read-across and the testing proposals was explained accordingly.

- **Molybdenum presentation:**
  - With regard to the Cruywagen and Heyns graph of speciation vs. pH, it was noted that the pH in the stomach with a hydrochloric acid concentration of around 0.5% is around 1 and not >4. Does the speciation of molybdate change under these conditions? At pH 4 the main species is the molybdate anion. At a lower pH, molybdate is protonated: there is a need to
understand the speciation of molybdate (and other metal ions) under gastric conditions. Biology selects the simple molybdate ion, so the molybdate equilibria will shift towards this anion as it is removed. With regard to the environment, it was recalled that, in water, there is a large number of counter-ions, humic acid, etc. that will affect that equilibrium. A question was asked about the fate of molybdate which is not absorbed: in what form does it appear in the ecosystem and what is its fate in sewage treatment and how does it impact on the use of sewage sludge?

- **Nickel presentation (revised section):**
  - How representative is synthetic sweat for the distribution of sweats in different people? Using the composition of the validated, regulatory EN1811 enables this representativity issue to be overcome. **Proposed action: better reflect on this in e.g. the “bioaccessibility roadmap currently prepared by industry**
  - What is the availability (or lack thereof) of reproductive toxicity data for the nickel subsulphide and nickel oxide and what is the basis for their current lack of classification as reproductive toxicants? In 2004-2006, the EU Classification and Labelling subcommittee revised or applied classifications to Ni-containing substances. Nickel chloride and nickel sulphate were classified as reproductive toxicants in animals based on substance-specific data. These classifications were then read-across to other water soluble Ni compounds and to Ni hydroxycarbonate (based on industry’s agreement to derogate to the worst case classification). Subsequently, Ni dihydroxide was read across to Ni hydroxycarbonate and was classified as a reproductive toxicant. Nickel metal, Ni sulphide, Ni subsulphide and Ni oxide were not classified as a reproductive toxicant by the C&L subcommittee. Though there was no robust reproductive animal study with these substances, the committee reasoned that toxicokinetic animal data demonstrated a >10-fold lower oral absorption of Ni from the water insoluble Ni substances than from the water soluble ones (as well as much reduced absorption after inhalation and dermal exposure) which would make it improbable, if not impossible, that the threshold for reproductive toxicity would be exceeded in human exposure scenarios.
  - How did nickel apply read-across for systemic effects from relative bio-elution data in artificial gastric fluid and acute LD50 for highly soluble nickel compounds (Repro cat 1B) to no Repro classification for slightly soluble or insoluble (higher acute LD50) nickel compounds? In order to extrapolate the effects of nickel sulphate to other nickel compounds, the relative bioaccessibility of Ni(II) from these compounds in gastro-intestinal fluids was assessed. While Ni release in gastric fluid from hydroxycarbonate was very high (and similar to that of Ni chloride), the release was very low from Ni dihydroxide and similar to that from Ni subsulphide and black Ni oxide. These data did not support a read across from hydroxycarbonate to Ni dihydroxide for the oral route of exposure. However that was not sufficient as there was no in vivo confirmation that the differences in gastric bioaccessibility would predict the difference in absorbed dose (systemic blood levels). The results of the acute toxicity studies by oral route with Ni hydroxycarbonate and dihydroxide reflected the observed differences in Ni bioaccessibility. This confirmed that 1) Ni
hydroxycarbonate could be predicted to have a similar acute bioavailability of Ni ion via oral route as Ni chloride and 2) Ni dihydroxide would be predicted to have a > 10-fold lower acute bioavailability of Ni ion via oral route and to have similar acute Ni bioavailability as Ni subsulphide or Ni oxide.

- What is the justification for considering that the relative blood Ni levels (bioavailable Ni) will be reflective of the relative Ni doses received by uterus and foetus? The reproductive effects observed after nickel exposure in rats are threshold effects that require Ni in blood (i.e., the absorbed dose that reaches the uterus) to achieve a certain level before the associated reproductive effects can manifest. In order to compare the potential effects of different nickel compounds the exact relationship between the levels of Ni in blood and foetus is not that important; the only assumption made is that there is a positive relationship between maternal blood Ni levels and foetus Ni levels. If maternal blood Ni levels are kept below those achieved at the threshold exposure level for reproductive effects, the foetus exposure levels can be expected to also be below the threshold.

- What is the justification for extrapolating a read-across from acute (e.g., LD50) to repeated exposure effects (e.g., reproductive effects)? There is sufficient TK data to demonstrate that the oral absorption of Ni (bioavailability of Ni) from Ni sulphate is the same after a single exposure as it is after repeated exposure (2 years). Therefore, all the information taken together supports: 1) Ni hydroxycarbonate to be predicted to have the same reproductive toxicity as Ni chloride and 2) Ni dihydroxide to behave similarly (gastric release, LD50) to Ni subsulphide and oxide compounds and therefore to be predicted to have the same reproductive toxicity profile as had been assigned to nickel oxide/subsulphide.

