

Hazard/Risk Assessment

Method Development for Determining the Removal of Metals from the Water Column under Transformation/Dissolution Conditions for Chronic Hazard Classification

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Abstract: An extension of the transformation/dissolution protocol (T/DP) was developed and evaluated as a tool to measure the removal of metals from the water column for chronic aquatic hazard classification. The T/DP extension (T/DP-E) consists of 2 parts: T/DP-E part 1, to measure metal removal from the water column via binding of metals to a substrate and subsequent settling, and T/DP-E part 2, to assess the potential for remobilization of metals following resuspension. The T/DP-E methodology (672-h [28-d] removal period, 1-h resuspension event, and 96-h resettling period) was tested using Cu, Co, and Sr solutions in the presence of a substrate. The metal removal rates varied from rapid removal for Cu to slower rates of removal for Co and Sr. The resuspension event did not trigger any increase in dissolved Cu, Co, or Sr. Additional 96-h experiments were conducted using dissolved Ni, Pb, Zn, and Ag and supported the conclusion that the T/DP-E is sufficiently robust to distinguish removal rates between metals with a wide range of reactivities. The proposed method provides a means to quantify the rate of metal removal from the water column and evaluate remobilization potential in a standardized and reliable way. *Environ Toxicol Chem* 2019;38:2032–2042. © 2019 SETAC

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INTRODUCTION

The transformation/dissolution protocol (T/DP), developed within the framework of the Organisation for Economic Co-operation and Development (2001) measures the rate and extent to which metal-bearing substances can produce soluble ionic species in aqueous media under a set of standard laboratory conditions. The data sets generated from the T/DP examination are then compared with ecotoxicity reference values to determine the short- and long-term potential aquatic hazard of the metal-bearing substance for hazard classification purposes. The T/DP has been included in the United Nations Globally Harmonized System (GHS) as Annex 10 (United Nations 2017). The T/DP has been successfully applied to derive hazard classifications on a wide range of metal-

bearing substances including metals, metal compounds, mattes, alloys, and concentrates (Skeaff et al. 2008, 2011; Skeaff and Beaudoin 2014; Huntsman et al. 2018).

The GHS (United Nations 2017) and the European Union's Classification, Labelling and Packaging (CLP; European Union 2013) hazard classification schemes include the concept of degradation, whereby rapid degradation from the water column for organics (>70% removal to CO₂ in 28 d) results in one category difference in the classification scheme. For metals and inorganic metal compounds, the rapid and irreversible removal of the metal from the water column can serve as a surrogate for the rapid degradation concept for organics. Unlike organic substances, metal ions are not degraded in the strict sense; but metals are transformed through a wide range of processes such as sorption, sedimentation, and mineralization. This can lead to a reduction in bioavailability and toxicity of the metal and, therefore, constitutes a reduction in long-term hazard potential (Burton et al. 2019, this issue).

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The aim of the present study was to evaluate if an extension of the T/DP (T/DP-E) could be used to assess the removal of metals from the water column in a standardized way, analogous to the concept of degradability for organic substances mentioned in the GHS (United Nations 2017) and CLP (European Union 2013) classification systems. To this end, the T/DP method was extended with 2 parts: part 1, to measure metal removal from the water column through binding to a substrate with subsequent settling (removal part), and part 2, to assess the remobilization of metals following resuspension of substrate (irreversibility part).

The experimental setup of this extension was developed to provide a standardized approach which includes the most relevant mechanisms for metal removal occurring in aquatic systems: 1) changes in metal speciation in the presence of a substrate and the associated removal attributable to these speciation changes, and 2) the potential for remobilization of metals on resuspension. Although several more complex processes may occur in real aquatic systems (see Burton et al. 2019 this issue), the experimental setup strikes a balance between 1) key processes, and 2) achieving a reasonable level of standardization and reproducibility. A standard methodology allows for metals to be assessed in a uniform, repeatable, and comparative way. This forms a key part of the rationale for assessing removal from the water column for metals. The following sections describe the protocol development, the rationale for selecting test parameters, and the method application and results for the T/DP-E. It is important to note that the original T/DP was not modified; therefore, all elements mentioned in the T/DP continue to be applicable.

MATERIAL AND METHODS

Proposed test method

The T/DP calls for the addition of 3 loadings of a metal-bearing substance into an aquatic medium, with agitation, followed by the evaluation of metal concentrations in solution over a 28-d (672-h) period. Introduced in the T/DP-E is the addition of substrate particles into a solution of a dissolved metal, with a period of rapid mixing and then settling.

One-liter Schott-Duran flasks were used for the T/DP-E, and all flasks were rinsed with approximately 200 mL of aqua regia to ensure that glassware used in the tests was

clean. Flasks were shaken and then allowed to stand for approximately 16 h in a fume hood. Flasks were then rinsed thoroughly with tap water and then with deionized water. Finally, flasks were filled with deionized water, capped, and stored until used.

The 10× diluted Organisation for Economic Co-operation and Development (OECD) 203 (Organisation for Economic Co-operation and Development 1992) medium at pH 6 was prepared in 20-L Nalgene carboys by adding the weighed reagents (Table 1) to deionized water and filtering through a 0.2- μ m 90-mm-diameter membrane filter, which also served to sterilize the medium. The composition of the 10× diluted OECD medium was obtained by applying the FactSage system (FactSage 2007) to calculate a pH of 6.09 when equilibrated with an atmosphere containing 0.5% CO₂ with the balance as air. The medium has low water hardness, is relatively dilute, and, as such, will have limited buffering capacity. As a guide for pH control, the T/DP proposes a variability of ± 0.2 pH units over a period of 1 wk. It also indicates that the pH should not be adjusted during the test using an acid or alkali. Consequently, no attempt was made to adjust pH during the T/DP-E tests.

A total of 9 Schott-Duran glass flasks containing 1 L of aqueous medium were conditioned to pH 6 by bubbling with 0.5% CO₂ with the balance as air for 24 h. Six of these flasks contained 10× diluted OECD 203 medium with an additional 1 mg/L concentration of the metal of interest, prepared from a soluble metal salt with a counter-ion that is not expected to affect the results of the test (Supplemental Data, Table S1). Of these 6 flasks, 3 had substrate added and 3 were substrate blanks, with no substrate added to correct for the dilution caused by sampling and replenishment of test medium during the experiment. The remaining 3 flasks of the 9 contained 10× diluted OECD 203 medium only for the procedural blanks. A procedural blank is processed identically to and at the same time as the other flasks. The blanks represent the background concentrations associated with factors such as glassware cleanliness, sampling, handling, and solution analysis. The test scheme is shown in Table 2. For the duration of the experiment, all flasks were bubbled with the gas containing 0.5% CO₂ with the balance air.

