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Running title: Activity-based environmental risk analysis of DEHP

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Abstract: This study applies a chemical activity-based approach to (i) evaluate environmental concentrations of di-ethylhexyl phthalate (DEHP) (n=23,651) and its metabolite mono-ethylhexyl phthalate (MEHP) (n=1,232) in 16 environmental media from 1,174 studies in the United States, Canada, Europe and Asia, and in-vivo toxicity data from 934 studies in 20 species as well as in-vitro biological activity data from USEPA's Toxicity Forecaster (ToxCast) and other sources; and (ii) conduct a comprehensive environmental risk analysis. The results show that the mean chemical activities of DEHP and MEHP in abiotic environmental samples from locations around the globe are 0.001 and 10^{-8}, respectively. This indicates that DEHP has reached on average 0.1% of saturation in the abiotic environment. The mean chemical activity of DEHP in biological samples is on average a 100-fold lower than that in abiotic samples, likely due to biotransformation of DEHP in biota. Biological responses in both in-vivo and in-vitro tests occur at chemical activities between 0.01-1 for DEHP and between approximately 10^{-6} and 10^{-2} for MEHP, suggesting a greater potency of MEHP compared to DEHP. Chemical activities of both DEHP and MEHP in biota samples were less than those causing biological responses in in-vitro bioassays without exception. A small fraction of chemical activities of DEHP in abiotic environmental samples (i.e., 4 to 8%) and none (0%) for MEHP were within the range of chemical activities associated with observed toxicological responses in in-vivo tests. The study illustrates the chemical activity approach for conducting risk analyses.

Keywords: Di-ethylhexyl phthalate, Mono-ethylhexyl phthalate, Chemical activity, Environmental risk assessment

INTRODUCTION

Di-ethylhexyl phthalate (DEHP) is a di-alkyl ester which is used worldwide, mainly as a plasticizer in composite materials such as polyvinyl chloride (PVC), polystyrene and polyacetates to make plastics soft and flexible [*1*]. The global production volume of DEHP is approximately 3 million tonnes/year [*2*]. DEHP is a very hydrophobic substance [*3*] with a potential for persistence [*4*] and bioaccumulation [*5*] in the environment. In biological organisms, DEHP is metabolized to mono-ethylhexyl phthalate (MEHP), which can be further degraded to phthalic acid and eventually to carbon dioxide and water [*6*]. Because of DEHP's high production volume and apparent persistence in the environment, DEHP is the subject of scientific and regulatory evaluations throughout the world. In these evaluations, risk assessment plays a key role. After decades of study, much data exist to conduct environmental risk assessments for DEHP [*7-9*]. However, the risk assessment remains challenging for several reasons. First, observations of environmental exposure (e.g. concentrations in food, water, air, sediment and soil) and in-vivo and in-vitro toxicity often involve measurements in different media. This precludes a direct comparison of many exposure and toxicity measures because such comparisons are akin to "comparing apples and oranges". A second challenge is that dosing levels aimed to detect toxicological responses in bioassays can exceed solubility limits of the chemical in the dosing media [*10*]. This can produce incorrect risk estimates because concentrations in excess of solubility do not normally exist in the environment. Incorrect risk estimates can also occur due to the ubiquitous nature of DEHP, which can cause error in environmental concentration measurements through background contamination [*11*]. A third challenge in risk assessments is accounting for cumulative risks resulting from exposure to multiple substances [*12*]. A fourth and emerging challenge is to incorporate in-vitro bioassay data from high-throughput and other tests in risk analyses involving contaminated water, sediment, soil or air. Currently, large amounts of information on the effects of environmental chemicals in cell-based and biochemical assays are being generated by the USEPA's Toxicity Forecaster (ToxCast) program [*13*], which is aimed at minimizing animal use and costs, and improving

toxicological insights. Efforts to relate the data from ToxCast's high-throughput in-vitro screening to exposure and toxicity in whole organisms are on-going [*14-18*] and may be facilitated by the application of a chemical activity-based approach. These challenges are not unique to the risk assessment of DEHP but affect the on-going risk assessment of commercial substances under the United Nations Stockholm Convention on Persistent Organic Pollutants [*19*], the European Union (EU) regulations on Registration, Evaluation, Authorization and Restriction of Chemicals (REACh) [*20*], the US Toxics Substances Control Act [*21*], the Canadian Environmental Protection Act [*22*] and similar regulatory programs throughout the world. It is the objective of this study to explore the application of chemical activity (also referred to as thermodynamic activity) to address some of these challenges and to improve the process of risk analysis for commercial chemicals. A chemical activity approach was used previously [*23*] to assess the environmental risks of another high-volume production substance, decamethylcyclopentasiloxane (D5), in support of a regulatory evaluation of D5 under the Canadian Environmental Protection Act. The present study documents the application of chemical activity to an environmental risk analysis of DEHP and its main metabolite MEHP.

THEORY

The chemical activity approach

The chemical activity approach to chemical risk analysis involves expressing exposure concentrations of a chemical in various environmental media and the biological response concentrations of that chemical in in-vitro and in-vivo experiments and field studies in terms of a common quantity (i.e. chemical activity), so that they can be compared using statistical methods. This approach and the closely related fugacity approach, both developed by Lewis [*24*,*25*], are widely used in chemical engineering, medicine, pharmacology and environmental chemistry. In medicine, the application of chemical activity was instrumental in predicting the action of general anesthetics in surgery [*26-28*]. In environmental chemistry, fugacity and chemical activity have also found useful applications as research tools to study both chemical dynamics in organisms and the environment [*29*-

32] as well as elucidating modes of toxic action [*33-35*]. Fugacity models (for organic compounds) and related aquivalency models (for metals) are widely used for assessing the distribution of a variety of chemical substances in the environment [*36-37*] and for environmental risk assessment [*23,38*].

Chemical Activity

The chemical activity of a substance in a medium is defined as the product of the activity coefficient (γ) and the concentration of the chemical in the medium expressed in terms of a mole fraction (x) in units of mol chemical/mol medium. For many neutral hydrophobic organic substances in dilute solutions, the activity coefficient can be approximated by the reciprocal of the chemical's maximum sorptive capacity or "solubility" of the chemical (X) in the medium involved, expressed in units of mol chemical/mol medium [*38*]. The chemical activity can therefore often be derived as the ratio of the chemical's concentration C (mol/m^3) and its solubility S (mol/m^3) in the medium in which it occurs [*38*]:

$$a = \gamma \cdot x = C/S \quad (1)$$

For substances, including DEHP, that are liquid at environmental temperatures, the chemical activity can range from 0 to its maximum value of 1. A chemical activity of 1 represents a thermodynamically ideal solution defined as the chemical in its pure, liquid or subcooled liquid (for solid chemicals) form.

A key property of chemical activity is that it is used to define a thermodynamic equilibrium as a situation where the chemical activities of the chemical in two or more media (e.g. media i and j) are equal [*39*]:

$$a_i = a_j \quad (2)$$

This is useful as it provides a method for relating and comparing activities in multi-media systems such as natural environments and for testing hypotheses of equilibrium partitioning in the environment. However, in the environment, physical and biological processes often interfere with the chemical's natural tendency to achieve an equilibrium and cause chemical activities to differ among media.

