Read across classification
How it should not be done
How it should be done

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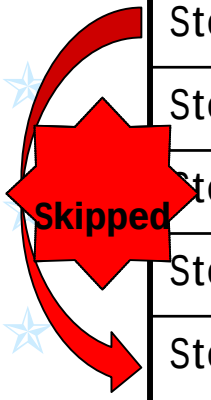
EC poor application of read across

- Built on water solubility similarity …
- … without water solubility data
- For 118 nickel compounds at a time across 4 water solubility categories
- Covering CMR, environmental toxicity, respiratory sensitization
- Assuming « ion theory » without evidence
- Skipping methodology steps

- Bad precedent
- Very good example on how it should NOT be done

OECD 9-step methodology

Step 1	Identification of structure-based category and its members
Step 2	Gather published and unpublished data for each category member
Step 3	Evaluate data for accuracy
Step 4	Construct a matrix of data availability
Step 5	Perform an internal assessment of the category
Step 6	Prepare category test plan
Step 7	Conduct necessary testing
Step 8	Perform an external assessment of the category
Step 9	Fill data gaps by read-across, extrapolation, interpolation, etc.



CONSEQUENCES OF POOR APPLICATION OF READ ACROSS

Significant downstream regulatory consequences

- REACH: SVHC – Authorisation
- Worker H&S measures, lower OEL (cf. chronic toxicity)
- Restrictions on investments – Seveso
- No Ecolabel
- Impact on green uses / procurement
- Ban on consumer sales
- Rising maritime transport costs (cf. aquatic toxicity)

Is the Ni example consistent with science ?

- NO !

  Read across does not a priori remove the need for testing in order to confirm or verify the validity of a proposed toxicological effect

- Specificity of metals must be taken into account
  - Water solubility alone is a poor surrogate for biological fluids
  - The categories of water solubility must be validated on the basis of data, to assess bioavailability, bioaccessibility and to distinguish between systemic and local toxicological effects

Water Solubility as a Basis for Extrapolating Biological Effects

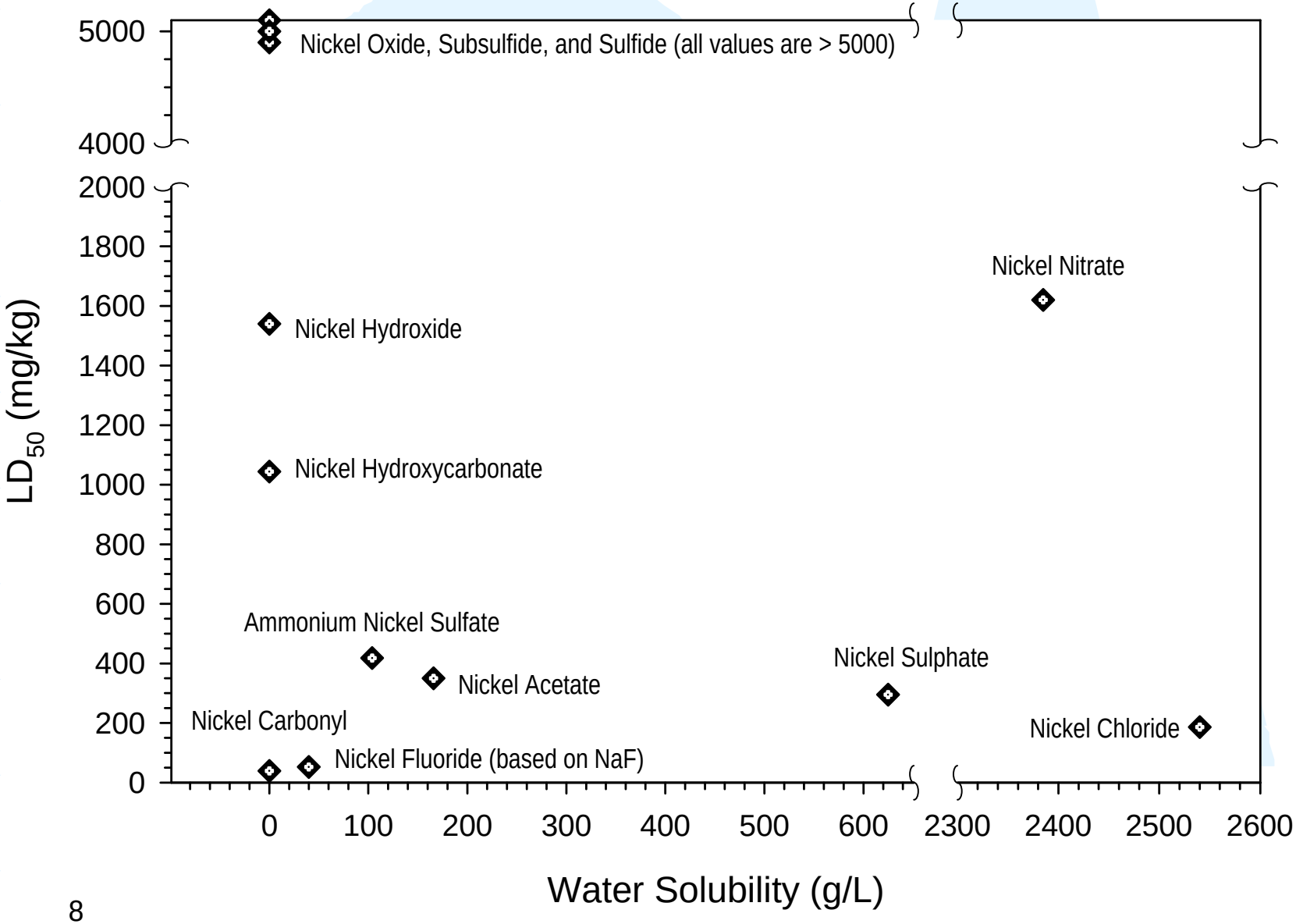
- To perform "read-across on the basis of water solubility alone" you have to:

1. Assume that absorption of compounds by all routes of exposure occur at pH 7
2. Assume that dissolution, corrosion, or solubilization of materials will be the same in sweat, gastric fluids, and lung fluids
3. Assume that all counter-ion have no intrinsic toxicity
4. Assume that all the toxicological endpoints of concern (*e.g.*, acute oral and inhalation toxicity, repeated dose toxicity, dermal sensitization and irritation, reproductive toxicity, mutagenicity, and carcinogenicity) are linearly related to water solubility and that this is true for all routes of exposure

Water Solubility as a Basis for Extrapolating Biological Effects

- These assumptions do not pass even simple verification tests !!
- For example, grouping of nickel compounds by water solubility would predict that a straight line could be drawn connecting all the points on a graph comparing water solubility against oral toxicity (LD_{50} data)
- When such data are plotted it is clear that there is no linear relationship between acute oral toxicity and water solubility of nickel compounds (see next slide)

Water Solubility vs. Oral LD_{50} for Nickel Compounds in Commerce in the EU



Alternative "Read-Across" Paradigm

- Use *in vitro* tests of the bioaccessibility of nickel ions in biological fluids as the tool to read-across individual toxicity endpoints from well studied reference compounds to poorly studied compounds

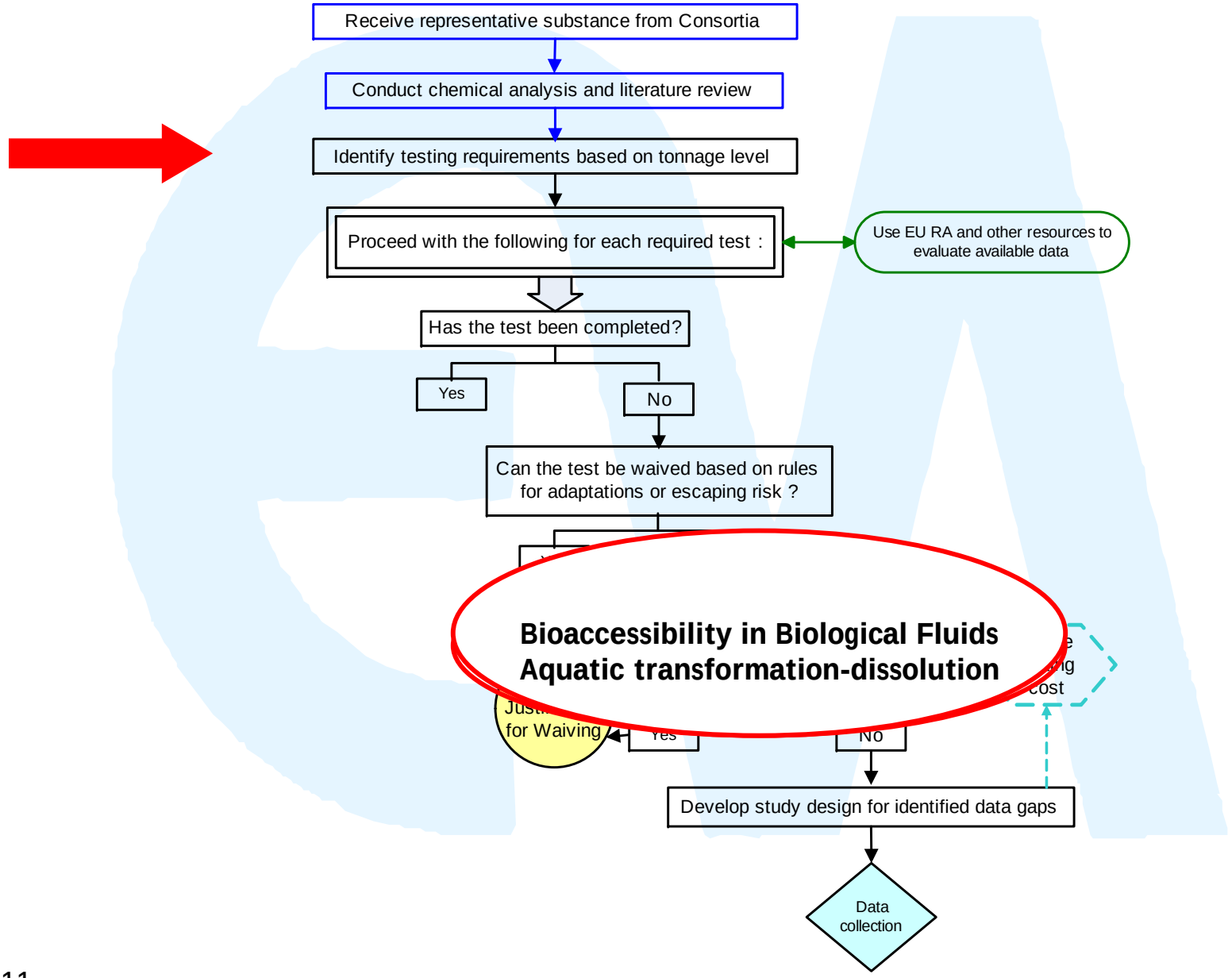
- Verify bioaccessibility read-across with acute *in vivo* tests

Alternative "Read-Across" Paradigm