At 0 h, prior to substrate addition, pH, temperature, and dissolved oxygen of the test solutions were measured with a HACH HQ40d multimeter with a pH probe (PHC10101) and

TABLE 1: Composition of 10× diluted Organisation for Economic Co-operation and Development 203 (International Organization for Standardization 6341) aqueous medium (no micronutrients) for a target pH of 6 (United Nations 2017, p. 523)^a

Component	MW	mg/L	Mg ²⁺	Ca ²⁺	Cl ⁻	HCO ₃ ⁻	SO ₄ ²⁻	Na ⁺	K ⁺	mmol/L
CaCl ₂ × 2H ₂ O	147.01	29.40		8	14.18					0.20
MgSO ₄ × 7H ₂ O	246.47	12.33	1.22				4.80			0.05
NaHCO ₃	84.01	6.48				4.70		1.77		0.077
KCl	74.55	0.58			0.27				0.30	0.0077
Totals (mg/L)			1.22	8.01	14.45	4.70	4.80	1.77	0.30	
mmol/L			0.05	0.20	0.41	0.077	0.05	0.077	0.0077	
Hardness as CaCO ₃ (mg/L)		25.0								

^aCalculated pH of medium when in equilibrium with 0.5% CO₂ balance air at 21.5 °C: 6.09 (FactSage 2007). MW = molecular weight.

TABLE 2: Transformation/dissolution protocol extension testing scheme at pH 6

Test no.	Cu			Co			Sr		
	Procedural blank: no metal; no substrate	Metal loading 1 mg/L, no substrate	Metal loading 1 mg/L, substrate loading 10 g/L	Procedural blank: no metal; no substrate	Metal loading 1 mg/L, no substrate	Metal loading 1 mg/L, substrate loading 10 g/L	Procedural blank: no metal; no substrate	Metal loading 1 mg/L, no substrate	Metal loading 1 mg/L, substrate loading 10 g/L
1	x								
2		x							
3			x						
4	x								
5		x							
6			x						
7	x								
8		x							
9			x						
10				x					
11					x				
12						x			
13				x					
14					x				
15						x			
16				x					
17					x				
18						x			
19							x		
20								x	
21									x
22							x		
23								x	
24									x
25							x		
26								x	
27									x

a dissolved oxygen probe (LD010101). At this point, triplicate 12-mL solution samples were drawn with a 15-mL syringe, passed through a 0.8/0.2- μ m Acrodisc syringe filter into a 15-mL sample tube, and acidified with 2 drops of Fisher trace metal-grade nitric acid (68–71%), which lowers the pH to approximately 1.5.

Ten grams of the air-dried substrate was then added to each of the 3 flasks containing 1 mg/L metal solution following the scheme shown in Table 2. Flasks were agitated on the orbital shaker for 1 min at 100 rpm to ensure mixing of the sediment in the solution. The pH, temperature, and dissolved oxygen of the test solutions were measured at each sampling time of 2, 6, 24, 48, 96, 168, 336, and 672 h and the solutions analyzed for the metal of interest. Selected samples were analyzed for total metal concentrations (including measurement of Fe as a fingerprint of the substrate). Flasks were replenished after each sampling point with the 10 \times diluted OECD 203 up to the 1-L mark to maintain the same substrate-to-solution ratio throughout the experiment.

The T/DP-E test is performed under static conditions, with the exception of the initial mixing (T/DP-E part 1) and the agitation for the resuspension event (T/DP-E part 2). Part 2 of the T/DP-E mimics a resuspension event, which aims to address metal remobilization under such conditions. After completion of the T/DP-E part 1, the flasks were agitated vigorously (e.g., in

an orbital shaker at 150 rpm for 1 h). The solutions were then allowed to react for 4 d (96 h) in static conditions. The solutions were sampled periodically at 0.5, 1, 2, 6, 24, 48, and 96 h after completion of the resuspension event. Samples were analyzed for dissolved metal concentrations, pH, dissolved oxygen concentration, and temperature; selected samples were analyzed for total metal concentrations.

Processing and analysis of substrates and solutions

Geochemical data provided by the Geological Survey of Canada of over 50 sediments collected from across Canada were evaluated for potential substrate use in the T/DP-E. The sediments, collected for exploration purposes from both streams and lakes, had been air-dried and stored in sample bags. Based on the geochemical composition, 3 sediments were then selected to represent a low binding substrate (LBS), a high binding substrate (HBS), and a substrate of intermediate composition (IBS). To remove the larger pebbles, shells, and twigs, the substrate was screened using a 14-mesh (1.41-mm) sieve; and the material passing the 14 mesh sieve was then used for the T/DP-E examination.

This substrate material was analyzed in triplicate by CanmetMINING's Analytical Services Group (ASG). The material was subjected to a microwave-accelerated 4-acid (HCl, HNO₃,

HF, and H₃BO₃) digestion system (Analytical Services Group 2013a), followed by analysis using an inductively coupled plasma atomic emission spectrometer (Varian Vista) for total Al, Mn, and Fe and using an inductively coupled plasma mass spectrometer (ICP-MS) for Co, Ni, Ag, Cu, Zn, and Pb (either a Thermo_Fisher X-series II or a Perkin Elmer Elan 6100). An additional aqua regia digestion followed by analysis for Fe was conducted to compare with the Fe values in the ARCHE Consulting (2013) report. Total carbon and total organic carbon (TOC) were measured using an ELTRA CS-2000 (Analytical Services Group 2013b). For the particle size analysis of the substrate, a Fritsch Analysette 22 Laser Particle Sizer Dry Dispersing was used. ICP-MS was used for the analysis of the solutions generated during the TD/P-E. During each analysis run, the ASG followed the established quality control measures (Supplemental Data, S1) to verify the instrument calibration and to monitor instrument stability.

Calculation of aqueous metal concentrations

For each individual test on each loading, a within-vessel average concentration was calculated as the arithmetic mean of the analytical determinations of the 3 sample solutions drawn at a particular sampling time. A between-vessel average was also calculated as the arithmetic mean of the average of the 3 within-vessel values. To calculate the net average concentration for each loading at each sampling time, the between-vessel average for the procedural blanks was subtracted from the corresponding concentrations in loaded tests. The standard deviations and the coefficients of variation were calculated.

Calculation of dilution factor for volume correction

The purpose of the substrate blank (1 mg/L metal solution in 10× diluted OECD 203 medium without substrate addition) was to allow for the calculation of a dilution factor. Each time a sample was taken, the solution was replenished with the 10× diluted pH 6 OECD medium. The dilution factor was calculated by correcting for the volume taken at each sampling time (see Supplemental Data, Table S2). This factor was then applied to the flasks with substrate loadings by multiplying the metal concentration by the dilution factor at any given time. This step is also useful to determine if metals are being removed from solution by variables other than the substrate (e.g., sorption onto the glass). If metal concentrations in the substrate blank remain stable after correction for dilution, then no other process is removing metals from solution.

