



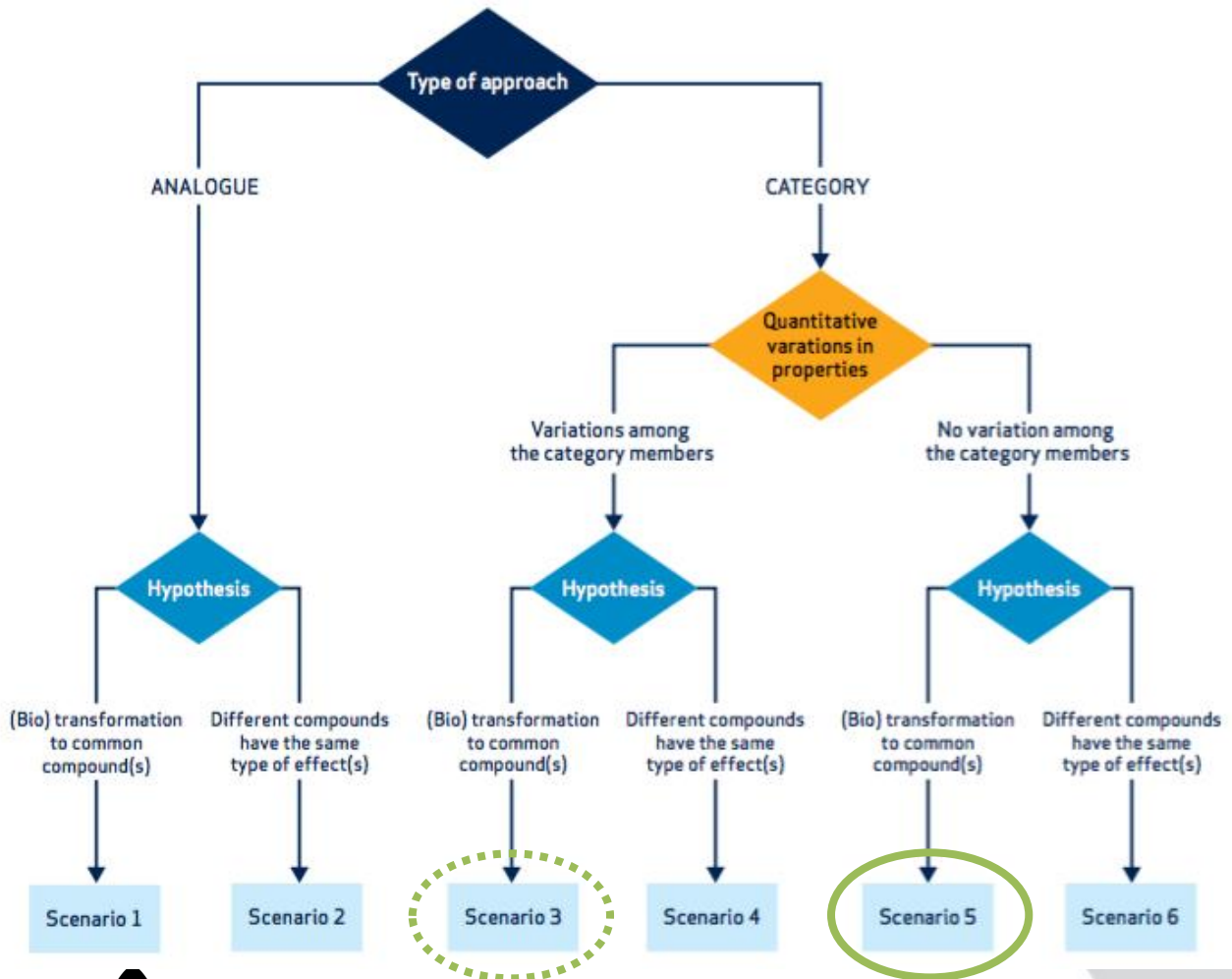
MISA Environment

READ ACROSS and RAAF

Koen Oorts, ARCHE Consulting



# ▶ Read-across Scenario



1. Many to many (category)<sup>2</sup>
2. Transformation to (release of) common metal ion (speciation controlled by environmental conditions)
3. Common transformation product, no variation among category members at equilibrium

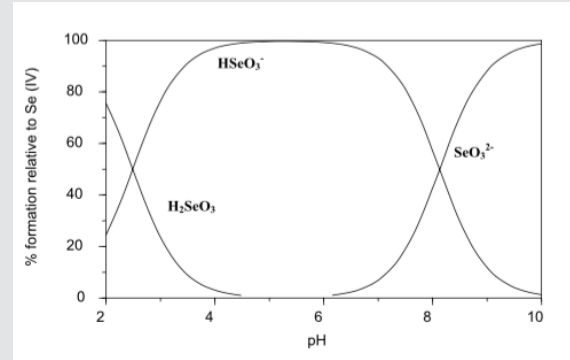
=> Scenario 5 (?)

## ▶ Read-across: solubility

- Toxicity occurs below solubility limit
  - ➔ Differences in solubility among Me compounds do not affect the results for environmental fate and (eco)toxicity
- Solubility below toxicity (ERV)
  - ➔ Transformation dissolution testing compared with ERV soluble Me substances

## ▶ Read-across: speciation

- Theoretical speciation diagrams



- Speciation measurements

➔ the properties of the receiving media determine the speciation of the released Me ion, regardless of the speciation of the compounds released.

## ► Read-across: counter-ions

- Negligible contribution of counter-ions to observed effects is justified when:
  - Abundance in natural environment > releases in metal compound corresponding to metal-PNEC ( $\text{Cl}^-$ ,  $\text{NO}_3^-$ ,  $\text{SO}_4^{2-}$ ,  $\text{NH}_4^+$ ,  $\text{Na}^+$ ,  $\text{Ca}^{2+}$ , ... based on e.g. FOREGS)
  - Low hazard profile compared to metal ion (e.g. acetate)
- Me compounds with other counter-ions are not part of overall category and assessed case by case (e.g. via assessment entity for both metal- and counter-ion)

# ► Interpreting RAAF jargon for metals

- *“(bio)transformation to common compounds”*
- *“structural similarity”*
  
- Metals dissociate in the environment
- Metal-ion is the cause of eco-toxicity
- (Redox) speciation is key in read-across justification
- ⇒ “Me-ion toxicity” concept
  - *~ = “common transformation product”*
  - *~ = “common functional group”*
  - *~ = “structural similarity”*
  
- **RAAF not completely fit for standard explanation of ion-toxicity concept**

# ► Generalisation of read across

- Metal ion-toxicity concept predicts endpoint information
- Same principle for Me-toxicity in water, sediment, soil, STP
- Same driver for assessment elements, e.g.:
  - Ecotoxicity
  - Bioaccumulation (ingestion? regulation?)
  - Environmental fate/partitioning /removal from water column
- Choice of most soluble source substance as worst case
- Generalisation among compartments?
  - (All data presented in RAAF-tables)

# Illustrative Example on the Read Across Assessment Framework for Environment

## MISA Environment Workshop

07 February 2019

Anne-Mari Karjalainen, Bram Versonnen,  
Marta Sobanska and Evita Luschutzky

## Read-across and grouping - Annex XI, section 1.5

*"Substances whose physicochemical, toxicological and ecotoxicological properties are likely to be similar or follow a regular pattern as a result of structural similarity may be considered as a group, or 'category' of substances"*

- Two conditions need to be fulfilled
  1. structural similarity between substances results in similar physicochemical and (eco)toxicological properties
  2. Properties of a substance may be predicted from other substances in the group
- Mechanistic explanation of why structural similarity is associated with similar properties and/or why structural dissimilarity does not matter.

## Read-Across Assessment Framework (RAAF)

- Structured framework to help in systematic
  - reporting of read-across assessments
  - evaluation of reliability of read-across
    - structured documentation of both processes (assessment tool)
    - transparency of both processes?
- ECHA's assessment of RA (using RAAF)
  - Under testing proposal evaluation (TPE) – plausibility of a (proposed) grouping approach
  - Under compliance check (CCH) – acceptability of an (existing) grouping approach

# Principles of RAAF and how they are used in the “hypothetical” example case

- Example case: RA “category” approach for Me-dioxide
- Source substances:
  - Me-trioxide
  - Sodium-Me



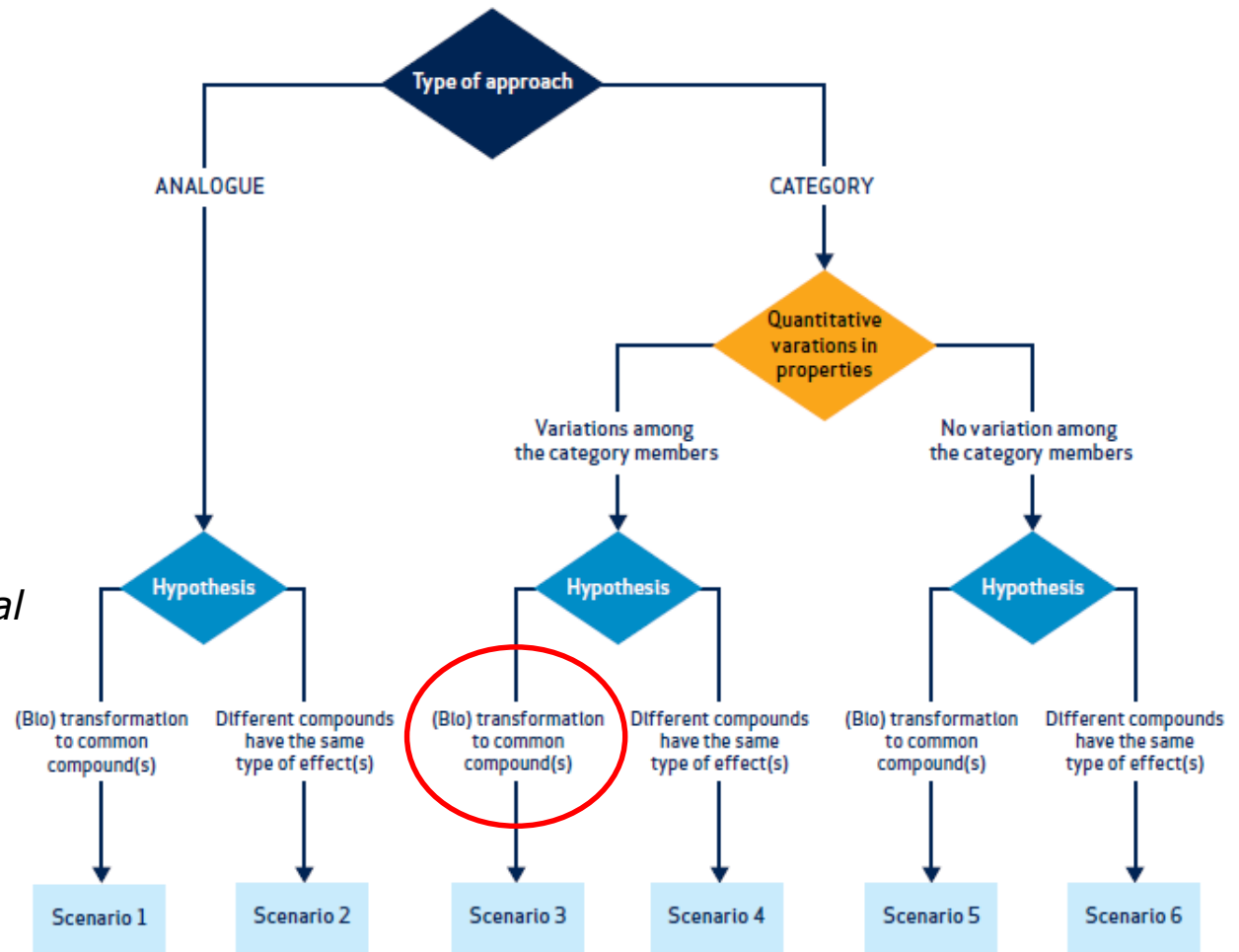
## RAAF – Six scenarios, two main groups

1. (Bio)transformation to common compounds
  - Analogue approach; equal or worst case (scenario 1)
  - Category approach; trend or no trend (scenarios 3 and 5)
  
2. Different compounds have the same type of effect
  - Analogue approach; equal or worst case (scenario 2)
  - Category approach; trend or no trend (scenarios 4 and 6)
  
- **Assessment elements (AEs)** help to cover the essential scientific elements that need to be included in a RA
  - Common AEs applicable to all scenarios
  - Scenario specific AEs

## Example case: Me-dioxide RA

- Prepared using RAAF
- Scenario 3 followed

*The properties of the substances are likely to be similar or follow a similar pattern as a result of presence of a common metal ion*



## Reporting of RA assessment

- How has the RA assessment been documented?
  - A justification document (section 13 of IUCLID?) or separate justifications in different endpoints?
  - Is there a (clear) hypothesis?

