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New Approach for Evaluating the Bioaccumulation Potential of Metals in the New Chemicals Division

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1. Summary

The bioaccumulation mechanism for metals differs from that for typical organic chemicals (i.e., lipophilic partitioning). Therefore, when evaluating metals in the New Chemicals Division (NCD), the standard lipid-based approach for assessing bioaccumulation potential based on bioconcentration factor (BCF) or bioaccumulation factor (BAF) measurements or estimations is not appropriate. Here, we present a new approach to evaluate the bioaccumulation potential of metals that can be applied in the new chemicals program. This approach does not quantitatively predict the metal's bioaccumulation potential but rather, it considers multiple lines of evidence and endpoints to determine whether the metal is likely to accumulate in a way that presents a concern for unreasonable risk. The weight of evidence (WoE) approach considers:

1. An assessment that integrates a literature review of available field measurements of metal concentrations in fish and shellfish with critical concentrations based on human chronic oral toxicity values criteria (i.e., non-cancer screening level, NCSL, and/or cancer slope factor, CSF).
2. A critical review of the test data, conclusions, and rationales related to bioaccumulation utilized by other programs, agencies, and organizations in their risk assessments for metals.
3. A critical review of relevant metal-specific information on bioconcentration, bioaccumulation, and trophic transfer available in peer-reviewed scientific literature.

2. NCD Assessment of Bioaccumulation

As part of the new chemical evaluation under TSCA, NCD's fate team typically assigns both a persistence (P) and a bioaccumulation (B) rating, consistent with EPA's 1999 PBT policy as outlined in the Federal Register.[1] The 1999 framework focused on standard organic chemicals and provides a bioaccumulation rating scheme based on BCF/BAF cutoffs. Accordingly, NCD rates standard organic chemicals as B1 (BCF/BAF < 1000), B2 (1000 ≤ BCF/BAF < 5000), or B3 (BCF/BAF > 5000), with a B2 or B3 rating being sufficient for the "B" portion of a PBT designation.

For chemicals that do not bioaccumulate via typical lipophilic partitioning, NCD fate assessors

[1] Category for Persistent, Bioaccumulative, and Toxic New Chemical Substances, 64 F.R. 60,194 (November 4, 1999). https://www.gpo.gov/fdsys/pkg/FR-1999-11-04/pdf/99-28888.pdf.

may deviate from B1-3 to assign a rating of either *B*low* or *B*high* to indicate their predicted bioaccumulation potential. "B*" denotes that the rating is for a chemical that does not bioaccumulate via lipophilic partitioning. The "low" or "high" designation indicates whether the chemical is expected to have low or high potential to bioaccumulate via other mechanisms. NCD does not have a formal cutoff between the "low" and "high" designation. However, a measured or estimated BCF or BAF > 1000, in combination with any additional information, has been considered as a screening level indication that a B*high rating may be warranted. The final designation is ultimately based on professional judgement.

In the TSCA new chemicals program, the bioaccumulation rating (i.e., B rating) serves two primary purposes: 1) to determine, in combination with the persistence (P) and toxicity (T) ratings, whether a new chemical substance is PBT and therefore subject to risk management, and 2) to assess potential risks to humans via fish and shellfish consumption. A rating of B*high is considered sufficient to contribute to a PBT designation.

Previously, NCD fate assessors have relied on available, measured BCF and/or BAF values to assign either a B*low or B*high rating when assessing a metal. However, the latest scientific data on bioaccumulation do not support the use of BCF or BAF when applied as generic threshold criteria for the hazard potential of inorganic metals in human and ecological risk assessment, as explained in Section 3.[2,3] Single-value BCF/BAFs and mechanistic bioaccumulation models for metals offer the most value for site-specific risk assessments when extrapolation across different exposure and environmental conditions is minimized.[2,3] Their utility is limited for national-scale risk assessments such as those performed within NCD.

3. Bioaccumulation Considerations for Metals

Metals are naturally occurring in the environment and vary in concentrations across geographic regions. Some metals are essential for maintaining the proper health of humans, animals, plants, and microorganisms. As a result, many species have evolved physiological or anatomical mechanisms to regulate accumulation and/or storage of certain metals, particularly essential metals and those that may mimic essential metals within the organism. In these species, homeostatic mechanisms can maintain optimal tissue levels over a range of exposures, even when exposure concentrations (e.g., in water, air, and/or food) exceed those normally encountered by the organism.[2] In contrast, certain metals can bioaccumulate to high levels in some aquatic organisms (for example zinc in barnacles and copper in crayfish) by active regulation due to species-specific physiological requirements, regardless of exposure level.[2] BCFs and BAFs cannot not distinguish between metals that are elevated to meet physiological requirements and those by which adverse effects may result when elevated.[4]

Unlike hydrophobic, nonionic organic chemicals, which generally cross biological membranes via passive diffusion, metals are taken up by a number of specific transport mechanisms. Some

[2] U.S. EPA. 2007. Framework for Metals Risk Assessment. EPA 120/R-07/001 March 2007. 172pp. www.epa.gov/sites/default/files/2013-09/documents/metals-risk-assessment-final.pdf

[3] McGeer JC *et al.* 2004. Issue paper on the bioavailability and bioaccumulation of metals. Submitted by Eastern Research Group (ERG) to the U.S. EPA on August 10, 2004. 126pp. www.epa.gov/sites/default/files/2014-11/documents/bio_final.pdf.

of these transport mechanisms involve binding with membrane carrier proteins, transport through hydrophilic membrane channels, and endocytosis. Passive diffusion is thought to be reserved for certain lipid soluble forms of metals, such as alky-metal compounds and neutral, inorganically complexed metal species (e.g., $HgCl_20$). The implication of these specific transport mechanisms is that metal bioaccumulation can involve saturable uptake kinetics, such that BCFs and BAFs depend on exposure concentration.[2] The existence of saturable uptake mechanisms, the presence of significant amounts of stored metal in organisms, and the ability of some organisms to regulate bioaccumulated metal within certain ranges are primarily responsible for the inverse relationship that has frequently been reported between BCFs/BAFs and metal exposure concentrations.[4,5] In these cases, higher BCFs or BAFs are associated with lower exposure concentrations and may be associated with lower tissue concentrations within a given aquatic BCF or BAF study. This is counter to the implicit assumption that higher BCFs or BAFs indicate higher metal hazard.[2] Using BCF and BAF data can lead to conclusions that are inconsistent with the toxicological data, as these values are sometimes highest (indicating hazard) at low exposure concentrations and are lowest (indicating low/no hazard) at high exposure concentrations, where impacts are likely.[4]