Merits of the chemical activity approach

Typically, environmental risk assessments involve a comparison of exposure and toxicity reference concentrations for a specific environmental medium (e.g. water). A simple comparison of exposure concentrations and toxicity reference concentrations cannot be used if the concentrations apply to different media. For example, the concentration of a chemical in fish or water cannot be directly compared to the concentration of that chemical in an in-vitro bioassay. Hence, in risk analyses involving concentrations in different media, there is a tendency to exclude data and information from risk assessments. By expressing chemical exposure and toxicity on a common basis, the chemical activity approach provides a method for including more information in a risk assessment than in a conventional concentration-based approach.

The chemical activity approach can also be useful in identifying erroneous data that should be avoided in an environmental risk analysis. Apparent chemical activities (derived from concentration observations or dosage levels in toxicity studies) greater than the maximum possible value of 1 indicate experimental artefacts, often due to dosing in excess of the chemical's solubility in the dosing medium and/or analytical error. For solid chemicals, the maximum chemical activity is a value F, which is often referred to as the fugacity ratio and is a function of the chemical's melting point (hence different for each solid chemical) but always less than 1 [*39*].

Another useful feature of the chemical activity approach in risk analysis is that non-polar narcosis, which is a mode of toxic action exhibited by many hydrophobic organic substances (and hence sometimes referred to as "baseline toxicity"), is associated with a relatively narrow range of

chemical activities between 0.01 to 0.09 [*33*,*34*]. In absence of toxicity data, chemical activities of environmental contaminants in excess of 0.01 can indicate a potential for toxic effects.

Another possible advantage of the activity approach, but one that requires further study, is that for chemicals with certain modes of toxic action (e.g. non-polar and polar narcosis), chemical activities appear to be additive [*33*,*34*]. This is likely relevant to the risk assessment for DEHP as there are several other phthalate esters including di-butyl-phthalate, butyl-benzyl-phthalate, di-iso-nonyl-phthalate and di-iso-decyl-phthalate that are mass produced and ubiquitous in the global environment.

Limitations of the chemical activity approach

The chemical activity approach assumes a linear relationship between activity and concentration. The existence of a linear activity-concentration relationship is generally accepted for dilute solutions of hydrophobic organic substances in water, but is less well characterized for organic media such as lipids, proteins and organic carbon, in which many organic chemicals tend to exhibit higher concentrations than those in water. However, in many cases it is reasonable to assume that such a linearity exists for many substances subject to environmental risk assessments. This is because environmental concentrations of many pollutants are often well below the mole fraction solubility x at which non-linearity between chemical activity and concentration is expected [*39*].

When comparing chemical activities between different media in a risk assessment it is important to avoid making erroneous assumptions regarding the occurrence of chemical equilibrium of DEHP between environmental media. This is especially important when comparing chemical activities in abiotic media (e.g. water, sediment, soil) to those in biotic media (biota) because biotransformation prevents a thermodynamic equilibrium and causes the chemical activity in organisms to be less than that in the medium to which the organism is exposed. Also, some chemicals (but not DEHP and MEHP) are known to biomagnify in organisms, causing the chemical activity in the organism to be greater than that in the medium to which the organism is exposed. For substances that undergo biotransformation (such as DEHP and MEHP), inappropriate equilibrium assumptions can be avoided

by limiting comparisons of activities among abiotic media (i.e. media external to the organism) and/or among biotic media (i.e. media internal to the organism). In certain circumstances, equilibrium assumptions can be a helpful and conservative tool in risk assessment. For example, for a chemical that is biotransformed, the activity in an exposure medium (e.g. water) can be viewed as a maximum value that will not be exceeded by the chemical activity in the organism.

In the calculation of chemical activities from reported concentrations, it is sometimes necessary to make assumptions regarding the effect of temperature, suspended solids concentrations, organic carbon content, lipid and protein contents of various biological media and other factors on the chemical activity because of a lack of relevant information. These assumptions (described for this study in the METHODS and the Supporting Information) are applied to improve the comparison between exposure and toxicity measurements from different studies, but also contribute uncertainty. In many risk assessments, concentration data are not routinely corrected for differences in the conditions among field and laboratory studies, hence also contributing uncertainty and error in risk assessments.

METHODS

Chemical Activity calculations

Methods for the calculation of chemical activities of DEHP and MEHP from reported concentrations of DEHP and MEHP in sample matrices can be found in [*40*]. Solubilities (mol/m^3) of DEHP in pure water (S_W), air (S_A) and lipids (S_L) were determined from the reported aqueous solubility, Henry's law constant (H) and octanol-water partition coefficient (K_{OW}), which have been compiled and reviewed [*3,6*]. The authors' recommended values for the chemical properties (Table S1) were used in the calculations. H and octanol-air partition coefficient (K_{OA}) values were adjusted for temperature as described in [*40*] to obtain temperature specific chemical activities in air. For MEHP, concentrations in air were not available, hence chemical activities in air were not calculated. The changes in S_W and *Kow* of liquid chemicals over the range of environmental temperatures are generally considered negligible [*37*]. Hence, no adjustments for temperature were made for these parameters. For the calculation of

chemical activities of DEHP in marine water or sediment, S_W and K_{OW} values were adjusted for salinity as described in [*40*]. Because MEHP is an acid and occurs in natural water with neutral pH levels mostly in ionized form, the pH specific aqueous solubility and octanol-water distribution coefficient *D* (instead of K_{OW}) were used for the activity calculations (Table S1). Sorptive capacities of DEHP and MEHP in proteins (S_P) and organic carbon (S_{OC}) were calculated as 0.05.S_L [*41*] and 0.35 S_L [*42*], respectively. For heterogeneous environmental media consisting of a combinations of media (e.g. surface water samples containing water and suspended solids, and biological media containing water, protein and lipid), the combined solubility (S_T) in the environmental matrix was determined as:

$$S_T = \sum_{j=1}^{m} \phi_j \, . S_j \qquad (3)$$

where ϕ_j is the volume fraction of each component *j* of a particular medium consisting of *m* components. Because the composition of most heterogeneous sample matrices was frequently unreported, we used generic values for the matrix composition to assess the sorptive capacities. Media-specific parameters used in the activity calculations for DEHP and MEHP are listed in Table S2.