- **Me-dioxide example**

- A justification document using the RAAF template covering all (relevant) endpoints (in section 13 of IUCLID)
- Clear hypothesis:

*The properties of the substances are likely to be similar or follow a similar pattern as a result of the presence of a common metal ion.*



*The read-across approach is based on the assumed presence, bioavailability and similar mobility of the common metal ion in environmental media following exposure to the different compounds of the metal in question. It is assumed that the bioavailability and mobility of the common metal ion determines the toxicity of the target and source compounds.*

*Assumed to be a reasonable approach as long as other components do not contribute to toxicity.*

## Assessment elements for scenario 3

AE #	AE type	AE Name
AE C.1	Common	Characterisation of source and target substances
AE C.2	Common	Structural similarity and dissimilarity within the category (category description)
AE C.3	Common	Link of structural similarities and structural differences with the proposed regular pattern (presence of hypothesis)
AE 3.1 AE 5.1	Scenario-specific	Formation of common (identical) and non-common compounds
AE 3.2 AE 5.2	Scenario-specific	Degradation of non-common compounds
AE 3.3 AE 5.3	Scenario-specific	Bioaccumulation potential of non-common compounds
AE 3.4 AE 5.4	Scenario-specific	Impact non-common compounds
AE C.4	Common	Impact of impurities on the prediction
AE C.5	Common	Consistency of properties in the data matrix
AE C.6	Common	Reliability and adequacy of the source data
AE C.7	Common	Bias that influences the prediction

## **(AE C.1) Characterisation of source & target substances (1/2)**

Have the substances been clearly identified & characterised allowing robust scientific assessment?

Composition/chemical formula, purity and impurities given for target and source substances?

- Target substance: required substance identity information given in the technical dossier, not in the RA report
- Source substances:
  - Identified only by name, EC and CAS numbers
    - Me-trioxide (EC No 123, Cas No 123) has been used for ecotoxicity endpoints and/or when data on the trioxide is missing sodium-Me-oxide (EC No 234, Cas No 234) has been used as the source.*
  - No specific information on purities/impurities under this AE
    - However, under AE C.4 Impact of impurities on the prediction given that
      - The target and source substances are expected to be of high purity, with no significant impurities affecting toxicity.*
    - And, purity of test materials given in the test material section in IUCLID


## **(AE C.1) Characterisation of source & target substances (2/2)**

### ECHA's assessment

- AE specifically identified, but not all elements of the AE covered
- Source substances not adequately characterised, and no information on their purity and impurities in the RA document
  - Could lead to failure of RA, but
  - here the substances are 'simple enough' to still allow assessment of RA, and
  - in the Endpoint Study Records (ESRs) given that test material 99.9 % pure.
- **Take home message:** All relevant information (especially the elements identified in the AEs) for both target and source substances should be given and (preferably) included in one location.

## (AE C.2) Structural similarity and dissimilarity; category description and supporting information (1/2)

Do the structural similarities and allowed dissimilarities identified in the category description apply to all category members? Need to identify:

- Common chemical structure (allowing prediction), and
  - allowed differences in chemical structures (not hampering prediction).
- Information provided:
    -  *The inorganic substances within the group share the metal constituent as a functional group. This shared constituent may lead to the release of metal ion assumed to be most commonly bioavailable ion and hence the most likely contributor to toxicity.*

- Information NOT provided:
  - Category definition/domain
  - Borders of the category
  - Explicit inclusion/exclusion criteria

 **Category description missing**

## **(AE C.2) Structural similarity and dissimilarity; category description and supporting information (2/2)**

### ECHA's assessment

- Common feature for the three substances in the group, 'the metal ion', clearly identified, however
  - as no category boundaries, exclusion/inclusion criteria given not clear why other compounds of the same metal not included in the category.
  - May create a bias, however no information under AE C.7 "Bias that influences the prediction".
  - Failure of RA? In some cases yes, if e.g. suspect that more toxic substances left out.
- **Scenario 1 Analogue**, (bio)transformation to common compounds, could have been better as the RA is rather of type many-to-one (one target, many source substances) than a category approach.

## **(AE C.3) Link of structural similarities and structural differences with the proposed regular pattern (1/5) (*Presence of hypothesis*)**

Does the Registrant provide a hypothesis/documentation for the read-across, including a scientific explanation that links the chemical structures to the prediction?

- Clear hypothesis given in the RA report (see also slide 9.)  
*properties...likely to be similar or follow a similar pattern due to common metal ion, its presence, bioavailability and similar mobility which determines toxicity...assumed that other components do not contribute to toxicity.*
- The justification addresses (1 to 4)
  1. Similarity in chemical composition (AE C.2, slides 10 and 11)
  2. Water solubility: *Me-dioxide and Me-trioxide have similar water solubilities as measured in the T/D study (OECD GD 29). This supports the RA. Due to its higher solubility sodium-Metal can be considered as the worst case approach with maximum metal release.*
  3. Environmental fate & distribution: *No data on behaviour of Me-dioxide is available, bioconcentration data has been read-across from metallic Me and sodium-Me-oxide. The soluble species released from the three compounds are assumed to be similar with similar behaviour in environment, even if the amount of soluble ions released is different.*

## **(AE C.3) Link of structural similarities and structural differences with the proposed regular pattern (2/5) (Presence of hypothesis)**

Does the Registrant provide a hypothesis/documentation for the read-across, including a scientific explanation that links the chemical structures to the prediction?

- The justification addresses (cont.)

#### 4. Aquatic speciation & bioavailability:

*In water the Me will be available in the highest oxidation state only. This was confirmed in the T/D studies where together with the rate and amount of the Me released, the soluble form of the Me was determined to justify that the dominant soluble form of the Me released from both water-soluble and poorly soluble compounds is identical.*

*The information on speciation supports the read-across approach.*

*While the amount of the dissolved Me as determined from the T/D studies varies, the information on speciation (ie that all metal are released in the same oxidation state) support the read-across. Data on the most soluble sodium-Me can be used as worst case approach.*

- Justification also includes formulas for the chemical reactions and TD data for each compound in the form of ESRs -> possible to assess validity of data

## **(AE C.3) Link of structural similarities and structural differences with the proposed regular pattern (3/5) (*Presence of hypothesis*)**

### ECHA's assessment

- Justification closely linked to the information obtained from the T/D studies
  - Information provided:
    - Amount, type and rate of formation of soluble metal at three different pHs given
    - Similarities in aquatic speciation and bioavailability explained and supported by data
    - Possible to assess the validity of all three T/D studies as ESRs provided
  - Information NOT provided/discussed in the justification:
    - Surface area/size of the particles assessed in the T/D studies
      - needed to be able to compare the results obtained
      - possible to assess hypothesis if cannot compare solubilities?
    - **Failure of RA?**



## **(AE C.3) Link of structural similarities and structural differences with the proposed regular pattern (4/5) (*Presence of hypothesis*)**

### ECHA's assessment

- Key information (surface area/size) missing from justification, but
  - For Me-dioxide and Me-trioxide info available in the T/D ESRs of the Me-dioxide and Me-trioxide.
    - The T/D results comparable as slightly higher surface area of the Me-dioxide particles correlate with its slightly higher solubility.
  - No information on size available for the sodium-Metal (in the dossier of target)
    - But information available in its registration dossier – larger size, still higher solubility supports hypothesis that sodium-Metal worst case in terms of solubility.
    - Can (should?) ECHA use information from another dossier in its evaluation? Confidentiality? Representability etc..?
    - Is the hypothesis acceptable when key information missing?

## **(AE C.3) Link of structural similarities and structural differences with the proposed regular pattern (5/5) (*Presence of hypothesis*)**

### ECHA's assessment

- Bioaccumulation
  - Only briefly addressed in the justification (slide 14).
  - Bioaccumulation study on sodium-Metal submitted as an ESR in dossier but not included in data matrix, discussion submitted in the respective endpoint summary.
    - Study conducted at concentration comparable to EC10 obtained from the long-term fish study on sodium-Metal, no significant bioaccumulation.
  - ECHA does consider that also this data should be included in the data matrix and that for the purpose of clarity read-across justification for all endpoints should be included in the RA report.

## **(AE C.4) Impact on impurities**

Has it been explained in the documentation whether the (identified) impurities have an impact on the prediction?

- Information provided

*Both the target and the source substances are expected to be of high purity, with no significant impurities affecting toxicity.*

- ECHA assessment

- No information on impurities of source substances provided but can be anticipated that due to the high purity of the test material the impurities of the source substances do not influence the prediction (please also see AE C.1 slides 11-12).
- The target substance is also of high purity and the total amount of impurities should not be above 0.1 %. The possible impurities have been identified.

## (AE C.5) Consistency of properties in the data matrix (1/2)

Is a data matrix for the category members available, and are there inconsistencies between the predicted property and related properties observed?

	1. Me-dioxide (registered substance)	2. Me-trioxide	3. sodium-Me-ion
Acute tox to inv	No effects observed	RA from 3.	No effects observed
Chronic tox to inv	RA from 3.	RA from 3.	No effects observed
Acute tox to fish	No effects observed	RA from 1.	No effects observed
Chronic tox to fish	RA from 3.	RA from 3.	21 d NOEC 34 mg/L
Algae	No effects observed	No effects observed	EC10 12 mg/L

*The effect concentration obtained from the chronic fish study...above the concentration of metal released in the T/D studies on Me-dioxide and Me-trioxide. Hence expected that soluble ions from Me-dioxide and Me-trioxide would not be released at levels that could cause chronic toxicity to aquatic organisms.*

## (AE C.5) Consistency of properties in the data matrix (2/2)

### ECHA's assessment

	1. Me-dioxide (registered substance)	2. Me-trioxide	3. sodium-Me-ion
Acute tox to inv	No effects observed	RA from 3.	No effects observed
Chronic tox to inv	RA from 3.	RA from 3.	No effects observed
Acute tox to fish	No effects observed	RA from 1.	No effects observed
Chronic tox to fish	RA from 3.	RA from 3.	21 d NOEC 34 mg/L
Algae	No effects observed	No effects observed	EC10 12 mg/L

- Useful to have data on all three substances on one trophic level.
- Test concentrations analytically verified – easy to compare with T/D data.
- The available data supports the read-across strategy, as hypothesised the data on sodium-Metal can be used as worst case approach.
- The data can hence be used to conclude that Me-dioxide and Me-trioxide do not cause risks to the aquatic environment.

## **(AE C.6) Reliability and adequacy of source data**

Are the source studies reliable and adequate and relevant for the purpose of the prediction?

- Information provided

The studies on source substances have been provided in the dossier as ESRs. Adequate Robust Study Summaries (RSSs) have been provided.

- ECHA assessment

- As given in Annex XI section 1.5., any data submitted needs to be adequate for classification and labelling and risk assessment.
- As RSSs are available it is possible to assess the validity of studies
- Adequate information on test material(s) is provided to assess impact of purities/impurities/composition on possibility to predict.
- Analytically verified test concentrations provided for all studies – possibility to compare with T/D data and to verify hypothesis based on solubility.