Method development rationale

Rationale for initial 1 mg/L dissolved metal concentration

In the original T/DP, the test is initiated by adding the solid metal-bearing test substance to the aquatic medium. However,

in the T/DP-E, the test is initiated with a solution of 1 mg/L of dissolved metal, prepared from a soluble salt with a counterion that is not expected to affect the results of the test. This modification was introduced to ensure that only metal removal was being measured, not a combination of both dissolution of the solid metal-bearing test substance and removal processes acting on the solubilized metal. The rationale for this approach is that in the T/DP, environmental toxicity and classification are determined at the level of substances. In contrast, metal removal from the water column can be determined at the level of dissolved metal ions. Therefore, it is appropriate for the development of the test method to initiate the test with a dissolved metal. When integrated in a testing scheme, the outcome of the T/DP will be used directly for the T/DP-E phase. The highest metal substance loading in the chronic hazard classification test scheme is 1 mg/L, and therefore, this was selected for this purpose as the target dissolved metal ion concentration.

Rationale for 10-g/L substrate addition

In aquatic systems, suspended solids are added from external sources (e.g., inflow and surface runoff) as well as internal sources (e.g., algal production, resuspension). Suspended solids leave the water column through settling and washout (in surface waters). In mechanistic lake models, solid balances considering the above sources/sinks are often assumed to be at steady state. Consequently, the suspended solids concentration is constant, and there is continual transport of solids out of the water column via settling (Thomann and Mueller 1987).

From the perspective of metal added to the water column, adsorption to suspended solids and subsequent settling represents a continual removal process. Added metal is transported on a continuous “solids conveyor belt” out of the water column. This flux of solids can be quantified by multiplying the suspended solids concentration (m_{s1}) by the settling velocity (w_s): $F_s = m_{s1} \times w_s$. Typical values for m_{s1} and w_s for a generalized water body in the EUSES model are 15 mg/L for the suspended solids concentration and 2.5 m/d for the settling velocity (European Commission 2004; European Chemicals Agency 2016). These values yield a solids flux (F_s) of 37.5 g/m²/d.

For practical purposes, the T/DP-E entails a single addition of substrate rather than a continuous addition. After mixing, this substrate is allowed to settle. Therefore, the substrate concentration in the experimental system is not constant but decreases from the initial value. The solids flux value was used to inform selection of an initial solids concentration that yields a similar integrated solid load leaving the water column. The T/DP-E (part 1) has a duration of 28 d. The integrated areal load of solids based on the solids flux is 1050 g/m². The required initial solids concentration to produce this integrated areal load in the T/D P-E (part 1) was evaluated by multiplying the integrated areal load value (1050 g/m²) by the ratio of the experimental flask area to experimental flask volume (0.00709 m²/L; see Supplemental Data, S2, for details). The resulting solids concentration was 7.4 g/L. A rounded value of 10 g/L was selected as the substrate loading rate to use in the T/DP-E experiments.

Rationale for the selection of an LBS

In the T/DP-E, a substrate that has relatively low or weak metal binding properties, an LBS, is used to be conservative with respect to metal removal. The substrate is characterized by comparison to typical European sediment characteristics (ARCHE Consulting 2013; Table 3). The main properties that drive metal binding were selected as Fe content and TOC content. It should be noted that pH and acid volatile sulfide (AVS) content also drive metal binding in sediments. These were not considered in the present study because 1) the pH is generally buffered at a given value in the T/DP-E, and 2) as a worst-case condition, the T/DP-E is conducted under oxic conditions, where AVS, which may bind metals, may not be present. The 10th percentile of the Fe concentrations (measured in aqua regia extract) and TOC concentrations are 0.75 and 1.3%, respectively (ARCHE Consulting 2013). Considering these substrate parameters, 3 substrates were identified, collected, and tested; and the LBS was selected based on the bulk chemistry (Table 4).

Substrates were evaluated under a variety of T/DP-E conditions, including different substrate loading levels (1 mg–10 g) and varying pHs and metal concentrations (P. Huntsman, unpublished data). As expected, pH control is an important criterion for the T/DP-E, just as in the T/DP. For some substrates, it was difficult to maintain pH of the solution at the target value of 6 for the entire duration of the experiment. This resulted in substrate LBS being selected for use in the T/DP-E in the majority of the experiments because pH did not increase above 6.

Rationale for experimental pH

The aqueous test media used in the T/DP and in the T/DP-E are based on the aquatic toxicity testing medium OECD 203 (International Organization for Standardization 6341). The pH of an aqueous solution is established by 1) its composition, 2) the composition of the atmosphere with which it is in equilibrium, and 3) any solids that may react with the solution. In the T/DP, the pH is controlled by equilibrating CO₂ with the bicarbonate in the media. The T/DP tests are carried out at a pH that maximizes the concentration of the dissolved metal ions within a pH range of 6 to 8.5. Extensive T/DP examinations of metal-bearing substances have shown that, for most metals, tests conducted at pH 6 result in greater metal dissolution rates than at higher pHs (Skeaff et al. 2008, 2011; Skeaff and Beaudoin 2014). Furthermore, ample data in real aquatic environments have demonstrated that, for most metals, the dissolution increases with decreasing pH (Burton et al. 2019, this issue and references therein). For this reason, the T/DP-E was developed at a relatively low pH of 6.

Rationale for static conditions during the metal-removal step

In the T/DP, the test solution is agitated throughout the test to maintain the flow of aqueous medium over the test substance. Because the T/DP-E is initiated with a solution of dissolved metal salt, this is no longer relevant. Furthermore, one of the aims of the T/DP-E is to evaluate settling of

a substrate under standardized conditions. Therefore, the T/DP-E test is performed under static conditions, with the exception of the initial mixing (T/DP-E part 1) and the agitation to mimic a resuspension event (T/DP-E part 2). It should be noted, however, that the continuous bubbling of the T/DP-E solution does induce some minimal mixing.

Rationale for resuspension conditions to check for potential remobilization

Surficial sediments may be resuspended and metals potentially remobilized into the overlying water. Sediment disturbances, such as resuspension attributable to propeller wash events and episodic hydrologic events, may expose sediments to surface waters with higher concentrations of dissolved oxygen; and oxidation of reduced ligands could increase metal solubility and cause toxicity to aquatic organisms (Chapman et al. 1998).

Evidence shows that metal oxides and oxyhydroxides play a role during resuspension events and acid-volatile sulfides may be oxidized during such events, but released metals are bound by the oxides and oxyhydroxides and returned to the sediment layer (Burton et al. 2019, this issue).