Environmental concentrations

Reported concentrations of DEHP (n=23,651 measurements) and MEHP (n=1,232 measurements) in 16 environmental media (i.e. outdoor air, sediment, surface water, soil, sludge, waste water treatment plant (WWTP) effluent, algae, plankton, invertebrates, fish, amphibians, birds, seals, meat for human consumption, milk and blood) from locations in the United States, Canada, Europe and Japan/Asia over the period between 1995 and July 2010 were compiled from 1,131 studies for DEHP and 43 studies for MEHP reported by Clark [*7*] and summarized in the Appendix of the Supporting Information. Clark [*7*] used the Klimisch evaluation scale [*43*] to evaluate all studies for data quality according to the following rankings: (1) reliable without restrictions (i.e., high quality studies with

precautions to prevent contamination; information on quality assurance/quality control measures (QA/QC) and blank corrections were provided; (2) reliable with restrictions (i.e. high quality studies but data were not corrected for blanks); (3) not reliable (i.e., studies lacking QA/QC measures; high concentrations noted in blanks; study included data which may not be representative of ambient conditions (e.g. studies that included samples from a known source of DEHP); or (4) unassignable/insufficient information available to categorize study (i.e., data were reported in government studies not available in English). Approximately 16% of the compiled DEHP concentration means were categorized as not reliable (category 3) [*7*] and are not included in the 1,131 DEHP exposure studies used in this analysis. Figure S1 illustrates that the exclusion of unreliable data does not have a significant effect on the distribution of the activities in any of the media except outdoor air. A number of reported aerial concentrations of DEHP were orders of magnitude greater than the vapor pressure of DEHP, corresponding to chemical activities greater than 1, which are not thermodynamically plausible. Data in category 4 were not excluded by Clark [*7*] and in this study because this would result in removal of some potentially high quality data. Non-detectable concentrations were set to one-half of the reported detection limit [*7*]. The mean DEHP and MEHP concentration from each study in categories 1, 2, and 4 was expressed in terms of a chemical activity. Median concentrations were used if mean values were not reported. If only a range of concentrations was reported, the highest value in the range was used. Compiled concentrations for each medium were represented by log-normal distributions. Geometric mean concentrations from 1122 and 36 studies were determined for DEHP and MEHP, respectively. All exposure data and the corresponding values of chemical activity are provided in the Appendix to the Supporting Information.

DEHP toxicity in-vivo

Hundreds of studies have investigated the biological responses of DEHP in live animals and in-vitro bioassays. Most of the in-vivo studies have been compiled and reviewed in two reports. First, as part of a DEHP risk assessment in 2008, the EU [*8*] reviewed acute and chronic toxicity studies of

DEHP in aquatic and terrestrial organisms. From a total of 148 studies, seven high quality no-observed-effect-concentrations (NOECs) were selected by the EU assessors to represent the range of effects in fish, amphibians, aquatic and soil invertebrates, microorganisms and higher plants exposed to DEHP via food, sediment, sludge or soil. These values are listed in Table S3.

Second, more than 750 studies reporting LC50 and lowest adverse effect level (LOAEL) values for DEHP effects in laboratory animals were reviewed by the US Consumer Product Safety Commission (CPSC) in 2010 [*9*]. From these, six studies with LOAEL values for reproductive, developmental and effects on rat livers were chosen by the CPSC assessors as representative toxicological endpoints and used in the calculation of acute, sub-chronic, and chronic acceptable daily intakes for the general population, children, and men and women of childbearing age. The selected LOAEL values are listed in Table S3. The 2008 EU risk assessment-selected NOECs [*8*] and the US CPSC-selected LOAELs [*9*] were expressed in terms of chemical activities using the same methods as those described above for the exposure concentrations.

DEHP toxicity in-vitro

The USEPA's Toxicity Forecaster (ToxCast) screening program included DEHP in 1,080 high-throughput screening assays (as of March 2016) for effects in-vitro using intact cells, membrane incubations, reporter gene assays, etc. The ToxCast data set consists of concentration-response profiles for each chemical-bioassay pair, and provides a determination of whether or not the chemical was active in each bioassay. Positive tests are reported as an AC50 value (i.e. nominal concentration, in μM, at which 50% of maximal biological activity was observed). DEHP was stated to biologically active in 40 ToxCast bioassays, of which 35 were performed using intact cells. The ToxCast AC50 values for DEHP in the 35 cell-based tests were converted to chemical activity using the equilibrium mass-balance model in [*44*], and by using DEHP's solubility in the bioassay's incubation medium according to Equation 3, where the components of the in-vitro system are albumin, lipid and water. The in-vitro incubation conditions used to calculate the activities are the same as those used in [*44*] and are assumed

to be identical across all assays. The details of the calculations are described in the Supporting Information and the values are listed in Table S4.

The chemical activity values obtained from ToxCast high-throughput screening technology were compared to results from in-vitro studies of the estrogenic effects of DEHP reviewed by Staples et al. [*10*], and DEHP's androgenic and thyroid activities reported in [*45*]. In total, five endpoints representative of DEHP effects observed in-vitro bioassays were expressed in terms of chemical activity (using the reported incubation parameters) and presented in Table S3.

MEHP toxicity in-vivo

To date, only five studies report on biological responses of MEHP in whole organisms: an LC50 value in carp [*46*] and four EC50 values for growth inhibition in algae [*47*], decreased luminescence in bacteria [*46*] and immobilization in Daphnia [*46,47*]. The metrics from in-vivo tests were converted to chemical activity as described in the Supporting Information and values are given in Table S5.

MEHP toxicity in-vitro

MEHP has been examined using the ToxCast technology using the same 1080 tests as those used for DEHP. MEHP was stated to be active in 31 tests, and 26 of these tests were cell-based. The AC50 values for MEHP provided in the ToxCast data set were converted from μM to chemical activity values as described in the Supporting Information. The ToxCast AC50 values expressed as chemical activity are given in Table S6. MEHP in-vitro effects on steroidogenesis or cytotoxicity were compiled from the primary literature, and results from 10 studies are given in Table S5. These EC50 and IC50 values were converted to chemical activity using reported incubation parameters as described in the Supporting Information.

RESULTS AND DISCUSSION

Ambient chemical activities of DEHP and MEHP

Concentrations (mol/m^3) of DEHP in air, water, sediment and soil from different locations around the world illustrate very large variations (Figure 1A). This variation reflects both differences in DEHP contamination levels among sample locations and differences in the affinity of DEHP for different media in the environment. Variations in chemical activities of DEHP among environmental media are much smaller than those for concentrations (Figure 1B). The variations in chemical activities of DEHP reflect mainly variations in DEHP contamination levels among the sampled media as differences in DEHP's affinity for different media is accounted for in the chemical activity metric. Despite large variations in chemical activities in individual media from different locations around the world, the geometric mean chemical activities of DEHP in ambient air, surface water, sediment and soil vary by only a factor of 6 and show no statistically significant differences in Student t-tests ($p<0.05$). The apparent similarity in geometric mean chemical activities of DEHP in different environmental media provides support for the role of equilibrium partitioning in environmental distribution. Equilibrium partitioning theory expects chemicals such as DEHP to naturally distribute in the environment according to their relative solubilities in environmental media. It also suggests that DEHP is persistent in the environment despite its high inherent biodegradability as an ester [*4*]. The high sorption affinity of DEHP to organic matter in particulates in water, sediments and soils and air and associated low bioavailability for microbial degradation may explain DEHP's high persistence in the environment [*4*]. The results show that the mean chemical activity of DEHP in samples collected from around the globe is approximately 0.001, indicating that DEHP has reached, on average, 0.1% of saturation and higher levels of saturation in many places of the world. This high chemical activity likely reflects the high global production volume of DEHP and the high persistence of DEHP in the environment.

Chemical activities of DEHP in biota and cow's milk also show large variations but are, on average, 100 fold lower than those in abiotic media (Figure 1B). The lower DEHP activities in biological media compared to those in abiotic media are consistent with the high degree of biotransformation of DEHP in organisms and humans [*6*]. Geometric mean chemical activities of DEHP among the sampled biological media show no statistically significant differences ($p > 0.05$). However, a food-web bioaccumulation study of DEHP in one particular location shows that lipid normalized concentrations of DEHP in biota (which is a proxy for chemical activity) follows a statistically significant decline in concentrations with increasing trophic level [*48*], indicating that DEHP does not biomagnify and is subject to trophic dilution in food-webs.