In addition, other biotic and abiotic factors influence metal bioavailability and bioaccumulation. Assimilation efficiencies can vary widely depending on the metal, its form and distribution in prey, species digestive physiology (e.g., gut residence time), environmental conditions, food ingestion rate, and metal concentration in the diet.[2] Considerable uncertainty can be associated with the application of literature-derived BCFs and BAFs for assessing the risks of metals, as variability in BCFs and BAFs for metals is known to be high. Much of this uncertainty results from bioavailability differences among the studies in which the BCFs or BAFs are measured (e.g., differences in water quality characteristics, metal speciation, and exposure pathways).[2]

Quantitative measures of trophic transfer (i.e., biomagnification factors [BMF] and trophic magnification factors [TMF]) are susceptible to many of the same complications discussed for BCF/BAF that lead to high variability and reduced utility beyond site-specific assessments. The availability of these measures is also limited for many metals.

Bioaccumulation and trophic transfer of metals can occur despite the fact that the movement of metals through the food web is complicated by factors of bioaccessibility, bioavailability, essentiality, regulation (uptake and internal distribution), detoxification, storage, and the natural adaptive capacity of organisms.[2] However, biomagnification (i.e., increases in concentration through multiple levels of the food web) is rare, with the exception of certain organometallic compounds, such as methylmercury, that can biomagnify many orders of magnitude in the aquatic food chain.[2,3] But lack of biomagnification cannot be interpreted as lack of exposure or concern via trophic transfer.[2] Even in the absence of biomagnification, organisms can bioaccumulate relatively large amounts of metals and become a significant source of dietary

[4] McGeer, JC *et al.* 2003. Inverse relationship between bioconcentration factor and exposure concentration for metals: implications for hazard assessment of metals in the aquatic environment. Environ Toxicol Chem 22(5): 1017-1037.
[5] Borgmann, U *et al*. 2004. Re-evaluation of metal bioaccumulation and chronic toxicity in *Hyalella azteca* using saturation curves and the biotic ligand model. Environ Pollut 131(3): 469-484.

metal to their predators.[6]

As a result of these numerous uncertainties, the application of measured BCF, BAF, or BMF values for metals is not appropriate beyond individual well characterized site- and food web-specific scenarios. The current science does not support the use of a single, generic threshold BCF/BAF/BMF value for a given metal as an indicator of that metal's hazard potential. For national-scale risk assessments, use of a single BCF/BAF/BMF value holds little utility due to high uncertainty that results from differences in bioavailability, exposure conditions, and species-specific factors that influence metal bioaccumulation by aquatic organisms.[2]

There are no simple metrics available that allow for the quantification of the potential for metal bioaccumulation.[2] Existing regulatory and scientific guidances do not provide a single quantitative approach for metals that is appropriately suited to the national-scale, screening-level assessment of bioaccumulation that is required for risk assessments in NCD. As described below, NCD has developed a WoE approach to assess the bioaccumulation potential of metals. Considering multiple lines of evidence will reduce uncertainty and allow for a more robust and scientifically supported assessment.

4. New Approach for Determining the Bioaccumulation Potential of Metals

This memorandum presents a WoE approach to consider the bioaccumulation potential of metals as part of the screening-level risk assessment of new chemicals under TSCA. This approach considers the following lines of evidence:

1. An assessment that integrates a literature review of available field measurements of metal concentrations in fish and shellfish with critical concentrations based on human chronic oral toxicity values (i.e., non-cancer screening level, NCSL, and/or cancer slope factor, CSF).
2. A critical review of the data, conclusions, and rationales related to bioaccumulation utilized by other programs, agencies, and organizations in their risk assessments for metals.
3. A critical review of relevant metal-specific information on bioconcentration, bioaccumulation, and trophic transfer available in peer-reviewed scientific literature.

NCD will review the complete body of evidence and assign a B rating of either B*low or B*high to each individual metal of interest. This approach does not quantitatively predict the metal's bioaccumulation potential; rather, it considers multiple lines of evidence and endpoints to determine whether the metal is likely to accumulate in a way that presents a concern for unreasonable risk.

4.1. Line of Evidence #1: Fish/Shellfish Tissue Concentrations Relative to Human Intake Criteria

The first line of evidence uses human chronic dietary toxicity values (i.e., RfD or CSF) to

[6] Reinfelder, JR *et al*. 1998. Trace element trophic transfer in aquatic organisms: a critique of the kinetic model approach. Sci Total Environ 219(213): 117-35.

determine whether individual metals pose a risk to human consumers based on literature-derived fish/shellfish tissue metal concentrations. Despite the uncertainties associated with bioavailability, bioaccumulation, and trophic transfer of dietary metals, the use of whole-body inorganic metal concentrations in prey species has utility to risk assessors for conservatively screening for exposure and risk to consumers.[2] Such an analysis can discriminate between metals that have the potential to cause effects via trophic transfer and metals that do not. Metals that bioaccumulate to levels in prey organisms (i.e., fish/shellfish) that cause impacts in predatory organisms (i.e., humans) are critical to identify and address through risk management.

This methodology does not provide a pure prediction of bioaccumulation, but rather integrates bioaccumulation and chronic toxicity data to examine metal accumulation in fish relative to human toxicity values. The methodology is analogous to the peer-reviewed methodology employed by EPA's Office of Water to generate their list of contaminants of concern to be included in fish tissue monitoring programs.[7] The scope of this line of evidence is limited to assessing risks to human consumers from the adult general population.

Metals have an abundance of published fish and shellfish tissue data available. These data span environmental exposure scenarios, including sites with wide ranging water chemistries, across geographical ranges with variations in background metal concentrations, and a variety of fish and shellfish species commonly consumed by humans. Thus, the data integrate many of the variables that affect organism tissue concentrations. The range and maximum tissue concentrations can be used as indicators of possible human exposure scenarios via fish and shellfish consumption for a given metal. Comparing this range to human dietary intake criteria (i.e., non-cancer and cancer screening levels and CSL) allows the assessor to consider both bioaccumulation and toxicity concurrently to answer the question of whether a given metal can accumulate in fish and shellfish to concentrations that pose a risk to human consumers within the bounds of reasonably anticipated environmental conditions. While not every possible environmental scenario can be represented by the available data, the approach provides a conservative, screening-level line of evidence that is an improvement over the previous reliance on BCFs/BAFs alone.