In summary, the suggestion that Ni dihydroxide may not warrant a classification for reproductive effects is based on a read across approach that considered relative oral bioaccessibility and LD50 data, and the similar acute and repeated dose kinetics for Ni (II) absorption. For the oral route, we confirmed that Ni hydroxycarbonate behaves like water soluble Ni compounds and concluded that Ni dihydroxide behaves more similarly to Ni oxide/subsulphide than to Ni chloride/ Ni sulphate. (For more detailed description, please see the Appendix sent with his report)

Proposed action: draw lessons from this case and further reflect on this in e.g. the ‘bioaccessibility roadmap currently prepared by industry’

- Cobalt presentation:
  - Cobalt relies on gastric bioaccessibility testing. Is this representative? What are the contributions of intestinal absorption; are there any data? It was suggested to clarify that the key read across assumption is that dissolution in gastric medium is the driver for gastrointestinal availability, making the metal available for absorption in the upper intestine/duodenum. This is an important case to make for all metals!
- Why was Co$_2$O$_4$ which has a mixed oxidation state, chosen? Reasons were that this compound has substantial market relevance and a large database, mostly concerning (II) oxidation state.

- **Vanadium presentation:**
  - V metal does not dissolve to any appreciable extent, is this due to the low loading? The loading is however standardised in most of our test systems to 100 mg/L. Whereas this may be considered quite low, it in fact addresses the poorly soluble substances; those that dissolve more readily may be considered more or less completely accessible.
  - Can one read across between IV or V without limits - are both interchangeable? For systemic toxicity? Yes, this has been verified in vivo.
  - Specify the form that enters the organism. This is primarily relevant for local effects; upon dissolution, the bioaccessibility data suggest that distinction between valences disappear...
### Annex 1: Attendance list

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<tr>
<th>Name</th>
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<tr>
<td>Mr Niklas ANDERSSON</td>
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<td>ECHA</td>
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<td>Ms Jana BALEJKOVA</td>
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<td>Slovak CA for Reach</td>
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<tr>
<td>Mr Peter BARICIC</td>
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<td>EC – DG Entreprise</td>
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<td>Dr Rodger BATTERSBY</td>
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<td>EBRC Consulting</td>
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<td>Dr Peter BOOGAARD</td>
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<td>Shell International</td>
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<tr>
<td>Ms Cecilia BOSSA</td>
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<td>Istituto Superiore di Sanita</td>
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<tr>
<td>Ms Sandra CAREY</td>
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<td>Molybdenum Consortium</td>
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<td>Mr Wim DE COEN</td>
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<td>ECHA</td>
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<tr>
<td>Ms Martina DRLICKOVA</td>
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<td>Slovak CA for Reach</td>
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<tr>
<td>Mr Andrew FASEY</td>
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<td>ECHA</td>
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<tr>
<td>Mr Sten FLODSTRÖM</td>
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<td>Swedish MSC Member</td>
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<tr>
<td>Ms Claudia FLOHR-BECKHAUS</td>
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<td>Knoell Consult GmbH</td>
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<tr>
<td>Mr Jörgen HENRIKSSON</td>
<td></td>
<td>Swedish MSC Member</td>
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<tr>
<td>Ms Diana KARITKINA</td>
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<td>Member State Committee Member</td>
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<td>Ms Anne-Mari KARJALAINEN</td>
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<td>ECHA</td>
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<td>Mr Derek KNIGHT</td>
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<td>European Chemicals Agency</td>
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<td>Mr Magnus LOFSTEDT</td>
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<td>Danish Environmental Protection Agency</td>
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<td>Dr Philippe MITCHELL</td>
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<td>Molybdenum Consortium</td>
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<tr>
<td>Dr Ruth MOELLER</td>
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<td>Centre de Recherche Public Henri Tudor</td>
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<tr>
<td>Dr Adriana Oller</td>
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<td>Ni Consortium</td>
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<tr>
<td>Mr Henrik TYLE</td>
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<td>Danish Environmental Protection Agency</td>
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<tr>
<td>Dr Steven VERBERCKMOES</td>
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<td>Co Consortium</td>
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<tr>
<td>Dr Violaine VEROUGSTRAETE</td>
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<td>Eurometaux</td>
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Annex 2: Short bio and contact details of the industry speakers