Figure 2A shows that there remains a paucity of MEHP concentration data relative to DEHP data. The average chemical activity of MEHP in the abiotic environment is approximately five orders of magnitude lower than that of DEHP (Figure 2B). This is likely caused by MEHP's low rate of formation in the ambient environment [*4*], due to the high hydrophobicity and sorption affinity to particulate matter of its precursor (DEHP), and MEHP's high inherent and apparent microbial biodegradability due to MEHP's low hydrophobicity and sorption affinity to organic matter at environmental pH levels [*4,49*]. Chemical activities of MEHP in tissues of organisms tend to be 1 to 10 fold greater than those in the abiotic environment. This is likely due to biotransformation of absorbed DEHP to MEHP in biota rather than through absorption of MEHP from the ambient environment.

Biological responses to DEHP and MEHP

Figure 3 illustrates that a large number of the toxicity studies compiled and reviewed by the EU [*8*] and CPCS [*9*] risk assessors applied DEHP dosing concentrations at apparent (but not real) chemical activities greater than 1. In these studies, dosing concentrations were above the aqueous solubility, often by many fold. Only approximately 30% and 60% of studies reviewed by the EU [*8*] and CPCS [*9*] risk assessors, respectively, were conducted at dosing levels below the solubility or sorptive capacity of DEHP in the dosing medium and hence at chemical activities of DEHP equal or

less than 1. This issue was also recognized by the EU risk assessment [*8*] and the US CPSC [*9*] resulting in the selection of respectively 7 and 6 no-observed and lowest-observed-adverse-effect levels that are below solubility and representative of DEHP in-vivo toxicity. The selected studies indicate that chemical activity ranges between 0.05-1 for no-observed-adverse-effects and 0.02-0.15 for lowest-observed-adverse-effects (Figure 1B). The range of abiotic chemical activities of 0.02 to 1 associated with in-vivo no- and lowest-observed-adverse-effect is similar to the biotic activity range of 0.01 to 1 associated with non-polar narcosis [*33,34*]. At first glance, this may indicate that DEHP causes biological responses through a non-specific mode of action similar to non-polar narcotics. However, biotransformation of DEHP in organisms causes chemical activities in organisms that are less than those in the media to which the organisms are exposed. Chemical activities of DEHP in test organisms exhibiting biological responses in the in-vivo studies can therefore be expected to be less than those in the dosing medium of the in-vivo toxicity tests and hence less than those associated with non-polar narcosis. This suggests that the observed biological responses of DEHP in the in-vivo tests may not be associated with non-polar narcosis.

In-vitro testing under the ToxCast program, showed biological activity of DEHP in 40 out of the 1080 (i.e. 3.7%) tests conducted. Chemical activities of DEHP associated with observed in-vitro biological responses in the ToxCast database range between 0.025 to 1.06. In-vitro toxicities in non-ToxCast studies range between 0.13 and 0.45 with the exception of one study that used a dosing concentration with an apparent chemical activity of approximately 4.5. The range of chemical activities associated with in-vitro biological activities (0.025-1) is in agreement with the range of chemical activities associated with the no and lowest-observed-adverse-effect levels in in-vivo studies (0.02-1). This suggests that less animal intensive in-vitro bioassays may be a reasonable alternative to in-vivo studies for DEHP. Also, the chemical activity approach may be a useful tool in the in-vitro to in-vivo extrapolation of biological responses. The range of chemical activities associated with biological activities in in-vitro tests also overlaps with the activity range associated with non-polar narcosis (0.01-

1). This suggests that biological activities in these bioassays may represent a mode of action described by non-polar narcosis. The possible lack or limited capacity for biotransformation in some of the bioassays may provide the circumstances for chemical activities of DEHP to reach the levels required for non-polar narcosis related biological activity. The observation that only a small fraction of the ToxCast in-vitro assays for DEHP shows biological activity indicates the importance of including appropriate receptors in testing protocols for conducting meaningful assessments. For very hydrophobic substances, such as DEHP, which have a high affinity for membranes and may elicit biological activity through interaction with membrane-bound receptors, the inclusion of membrane associated receptors may be appropriate.

In ToxCast in-vitro bioassays, MEHP was found to be biologically active in 31 of the 1080 tests (i.e., 2.9%). In the in-vitro tests using intact cells (n=26 tests), MEHP exhibited responses at chemical activities ranging between 0.000003 to 0.005, which are lower than those observed for DEHP in in-vitro tests. Chemical activities corresponding with acute mortality of MEHP in in-vivo studies ranged between 0.0005 to 0.01, hence also lower than those of DEHP. Both in-vivo and in-vitro studies indicate a toxicological potency of MEHP greater than that of DEHP. The activity ranges for responses in in-vivo and in-vitro tests overlap (Figure 2B) indicating that biological activity of MEHP in in-vivo and in-vitro tests occurs within a similar chemical activity range of 10^{-6} to 10^{-2}. This finding also supports the replacement of in-vivo tests by in-vitro tests and encourages further research into the application of chemical activity for in-vitro to in-vivo extrapolation of biological responses. However, the fact that only 2.9% of ToxCast in-vitro bioassays show biological activity illustrates the importance of choosing appropriate receptors in in-vitro testing protocols.

Risk

Risk is defined here as the fraction of studies reporting concentrations that correspond to chemical activities of DEHP or MEHP in environmental media that are within the range of chemical activities associated with in-vivo or in-vitro biological effects. Figure 4A illustrates that in only 39 out

of 934 studies (or 4.2% of the available ambient exposure studies) external chemical activities are within the range of no-observable effects established by the EU risk assessment [*8*] while 76 out of 934 studies (or 8.1%) exhibit mean DEHP activities within the range of lowest-observed-effects-levels identified by the US CPSC [*9*]. The mean global DEHP activity in environmental media external to organisms is well below the chemical activity ranges associated with lowest-observed-effects-levels and no-observed-effects-levels.

In all of the 197 studies involving biota sampling (i.e. 100%), mean chemical activities of DEHP in biota are below those associated with AC50 values determined by high-throughput ToxCast assays (Figure 4B) and conventional in-vitro bioassays for cytotoxicity, estrogenic, androgenic and anti-thyroid hormone activity. These results indicate that DEHP concentrations in biota at the study locations were not at concentrations that are associated with known biological effects in in-vitro studies.

MEHP activities in surface water, sediment and waste water treatment effluents are orders of magnitude below chemical activities associated with toxicological effects of MEHP in daphnia and fish (Figure 4C). This indicates that uptake of MEHP from the ambient environment is likely insignificant and can be ignored in risk analyses. Chemical activities of MEHP in wildlife species and human tissue are in all cases (Figures 4D) lower than those associated with in-vitro bioassay responses. This suggests that MEHP concentrations in biological samples that were investigated are not at levels that may be of concern.

It should be stressed that one of the key limitations of the present study is the exclusion of phthalate esters other than DEHP and MEHP in the risk analysis. The chemical activity approach may provide a methodology for doing a combined risk assessment for multiple phthalate esters that will be explored in future work.

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

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Data Availability—Data can be obtained by accessing the cited papers, and from the SI and the Appendix to the SI.