4.1.1. Step 1: Literature search for metal concentrations in fish and shellfish tissue

The NCD fate assessor first performs a literature search for available field measurements of fish and shellfish tissue concentrations for the metal of interest. To be considered in the analysis, studies must include the identity of the metal tested, fish/shellfish tissue metal concentrations, the identity of the tissue in which the metals were measured, the study species, and the collection site's general location and description. Studies not including all these criteria are excluded from the analysis. Studies without an indication of whether reported concentrations were on a wet or dry weight basis may still be included. The conservative assumption that the measurements are on a wet weight basis will be made and the uncertainty will be noted in the database.

Measurements in fish muscle (i.e., fillet) or whole soft bodies of shellfish will be preferred as these are the most common tissues consumed by the general population. In the absence of

[7] U.S. EPA Office of Water. 2024. Contaminants to Monitor in Fish and Shellfish Advisory Programs: Compilation of Peer Review-Related Information. EPA 823-R-24-001. July 2024. www.epa.gov/system/files/documents/2024-06/contaminants-monitor-fish-peer-review-package.pdf.

sufficient data in preferred tissues, whole body fish metal concentrations may be included in the assessment. Whole body metal concentrations are often higher than those in the edible portion of fish, so their inclusion represents a more conservative assessment of bioaccumulation potential. Studies included are limited to field studies to best reflect ambient conditions (i.e., laboratory studies excluded) and should include only fish and shellfish species representative of those consumed by humans. Tissue concentration should be considered on a wet-weight basis. If the tissue concentration is reported on a dry-weight basis (C_{DW}), it can be converted to an equivalent wet-weight concentration (C_{WW}) according to the equation:

$$C_{WW} = C_{DW} * 0.2 \qquad \text{[Equation 1]}$$

The calculation assumes 80% moisture content (20% dry mass) in fish and shellfish tissues. A moisture content of 80% was chosen as a reasonable estimate appropriate for screening-level purposes based on measured raw fish and shellfish values (Table 1).

Table 1: Percent moisture measured in raw fish and shellfish[8]

Organism Type	**Number of Species Measured**	**Percent Moisture (%)**		
		Mean	**Median**	**Range**
Fish	77	75.7	76.4	63.6 – 83.2
Shellfish	19	80.0	80.3	74.1 – 86.2

When tissue concentrations are very near the human toxicity concentrations, species-specific moisture content information can be applied for a more precise estimate. These values are available for a variety of commonly consumed by humans in the U.S.[8]

Ideally, the waters from which the organisms were collected should span a broad geographical range to capture populations that are adapted to a variety of background metal concentrations. Field studies should represent environmental contamination scenarios ranging from background metal concentrations to sites anticipated to be highly contaminated by the metal of interest. As many water body types as possible should be included to cover a wide range of water chemistries. If the available data for a metal are narrower in scope, this will be noted in the assessment and considered when interpreting the conclusions of the line of evidence.

All data extracted from the literature will be put into a database that includes relevant information such as:

- Identity of metals measured
- Fish or shellfish tissue mean metal concentration
- Fish or shellfish tissue metal concentration range
- Concentration basis: wet weight or dry weight
- Tissue type (e.g., muscle, whole body)
- Test organism
- Water type: freshwater vs. marine
- Geographic location/further site details

[8] U.S. Environmental Protection Agency. 2011. Exposure Factors Handbook: 2011 Version. EPA/600/R-09/052F. https://www.epa.gov/expobox/exposure-factors-handbook-2011-edition

- Study literature reference
- Additional notes to assist in the interpretation of the study results

Each database entry represents the mean concentration in one species at one location within a given study. Thus, if there are multiple species or sampling locations in a single study, there may be multiple database entries related to that study.

4.1.2. Step 2: Determination of the human chronic oral reference dose (RfD) and cancer slope factor (CSF)

NCD will identify a reference dose (RfD, mg/kg/day) for each metal for use in the methodology. The RfD will be based on a no observed adverse effect level (NOAEL), lowest observed adverse effect level (LOAEL), or benchmark dose for non-cancer endpoints and the associated safety factor (SF), as detailed in existing EPA guidances.[9]

For cancer endpoints for which a linear low dose extrapolation is appropriate, NCD will utilize an oral cancer slope factor (CSF), when available. The CSF is an estimate of the increased cancer risk from oral exposure to a dose of 1 mg/kg-day for a lifetime and can be multiplied by an estimate of lifetime exposure (in mg/kg-day) to estimate the lifetime cancer risk.

4.1.3. Step 3: Translation of the RfD and CSF into acceptable fish/shellfish tissue concentrations for human consumption (CNSL and CSL)

The fish tissue concentration corresponding to the RfD for non-cancer endpoints (i.e., the non-cancer screening level, NCSL) can be calculated using the RfD (mg/kg/day) for a given metal along with the consumer body weight (BW, kg) and fish consumption rate (FCR, g/day).

$$NCSL = [(RfD * BW) / FCR] * 1000 \qquad \text{[Equation 2]}$$

The NCSL (µg/g) thus represents the predicted maximum fish tissue (e.g., muscle) concentration of the metal of interest that can be consumed over a lifetime by a human consumer of a given BW and FCR with no expected adverse health impact.

For each contaminant with a cancer slope factor (CSF), NCD will also calculate a cancer screening level (CSL) using the following equation:

$$CSL = (CRL * BW) / (CSF * FCR) \qquad \text{[Equation 3]}$$

CRL is the cancer risk level and represents the increased lifetime risk of developing cancer from exposure to a substance. Thus, the CSL represents the fish tissue concentration of a given substance that will result in an increased cancer risk (e.g, 1 in 1,000,000 increase if a CRL of 10^{-6} is utilized) from a lifetime of fish consumption by a human consumer of a given BW and FCR.

The BW and FCR utilized will be selected to be consistent with the current methodologies of

[9] U.S. EPA. A Review of the Reference Dose and Reference Concentration Processes. U.S. Environmental Protection Agency, Risk Assessment Forum, Washington, DC, EPA/630/P-02/002F, 2002. https://www.epa.gov/sites/default/files/2014-12/documents/rfd-final.pdf.