## **(AE C.7) Bias that influences the prediction**

Is there apparent evidence that additional substances exist which are currently not used in the analogue approach/category and which arguably could be included?

- Information provided
  - No specific discussion on whether other compounds of the same metal could have been included in the read-across approach even when indicated that this AE addressed (see also slide 14).
- ECHA assessment: deviation acceptable due to
  1. The T/D data on the target substance and the more soluble source allow comparison of both the levels released and speciation of the metal released.
  2. The ecotoxic data provided on the target substance, the trioxide of similar solubility and the more soluble form sodium-Metal, together with the T/D data are considered sufficient to conclude that aquatic effects would not be caused at levels of metal released from the target.
    - Data on another (soluble) species is not considered to contribute further.

## AEs not addressed in the example

- AEs 3.1 to 3.4 on impact on non-common compounds skipped
  - have only hypothesised that read-across based on solubility “*is a reasonable approach as long as other constituents do not contribute to toxicity*”
    - But in essence the impact of non-common compounds on the prediction not addressed.
    - The substances are ‘simple enough’ to make the omission not critical, however in many other cases such omission would lead to failure of the read-across.
- Impact of all components/counterion(s) should be fully addressed
  - T/D data for e.g. multielement compound should include results for all elements
  - Hypothesis and data provided as support should address also the counterion toxicity (separate aquatox data for each element may be required)
  - Impurities?

# **Principles of RAAF and how they are used in the “hypothetical” example case**

- Summary and conclusions**



## Summary and Conclusions

- ✓ Adequate documentation of the approach has been provided
  - However not all relevant information included in the RA report, but elsewhere in dossier, and
  - some information only outside of the dossier (possible for ECHA to use?)
- ✓ Source data is valid, sufficient information provided
  - Data acceptable for classification and labelling
- ✓ Evidence supports hypothesis.
  - Source substances not adequately characterised
  - Not explained why only these three substances grouped together
- Read-across acceptable with deviations (?) or fails (?) as all relevant information not included in the technical dossier under assessment

# Thank you!

ECHA RAAF:

[https://echa.europa.eu/documents/10162/13628/raaf\\_en.pdf](https://echa.europa.eu/documents/10162/13628/raaf_en.pdf)

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# Metals read-across: Cobalt category

W Stubblefield, K Belska, P Marsh,  
and P Campbell

MISA 2 (Helsinki, Finland)  
8 February 2019

# Co REACH substances

- ▶ Covers 26 substances in total
- ▶ Mostly mono-constituent substances
- ▶ Broad range of different substance types
  - Cobalt metal
  - Cobalt oxides, hydroxides, 'inorganic' salts (x12)
  - Cobalt salts of organic acids (x11)
    - Divalent cobalt salts
    - Mainly carboxylic acids
    - Different carbon chains
      - Chain length from C2 (acetate) to C18 (e.g. stearate, resin acids)
      - Straight and branched chains

# Cobalt ecotoxicity database

- ▶ “State-of-the-science” approach

*“The toxic metal [for testing] should be added in the form of an inorganic salt having relatively high solubility. Nitrate salts are generally acceptable; chloride and sulphate salts of many metals are also acceptable.” (US EPA 1985)*

- Most testing conducted with cobalt dichloride salt

- ▶ Robust dataset

- Freshwater: >120 studies on 12 species
- Marine water: 10 species
- Soils: 141 studies on 14 species / microbial processes
- TDP (OECD 29) data for 13 different Co materials

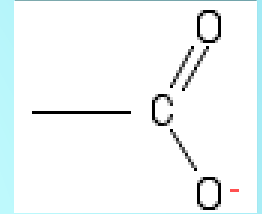
Organism	Common name	# chronic studies
<i>Hyalella azteca</i>	Isopod	1
<i>Lemna minor</i>	Duckweed	6
<i>Ceriodaphnia dubia</i>	Water flea	42
<i>Lymnaea stagnalis</i>	Snail	5
<i>Daphnia magna</i>	Water flea	15
<i>Pseudokirchneriella subcapitata</i>	Algae	31
<i>Aelosoma sp.</i>	Oligochaete	1
<i>Chironomus tentans</i>	Midge	1
<i>Pimephales promelas</i>	Fathead minnow	1(25)
<i>Brachionus calyciflorus</i>	Rotifer	15
<i>Danio rerio</i>	Zebrafish	1
<i>Oncorhynchus mykiss</i>	Rainbow trout	1

# Read-across justification

- ▶ All substances in cobalt category liberate  $\text{Co}^{2+}$ 
  - $\text{Co(II)}$  is the dominant species in oxygenated systems of neutral to acidic pH
- ▶ Ecotoxicity driven by cobalt ion
  - High toxicity to several species
  - Cobalt(II) ion is highly ecotoxic
    - PNEC (freshwater) =  $0.7 \mu\text{g Co/L}$
- ▶ Counter-ions do not contribute significantly to toxicity (discussed later in detail)
  - Many are ubiquitous in the environment
  - Some are essential fatty acids and/or derived from natural products

# Cobalt Organic Metal Salts (OMS) hypothesis

- ▶ Inorganic salts of cobalt with organic acids
  - OECD definition “organic metal salts”
  - Not “organometallic” compounds
  - Ionic bonding between  $\text{Co}^{2+}$  and two carboxylate  $[-\text{COO}]^-$  ions
- ▶ Cobalt OMS dissociate under most environmental conditions
  - Equilibrium between total dissociation and complex formation with available dissolved ligands



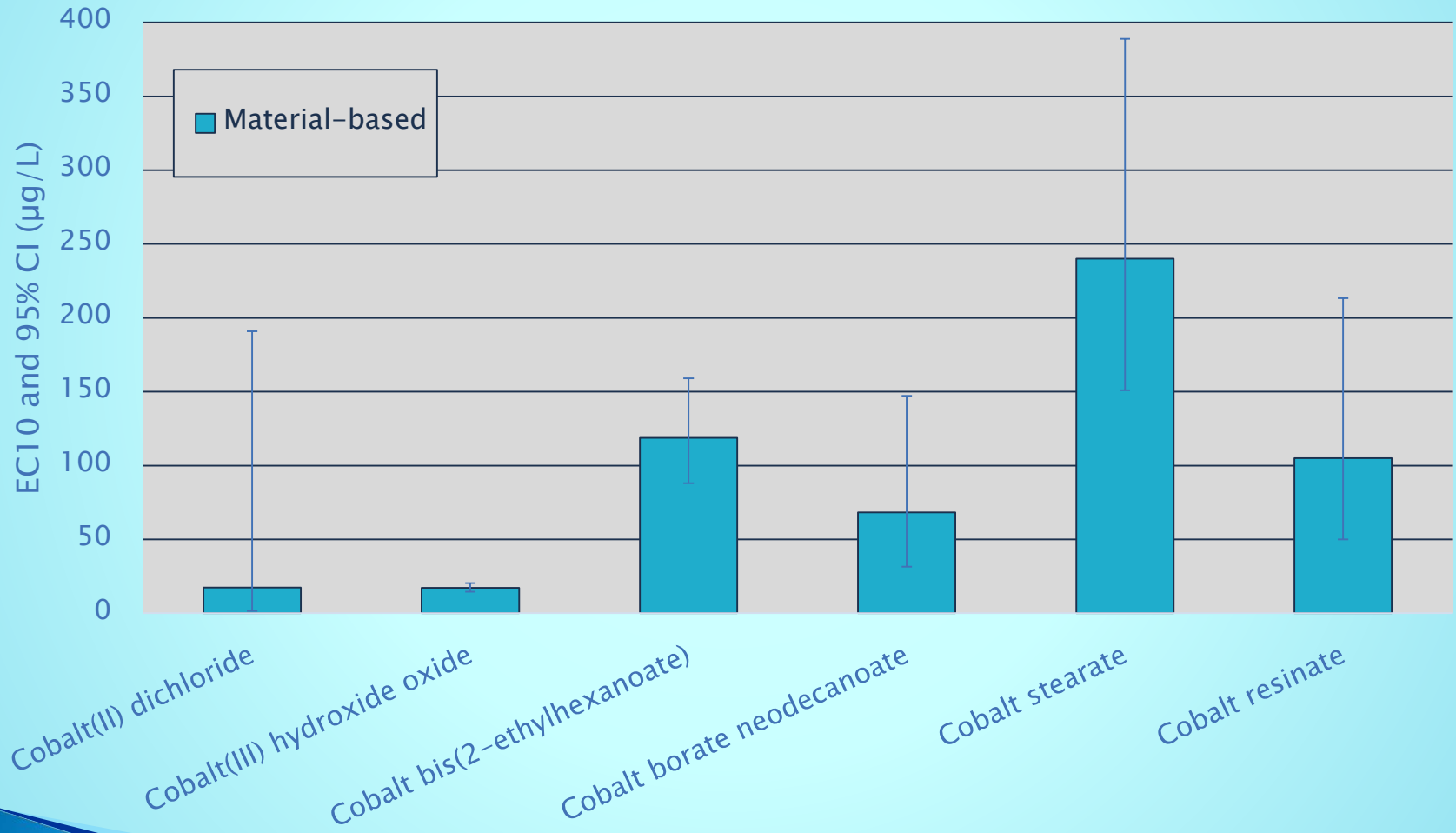
# Research programme

- ▶ Chronic toxicity tests with algae, invertebrate, and fish were conducted with a range of representative cobalt organic salts
- ▶ Test conditions were the same, within a species, to eliminate concerns associated with variability due to differences in Co bioavailability
- ▶ Total and dissolved Co concentrations measured in all tests
- ▶ Results of all tests were compared with data from soluble metal salt (cobalt dichloride)