REFERENCES

1. Stanley MK, Robillard KA, Staples CA. 2003. Introduction. In Staples CA ed, *Phthalate Esters: The Handbook of Environmental Chemistry*, Vol. 3 Anthropogenic Compounds, Part Q. Springer-Verlag, Heidelberg, Germany, pp 1-7.

2. Plasticizers. 2013. Report Number 576.0000. Chemical Economics Handbook, IHS Chemical Publication. Available from: http://www.ihs.com/products/chemical/planning/ceh/plasticizers.aspx.

3. Cousins I, Mackay D. 2000. Correlating the physical-chemical properties of phthalate esters using the 'three solubility' approach. *Chemosphere* 41:1389-1399.

4. Kickham P, Otton SV, Moore MM, Ikonomou MG, Gobas FAPC. 2012. Relationship between biodegradation and sorption of phthalate esters and their metabolites in natural sediments. *Environ Toxicol Chem* 31:1730-1737.

5. Gobas FAPC, Mackintosh CE, Webster G, Ikonomou MG, Parkerton TF, Robillard KA. 2003. Bioaccumulation of phthalate esters in aquatic food-webs. In Staples CA ed, *Phthalate Esters: The Handbook of Environmental Chemistry*, Vol. 3 Anthropogenic Compounds, Part Q. Springer-Verlag, Heidelberg, Germany, pp 201-225.

6. Staples CA, Peterson DR, Parkerton TF, Adams WJ. 1997. The environmental fate of phthalate esters: A literature review. *Chemosphere* 35:667-749.

7. Clark KE. 2010. Report on update to the phthalate ester concentration database–2010. Reference No. PE-143.0-FIN-BEC Amendment 10, American Chemistry Council Environmental Research Task Group of the Phthalate Esters Panel, Arlington VA.

8. European Union risk assessment report on bis(2-ethylhexyl)phthalate (DEHP). 2008. EUR 23384 EN Volume 80. Pakalin S, Aschberger K, Cosgrove O, Lund B-O, Paya-Perez A, Vegro S, eds. European Chemicals Bureau, Luxembourg. 575 pp. Available from: http://echa.europa.eu/documents/10162/e614617d-58e7-42d9-b7fb-d7bab8f26feb.

9. Carlson KR. 2010. Toxicity review of di(2-ethylhexyl) phthalate (DEHP). US Consumer Product Safety Commission, Memorandum to MA Babich. April 2010. 317 pp. Available from: https://www.cpsc.gov/content/toxicity-review-of-dehp.

10. Staples CA, Guinn R, Kramarz K, Lampi M. 2011. Assessing the chronic aquatic toxicity of phthalate ester plasticizers. *Hum Ecol Risk Assess* 17:1057-1076.

11. Ikonomou MG, Kelly BC, Blair JD, Gobas FAPC. 2012. An interlaboratory comparison study for the determination of di-alkyl phthalate esters in environmental and biological samples. *Environ Toxicol Chem* 31:1948-1956.

12. National Research Council Final Report. 2008. Phthalates and cumulative risk assessment: The tasks ahead. The National Academies Press, Washington, DC. Available from: http://www.nap.edu/catalog.php?record_id=12528#toc.

13. Richard AM, Judson RS, Houck KA, Grulke CM, Volarath P, Thillainadarajah I, Yang C, Rathman J, Martin MT, Wambaugh JF, Knudsen TB, Kancherla J, Mansouri K, Tier G, Williams AJ, Little SB, Crofton KM, Thomas RS. 2016. The ToxCast chemical landscape - Paving the road to 21st century toxicology. *Chem Res Toxicol* 29:1225-1251.

14. Yoon M., Campbell JL, Andersen ME, Clewell HJ. 2012. Quantitative in vitro to in vivo extrapolation of cell-based toxicity assay results. *Crit Rev Toxicol* 42:633-652.

15. Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. 2013. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. *Chem Res Toxicol* 26:878-895.

16. Thomas RS, Philbert MA, Auerbach SS, Wetmore BA, Devito MJ, Cote I, Rowlands JC, Whelan MP, Hays SM, Andersen ME, Meek MEB, Reiter LW, Lambert JC, Clewell HJ, Stephens ML, Zhao QJ, Wesselkamper SC, Flowers L, Carney EW, Pastoor TP, Petersen DD, Yauk CL, Nong A. 2013. Incorporating new technologies into toxicity testing and risk assessment: Moving from 21st century vision to a data-driven framework. *Toxicol Sci* 136:4-18.

17. Wetmore BA. 2015. Quantitative in vitro-to-in vivo extrapolation in a high-throughput environment. *Toxicology* 332:94-101.

18. Wetmore BA, Wambaugh JF, Ferguson SS, Li L, Clewell HJ, Judson RS, Freeman K, Bao W, Sochaski MA, Chu TM, Black MB, Healy E, Allen B, Andersen ME, Wolfinger RD, Thomas RS. 2013. Relative impact of incorporating pharmacokinetics on predicting in vivo hazard and mode of action from high-throughput in vitro toxicity assays. *Toxicol Sci* 132:327-346.

19. Final Act of the Conference of Plenipotentiaries on The Stockholm Convention on Persistent Organic Pollutants. 2001. United Nations Environment Program, Geneva, Switzerland. 44 pp.

20. Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC of the European Parliament and of the Council and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC. 2006. The European Parliament and the Council of the European Union. 673 pp.

21. Toxic Substances Control Act. 2016. US Environmental Protection Agency, Washington, DC.

22. Canadian Environmental Protection Act. 1999. Government of Canada, Canada Gazette Part III 22(3).

23. Gobas FAPC, Xu S, Kozerski G, Powell DE, Woodburn KB, Mackay D, Fairbrother AE. 2015. Fugacity and activity analysis of the bioaccumulation and environmental risks of decamethylcyclopentasiloxane (D5). *Environ Toxicol Chem* 34:2723-2731.

24. Lewis GN.1901. The law of physico-chemical change. *Proc Am Acad Arts Sci* 37:49-69.

25. Lewis GN. 1907. Outlines of a new system of thermodynamic chemistry, *Proc Am Acad Arts Sci* 43:259-293.

26. Overton E. 1896. Uber die osmotischen Eigenschaften der Zelle in ihrer Bedeutung fur die Toxikologie und Pharmakologie, Vierteljahrsschr. *Naturforsch Ges Zuerich* 41:383-406.

27. Meyer H. 1899. Zur Theorie der Alkoholnarkose, *Arch Exp Pathol Pharmakol* 42:109-118.

28. Ferguson J. 1939. The use of chemical potentials as indices of toxicity, *Proc R Soc London B, Biol Sci* 127:387-404.

29. Burkhard LP, Arnot JA, Embry MR, Farley KJ, Hoke RA, Kitano M, Leslie HA, Lotufo GR, Parkerton TF, Sappington KG, Tomy GT. 2012. Comparing laboratory and field measured bioaccumulation endpoints. *Integ Environ Assess Manag* 8:17-31.

30. Connolly JP, Pedersen CJ. 1988. A thermodynamic-based evaluation of organic chemical accumulation in aquatic organisms. *Environ Sci Technol* 22:99-103.

31. Gobas FAPC, Wilcockson JWB, Russell RW, Haffner GD. 1999. Mechanism of biomagnification in fish under laboratory and field conditions. *Environ Sci Technol* 33:133-141.