NCD at the time of the assessment. These values can easily be adapted within the methodology as needed, such as if NCD updates the FCR applied to the adult general population or if there is a need to apply the approach to other populations, such as potentially exposed or susceptible subpopulations (PESS). At this time, NCD Exposure assessors consider the adult general population when assessing risks due to fish/shellfish consumption and employ a BW of 80 kg and a FCR of 7.5 g/day. These values come from the 2014 EFAST Manual,[10] and were derived from 2011 Exposure Handbook.[8] The fish consumption rate includes consumption of both fish and shellfish.

NCD may also run a sensitivity analysis of the results by additionally calculating NCSL and CSL using an alternate FCR, such as the 22 g/day rate utilized for the general population by EPA's Office of Water (OW) in their assessments.[11] This will provide information on whether the conclusions of the assessment would be impacted if NCD chooses to align the NCD fish consumption rate with that of OW in the future.

4.1.4. Step 4: Comparison of the NCSL and CSL to the literature-derived fish/shellfish tissue concentrations

The NCSL and CSL are next compared to the literature-derived fish/shellfish tissue concentrations to ascertain whether there is a likelihood that a human fish/shellfish consumer may consistently be exposed to fish concentrations exceeding the NCSL/CSL over a lifetime of fish consumption. Consistent exceedances of the either value (i.e., fish/shellfish tissue concentrations are greater than the NCSL or CSL for a significant portion of the measured values extracted from the peer-reviewed literature) may indicate that a human consumer has the potential to exceed the metal RfD via dietary exposure. Factors to consider in making this determination include:

- What proportion of the fish tissue concentrations found in the literature exceed the NCSL and/or CSL?
- Are the exceedances mean concentrations or single tissue concentrations measured in the study?
- What were the environmental and site conditions under which the NCSL/CSL exceedances were measured?
- Are the species with measured NCSL/CSL exceedances widely consumed or only rarely, or are they only local to a geographic region outside the U.S.?
- Are there any other factors that make the data less representative, valid, or applicable?

Additional information to assist in the interpretation of the results may include whether there are any reports in the literature of human exposures to the metal via fish consumption leading to adverse health outcomes or human body burdens of the metal above levels of concern, as well as whether any regulatory actions (e.g., advisories) have been issued for the metal with regards to fish consumption.

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[10] U.S. Environmental Protection Agency. 2018. Exposure and Fate Assessment Screening Tool (E-FAST) 2014 Version Documentation Manual. Prepared by Versar, Inc. February 2018 under EPA Contract No. EP-W-16-009. 187 pp.

[11] U.S. Environmental Protection Agency, Office of Water. Human Health Ambient Water Quality Criteria: 2015 Update. EPA 820-F-15-001. June 2015. https://www.epa.gov/sites/default/files/2015-10/documents/human-health-2015-update-factsheet.pdf

4.2. Line of Evidence #2: Benchmarking Against Other Agencies/Organizations

The second line of evidence uses existing risk assessments for metals to inform NCD's assessment of bioaccumulation potential. For each metal assessed, existing risk assessments that have been performed by other agencies or other EPA offices will be located and reviewed. This may include:

- Agency for Toxic Substances and Disease Registry (ATSDR) Toxicological Profiles
- Priority Substances List Assessment Reports under the Canadian Environmental Protection Act (CEPA)
- Environment and Climate Change Canada/Health Canada (ECCC/HC) Screening Assessments
- European Union (EU) Risk Assessment Reports
- Organization for Economic Cooperation and Development (OECD) Assessment Profiles
- EPA Water Quality Criteria documents (human health and/or aquatic life)
- European Chemicals Agency (ECHA) dossiers
- Environmental Health Criteria Monographs from the World Health Organization's International Programme for Chemical Safety (WHO/IPCS)
- Risk assessments from states

For each available assessment identified, any information pertinent to bioaccumulation will be extracted and summarized including any conclusions about bioaccumulation potential and the accompanying rationale.

4.3. Line of Evidence #3: Literature Review of Pertinent Metal-Specific Bioaccumulation Information

The third line of evidence uses studies from the peer-reviewed literature to compile information on the bioaccumulation characteristics of each metal, such as whether they are subject to homeostatic regulation and/or show consistent evidence of biomagnification or biodilution in aquatic food webs.

As described above, individual BCF, BAF, and BMF values found in the literature have little utility for the purposes of NCD's broad-scale risk assessments. However, more general information about the bioaccumulation behavior of specific metals can contribute to the WoE used to determine a B rating. For example, if an inverse relationship between BCF and exposure concentration in the water is consistently observed for a given metal, this suggests that the metal undergoes homeostatic regulation, lending support to a conclusion that the metal has low bioaccumulation potential.[4] Similarly, a body of literature demonstrating no significant relationship between the organism metal concentration and trophic level within food webs could suggest that the metal is unlikely to undergo trophic transfer in similar food webs, lending support to a low bioaccumulation potential rating.

A literature search will be conducted for each metal and relevant information for aquatic food webs will be extracted and summarized. The focus will be to derive general information on the bioaccumulation process and its controls (e.g., homeostatic regulation) for a given metal, rather than to identify quantitative BCF and BAF values.

5. Situations in Which There is Incomplete or Conflicting Information

EPA has a long history of using WoE approaches to support scientific conclusions for risk assessment.[12-15] This document provides a reasonable, scientifically supportable approach using three lines of evidence:

1. An assessment that integrates a literature review of available field measurements of metal concentrations in fish and shellfish with critical concentrations based on human chronic oral toxicity values criteria (i.e., non-cancer screening level, NCSL, and/or cancer slope factor, CSF).
2. A critical review of the test data, conclusions, and rationales related to bioaccumulation utilized by other programs, agencies, and organizations in their risk assessments for metals.
3. A critical review of relevant metal-specific information on bioconcentration, bioaccumulation, and trophic transfer available in peer-reviewed scientific literature.

EPA recognizes that there may be metals for which data from one or more lines of evidence are unavailable, of reduced quantity or quality and/or provide conflicting evidence. The quality and adequacy of the data will be considered, and any data gaps or uncertainties will be considered and described transparently. Where possible and appropriate, NCD will fill data gaps with information available for similar metals (i.e., chemically similar, close neighbors in the periodic table). In some cases, NCD may need to rate the metal as "BU", indicating the bioaccumulation potential is unknown (U), to ensure its risks can be conservatively managed.

6. Example of the Application of the WoE Approach to Cobalt

Cobalt is highlighted here to demonstrate the application of the WoE approach to a metal of interest to NCD.