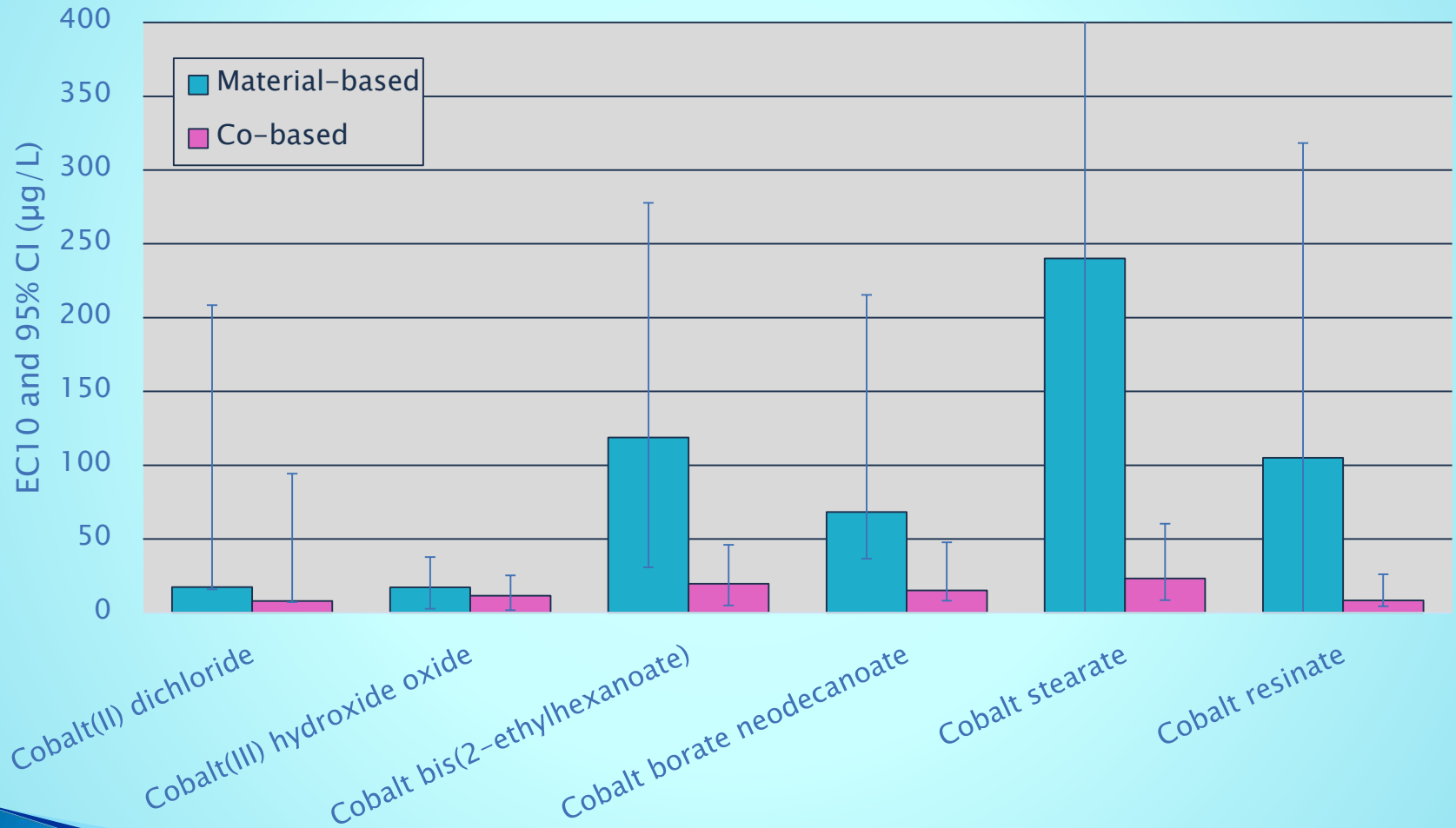
# Test methods

	<b>Water flea</b> <i>Ceriodaphnia dubia</i>	<b>Fathead minnow</b> <i>Pimephales promelas</i>	<b>Algae</b> <i>Pseudokirchneriella subcapitata</i>
Test protocol	USEPA (2002)	ASTM 1241, OECD 210	OECD 201
Test duration	7 days	34 days	72 hours
Culture and Test medium	Synthetic moderately hard water, 108 mg/L as CaCO <sub>3</sub>	Lab Blended H <sub>2</sub> O (Well water blended with reverse osmosis H <sub>2</sub> O); Hardness ~100 mg/L as CaCO <sub>3</sub>	Algal growth media without EDTA, prepared with deionized water and nutrient stock solutions
Endpoints	Survival and Reproduction	Survival and Growth	Growth rate and Yield
Tested life stage	< 24 hour old neonates	Fertilized eggs	3-7 day old culture