Benefits of the "bioaccessibility" approach:

1. Bioaccessibility of nickel ions is assessed in biologically relevant fluids which correlate to the primary routes of exposure (*e.g.*, gastric fluid, lung lavage, sweat, and lysosomal fluid)
2. Verification testing will determine if the counter-ion of a compound has intrinsic toxicity
3. Toxicological endpoints of concern can be correlated with the route of exposure that results in the greatest risk of that particular toxicity occurring
4. Does not require that all the toxicity endpoints for any reference compound be "read-across" to the compound being studied; read-across is performed on an endpoint by endpoint basis

Strategy for Nickel Consortia Bioaccessibility Testing



Strategy for Nickel Consortia Bioaccessibility Testing

Endpoint-Specific Read-across

- Start with source substances, those for which obligatory toxicology information already exists
- Develop biologically relevant baseline data for the source substances
- Compare the same data for the target substance with the baseline data
- Identify the source substance which provides the most accurate comparison and read-across to its respective toxicology

Strategy for Nickel Consortia Bioaccessibility Testing

Performing a chemical category read-across to similar data rich substances requires:

Tier 1

- *In vitro* dissolution kinetics data in biological fluids
- Knowledge of counter-ion toxicity

Tier 2

- Verification of read-across assumptions
  - *in vivo* toxicity testing (*e.g.* acute oral)
  - *in vivo* toxicokinetics analysis