6.1. Cobalt Line of Evidence #1: Fish/Shellfish Tissue Concentrations Relative to Human Intake Criteria

NCD conducted an extensive literature review and extracted data for cobalt studies with reported fish and shellfish tissue concentration data meeting the criteria outlined above from 38 peer-reviewed studies, providing 264 reported values representing cobalt tissue concentrations in 246 species/location combinations (see Appendix).

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[12] U.S. EPA (Office of Pesticide Programs). Guidance on Use of Weight of Evidence When evaluating the Human Carcinogenic Potential of Pesticides. June 2023. https://www.epa.gov/system/files/documents/2023-06/2023%20CARC%20WoE%20Guidance.pdf.

[13] U.S. EPA (Office of Research and Development). Application of Weight-of-Evidence Methods for Transparent and Defensible Numeric Nutrient Criteria. May 2024. https://www.epa.gov/system/files/documents/2024-05/woe_nnc_508_final.pdf.

[14] U.S. EPA. Weight of Evidence in Ecological Assessments. December 2016. EPA/100/R-16/001. https://www.regulations.gov/document/EPA-HQ-OPPT-2016-0654-0100.

[15] OECD (2019), Guiding Principles and Key Elements for Establishing a Weight of Evidence for Chemical Assessment , OECD Series on Testing and Assessment, No. 311, OECD Publishing, Paris, https://doi.org/10.1787/f11597f6-en.

NCD identified an RfD of $0.00\overline{3}$ mg/kg BW/day based on polycythemia.[16] This translates to a NCSL of 36 µg/g using Equation #3 for a consumer with a body weight of 80kg at a fish consumption rate of 7.5g/day, the values currently utilized in NCD risk assessments. An oral CSF could not be established due to inadequate evidence for carcinogenicity of cobalt and cobalt compounds by the oral route of exposure.[17] Therefore, a CSL was not calculated for comparison to fish/shellfish tissue concentrations. Figure 1 compares the fish/shellfish tissue concentrations from the literature to the NCSL, with the blue bars representing fish tissue concentrations for each of the 264 species/location combination measurements and the yellow line indicating the NCSL for cobalt at a FCR of 7.5 g/day.

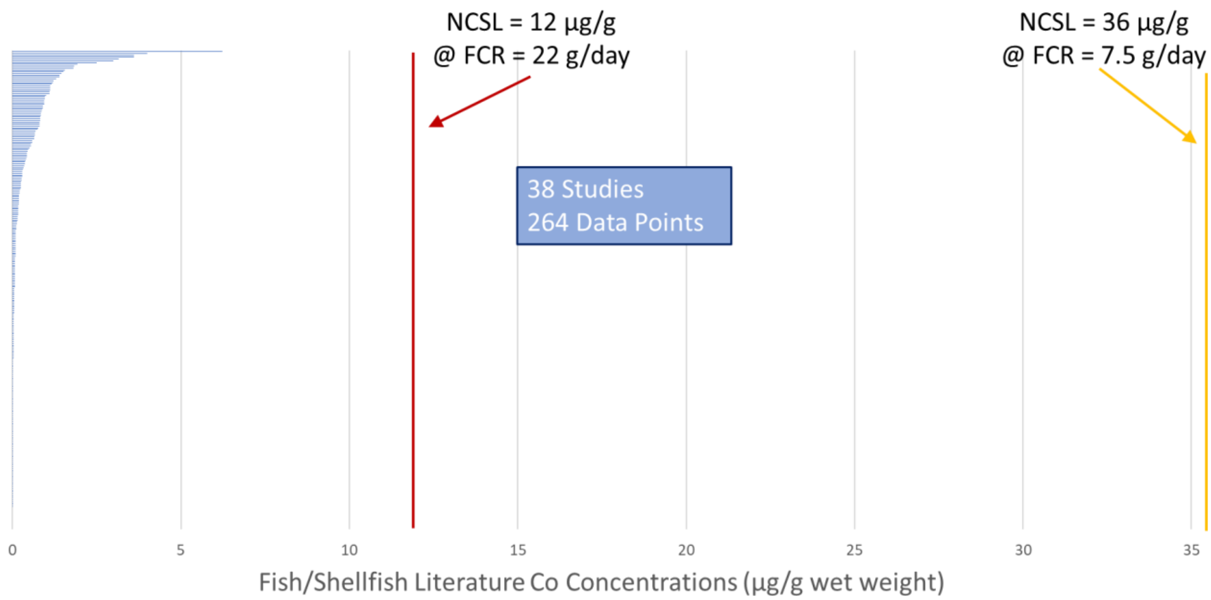

Figure 1: Literature values of cobalt fish tissue concentrations relative to the non-cancer screening level (NCSL) at a FCR of 7.5 g/day (yellow line; value currently utilized in NCD assessments) and 22 g/day (red line; value currently utilized in EPA Office of Water assessments)

All fish and shellfish tissue concentrations across species and environmental and contamination conditions were approximately an order of magnitude or more below the NCSL. Over 75% of reported values were at least two orders of magnitude below the NCSL. Evidence from a wide variety of field conditions indicates that cobalt is unlikely to bioaccumulate in fish or shellfish species representative of those consumed by humans to a concentration exceeding the NCSL.

In addition, no current or historical U.S. fish consumption advisories based on cobalt were found.[18] EPA's database of U.S. fish tissue data collected by States and Tribes for fish

[16] Davis JE, Fields JP. 1958. Experimental production of polycythemia in humans by administration of cobalt chloride. Proc Soc Exp Biol Med 99(2): 493-495.
[17] California Environmental Protection Agency. 2010. Cobalt and Cobalt Compounds Cancer Inhalation Unit Risk Factors: Technical Support Document for Cancer Potency Factors Appendix B. https://oehha.ca.gov/media/downloads/crnr/cobaltcpf100220.pdf.
[18] https://fishadvisoryonline.epa.gov/Advisories.aspx. Accessed 10/23/2024.

consumption advisories contains 43 measurements of cobalt in fish tissue.[19] The highest reported concentration is 1.95 µg/g wet weight, well below the NCSL of 36 µg/g. No reports of adverse health impacts in humans linked to consuming cobalt-contaminated fish were found. Further, cobalt is not included in the EPA Office of Water's list of contaminants to monitor in fish and shellfish advisory programs.[7,20] This information supports the conclusion that there is a low likelihood that human fish/shellfish consumers are consistently exposed to cobalt via fish consumption at levels exceeding the NCSL.