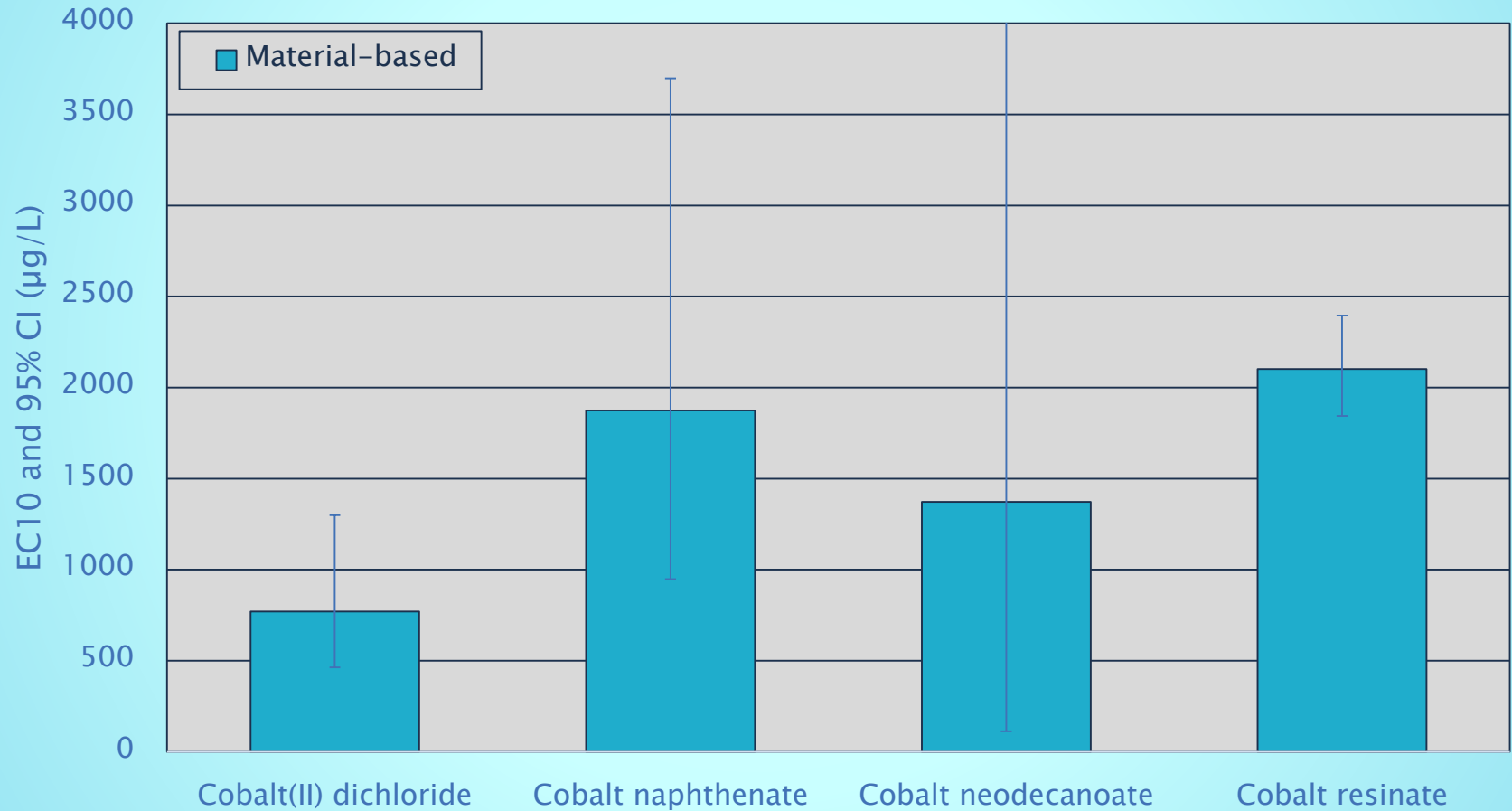
# *Ceriodaphnia dubia* chronic toxicity



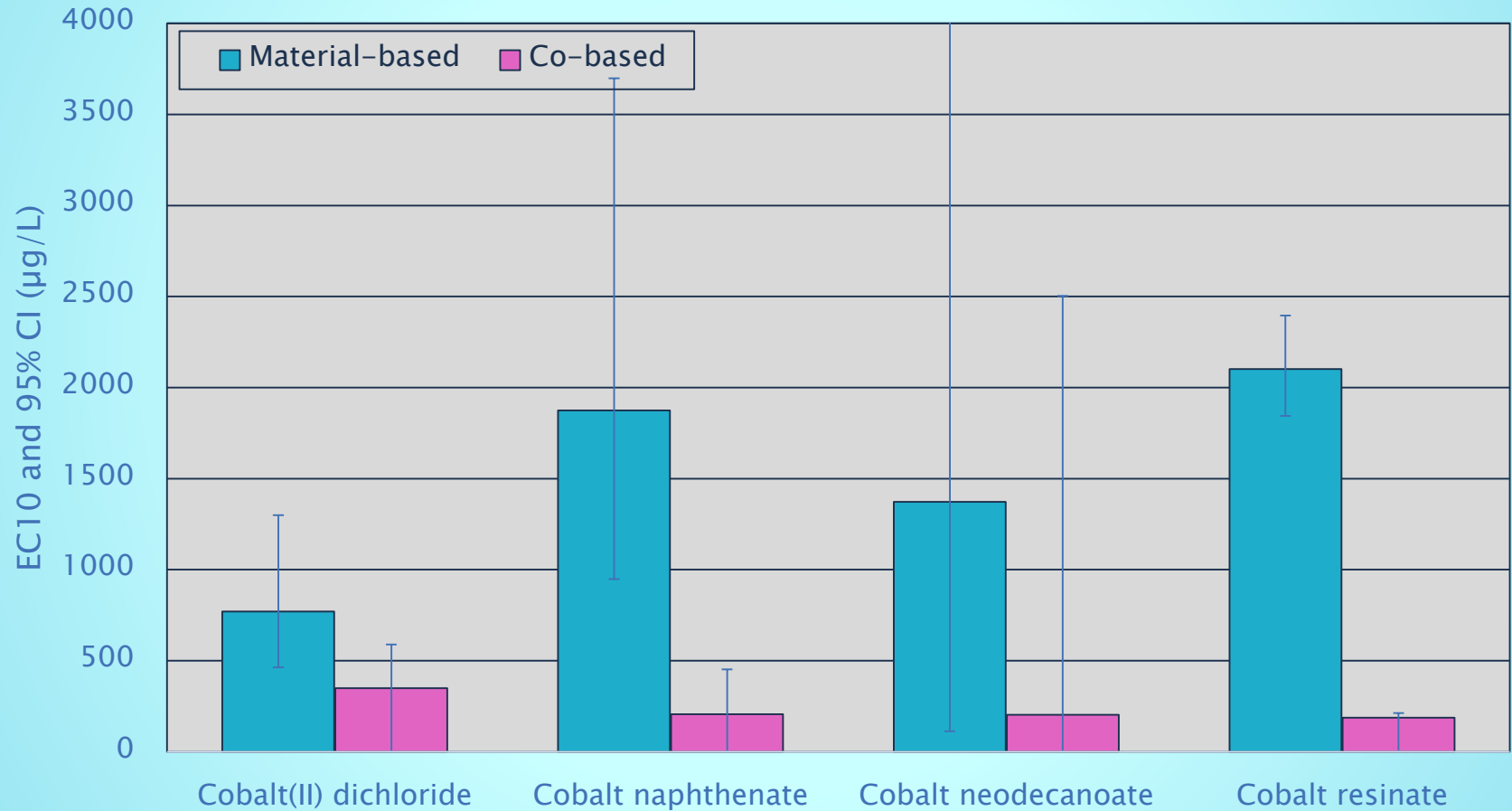
# *Ceriodaphnia dubia* chronic toxicity



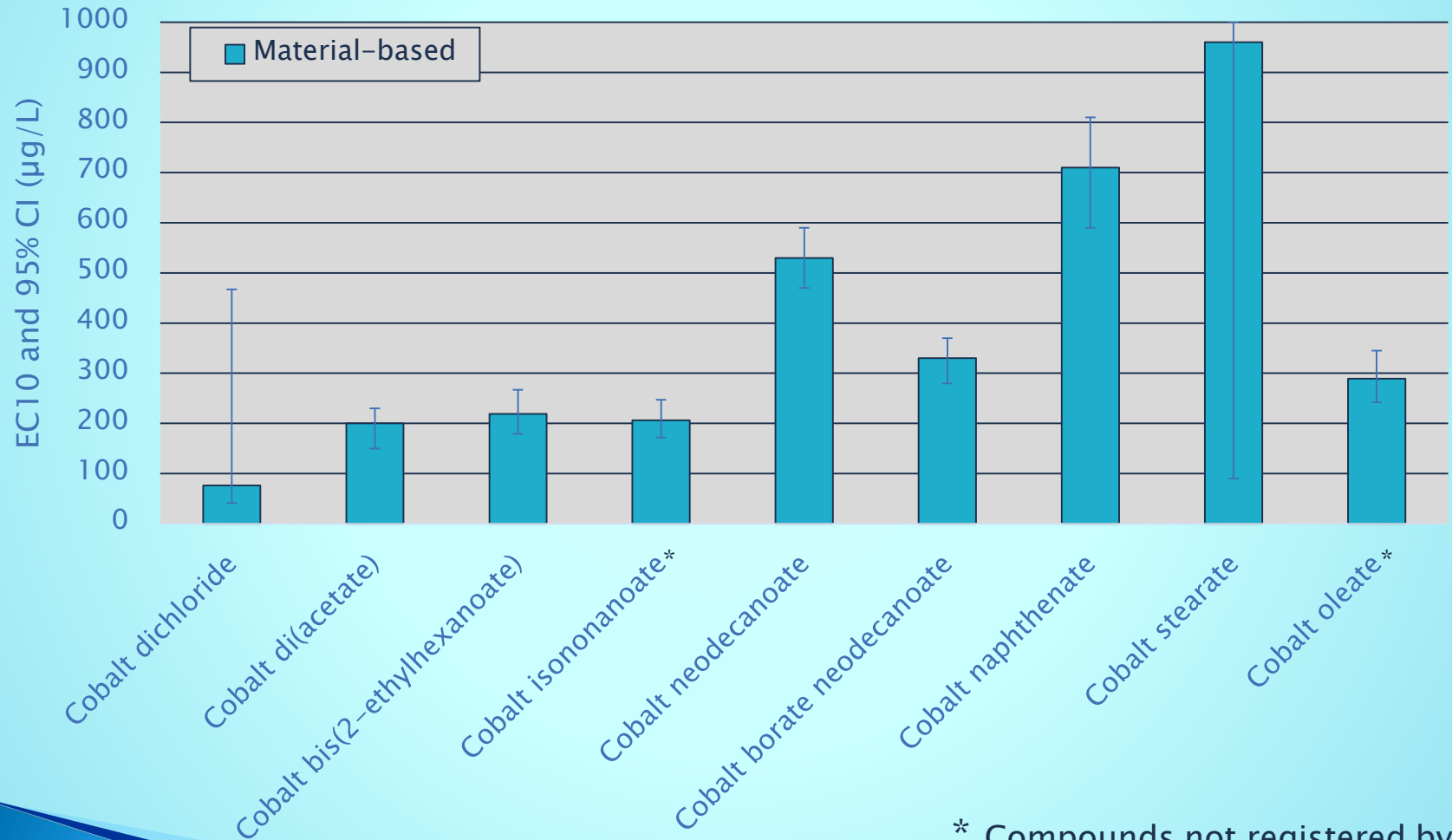
# Fathead minnow (*Pimephales promelas*) ELS chronic toxicity



# Fathead minnow (*Pimephales promelas*) ELS chronic toxicity

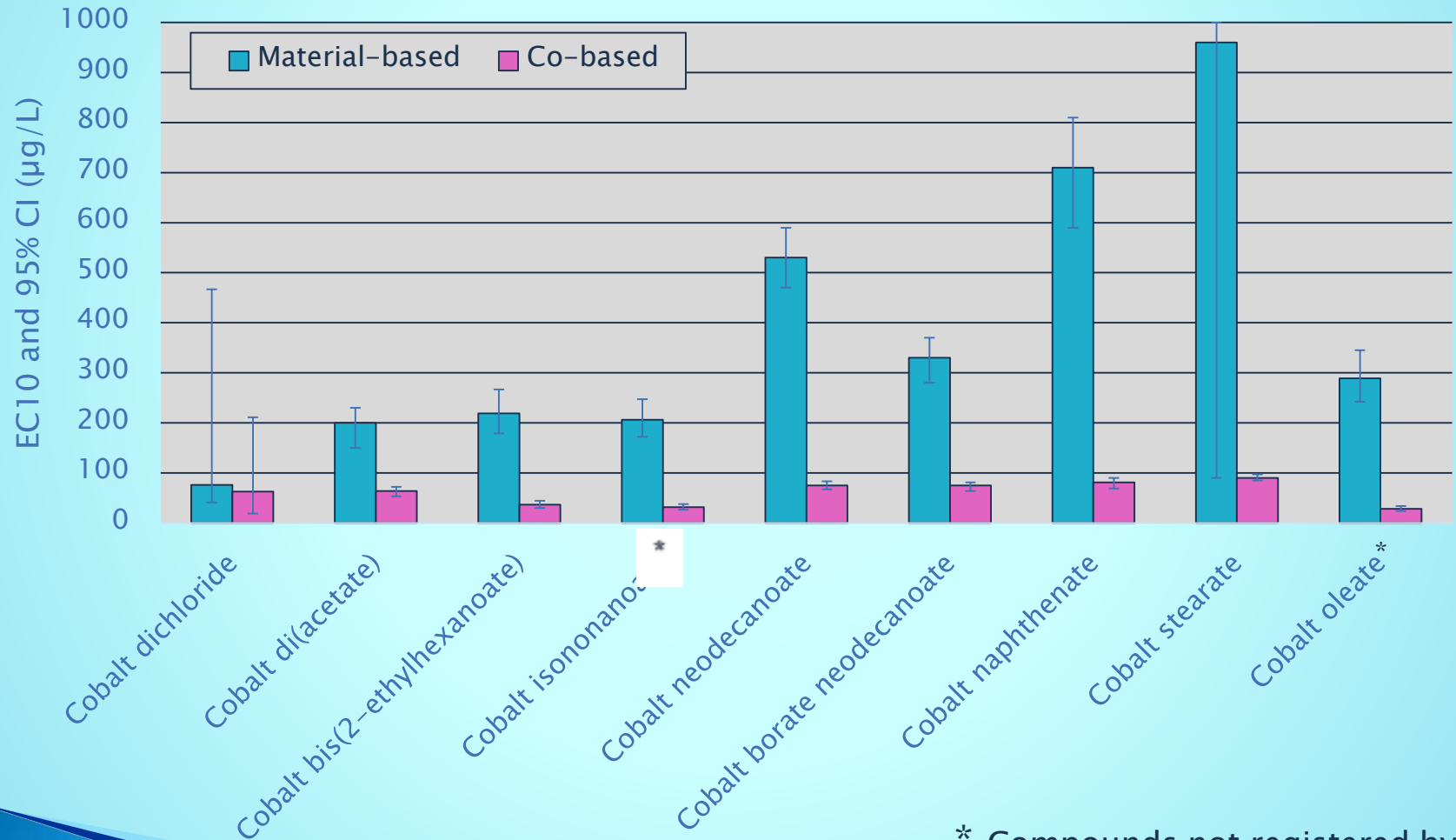


# Chronic algal data (*Pseudokirchneriella subcapitata*)



\* Compounds not registered by the Cobalt REACH Consortium

# Chronic algal data (*Pseudokirchneriella subcapitata*)



\* Compounds not registered by the Cobalt REACH Consortium

# Conclusions from empirical data

- ▶ Endpoint responses (e.g.,  $LC_{50}$ ,  $EC_{10}$ ) vary greatly when based on concentrations of total compound
- ▶ No statistical differences noted between “reference” metal salt/“source substance” (i.e., cobalt dichloride) and organic metal salts (OMS\*) (e.g., cobalt carboxylate salts) when based on measured dissolved Co concentration
- ▶ Results from cobalt dichloride tests are predictive of organic metal salts when analysis is based on dissolved Co concentrations

\* As defined in OECD 212 guidance on “Assessing the risks of organic metal salts”

How do we deal with the possible contribution of counter-ions (i.e., anions) to the toxicity of metal salts?

# Data from major ion research

SETAC PRESS

Environmental Toxicology and Chemistry, Vol. 35, No. 12, pp. 3039-3057, 2016  
Published 2016 SETAC  
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## THE ACUTE TOXICITY OF MAJOR ION SALTS TO CERIODAPHNIA DUBIA: I. INFLUENCE OF BACKGROUND WATER CHEMISTRY

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(Submitted 3 February 2016; Returned for Revision 4 March 2016; Accepted 10 May 2016)

**Abstract:** The ions  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Cl}^-$ ,  $\text{SO}_4^{2-}$ , and  $\text{HCO}_3^-/\text{CO}_3^{2-}$  (referred to in the present study as "major ions") are present in all freshwaters and physiologically required by aquatic organisms but can increase to harmful levels from a variety of anthropogenic activities. It is also known that the toxicities of major ion salts can vary depending on the concentrations of other ions, and understanding these relationships is key to establishing appropriate environmental limits. The authors present a series of experiments with *Ceriodaphnia dubia* to evaluate the acute toxicity of 12 major ion salts and to determine how toxicity of these salts varies as a function of background water chemistry. All 10 salts that showed toxicity also showed some degree of reduced toxicity as the lowest median lethal concentrations found for K salts. These experiments also indicated multiple mechanisms of toxicity, whereas the toxicities of K salts were primarily water increased. Experiments that independently varied Ca:Mg ratio, Na:K ratio, Cl:SO<sub>4</sub> ratio, and alkalinity/pH demonstrated that concentration was the primary factor influencing the toxicities of Na and Mg salts, whereas the toxicities of K salts were primarily influenced by the concentration of the anions and were well correlated with osmolality. Understanding these relationships between Na salts also reflected an influence of the anions and were well correlated with osmolality. Understanding these relationships between major ion toxicity and background water chemistry should aid in the development of sensible risk-assessments and regulatory standards. *Environ Toxicol Chem* 2016;35:3039-3057. Published 2016 Wiley Periodicals Inc. on behalf of SETAC. This article is a US government work and, as such, is in the public domain in the United States of America.

**Keywords:** Aquatic toxicology  
Major ions  
*Ceriodaphnia dubia*

Toxicity mechanism  
Dose-response modeling

AND PIMMINS (MINNOWS)

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(Received 29 April 1996; Accepted 20 February 1997)

**Abstract—**Toxicity of fresh waters with high total dissolved solids has been shown to be dependent on the specific ionic composition of the water. To provide a predictive tool to assess toxicity attributable to major ions, we tested the toxicity of over 2,900 ion solutions using the daphnids, *Ceriodaphnia dubia* and *Daphnia magna*, and fathead minnows (*Pimephales promelas*). Multiple logistic regression was used to relate ion composition to survival for each of the three test species. In general, relative ion toxicity was  $\text{K}^+ > \text{HCO}_3^- \approx \text{Mg}^{2+} > \text{Cl}^- > \text{SO}_4^{2-}$ ;  $\text{Na}^+$  and  $\text{Ca}^{2+}$  were not significant variables in the regressions, suggesting that the toxicity of  $\text{Na}^+$  and  $\text{Ca}^{2+}$  salts was primarily attributable to the corresponding anion. For *C. dubia* and *D. magna*, toxicity of  $\text{Cl}^-$ ,  $\text{SO}_4^{2-}$ , and  $\text{K}^+$  was reduced in solutions enriched with more than one cation. Final regression models showed a good quality of fit to the data ( $R^2 = 0.767-0.861$ ). Preliminary applications of these models to field-collected samples indicated a high degree of accuracy for the *C. dubia* model, while the *D. magna* and fathead minnow models tended to overpredict ion toxicity.