METHOD APPLICATION AND RESULTS

Experiments were conducted on Cu, Co, and Sr using the T/DP-E parts 1 and 2 and consisted of a 28-d (672-h) full test removal period, a 1-h resuspension event, and a 96-h period to assess remobilization and irreversibility. Additional 96-h removal experiments were conducted on Cu, Co, Ni, Zn, Pb, and Ag to determine the applicability of the method to a range of metals.

Substrate analysis

Results from the geochemical analyses of 3 substrates are found in Table 4. Based on TOC and percentage Fe values, the LBS was found to be a good match with the 10th percentile of typical European sediment characteristics (ARCHE Consulting 2013; Table 3). In addition, the IBS and HBS substrates correspond well with the ARCHE Consulting (2013) average and 90th percentile values, respectively. From the results of the particle size analysis, the LBS could be described as a medium sand, the IBS as a coarse sand, and the HBS as predominantly silt and clay (Supplemental Data, Table S3).

TABLE 3: Aqua regia extractable average concentrations of Fe and total organic carbon (TOC) average concentrations in European sediments

Percentile	Fe (wt%)	TOC (%)
P10	0.75	1.3
Average	2.25	3.7
P90	4	6.7

From ARCHE Consulting (2013).

TABLE 4: Results of substrate geochemical analysis

Substrate	Type	Fe (wt%)	TOC (%)	Total carbon (%)	Co (ppm)	Mn (ppm)	Ni (ppm)	Ag (ppm)	Cu (ppm)	Zn (ppm)	Pb (ppm)
LBS	Stream	1.56	1.2	1.1	11.9	506	34	0.05	18	54.7	13.5
IBS	Stream	3.61	2.5	3.4	17.4	1413	62	0.26	17		21.4
HBS	Lake	5.98	3.3	3.1	36.3	1147	94.6	0.23	87	14.5	15.0

HBS = high binding substrate; IBS = intermediate binding substrate; LBS = low binding substrate.

Results for Cu removal and remobilization

A summary of dissolved and total metal concentrations corrected for dilution for the 1-mg/L Cu, Co, and Sr solutions with the LBS can be found in the Supplemental Data (Table S4).

Measurements taken during the Cu test show that dissolved oxygen varied between 7.8 and 9.3 mg/L (average 8.2 mg/L). The average temperature during the same time period was 22.5 °C, with a range of 20.0 to 23.2 °C. The average pH in the 3 procedural blanks (10× diluted OECD pH 6 medium, no metal and no substrate) was 6.10, with a range of 5.90 to 6.28. The average pH for the flasks with 10 g of the LBS was 6.05, with a range of 5.82 to 6.21.

For the 3 procedural blanks, with the exception of elevated Cu readings at 0 and 2 h, which may be attributable to carry-over on the ICP-MS, the average absolute concentration of Cu was ≤1.0 µg/L throughout the 672-h test.

For the 1-mg/L Cu solutions with the 10-g/L LBS loading, the net average aqueous (aq) Cu concentration at 0 h was 915 µg/L ($\sigma\%$ = 2). This decreased to 198 µg/L ($\sigma\%$ = 33) by 96 h, which would represent a 78% removal of Cu over 96 h. By 672 h, the $\text{Cu}_{(\text{aq})}$ concentration was 44 µg/L ($\sigma\%$ = 8), which would represent 95% removal of Cu. Dissolved Fe concentrations were also measured, peaking at 6 h at 24 µg/L (Figure 1). Measurements of Fe were considered optional in the present study but do provide an additional weight of evidence, particularly as a marker for substrate resuspension.

At select times, samples were also analyzed for total Cu and total Fe (Supplemental Data, Table S4). Initially, total Cu is greater than dissolved Cu, indicating that some fraction of the Cu in the system is associated with particles. At 96 h, the 2 values are more comparable. At 672 h, essentially all of the water column Cu is dissolved. However, total Fe concentrations of 439 µg/L were measured at 2 h, suggesting that fine-grained Fe-bearing minerals were still in solution.

After the 672-h reading was taken, the flasks were shaken for 1 h. The shaking was stopped, and samples were collected within 0.5 h and subsequently at 1, 2, 6, 24, 48, and 96 h. The net corrected average $\text{Cu}_{(\text{aq})}$ concentration at 672 h (or 0 h before resuspension) was 44 µg/L ($\sigma\%$ = 8). After shaking, this increased slightly to 53 µg/L ($\sigma\%$ = 59) at 1 h (after resuspension) but then continued to decline to 33 µg/L ($\sigma\%$ = 14) by 96 h after resuspension (Figure 1). Therefore, it is shown that the resuspension of the substrate does not result in a release of Cu ions into solution. Total Cu concentrations at 0.5 h after resuspension increase to 105 µg/L compared to the dissolved concentration of 34 µg/L. However, total Fe concentrations increase to 1980 µg/L at the same sampling time compared to the dissolved concentrations of 41 µg/L.

Results for Co removal and remobilization

Measurements during the entire test show that dissolved oxygen varied between 8.0 and 9.0 mg/L (average 8.4 mg/L). The average temperature during the same time period was 21.5 °C, with a range of 19.1 to 22.1 °C. The average pH for the flasks with 10 g of the LBS was 6.12, with a range of 5.94 to 6.29.

For the 3 procedural blanks, with the exception of elevated Co readings at 0 and 2 h (as with Cu), which may be attributable to carryover on the ICP-MS, the average absolute concentration of Co was ≤1.2 µg/L throughout the 28-d test.

For the 1-mg/L Co solutions with the 10 g/L LBS loading, the net average $\text{Co}_{(\text{aq})}$ concentration at 0 h was 983 µg/L ($\sigma\%$ = 1; Figure 2). This decreased to 533 µg/L ($\sigma\%$ = 2) by 96 h, which would represent a 46% removal of Cu (corrected for dilution) over 96 h. By 672 h, the $\text{Co}_{(\text{aq})}$ concentration was 453 µg/L ($\sigma\%$ = 7, corrected for dilution), which would represent 54% removal of Co. Dissolved Fe concentrations were also measured, peaking at 6 h at 17 µg/L. At select time periods, samples were also collected for total Co and total Fe. Total Co concentrations in the 96-h samples are slightly greater than the dissolved Co, suggesting some particulate Co is still in suspension. Total Fe concentrations are higher than the equivalent dissolved concentration (Supplemental Data, Table S4).

After the 672-h reading was taken, the flasks were shaken for 1 h. The net corrected (for dilution average) $\text{Co}_{(\text{aq})}$ concentration at 672 h (0 h before resuspension) was 453 µg/L ($\sigma\%$ = 7). After the resuspension, this continued to decline to 416 µg/L ($\sigma\%$ = 3) by 96 h (after resuspension). Therefore, it is shown that resuspension of the substrate does not release Co ions into solution.