A sensitivity analysis was conducted and led to the same overall conclusion. When OW's current FCR of 22 g/day is considered, rather than NCD's 7.5 g/day, the NCSL decreases from 36 µg/g to 12 µg/g (red line in Figure 1). All fish and shellfish tissue concentrations extracted from the peer-reviewed literature were below this more conservative NCSL. Measurements available in the EPA's database of U.S. fish tissue data collected by States and Tribes for fish consumption advisories were also all below 12 µg/g.

6.2. Cobalt Line of Evidence #2: Benchmarking Against Other Agencies/Organizations

NCD searched programs/agencies to identify existing risk assessments containing information on cobalt bioaccumulation. The search results are shown in Table 2, including if information was available for cobalt and which contained a discussion of cobalt bioaccumulation. NCD found four existing risk assessments that included a discussion of bioaccumulation, all of which concluded that the bioaccumulation and biomagnification potential for cobalt is low. Details of each risk assessment are summarized below.

Table 2: Availability of Existing Risk Assessments for Cobalt

Risk Assessment	Available?	Contains Bioaccumulation Discussion?
ATSDR Toxicological Profile	Y	Y
Priority Substances List Assessment Report under CEPA	N	N/A
ECCC/HC Screening Assessment	Y	Y
EU Risk Assessment Report	N	N/A
OECD Assessment Profile	Y	Y
EPA Water Quality Criteria Document	N	N/A
ECHA dossier	Y	Y
WHO/IPCS Environmental Health Criteria Monograph	N	N/A
Risk assessments from states	N	N/A

The Agency for Toxic Substances and Disease Registry (ATSDR) Toxicological Profile for Cobalt states that mollusks accumulate little cobalt in their edible parts (BCF = 1 to 300 in soft tissue), citing two references.[21] The ATSDR profile also reports cobalt concentrations from literature in some species. In the studies cited by ATSDR, fish cobalt concentrations were <1 µg/g wet weight from three studies in freshwater fish and from two studies on marine fish, consistent with the conclusion from Line of Evidence #1 that fish tissue concentrations are not

[19] https://fishadvisoryonline.epa.gov/FishTissue.aspx. Accessed 10/4/2024.
[20] U.S. EPA Office of Water. 2024. Fact Sheet: Contaminants to Monitor in Fish and Shellfish Advisory Programs. EPA-823-F-24-011. https://www.epa.gov/system/files/documents/2024-06/contaminants-monitor-fish-factsheet-july2024.pdf
[21] ATSDR. 2023. Toxicological Profile for Cobalt: Draft for Public Comment. https://www.atsdr.cdc.gov/ToxProfiles/tp33.pdf.

likely to exceed the NCSL of 36 µg/g. The ATSDR profile also described a study in an amphipod-fish-seabird food web in Antarctica that showed that cobalt concentrations did not increase with trophic level, indicating no biomagnification. **ATSDR concluded that cobalt does not biomagnify.**

The Environment and Climate Change Canada (ECCC) and Health Canada (HC) Screening Assessment states that cobalt and soluble cobalt compounds do not meet the bioaccumulation criteria as set out in the Persistence and Bioaccumulation Regulations of CEPA (i.e., BAF > 5000).[22] The ECCC/HC assessment's discussion of bioaccumulation in aquatic systems begins by acknowledging that BCF and BAF are considered to have "little usefulness in predicting metal hazards." Despite this, ECCC/HC do report literature BAF values from 20 references for various species of algae, invertebrates, fish, and zooplankton for marine and fresh water ranging from 7.4 to 3110, with a mean value of 878 and a median value of 720. The raw data were not presented in the assessment, so the BAF ranges for specific taxa could not be determined. ECCC/HC did note that no groups of organisms seemed to have higher BCF/BAF than others. The assessment also cites four studies reporting zooplankton-fish BMFs (marine and freshwater) ranging from 0.004-0.087. ECCC/HC also cite four TMF studies that showed no statistically significant relationship between cobalt concentration and nitrogen stable isotopes in food webs. **Based on these results, ECCC/HC concluded that the bioaccumulation potential of cobalt in natural ecosystems is relatively low and that cobalt does not present a risk for biomagnification.**

The Organization for Economic Cooperation and Development (OECD) Assessment Profile provides a brief discussion on bioaccumulation, beginning with a statement that cobalt uptake is expected to be regulated to some extent by many organisms through mechanisms of homeostasis and detoxification because it is an essential micronutrient for bacteria, plants and animals.[23] OECD cites the same BAF and BMF studies and values reported in the ECCC/HC Screening Assessment.[22] No additional information is provided. **Considering these values and regulation mechanisms for cobalt in most organisms, OECD expects the bioaccumulation and biomagnification potentials of cobalt in aquatic ecosystems to be low.**

The European Chemicals Agency (ECHA) Dossier for cobalt discusses the essentiality and active regulation of cobalt by homeostatic mechanisms in plants and animals. According to the Dossier, existing information suggests that cobalt does not biomagnify, as with most metals; rather, cobalt exhibits biodilution, particularly in upper levels of both aquatic and terrestrial food chains.[24] From a review of 54 studies, ECHA found that cobalt accumulates from water to plants in aquatic systems (BCF >100 to 5000); however, higher trophic levels show reduced accumulation: BCF ≤ 515 for invertebrates, with both freshwater and marine fish showing BCF/BAF < 10. ECHA also cites a marine trophic transfer study that reported trophic transfer values <1 based on cobalt tissue concentrations across a number of trophic pathways,

[22] ECCC/HC. 2017. Screening Assessment Cobalt and Cobalt-Containing Substances. https://www.canada.ca/content/dam/eccc/migration/ese-ees/dceb359c-245f-4a06-b2e5-62887d47c806/en_cobalt-20fsar-20final-20mai-2025-202017-20.pdf.
[23] OECD. 2010. Initial Targeted Assessment Profile (Human Health and Environment). https://hpvchemicals.oecd.org/ui/handler.axd?id=e6f30459-3de7-402f-a7b6-9b394f34efe1.
[24] https://chem.echa.europa.eu/100.028.325/overview?searchText=cobalt. Accessed 10/25/2024.

incorporating phytoplankton, zooplankton, sea bream and sea bass. **The ECHA Dossier concludes that cobalt does not biomagnify through either freshwater or marine trophic food webs.**