32. Nfon E, Cousins IT. 2007. Modelling PCB bioaccumulation in a Baltic food web. *Environ Pollut* 148:73-82.

33. Schmidt SN, Mayer P. 2015. Linking algal growth inhibition to chemical activity: Baseline toxicity required 1% of saturation. *Chemosphere* 120:305-308.

34. Mackay D, Arnot JA. 2011. The application of fugacity and activity to simulating the environmental fate of organic contaminants. *J Chem Eng Data* 56:1348-1355.

35. Di Toro DM, CS Zarba, DJ Hansen, WJ Berry, RC Swartz, CE Cowan, SP Pavlou, HE Allen, NA Thomas, PR Paquin. 1991. Technical basis for the equilibrium partitioning method for establishing sediment quality criteria. *Environ Toxicol Chem* 11:1541-1583.

36. Mackay D. 2001. Multimedia environmental models: The fugacity approach. 2nd Edition. Lewis Publishers, Boca Raton, FL. 261 pp.

37. Schwarzenbach RP, Gschwend PM, Imboden DM. 2003. Environmental Organic Chemistry. John Wiley and Sons, Hoboken, NJ. 1313 pp.

38. Mackay D, Arnot JA, Wania F, Bailey RE. 2011. Chemical activity as an integrating concept in environmental assessment and management of contaminants. *Integ Environ Assess Manag* 7:248-255.

39. Prausnitz JM. 1969. Molecular thermodynamics of fluid-phase equilibria. Prentice-Hall, New Jersey.

40. Gobas FAPC, Otton SV, Tupper-Ring LF, Crawford MA. 2015. Chemical activity calculator for solid and liquid non-polar organic chemicals in environmental media. Environmental Toxicology Research Group at Simon Fraser University, Burnaby, BC. Available from: http://www.rem.sfu.ca/toxicology/models/activity-calculator/.

41. DeBruyn AMH, Gobas FAPC. 2007. The sorptive capacity of animal protein. *Environ Toxicol Chem* 26:1803-1808.

42. Seth R, Mackay D, Muncke J. 1999. Estimating the organic carbon partition coefficient and its variability for hydrophobic chemicals. *Environ Sci Technol* 33:2390-2394.

43. Klimisch HJ, Andreae M, Tillman U. 1997. A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regul Toxicol Pharmacol* 25:1-5.

44. Armitage JM, Wania F, Arnot JA. 2014. Application of mass balance models and the chemical activity concept to facilitate the use of in vitro toxicity data for risk assessment. *Environ Sci Technol* 48:9770-9779.

45. Shen OX, Du GZ, Sun H, Wu W, Jiang Y, Song L, Wang X. 2009. Comparison of in-vitro hormone activities of selected phthalates using reporter gene assays. *Toxicol Lett* 191:9-14.

46. Scholz N. 2003. Ecotoxicity and biodegradation of phthalate monoesters. *Chemosphere* 53:921-926.

47. Jonsson S, Baun A. 2003. Toxicity of mono- and diesters of o-phthalic esters to a crustacean, a green alga, and a bacterium, *Environ Toxicol Che*m 22:3037–3043.

48. Mackintosh CE, Maldonado J, Hongwu J, Hoover N, Chong A, Ikonomou MG, Gobas FAPC. 2004. Distribution of phthalate esters in a marine aquatic food web: Comparison to polychlorinated biphenyls, *Environ Sci Technol* 38:2011–2020.

49. Otton SV, Sura S, Blair J, Ikonomou MG, Gobas FAPC. 2008. Biodegradation of mono-alkyl phthalate esters in natural sediments. *Chemosphere* 71:2011-2016.

Figure 1. Concentrations (mol/m^3) (**A**) and corresponding chemical activities (unitless) (**B**) of DEHP in various environmental, abiotic and biotic samples from locations around the world [*7*]. Each data point is the mean or median value of concentration observations in one study. The number of studies is given in brackets. Geometric means of chemical activities of DEHP in the various media are indicated by the horizontal bars. The red line indicates the maximum possible chemical activity (a=1). The range of chemical activities associated with selected biological response endpoints are indicated by the rectangles. Biological responses included are the no-observed-effects-concentrations for DEHP effects in fish, amphibians, invertebrates, bacteria and plants exposed via diet, sludge, sediment or soil reviewed and recommended in [*8*]; the lowest-observed-adverse-effects-levels for effects of DEHP on liver, reproduction and development in rats after oral exposure as reviewed and recommended in [*9*]; in-vitro bioassay responses of estrogenic, androgenic and thyroid activities [*10,45*]; and AC50s from the ToxCast screening program.

Figure 2. Concentrations (mol/m^3) (A) and corresponding chemical activities (unitless) (B) of MEHP in various environmental, abiotic and biotic samples from locations around the world [*7*]. Each data point is the mean or median value of concentration observations in one study. The number of studies is given in brackets. Geometric means of chemical activities of MEHP in the various media are indicated by the horizontal bars. The red line (a=1) indicates the maximum possible chemical activity. The range of chemical activities associated with selected biological response endpoints are indicated by the rectangles. Biological responses included are LC50 and EC50s in aquatic organisms [*46,47*]; in-vitro bioassay effects of MEHP on steroidogenesis or cytotoxicity; and AC50s from the ToxCast screening program.

Figure 3. Cumulative probability distributions of apparent chemical activities corresponding with biological response endpoints of DEHP in various studies. Grey circles represent NOEC values reported in 148 studies, from which EU risk assessors [*8*] selected 7 NOEC values for fish, amphibians, invertebrates, microorganisms and plants exposed via diet, sediment, soil, or sludge (green circles) to represent the toxicity of DEHP. Grey triangles are LOAEL values derived in 451 studies of effects on testes, ovary, development, reproduction or liver in rats exposed to DEHP via the diet or gavage, from which assessors from the US Consumer Products Safety Commission [*9*] selected 6 values representative of biological effects in rats (yellow triangles). Black circles represent AC50s of DEHP from the ToxCast data base. The red line indicates maximum possible chemical activity value of 1.

Figure 4. Cumulative probability distributions of chemical activities of DEHP in abiotic media (A) and biotic media (B), and of MEHP in abiotic media (C) and biotic media (D) in relation to the range of chemical activities associated with biological responses in in-vivo and in-vitro tests. Data points represent the following: in panel A, DEHP external exposures (black circles), NOEC values for DEHP from the EU risk assessment [*8*] (blue circles), LOAELS for DEHP from the US CPSC [*9*] (blue triangles); in panel B, DEHP internal exposures (black circles), EC50/IC50 values for in vitro effects of DEHP (green squares), ToxCast AC50 values for DEHP (green diamonds); in panel C, MEHP

exposures (gray circles), EC50/LC50 values of toxicological effects of MEHP in daphnia and fish (blue squares); in panel in panel D, MEHP internal exposures (gray circles), EC50/IC50 values for in vitro effects of MEHP (green squares), ToxCast AC50 values for MEHP (green diamonds).