- **Adriana R. Oller (NIPERA).**

  Adriana Oller is originally from Argentina, where she obtained a Master’s degree in Biochemistry from Buenos Aires University in 1980. In 1983, after completing a two-year residency in Toxicology and Forensic Chemistry at the School of Pharmacy and Biochemistry (Buenos Aires University), Adriana emigrated to the United States, where she completed a Ph.D. in Genetic Toxicology at the Massachusetts Institute of Technology (Cambridge, MA) in 1989. Adriana continued her genetic toxicology research on spontaneous mutations and DNA repair at the Lineberger Cancer Research Center in Chapel Hill and the U.S. National Institutes of Environmental Health Sciences (NIEHS) in Research Triangle Park (North Carolina). In 1994, she joined the staff of the Nickel Producer Environmental Research Association (NIPERA, Inc).

  Contact details: Adriana Oller, Nickel Producers Environmental Research Association (NIPERA). 2525 Meridian Parkway, Suite 240 Durham, NC 27713 (US)
  Telephone: +1 (919) 595-1945 / Email: aoller@nipera.org

- **Rodger Battersby (EBRC).**

  Rodger Battersby has a background in Chemistry and Biochemistry. He has headed the EBRC consultancy offices for 20 years. EBRC provides consulting services with a focus on the chemical, biocidal and agrochemical industries. EBRC’s portfolio includes a number of metals for which they provide(d) support for REACH registration in 2010 and now again in 2013.

  Contact details: Rodger Battersby, EBRC Consulting GmbH. Raffaelstrasse. 430177 Hannover (Germany)
  Telephone: +49 511 8983890 / Email: rvb@ebrc.de

- **Steven Verbeckmoes (Umicore)**

  After completing his studies in biochemical engineering in 1997, Steven Verberckmoes obtained a PhD in medical sciences at the University of Antwerp in 2005 after investigating the effect of strontium on bone development and mineralization. From 2005-2008 he took the position as post-doctoral researcher at the University of Antwerp in the Department of Pathophysiology on the topic of characterization of the mineral phase of various calcified tissues. In 2008 Steven joined Umicore as toxicologist in the central R&D organization, where he is involved in the scientific development of the regulatory dossiers of (metal-containing) chemicals. In this role, he is part of the scientific working groups of the Cobalt REACH Consortium, where the grouping and read-across strategy on cobalt substances is being developed.

  Contact details: Steven Verbeckmoes, Umicore. Kasteelstraat 7 - B-2250 Olen, Belgium
  Telephone: +32 14 24 52 87 / Email: steven.verberckmoes@umicore.com
o Philip Mitchell

Inorganic chemist, a Chemistry Professor, University of Reading, UK, now Emeritus. Did a doctorate at the University of Oxford with Professor RJP Williams on the Bioinorganic Chemistry of Molybdenum.

He has worked on molybdenum chemistry and applications especially in lubrication and catalysis, molybdenum biochemistry, and also on neutron scattering and computational modeling of inorganics. Consultant to IMOA, Climax Molybdenum, International Tungsten Industry Association

Contact details: p.c.h.mitchell@reading.ac.uk

o Sandra Carey

Sandra has worked for many years in the mining industry, including 10 years as IMOA’s Health and Environment Executive and 6 years as Project Manager for the REACH Molybdenum Consortium. In the recent past she presented on ‘SIEF Management’ and ‘Lessons Learned as an Lead Registrant’ at the ECHA First Lead Registrant Workshop, and also at the ECHA/Commission REACH Conference in Brussels in September last year.

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o Violaine Verougstraete

Violaine studied medicine and toxicology at the Catholic University of Louvain, did a DEA in Public Health and obtained her PhD in Public Health in 2005 from the Catholic University of Louvain (Belgium). She worked as a researcher at the Industrial Toxicology and Occupational Medicine Unit of the Catholic University of Louvain for 8 years. She collaborated in the EU Risk Assessment « Cadmium and Cadmium Oxide ». Between May 2005 and December 2011, she worked for Eurometaux as Health and Alloys Manager. Her main task consisted of coordinating Eurometaux’s scientific activities and projects, e.g. the HERAG and MERAG projects on risk assessment methodologies for metals, the GHS Joint Project, and human/environmental toxicology-related activities. With regard to REACH and CLP more specifically, she coordinates work on Exposure Scenarios, Exposure Modelling and Classification Tools, as well as technical projects on metal specificities backing up the registration and notification dossiers. She attends ECHA Risk Assessment Committee meetings as a regular stakeholder. She has been EHS Director at Eurometaux since January 1, 2012.

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- Vanadium consortium: Ms Astrid Voigt, EBRC Consulting GmbH Raffaelstrasse. 430177 Hannover
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