6.3. Cobalt Line of Evidence #3: Literature Review of Pertinent Metal-Specific Bioaccumulation Information

NCD identified ten studies in the peer-reviewed literature that measured trophic transfer of cobalt and found no evidence of biomagnification in aquatic food webs (Table 3). Five studies, three in marine and two in freshwater food webs, observed trophic dilution of cobalt, indicated by an inverse correlation between organismal cobalt concentrations and trophic level, as indicated by stable isotope measurements (i.e., $\delta^{15}N$).[25-29] Chouvelon *et al*. (2019) did not measure $\delta^{15}N$ directly, but found continuously decreasing cobalt concentrations and BAFs from phytoplankton to zooplankton to fish in a marine food web, also indicating trophic dilution of cobalt.[30] The remaining four studies, three in marine food webs and one in a freshwater food web, found no correlation between organismal cobalt concentrations and $\delta^{15}N$, indicating a lack of cobalt biomagnification.[31-34] None of the studies identified observed evidence of biomagnification in aquatic food webs.

[25] Asante, K.A., et al. 2008. Trace elements and stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) in shallow and deep-water organisms from the East China Sea. Environmental pollution, 156(3), pp.862-873.
[26] Balzani, P., et al. 2021. Combining metal and stable isotope analyses to disentangle contaminant transfer in a freshwater community dominated by alien species. Environmental Pollution, 268, p.115781.
[27] Briand, M.J., et al. 2018. Tracking trace elements into complex coral reef trophic networks. Science of the Total Environment, 612, pp.1091-1104.
[28] Fey, P., et al. 2019. Does trophic level drive organic and metallic contamination in coral reef organisms?. Science of the Total Environment, 667, pp.208-221.
[29] Revenga, J.E., et al. 2012. Arsenic, cobalt and chromium food web biodilution in a Patagonia mountain lake. Ecotoxicology and Environmental Safety, 81, pp.1-10.
[30] Chouvelon, T., et al. 2019. Patterns of trace metal bioaccumulation and trophic transfer in a phytoplankton-zooplankton-small pelagic fish marine food web. Marine Pollution Bulletin, 146, pp.1013-1030.
[31] Campbell, L.M., et al. 2005. Mercury and other trace elements in a pelagic Arctic marine food web (Northwater Polynya, Baffin Bay). Science of the Total Environment, 351, pp.247-263.
[32] Erasmus, A., et al. 2020. Trophic transfer of pollutants within two intertidal rocky shore ecosystems in different biogeographic regions of South Africa. Marine Pollution Bulletin, 157, p.111309.
[33] Ikemoto, T., et al. 2008. Biomagnification of trace elements in the aquatic food web in the Mekong Delta, South Vietnam using stable carbon and nitrogen isotope analysis. Archives of environmental contamination and toxicology, 54, pp.504-515.
[34] Nfon, E., et al. 2009. Trophodynamics of mercury and other trace elements in a pelagic food chain from the Baltic Sea. Science of the Total Environment, 407(24), pp.6267-6274.

Table 3: Summary of Studies that Examine the Trophic Transfer of Cobalt (Co)

Organism Description	Location	Freshwater/ Marine	Reference	Evidence	Conclusion
35 fish species and 15 invertebrate species of diverse feeding strategies	East China Sea	Marine	Asante *et al.* (2008)[25]	Significant negative correlation between trophic level ($\delta^{15}N$) and organismal Co concentrations.	Co undergoes trophic dilution in the food web studied.
Three crustacean and eight fish species of various trophic levels	Arno River (central Italy)	Freshwater	Balzani *et al.* (2021)[26]		
Primary producers, consumers (herbivorous, omnivorous and carnivorous invertebrates) and high-level predators (anguilliform fish)	Lagoon of New Caldonia (South Pacific)	Marine	Briand *et al.* (2018)[27]		
Ice algae, three species of zooplankton, Arctic cod (Boreogadus saida), ringed seals (Phoca hispida) and eight species of seabirds	Northwater Polynya, Baffin Bay (arctic Ocean)	Marine	Fey *et al.* (2019)[28]		
Plankton, benthic invertebrates, forage fish, and, plants	LakeMoreno, Patagonia, Argentina	Freshwater	Revenga *et al.* (2012)[29]		
Phytoplankton, zooplankton, and pelagic fish (European sardine and anchovy)	Gulf of Lions, NW Mediterranean Sea	Marine	Chouvelon *et al.* (2019)[30]	Decreasing Co BAF with increasing general trophic level.	
Ice algae, three species of zooplankton, Arctic cod (Boreogadus saida), ringed seals (Phoca hispida) and eight species of seabirds	Northwater Polynya, Baffin Bay (Arctic Ocean)	Marine	Campbell *et al.* (2005)[31]	No significant correlation between trophic level ($\delta^{15}N$) and organismal Co concentrations.	Co does not biomagnify in the food web studied.
37 species of algae, invertebrates, and (i.e. primary producers, primary consumers, secondary consumers and tertiary consumers)	South African coast (Indian Ocean)	Marine	Erasmus *et al.* (2020)[32]		
Particulate organic matter (POM), phytoplankton, gastropod (1 species), crustaceans (5 species), and fish (15 species)	Mekong Delta, South Vietnam	Freshwater	Ikemoto *et al.* (2008)[33]		
Phytoplankton, zooplankton, mysis and herring	Baltic Sea	Marine	Nfon *et al.* (2009)[34]		

Jeffree *et al*. (2014) measured metal concentrations, including cobalt, in two fish species along a contamination gradient within a mining impacted river in Australia.[35] Populations of both fish species exposed to the highest concentrations of mine-related metals (cobalt, copper, lead, manganese, nickel, uranium, and zinc) in surface water and sediment had the lowest tissue (bone, liver, and muscle) concentrations of these metals. The authors explored several hypotheses for the observation and concluded that the most plausible interpretation is that populations of both fish species have modified kinetics within their metal bioaccumulation physiology, via adaptation or tolerance responses, to reduce their body burdens of metals.

6.4. Cobalt Conclusions from the WoE Approach

EPA used a WoE approach to determine the bioaccumulation potential of cobalt for the purposes of TSCA new chemicals risk assessments. All three lines of evidence support a low concern for cobalt bioaccumulation in aquatic food webs.