Total Co concentrations at 0.5 h after resuspension increase to 517 µg/L compared to the dissolved concentration of 450 µg/L. However total Fe concentrations increase to 1560 µg/L at the same sampling time compared to the dissolved Fe concentrations of 36 µg/L (Supplemental Data, Table S4).

Results for Sr removal and remobilization

Measurements during the entire test show that dissolved oxygen varied between 8.1 and 9.0 mg/L (average 8.5 mg/L). The average temperature during the same time period was 21.7 °C, with a range of 20.6 to 22.5 °C. The average pH for the flasks with 10 g of the LBS was 5.90, with a range of 5.61 to 6.19.

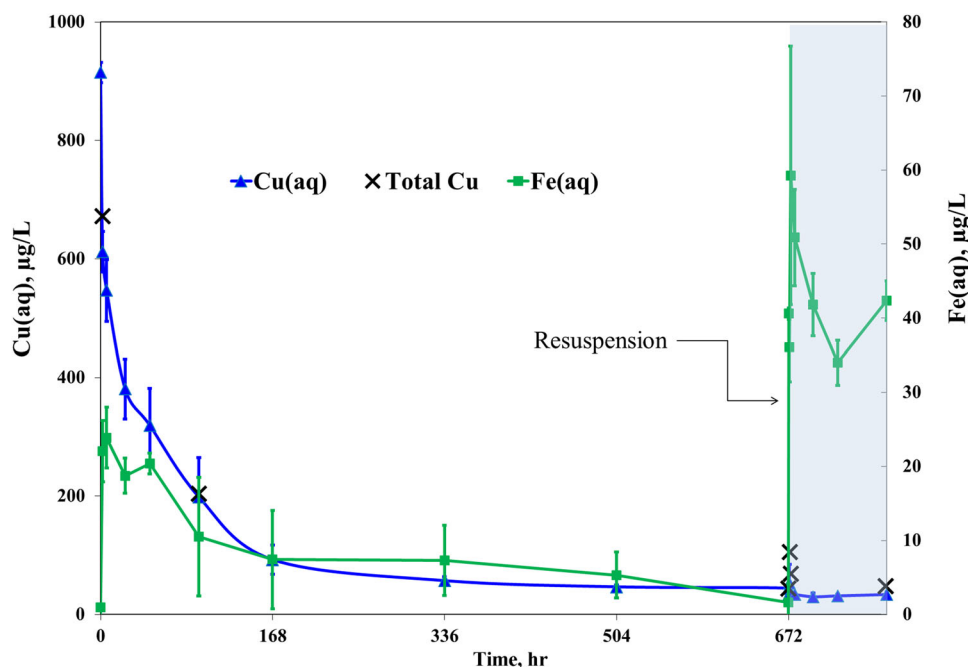


FIGURE 1: Net corrected aqueous Cu and dissolved Fe data for the 1-mg/L Cu solutions at pH 6 using the low binding substrate for the extended transformation/dissolution protocol parts 1 and 2. Blue shaded area indicates the resuspension event.

For the 3 procedural blanks, the average absolute concentration of Sr was ≤ 1.8 $\mu\text{g/L}$ throughout the 672-h test. For the 1-mg/L Sr solutions with the 10-g/L LBS loading, the net average $\text{Sr}_{(\text{aq})}$ concentration at 0 h was 916 $\mu\text{g/L}$ ($\sigma\% = 2$). This decreased to 581 $\mu\text{g/L}$ ($\sigma\% = 3$) by 96 h (Figure 3), which would represent a 37% removal of Sr. By 672 h, the $\text{Sr}_{(\text{aq})}$ concentration was 541 $\mu\text{g/L}$ ($\sigma\% = 5$), which would represent 41% removal of Sr. The average absolute Sr concentration in substrate blank

flasks (Sr solution with no substrate) decreased from 913 to 628 $\mu\text{g/L}$ over the course of 672 h, primarily because of sampling and the addition of the 10 \times diluted OECD pH 6 medium. The corrected Sr concentration in the substrate blank at 672 h is 802 $\mu\text{g/L}$, which would suggest that some Sr (approximately 5–10%) is being removed by a mechanism other than dilution, possibly sorption onto the glass or precipitation. The corrected (for both dilution and additional losses) 672-h removal of Sr is

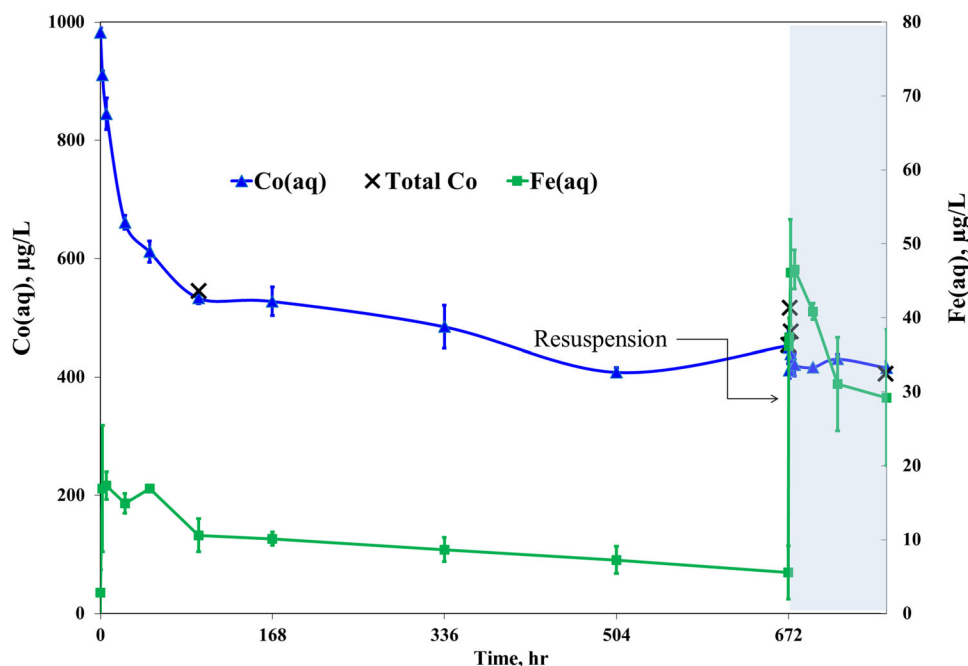


FIGURE 2: Net corrected aqueous Co and dissolved Fe data for the 1-mg/L Co solutions at pH 6 using the low binding substrate for the extended transformation/dissolution protocol parts 1 and 2. Blue shaded area indicates the resuspension event.

34%. Dissolved Fe concentrations were also measured, peaking at 6 h at 21 $\mu\text{g/L}$. At select time periods, samples were also collected for total Sr and total Fe. Total Sr values are generally slightly higher than dissolved values, whereas total Fe concentrations are consistently higher than the dissolved Fe values (Supplemental Data, Table S4).