**Keywords—**Ions Total dissolved solids Salinity Toxicity *Ceriodaphnia dubia*

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## THE ACUTE TOXICITY OF MAJOR ION SALTS TO CERIODAPHNIA DUBIA. II. EMPIRICAL RELATIONSHIPS IN BINARY SALT MIXTURES

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(Submitted 16 August 2016; Returned for Revision 17 September 2016; Accepted 27 October 2016)

**Abstract:** Many human activities increase concentrations of major geochemical ions ( $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Cl}^-$ ,  $\text{SO}_4^{2-}$ , and  $\text{HCO}_3^-/\text{CO}_3^{2-}$ ) in freshwater systems, and can thereby adversely affect aquatic life. Such effects involve several toxicants, multiple toxicity mechanisms, various ion interactions, and widely varying ion compositions across different water bodies. Previous studies of individual salt toxicities have defined some useful relationships; however, adding single salts to waters results in atypical compositions and does not fully address mixture toxicity. To better understand mechanisms and interactions for major ion toxicity, 29 binary mixture experiments, each consisting of 7 to 8 toxicity tests, were conducted on the acute toxicity of major ion salts and mannitol to *Ceriodaphnia dubia*. These tests showed multiple mechanisms of toxicity, including: 1) nonspecific ion toxicity, correlated with osmolality and to which all ions contribute; and 2) cation-dependent toxicities for potassium (K), magnesium (Mg), and calcium (Ca) best related to their chemical activities. These mixture studies primarily operate independently, except for additive toxicity of Mg-dependent and Ca-dependent toxicities. These mechanisms of toxicity, including: 1) nonspecific ion toxicity, and Mg salt toxicities and Na on K salt toxicity, and further indicated lesser ameliorative effects of Ca on K salt toxicity and Mg on Na salt toxicity. These results provide a stronger basis for assessing risks from the complex mixtures of ions found in surface waters. *Environ Toxicol Chem* 2017;36:1525-1537. Published 2016 Wiley Periodicals Inc. on behalf of SETAC. This article is a US government work and, as such, is in the public domain in the United States of America.

**Keywords:** Aquatic toxicology  
Toxicity mechanisms  
Concentration addition  
Independent action  
Chemical activity

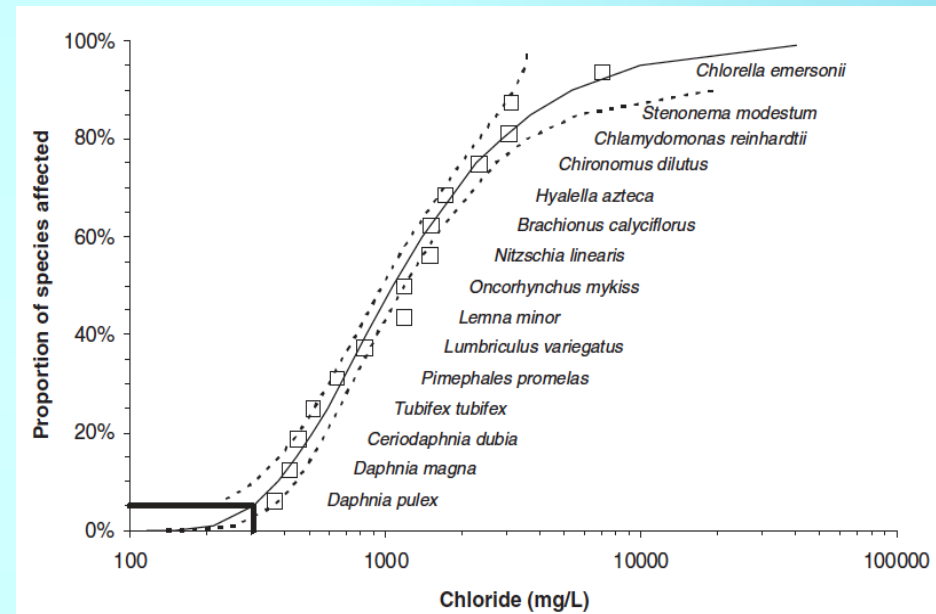
# LC<sub>50</sub> values for select species (as total ion concentration in mg/L)

	<i>Ceriodaphnia</i>	<i>Daphnia magna</i>	Fathead minnow
NaCl	1960	4770	6390
Na <sub>2</sub> SO <sub>4</sub>	3080	4580	7960
NaHCO <sub>3</sub>	1020	1640	<850
KCL	630	660	880
K <sub>2</sub> SO <sub>4</sub>	<680	720	680
KHCO <sub>3</sub>	630	650	<510
CaCl <sub>2</sub>	1830	2770	4630
CaSO <sub>4</sub>	>1910	>1970	>1970
MgCl <sub>2</sub>	880	1330	2120
MgSO <sub>4</sub>	1770	1820	2820

Data source: Mount et al. 1997.  
*Environ. Toxicol. Chem.* 16:2009–  
2019.

# Chronic toxicity of chloride

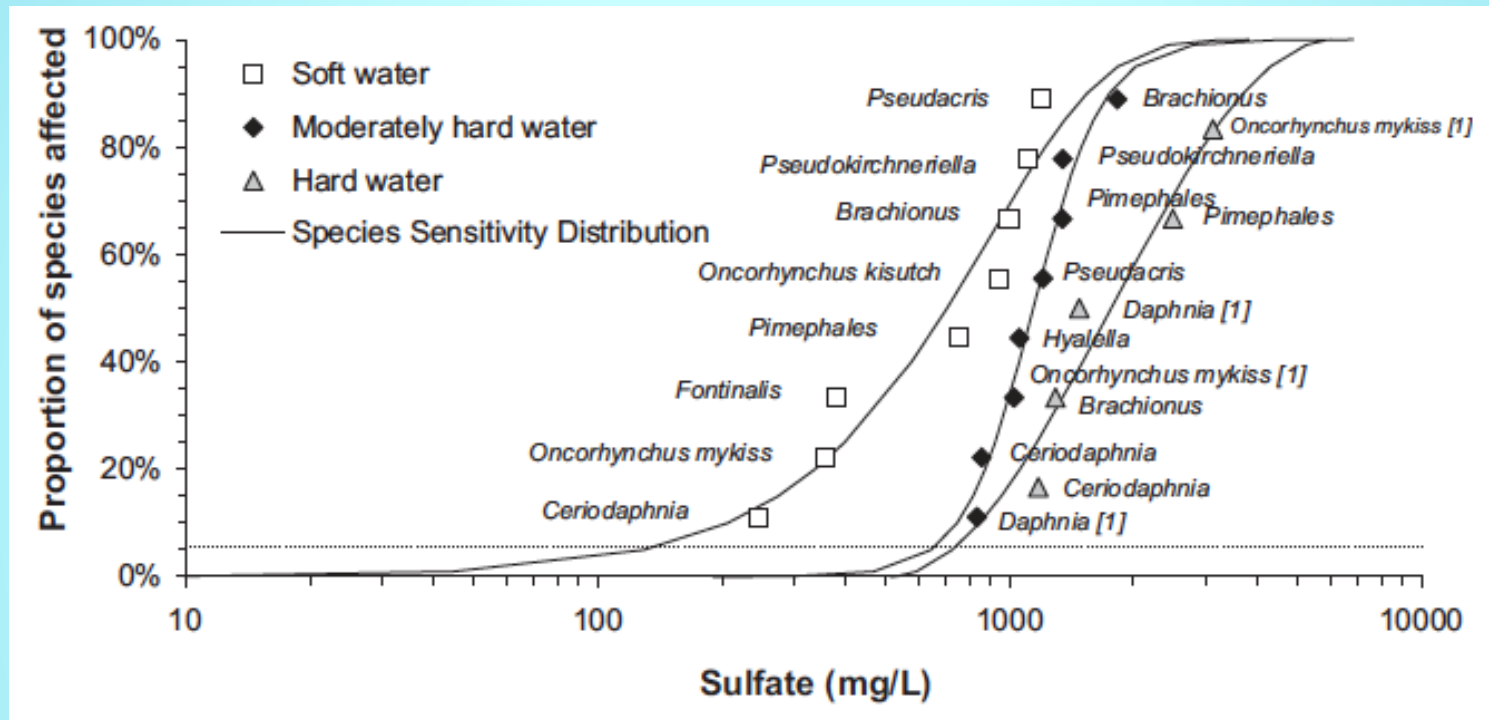
Species	IC25 (mg/L)
<i>Ceriodaphnia dubia</i>	454 (251-819)*
<i>Daphnia magna</i>	421 (262-825)
<i>Oncorhynchus mykiss</i>	1,174 (733-1,344)
<i>Pimephales promelas</i>	704 (486-973)
<i>Lumbriculus variegatus</i>	825 (549-1,256)
<i>Tubifex tubifex</i>	606 (391-632)
<i>Chironomus dilutus</i>	2,590 (2,118-2,590)
<i>Hyalella azteca</i>	1,705 (440-1,907)
<i>Brachionus calyciflorus</i>	1,505 (540-1,670)



HC<sub>5</sub> (and 95% confidence intervals) calculated on this basis was 307 (217 to 369) mg/L.

Data source: Elphick et al. 2011. *Environ. Toxicol. Chem.* 30:239-246.

# Chronic toxicity of sulfate



Species sensitivity distributions for sulfate under soft (10–40 mg/L), moderately hard (80–100 mg/L), and hard water (150–250 mg/L) conditions. Result in guidelines for soft, moderately hard, and hard water conditions of 129, 644, and 725 mg/L sulfate, respectively.

Data source: Elphick et al. 2011. *Environ Toxicol Chem.* 30:247–253.

# Organic acid data

Material	Species	Endpoint
Acetic acid	Bluegill	96h LC <sub>50</sub> 75 mg/L
Acetic acid	Daphnia magna	48h EC <sub>50</sub> 65 mg/L
Acetic acid	Scenedesmus sp.	8-d EC <sub>50</sub> 4000 mg/L
Propionic acid, Na salt	Bluegill	96h LC <sub>50</sub> 85 mg/L
Propionic acid, Na salt	Rainbow trout	96h LC <sub>50</sub> 67 mg/L
Propionic acid	Daphnia magna	48h EC <sub>50</sub> 22.7 mg/L
Propionic acid	Scenedesmus sp.	72h EC <sub>50</sub> 43–46 mg/L
Stearic acid	Coho salmon	96h LC <sub>50</sub> 12 mg/L
Tall oil fatty acids	Fathead minnow	96h LC <sub>50</sub> >1000 mg/L
Tall oil fatty acids	Daphnia magna	48h EC <sub>50</sub> >1000 mg/L
Tall oil fatty acids	S. capricornutum	72h EC <sub>50</sub> 855 mg/L
Fatty acids, C16–C18	Golden orfe	96h LC <sub>50</sub> >1000 mg/L
Fatty acids, C16–C18	Rainbow trout	96h LC <sub>50</sub> >100 mg/L
Fatty acids, C16–C18	Daphnia magna	48h EC <sub>50</sub> >1000 mg/L
Fatty acids, C16–C18	S. capricornutum	72h EC <sub>50</sub> >1000 mg/L