- *LoE 1 (Fish/shellfish tissue concentrations relative to human intake criteria)*: There is a low likelihood that human consumers are consistently exposed to cobalt via fish/shellfish consumption at levels exceeding the non-cancer screening level (NCSL) over a lifetime of consumption.
- *LoE 2 (Benchmarking against other agencies/organizations)*: Four out of four available risk assessments concluded that the bioaccumulation and biomagnification potential for cobalt is low.
- *LoE 3 (Literature review of pertinent metal-specific bioaccumulation information)*: Ten EPA-reviewed studies indicated either trophic dilution in aquatic food webs or no relationship between cobalt concentrations in organisms and δ15N (an indicator of trophic level); none found evidence of biomagnification. Another study documented an inverse relationship between cobalt concentrations in fish and environmental contamination levels, suggesting active homeostatic regulation of the metal in these species.

Based on the weight of evidence, cobalt has a low potential to bioaccumulate and is therefore assessed as **B*low** rating in NCD risk assessments.

[35] Jeffree, R. A., S. J. Markich and J. R. Twining (2014). "Diminished Metal Accumulation in Riverine Fishes Exposed to Acid Mine Drainage over Five Decades." PLOS ONE 9(3): e91371.

Appendix

Literature with Cobalt Fish or Shellfish Tissue Concentration Data Used for Line of Evidence #1 (Fish and Shellfish Tissue Concentrations Relative to Human Intake Criteria)

1. Anandkumar, A., R. Nagarajan, K. Prabakaran, C. H. Bing and R. Rajaram (2018). "Human health risk assessment and bioaccumulation of trace metals in fish species collected from the Miri coast, Sarawak, Borneo." Mar Pollut Bull **133**: 655-663.
2. Andreji, J., Stránai, I., Massányi, P., & Valent, M. (2005). “Concentration of selected metals in muscle of various fish species.” Journal of environmental science and health **40(4):** 899-912.
3. Asante, K. A., T. Agusa, H. Mochizuki, K. Ramu, S. Inoue, T. Kubodera, S. Takahashi, A. Subramanian and S. Tanabe (2008). "Trace elements and stable isotopes (delta13C and delta15N) in shallow and deep-water organisms from the East China Sea." Environ Pollut **156**(3): 862-873.
4. Badsha, K. S. and C. R. Goldspink (1988). "Heavy metal levels in three species of fish in Tjeukemeer, a Dutch polder lake." Chemosphere **17**(2): 459-463.
5. Balzani, P., P. J. Haubrock, F. Russo, A. Kouba, P. Haase, L. Vesely, A. Masoni and E. Tricarico (2021). "Combining metal and stable isotope analyses to disentangle contaminant transfer in a freshwater community dominated by alien species." Environ Pollut **268**(Pt B): 115781.
6. Barlas, N. (1999). "A pilot study of heavy metal concentration in various environments and fishes in the Upper Sakarya River Basin, Turkey." Environmental Toxicology **14**(3): 367-373.
7. Bouchoucha, M., R. Chekri, A. Leufroy, P. Jitaru, S. Millour, N. Marchond, C. Chafey, C. Testu, J. Zinck, P. Cresson, F. Miralles, A. Mahe, N. Arnich, M. Sanaa, N. Bemrah and T. Guerin (2019). "Trace element contamination in fish impacted by bauxite red mud disposal in the Cassidaigne canyon (NW French Mediterranean)." Sci Total Environ **690**: 16-26.
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9. Chouvelon, T., E. Strady, M. Harmelin-Vivien, O. Radakovitch, C. Brach-Papa, S. Crochet, J. Knoery, E. Rozuel, B. Thomas, J. Tronczynski and J. F. Chiffoleau (2019). "Patterns of trace metal bioaccumulation and trophic transfer in a phytoplankton-zooplankton-small pelagic fish marine food web." Mar Pollut Bull **146**: 1013-1030.
10. Erasmus, A., Y. Ikenaka, S. M. M. Nakayama, M. Ishizuka, N. J. Smit and V. Wepener (2020). "Trophic transfer of pollutants within two intertidal rocky shore ecosystems in different biogeographic regions of South Africa." Mar Pollut Bull **157**: 111309.
11. Gashkina, N. A. and T. I. Moiseenko (2020). "Influence of Thermal Pollution on the Physiological Conditions and Bioaccumulation of Metals, Metalloids, and Trace Metals in Whitefish (Coregonus lavaretus L.)." Int J Mol Sci **21**(12).
12. Gebrekidan Asgedom, A., M. Berhe Desta and Y. Weldegebriel Gebremedh (2013). "Bioaccumulation of Heavy Metals in Fishes of Hashenge Lake, Tigray, Northern Highlands of Ethiopia." American Journal of Chemistry **2**(6): 326-334.

13. Greig, R. A. and J. Jones (1976). "Nondestructive neutron activation analysis of marine organisms collected from ocean dump sites of the middle eastern United States." Arch Environ Contam Toxicol **4**(4): 420-434.
14. Hantoush, A., G. Al-Najare, A. Amteghy, H. Al-Saad and K. Ali (2012). "Seasonal variations of some trace elements concentrations in Silver Carp Hypophthalmichthys molitrix Consolidated from farms in central Iraq." Marsh Bulletin **7**: 126-136.
15. Hellou, J., L. L. Fancey and J. F. Payne (1992). "Concentrations of twenty-four elements in bluefin tuna, Thunnus thynnus from the Northwest Atlantic." Chemosphere **24**(2): 211-218.
16. Ikemoto, T., N. P. Tu, N. Okuda, A. Iwata, K. Omori, S. Tanabe, B. C. Tuyen and I. Takeuchi (2008). "Biomagnification of trace elements in the aquatic food web in the Mekong Delta, South Vietnam using stable carbon and nitrogen isotope analysis." Arch Environ Contam Toxicol **54**(3): 504-515.
17. Jayaprakash, M., R. S. Kumar, L. Giridharan, S. B. Sujitha, S. K. Sarkar and M. P. Jonathan (2015). "Bioaccumulation of metals in fish species from water and sediments in macrotidal Ennore creek, Chennai, SE coast of India: A metropolitan city effect." Ecotoxicol Environ Saf **120**: 243-255.
18. Jeffree, R. A., S. J. Markich and J. R. Twining (2014). "Diminished Metal Accumulation in Riverine Fishes Exposed to Acid Mine Drainage over Five Decades." PLOS ONE **9**(3): e91371.
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