Immediately after the resuspension, the net corrected average $\text{Sr}_{(\text{aq})}$ concentration was 508 $\mu\text{g/L}$ ($\sigma\% = 1$) compared to 541 $\mu\text{g/L}$ before, suggesting that the agitation increased metal removal and that the resuspension of the substrate did not release Sr ions into solution. Total Sr concentrations at 0.5 h after resuspension increase slightly to 517 $\mu\text{g/L}$ compared to the dissolved concentration of 508 $\mu\text{g/L}$. However, total Fe concentrations increase to 1090 $\mu\text{g/L}$ at the same sampling time compared to the dissolved Fe concentrations of 41 $\mu\text{g/L}$.

Further application of the method

A series of 96-h experiments were conducted on Cu, Co, Ni, Zn, Pb, and Ag. The purpose of this was to determine, in a shorter time frame than the full 672-h test, if the method is sufficiently robust to distinguish between those metals that are rapidly removed from the water column and those that are not. In all other aspects, the setup remained identical to the 672-h test.

For the Cu 96-h experiment using the LBS, 78% removal of Cu was observed compared to 87% removal at the 96-h sampling point in the 672-h experiment. The experiment was repeated at the University of Michigan, and 83% Cu was removed within 96 h. For Co, using the LBS, 46% removal of Co was observed both when the experiment was run and stopped at 96 h and at 96 h when the experiment was run for the full 672 h. For Ni, using the LBS, 55% removal was observed

when the experiment was run at CanmetMINING and 61% when it was run at the University of Michigan. This would suggest good reproducibility of the test method between laboratories.

Removal of Ni was examined over a 96-h period using the 3 different substrates: LBS, IBS, and HBS. Measurements show that dissolved oxygen varied between 7.9 and 8.6 mg/L (average 8.4 mg/L). The average temperature was 21.6 $^{\circ}\text{C}$, with a range of 20.5 to 22.0 $^{\circ}\text{C}$. The average pH in the 3 procedural blanks (10 \times diluted OECD pH 6 medium) was 6.06, with a range of 5.97 to 6.17. The average pH in the 3 substrate blanks (1 mg/L Ni solution with no substrate) was 6.14, with a range of 6.04 to 6.20. The measured pH values averaged among each substrate loading are plotted as a function of time in Figure 4A. The graph clearly demonstrates that although addition of IBS results in a pH increase to 6.6 and addition of HBS results in a decrease in pH to 5.6, the samples with LBS remained stable in the range 5.9 to 6.1.

For the 3 procedural blanks, the average absolute concentration of Ni was ≤ 1.5 $\mu\text{g/L}$ throughout the T/DP-E examination. The kinetic data for $\text{Ni}_{(\text{aq})}$ are presented in Figure 4B. For the 1-mg/L Ni solutions with the 10-g/L LBS loading, the $\text{Ni}_{(\text{aq})}$ concentration at 0 h was 959 $\mu\text{g/L}$ ($\sigma\% = 2$). This decreased to 429 $\mu\text{g/L}$ ($\sigma\% = 4$) by 96 h, which would represent 55% removal of Ni. With the 10-g/L IBS loading, the $\text{Ni}_{(\text{aq})}$ concentration at 0 h was 960 $\mu\text{g/L}$ ($\sigma\% = 1$). By 96 h, the $\text{Ni}_{(\text{aq})}$ concentration was 214 $\mu\text{g/L}$ ($\sigma\% = 8$), which would represent 78% removal of Ni. For the 1-mg/L Ni solutions with 10 g/L of sediment HBS, the $\text{Ni}_{(\text{aq})}$ concentration at 0 h was 965 $\mu\text{g/L}$ ($\sigma\% = 2$). By 96 h, the $\text{Ni}_{(\text{aq})}$ concentration was 492 $\mu\text{g/L}$ ($\sigma\% = 5$), which would represent 49% removal of Ni over 96 h.

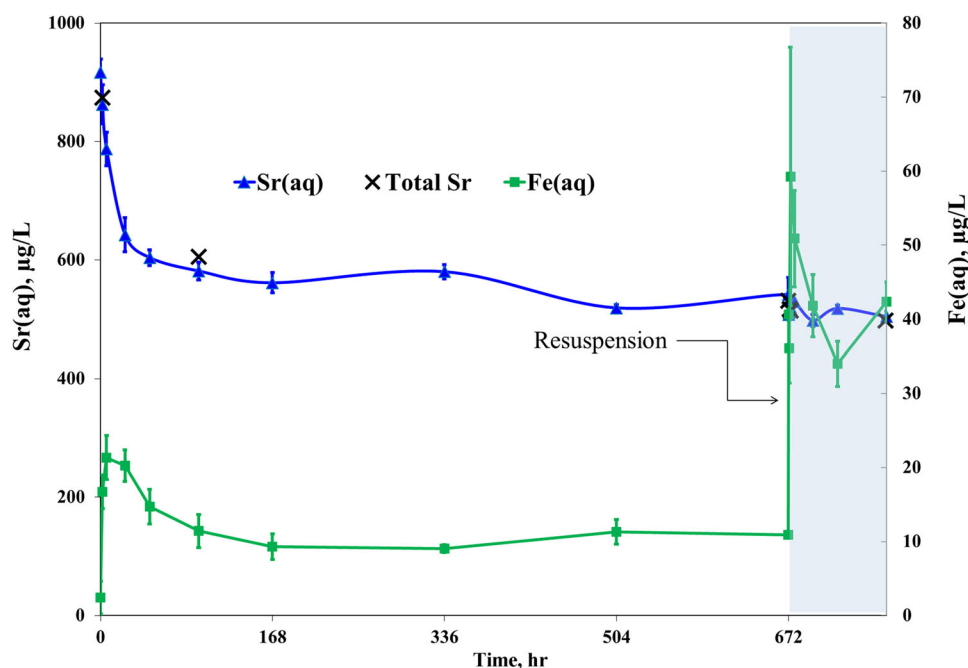


FIGURE 3 Net corrected aqueous Sr and dissolved Fe data for the 1-mg/L Sr solutions at pH 6 using the low binding substrate for the extended transformation/dissolution protocol parts 1 and 2. Blue shaded area indicates the resuspension event.

A regression analysis of the Ni kinetic data was performed, followed by extrapolation to 672 h using TableCurve 2D (Ver 5.01.01). Comparing the 672-h Ni concentrations derived from the regression analysis and extrapolation, the LBS is predicted to be the worst performer (lowest % removal) with respect to metal removal, although at 96 h it is the HBS that has the lowest % metal removal.