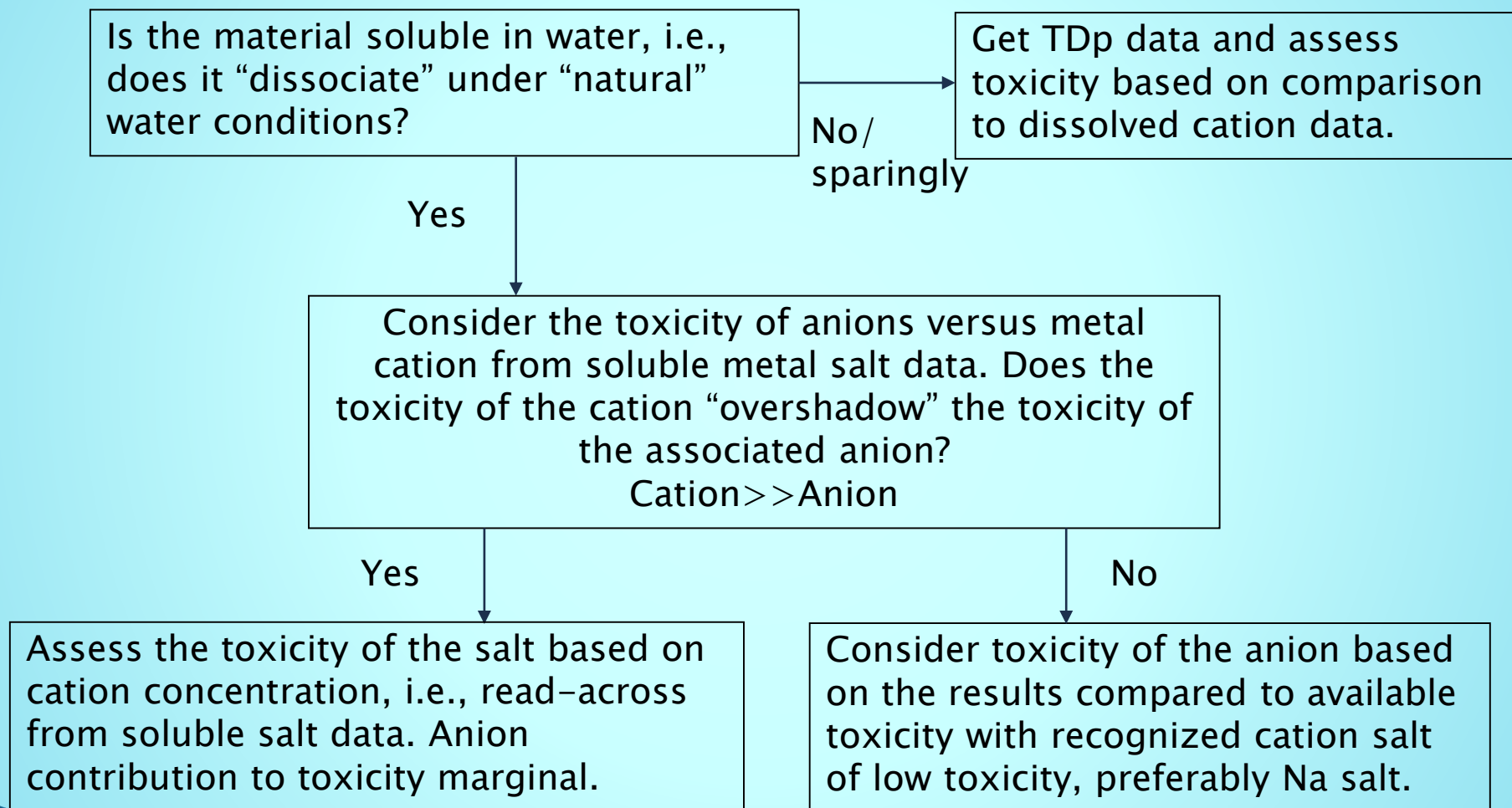
# Conclusions

- ▶ Results indicate that the typical anions of soluble inorganic metal salts (e.g., Cl, SO<sub>4</sub>, NO<sub>3</sub>, HCO<sub>3</sub>) are acutely and chronically toxic to fish and invertebrates in **mg/L** concentrations
  - Many cationic metals are chronically toxic in the **µg/L** range
  - Toxicity evaluations for cationic metals should be conducted with soluble metal salts that have anionic moieties that contribute little to no toxicity (e.g., Cl and NO<sub>3</sub>)
    - Consistent with current state-of-the-science and regulatory recommendations

# Conclusions

- ▶ If evaluating the toxicity of anions, tests should be conducted with a cation salt that contributes minimally to the toxicity of the salt
  - Na salts are preferred

# Proposed approach



# Status of Co env read-across

- ▶ The category consists of inorganic cobalt substances and cobalt salts of organic acids for which environmental toxicity is governed by the cobalt cation
- ▶ Each of the “Common and Scenario specific Assessment Elements” have been addressed in the read-across justification
  - Descriptive summary text contained in Sections 4 and 7 of CSR
  - Full text in RAAF format as stand-alone Appendix to CSR – currently covers only Scenario 5 used for risk assessment



# Selection of scenarios

- ▶ Risk assessment: Scenario 5
  - Assessment of effects of dissolved  $\text{Co}^{2+}$  (soluble salt used as test item to deliver Co ion)
  - Exposure measured as dissolved  $\text{Co}^{2+}$  in solution
  - Assessment of all compounds based on same ion → no variation in strength of effect
- ▶ Classification: Scenario 3
  - ERV based on toxicity of Co ion
  - Strength of effect for classification varies based on solubility, in a regular pattern
  - Transformation/dissolution used to classify as outlined in CLP guidance Annex IV



# Potential weaknesses / data insufficiencies

- ▶ Formation of common and non-common compounds
  - All studies monitored “dissolved” (i.e., <0.45 μm) cobalt concentrations. *No monitoring of aqueous complexes of cobalt and counter-ion.*
  - To address this we proposed to use the complexation constant, for example:



(i.e. formation of the dissolved organic-metal complex in solution)

- Proposed use of model approach (MINEQL and Visual MINTEQ). Model databases include only acetate and propionate carboxylate salts for Co. Also searched the IUPAC database; it included additional carboxylates (e.g., hexanoate; stearate; decanoate)... *but there were no data for their interaction with Co.*



# Vanadium substances – read-across approach for ecotoxicity data

**MISA Meeting**  
**6 February 2019**

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Astrid Voigt, Ph.D.  
EBRC Consulting GmbH  
Hannover

# Vanadium substances & grouping

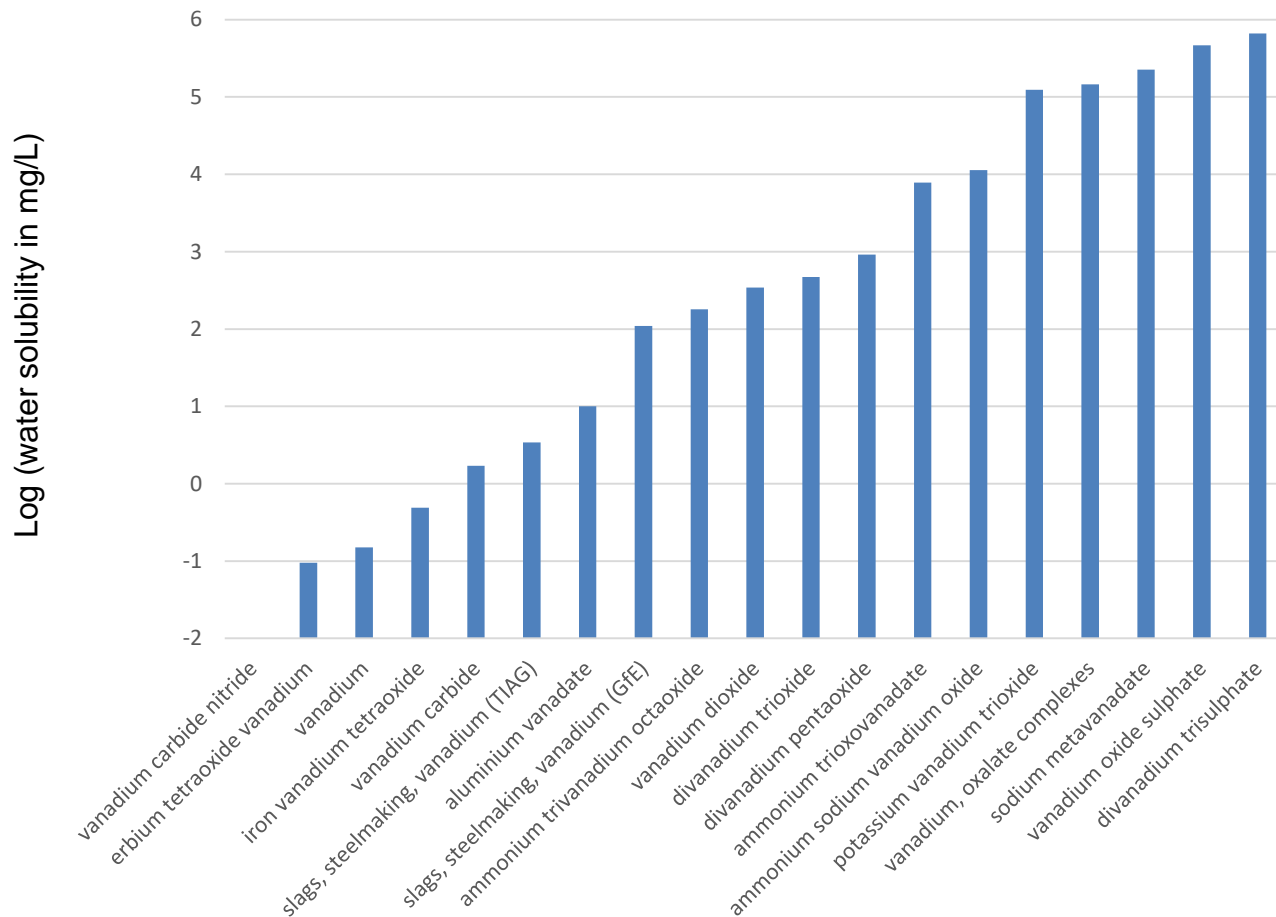
## Vanadium substance portfolio:

- metal & interstitial carbides (VC, VCN)
- tri-, tetra-, pentavalent oxides & salts (vanadates, sulfates)

## Tentative grouping based on solubility of V substances:

- sparingly soluble metal & carbides
- sparingly soluble tri-, tetra-, pentavalent V substances
- soluble tri-, tetra-, pentavalent V substances

# Vanadium substances - water solubility



Difference:  
>  $6.6 \times 10^7$

## Transformation/dissolution in environmental media

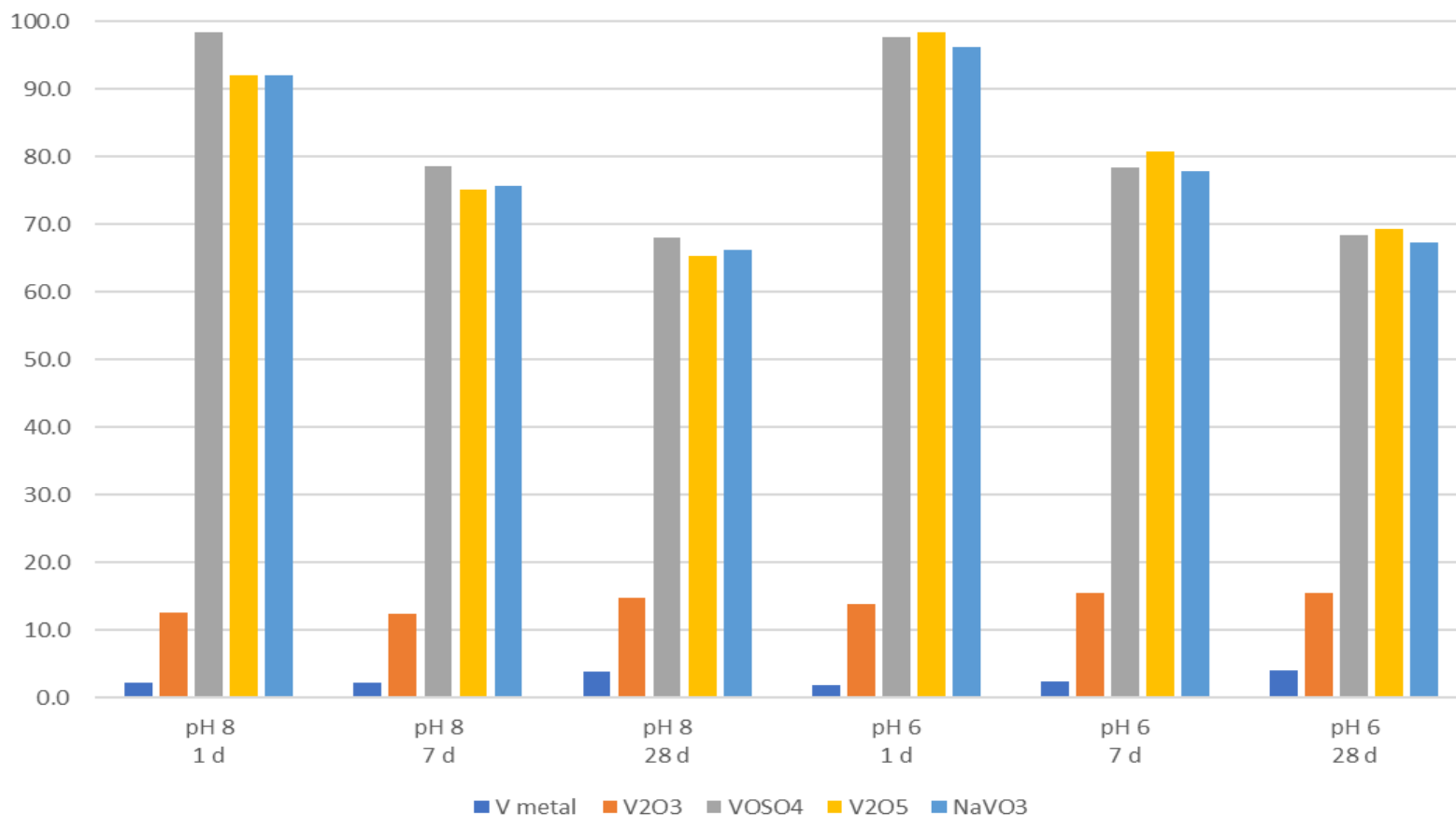


- T/Dp: OECD TG 29
- Loading: 1 mg/L at pH 6 & pH 8
- Analysis of dissolved V plus speciation after 2, 6, 24, 96, 168, 336, 504 & 672 h
- Dissolved total V quantified by ICP-MS or ICP-OES
- V (IV) & V (V) species separated by HPLC & quantified by ICP-MS
- V (III) calculated as difference between V total & sum of V (IV) & V (V)