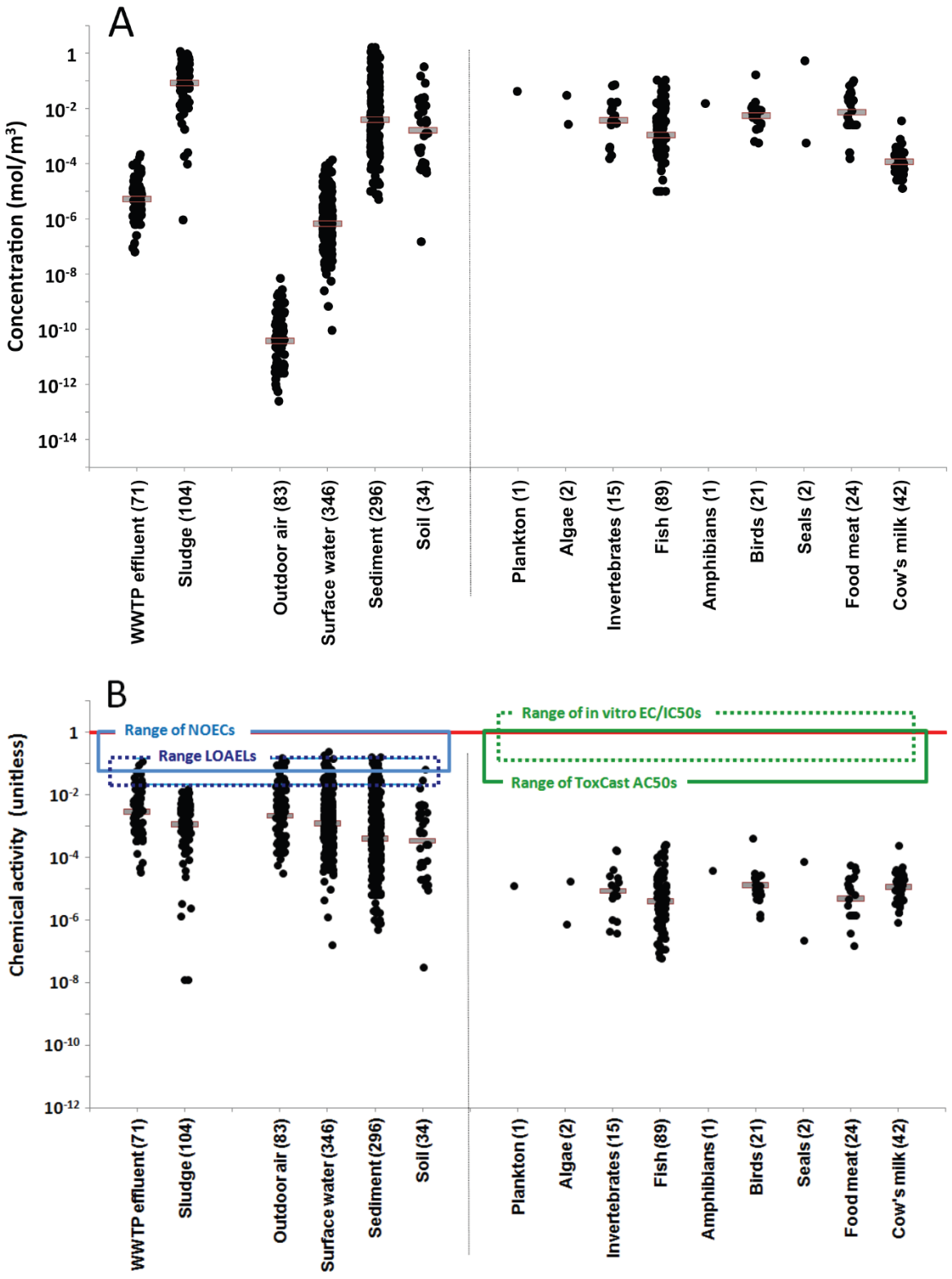

Figure 1

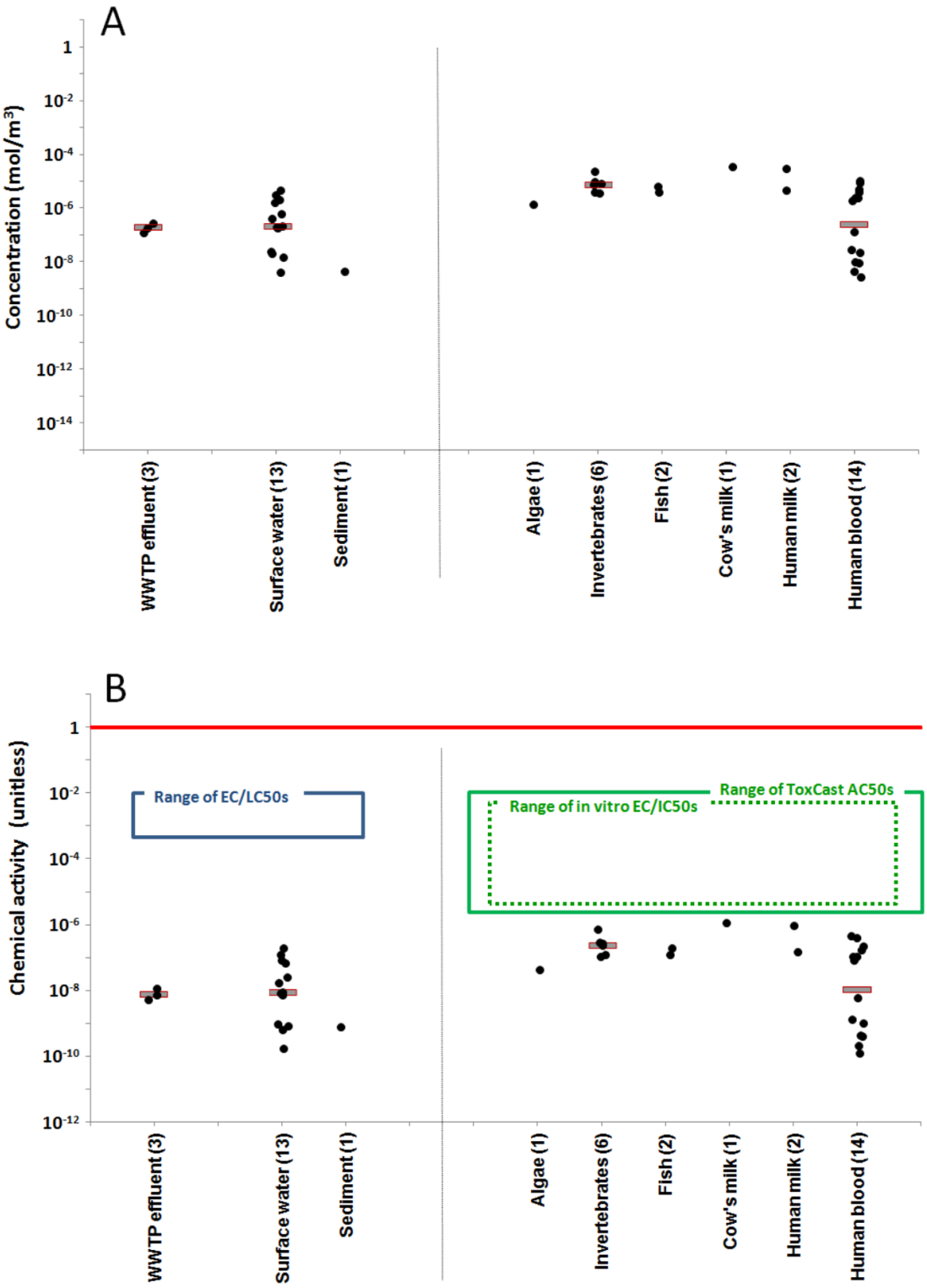

Figure 2