Strategy for Nickel Consortia Bioaccessibility Testing

- Source (reference) substances already tested:
  - Nickel sulfate
  - Nickel chloride (*underway*)
  - Nickel subsulfide
  - Nickel oxide (green)
  - Nickel metal

- Tested in 6 biological fluids with an acute *in vivo* test:
  - Gastric fluid
  - Intestinal fluid
  - Alveolar fluid
  - Interstitial fluid
  - Lysosomal fluid
  - Sweat

Strategy for Nickel Consortia Bioaccessibility Testing

- Read-across substances undergoing bioaccessibility testing :
  - Nickel hydroxycarbonate
  - Nickel sulphamate
  - Nickel acetate
  - Nickel dihydroxide
  - Nickel oxide (green)
  - Nickel oxide (black)
  - Nickel fluoride
  - Ferronickel
  - Nickel matte (sulfidic-low copper)
  - Nickel matte (sulfidic-high copper)
  - Nickel matte (metallic)
  - Nickel sulphide
- Tested in 6 biological fluids with *in vivo* verification tests (results available by Q2 2009)

Bioaccessibility Data and Carcinogenicity

- 118 Ni compounds classified as carcinogenic class 1&2 based on poor application of read-across approach using simple water solubility data and unjustified assumptions
- Industry needs to prove these substances are not carcinogen class 1&2 …
- … by creating bioaccessibility data, cell transformation data and collecting mechanistic information

➔ BURDEN OF DISPROOF IS ON INDUSTRY

CONCLUSION

- Using water solubility as a grouping criteria for read-across is a too simplistic approach
  - False positive errors will occur by reading-across all the classifications from a heavily classified reference chemical to a chemical which may respond very differently under biological conditions
  - False negative errors will occur by reading-across all the classifications from a non-toxic and unclassified reference chemical to a chemical which may cause toxicity under biological conditions
- Read-across will never be “foolproof”, but starting with biologically relevant grouping data (*e.g.*, bioaccessibility in biological fluids) will reduce errors in classification

Industry should invest in good scientific evidence to substantiate the read-across approach and eventual classification.
This will prevent future misuse in the name of absence of good quality data

ACTION

- Fixing the theory
  Scientific literature on the sound scientific application of read across to metals

- Fixing the practice
  Workshop to seek a multi-metallic agreement on the practical application of read across classification for metals, in the framework of REACH (early July 2009)

- Disseminating the right methodology
  Meeting scientific community across the EU and in non EU regions