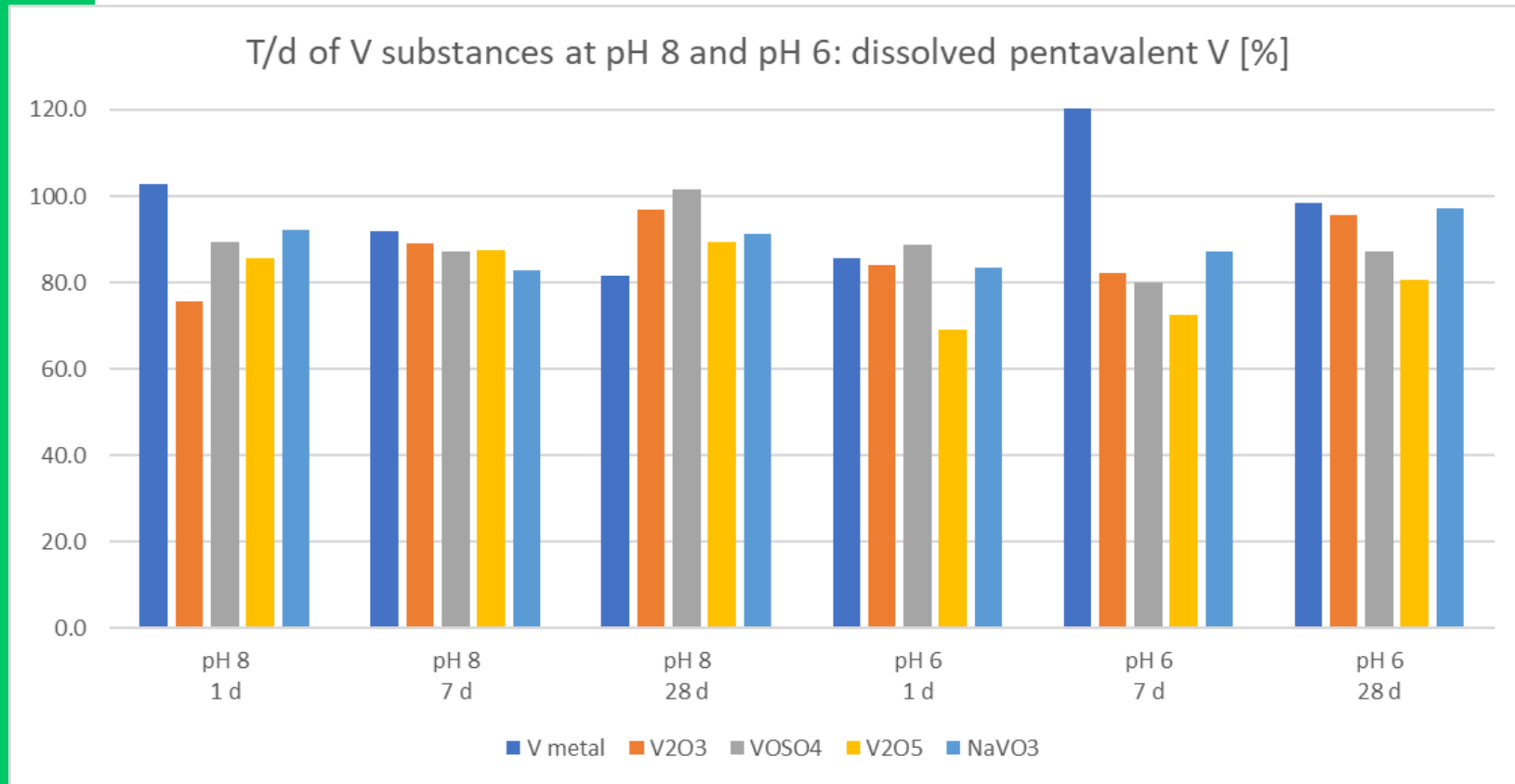
### Selected vanadium substances:

- elemental: V metal (99.1% V)
- tetravalent:  $\text{VO}_2$  (31.0% V)
- Trivalent:  $\text{V}_2\text{O}_3$  (67.9% V)
- Pentavalent:  $\text{NaVO}_3$  (39.7% V)  
 $\text{V}_2\text{O}_5$  (56.0% V)

T/d of V substances at pH 8 and pH 6: relative total dissolved V [%]

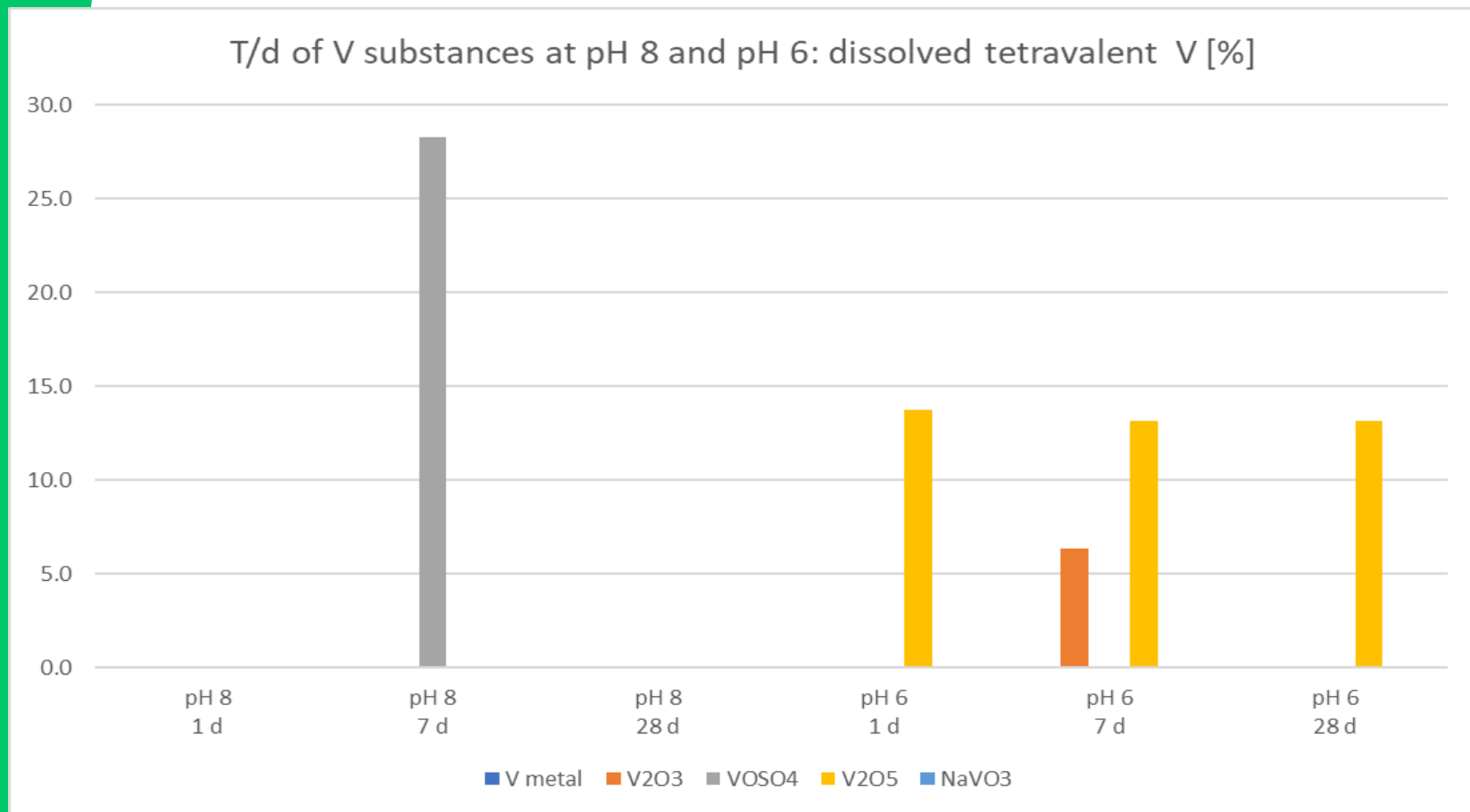


- Poorly soluble: V, V<sub>2</sub>O<sub>3</sub>
- soluble substances: decrease of V concentrations with time due to media replenishment after sampling
- soluble: VOSO<sub>4</sub>, V<sub>2</sub>O<sub>5</sub>, NaVO<sub>3</sub>



→ Dissolved V steadily in pentavalent form  $\geq 70 - 100\%$

→ No increase/decrease of relative V (V) concentrations with time



- Tetravalent V mostly < LOD/LOQ
- No increase/decrease of relative V (IV) concentrations with time
- V (IV) concentrations not steady over time (only sporadic)

## Read-across conclusions for V in environmental media

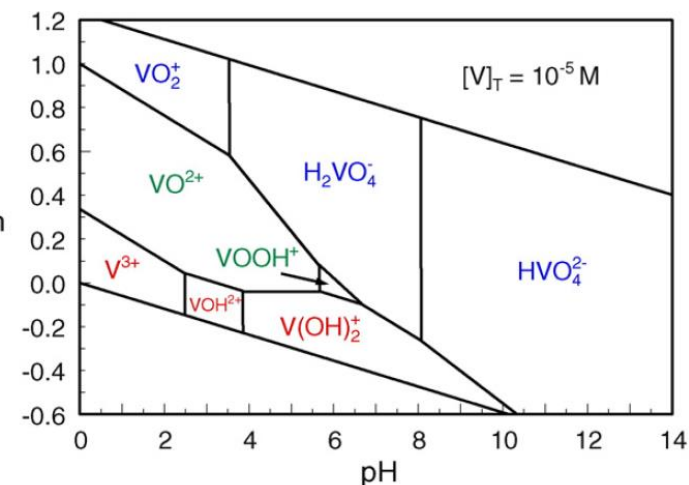
- vanadium may occur under environmentally relevant conditions in 3 oxidation states (+III, +IV & +V), however:
  - V (III) occurs only under strongly reducing conditions
  - V (IV) as vanadyl is only stable at low pH and/or mildly reducing conditions, notably at high organic matter content
  - V (V) as vanadate is predominant under most environmental conditions
- In T/Dp: regardless of different redox of V, at pH 6 & 8 dissolved V is nearly exclusively in pentavalent form

→ V speciation depends on environmental conditions

but not on valence of V origin

→ Solubility but not valence of V substance affect ecotoxicity Eh

→ Unlimited read-across between V substances



Thank you. Any questions?