Additional 96-h removal experiments were conducted using solutions of Zn, Pb, and Ag and using the LBS. For procedural blanks, the average absolute concentration of Zn was ≤ 6.2 $\mu\text{g/L}$, that of Pb was ≤ 5.8 $\mu\text{g/L}$, and that of Ag was ≤ 0.02 $\mu\text{g/L}$ throughout the T/DP-E examination.

For the 1-mg/L Zn solutions with the 10-g/L LBS loading, the $\text{Zn}_{(\text{aq})}$ concentration at 0 h was 1135 $\mu\text{g/L}$ ($\sigma\% = 2$). This decreased to 492 $\mu\text{g/L}$ ($\sigma\% = 3$) by 96 h, which would represent 57% removal of Zn over 96 h (Figure 5A).

The $\text{Pb}_{(\text{aq})}$ concentration at 0 h was 640 $\mu\text{g/L}$ ($\sigma\% = 1$). This decreased to 32 $\mu\text{g/L}$ ($\sigma\% = 35$) by 96 h, which would represent 95% removal of Pb over 96 h (Figure 5B), mostly within the first 2 h of the experiment. It is important to note that although sufficient $\text{Pb}(\text{NO}_3)_2$ was added to the 10 \times diluted OECD aquatic medium to make a 1-mg/L Pb solution, it is believed that a quantity of Pb ions precipitated out of solution. Unlike the Ag solution, however, this solution was not filtered prior to the T/D examination. However, the fact that the substrate blank (corrected for dilution and the blank) remained stable over the 96-h period would suggest that no further precipitation of Pb occurred which could be mistaken for Pb removal by the sediment.

It is important to note that because of the precipitation of Ag from solution, it was not possible to prepare a solution of a concentration >0.1 mg/L. Sufficient AgNO_3 was added to the 10 \times diluted OECD aquatic medium to prepare a 1-mg/L solution, but this was then filtered to remove any precipitate. There is a very slight decline of Ag in the corrected sediment blank from 50 to 46 $\mu\text{g/L}$, which might suggest some further, but very minor, precipitation. For the 1-mg/L Ag solutions with the 10-g/L LBS

loading, the $\text{Ag}_{(\text{aq})}$ concentration at 0 h was 50.2 $\mu\text{g/L}$ ($\sigma\% = 1$). This decreased to 5.5 $\mu\text{g/L}$ ($\sigma\% = 29$) by 96 h, which would represent 89% removal of Ag over 96 h (Figure 5C).

DISCUSSION

The present study was designed to assess whether an extension to the T/DP could be used to measure the rate and extent of metal removal from the water column and the potential for remobilization. It was recognized that the T/DP-E needed to be sufficiently robust to distinguish between metals with a wide range of reactivities to yield consistent hazard classification outcomes for each metal. The primary difference between the T/DP and the T/DP-E is that the former measures the reaction kinetics of metal release from a metal-bearing substance into an aquatic medium, whereas the latter measures metal removal in the presence of a substrate.

Previous studies (Skeaff et al. 2008, 2011; Skeaff and Beaudoin 2014; Huntsman 2018) have demonstrated that properly equipped and operated laboratories can apply the T/DP to metal-bearing substances to yield acute and chronic aquatic hazard classification outcomes that are reliable, consistent, and in agreement with other laboratories. In the development of the T/DP-E, it was therefore important to maintain as many elements of the original T/DP as possible. The procedure for blanks and replicates, in addition to the schedule for sampling, remained the same to maintain the same level of robustness.

As expected, pH control is an important criterion for the T/DP-E. With some substrates, it was difficult to maintain pH of the solution at the target value of 6 for the entire duration of the experiment. Higher pH values, near pH 7, increase metal partitioning as complexation sites increase. This phenomenon supported selection of the LBS for use in the T/DP-E because pH did not increase above 6.

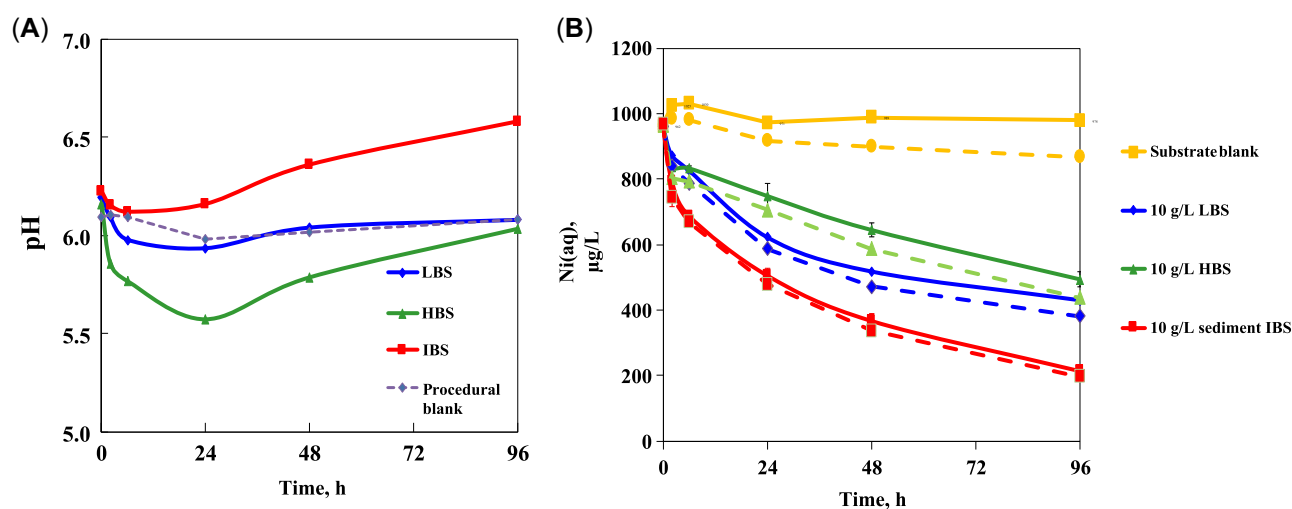


FIGURE 4: (A) Variation in measured pH as a function of time for the 1-mg/L Ni solutions with the 3 different substrates and (B) net kinetic aqueous Ni data for the 1-mg/L transformation/dissolution solutions at pH 6 in the presence of 3 different substrates. Dashed lines indicate results uncorrected for the dilution. HBS = high binding substrate; IBS = intermediate binding substrate; LBS = low binding substrate.