Addendum

Evidence of misleading classifications on the basis of read across

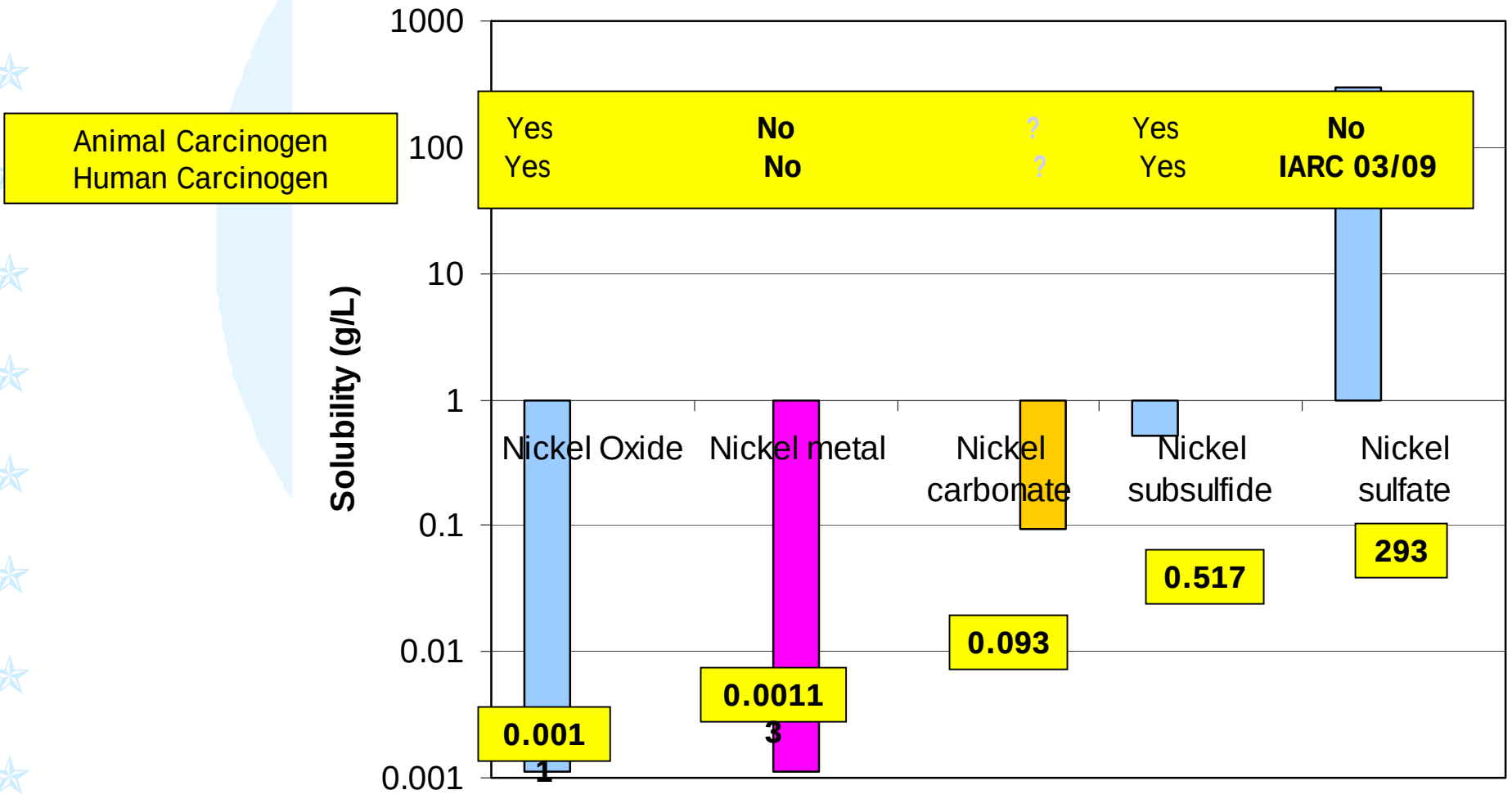
Environment

Looks OK, BUT

- Transformation/Dissolution Protocol applied to Ni matte revealed that water solubility-based read across is not validated by testing by a 100 order of magnitude

➔ Since the methodology does not work for environment, it reinforces the need for scientific justification via testing for Human Health

Nickel Carcinogenicity and Solubility



Carcinogenicity

- Some insoluble and soluble Ni are Carc. Cat. I
  - BUT : Two separate modes of action:
    - Insoluble : bioavailability of Ni ion to the critical site in the target cells in the lung
      Ni substances with very low bioavailability may not cause tumors at all !
    - Soluble : solubility is high, but bioavailability is poor due to poor uptake of the Ni ion in the cells
  - Ni Carbonyl, very soluble, is classified Carc. 3 !
  - Uncertainty on how organic compounds will be metabolized or whether “nickel” will be bioavailable at all

Mutagenicity
No sufficient evidence for read across

- Conflicting evidence for in vivo mutagenicity for Ni salts
- Specialized experts concluded no sufficient evidence for Ni carbonate
- The proposal does not check whether free nickel ion is even released from organonickel compounds

Reproductive toxicity

- This is a threshold-based toxicity : the Ni ion must be bioavailable in sufficient concentrations to the target organs
- The proposal needs to consider some toxicokinetic data on the absorption, bioavailability, and metabolism of each compounds

Acute toxicity

- Similar water solubility compounds were here classified differently
  - NiCl2 : T/R23/25
  - NiSO4 : Xn/R20/22
  - NiNO3 : Xn/R20/22 and 7 times more toxic than NiSO4
- Ni ion availability for organonickel compounds was not considered

Irritation

- This endpoint depends on the water solubility but also on the bioavailability and irritancy of the anion
- Specific concentration limit for NiSO4 was based on data.
- Eye irritation classification for Ni nitrate, despite similar solubility as NiSO4

Skin sensitization
Consideration of bioavailability is needed

- This endpoint depends entirely on bioavailability of the Ni ion on the skin
- It is not known whether the nickel ion may be released from water soluble organonickel compounds