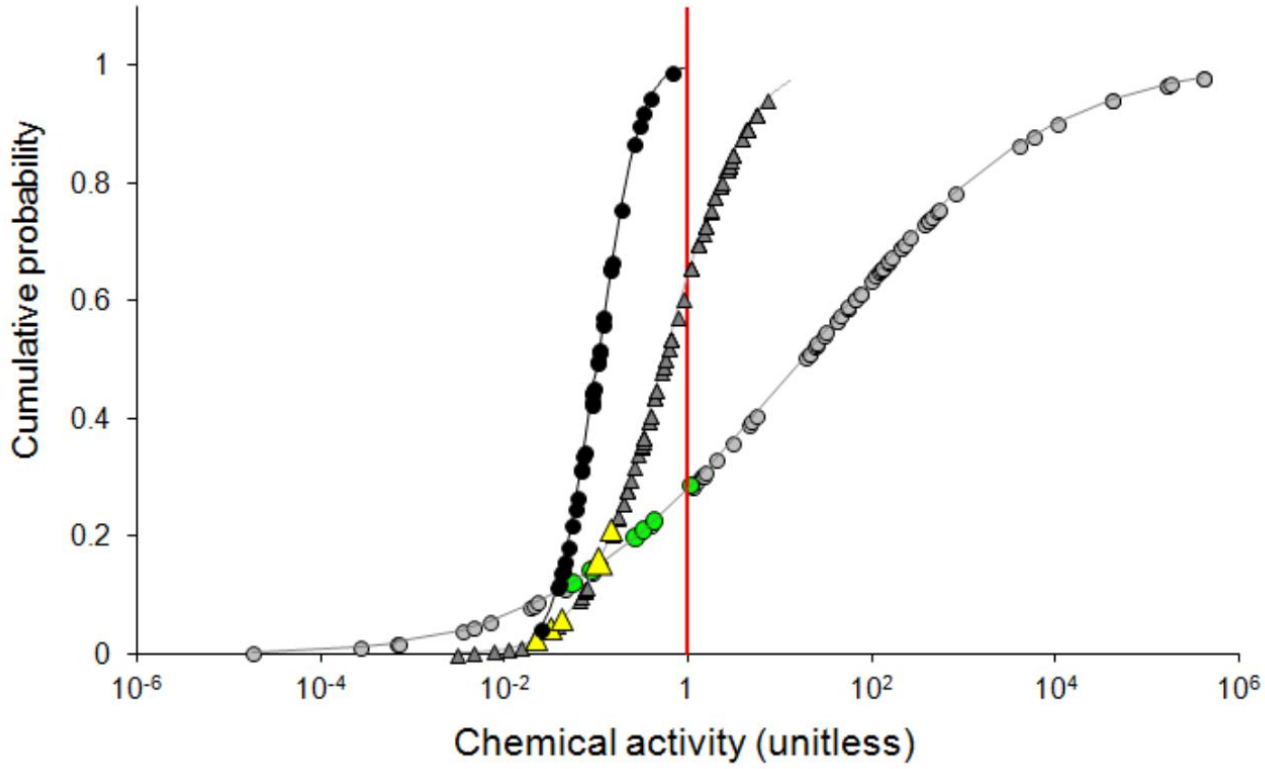

Figure 3

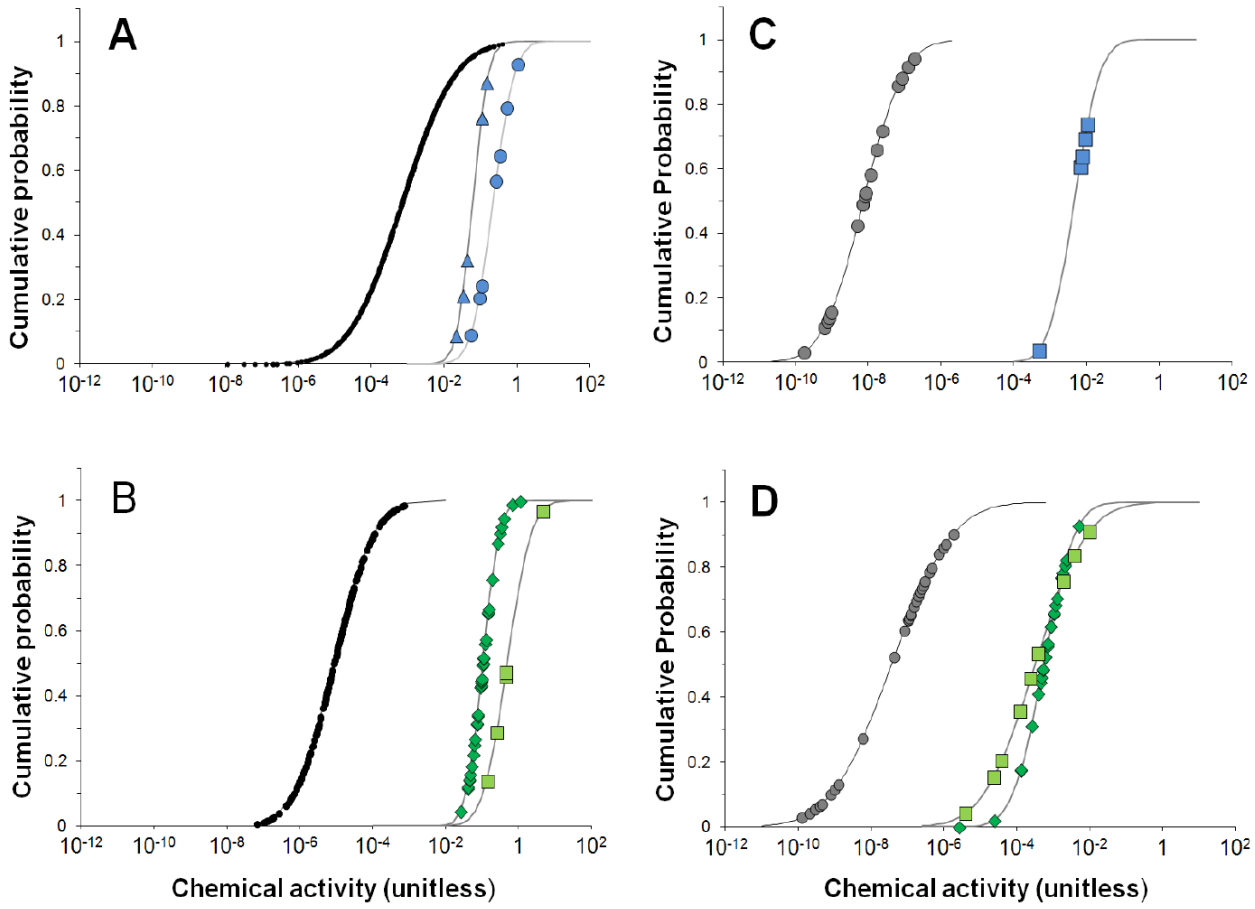

Figure 4