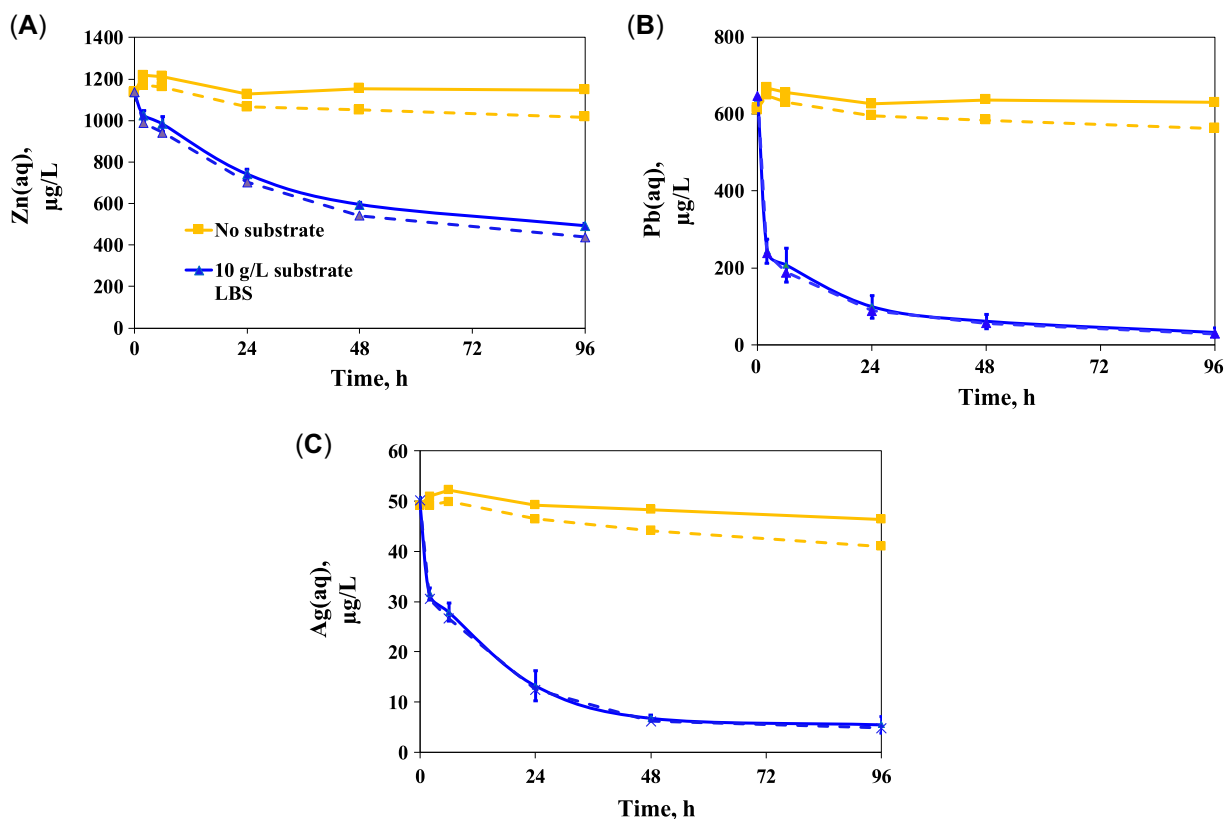


FIGURE 5: Net transformation/dissolution kinetic aqueous Zn (A), Pb (B), and Ag (C) data for the 1-mg/L solutions at pH 6 in the presence of no substrate (orange line) and low binding substrate (blue line). Dashed lines indicate results uncorrected for the dilution.

In many aquatic systems, anoxic or anaerobic conditions prevail in buried sediments and gradients in oxygen availability develop. The proposed test method needed to strike a balance between 1) recognizing the complexity of aquatic systems, and 2) standardization of the conditions, repeatability, reproducibility, and comparability between different metals. The test method does include the relevant processes for metal removal (binding to substrate, settling, resuspension), but anoxic reactions are excluded. This may lead to an underestimation of the removal and detoxification of some metals (those that are strongly removed under anoxic conditions) in aquatic systems.

From the 96-h Ni removal data using the 3 different substrates, it is clear that substrate selection is a key factor influencing the outcome of the experiments. For the T/DP-E to become an accepted “standard” methodology, a universally accepted, widely available substrate must be identified. Ongoing research is focusing on identifying the properties of an appropriate natural or artificial substrate for global use in hazard classification under the GHS (United Nations 2017) or CLP (European Union 2013). The research is also looking to identify optimal parameters describing a suitable LBS for use in the T/DP-E to allow for laboratories to identify a local sediment for use in the protocol. The parameters used to select should be simple from an analytical perspective, to allow for a wide range of laboratories to identify local, suitable sediments.

Results from the T/DP-E part 1 with the initial 1-mg/L Cu solution demonstrate that Cu reaches 95% removal from the water column within the 672-h testing period using the LBS. The Cu test did not demonstrate remobilization of Cu during the resuspension event, unlike dissolved and total Fe concentrations, which increased following resuspension. Concentrations of Fe also increased following resuspension with the Co and Sr experiments.

Following the same procedure for Sr as for Cu, it was shown that only 34% of Sr was removed by binding to the substrate. This clearly demonstrates that the newly developed T/DP-E is sufficiently robust to distinguish between those metals that are rapidly removed from the water column and those that are not. This was demonstrated again with the 96-h experiments with Pb and Ag showing a very rapid and then readily perceivable reduction in removal rates between days 1 and 4, with dissolved concentrations appearing to start leveling off as equilibrium is approached. The experimental data for Ni and Zn do not demonstrate this same effect.

Based on the data of the present study, a numerical model was developed to provide mechanistic insight into important processes controlling dissolved metal removal from the water column during the experiments performed using the T/DP-E part 1 (see Burton et al. 2019, this issue). This model indicates that, depending on the rate of metal adsorption (relative to the rate at which substrate particles settle), some of the metal in the water column adsorbs to the particles and settles with them. Once most of the particles have

settled from the water column, the dissolved and total metal concentrations become equal, indicating that essentially all water column metal is dissolved. Despite the lack of suspended particles in the water column, metal removal continues via transport to and direct adsorption by the settled substrate particles which form the sediment layer. So, the key phenomena responsible for removal of metals were 1) kinetically controlled adsorption to rapidly settling substrate particles, and 2) transport to and direct adsorption by the settled substrate particles.

CONCLUSIONS

Dominant processes affecting metal fate in aquatic environments are effectively demonstrated in the T/DP-E, including such processes as metal binding to suspended solids with associated complexation and transformation processes rapidly occurring, settling of metal associated suspended solids, and initial diagenesis reactions resulting in decreased remobilization potential.

The present study was aimed at extending the T/DP to establish the rate and extent of partitioning of metals from the water column so that the resulting data can be used in metals chronic hazard classification. Through the addition of a substrate, data on water column residence times and processes can be generated. The data from the present study support the use of the proposed T/DP-E, thus providing a means to quantify the rate of metal removal from the water column and addressing this important process in the determination of the chronic aquatic hazard classification of metals.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.4471.

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Data Accessibility—Data, associated metadata, and calculation tools are available from the corresponding author (phillippa.huntsman@canada.ca).

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