SETAC PRESS

Environmental Toxicology and Chemistry, Vol. 36, No. 6, pp. 1483–1492, 2017

Printed in the USA

CHEMICAL ACTIVITY–BASED ENVIRONMENTAL RISK ANALYSIS OF THE PLASTICIZER DI-ETHYLHEXYL PHTHALATE AND ITS MAIN METABOLITE MONO-ETHYLHEXYL PHTHALATE

Frank A.P.C. Gobas,*†‡ S.Victoria Otton,† Laura F. Tupper-Ring,† Meara A. Crawford,† Kathryn E. Clark,§ and Michael G. Ikonomou‖

†School of Resource and Environmental Management, Simon Fraser University, Burnaby, British Columbia, Canada
‡Department of Biological Sciences, Simon Fraser University, Burnaby, British Columbia, Canada
§BEC Technologies, Aurora, Ontario, Canada
‖Institute of Ocean Sciences, Ocean Sciences Division, Fisheries and Oceans Canada, Sidney, British Columbia, Canada

(Submitted 2 September 2016; Returned for Revision 30 September 2016; Accepted 14 November 2016)

Abstract: The present study applies a chemical activity–based approach to: 1) evaluate environmental concentrations of di-ethylhexyl phthalate (DEHP; $n = 23\,651$) and its metabolite mono-ethylhexyl phthalate (MEHP; $n = 1232$) in 16 environmental media from 1174 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 the US Environmental Protection Agency's Toxicity Forecaster and other sources; and 2) 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 100-fold lower than that in abiotic samples, likely because of biotransformation of DEHP in biota. Biological responses in both in vivo and in vitro tests occur at chemical activities between 0.01 to 1 for DEHP and between approximately 10^{-6} and 10^{-2} for MEHP, suggesting a greater potency of MEHP compared with DEHP. Chemical activities of both DEHP and MEHP in biota samples were less than those causing biological responses in the in vitro bioassays, without exception. A small fraction of chemical activities of DEHP in abiotic environmental samples (i.e., 4–8%) and none (0%) for MEHP were within the range of chemical activities associated with observed toxicological responses in the in vivo tests. The present study illustrates the chemical activity approach for conducting risk analyses. *Environ Toxicol Chem* 2017;36:1483–1492. © 2016 SETAC

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

INTRODUCTION

Di-ethylhexyl phthalate (DEHP) is a di-alkyl ester that is used worldwide, mainly as a plasticizer in composite materials such as polyvinyl chloride, polystyrene, and polyacetates to make plastics soft and flexible [1]. The global production volume of DEHP is approximately 3 million tonne/yr [2]. Di-ethylhexyl phthalate 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 its 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 as a result of 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 US Environmental Protection Agency's Toxicity Forecaster (ToxCast) program [13], which aims to minimize animal use and costs and improve toxicological insights. Efforts to relate the data from ToxCast's high-throughput in vitro screening to exposure and toxicity in whole organisms are ongoing [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. They affect the ongoing risk assessment of commercial substances under the United Nations Stockholm Convention on Persistent Organic Pollutants [19]; the European Union regulations on Registration, Evaluation, Authorization and Restriction of Chemicals (REACh) [20]; the US Toxic Substances Control Act [21]; the Canadian Environmental

This article includes online-only Supplemental Data.

* Address correspondence to gobas@sfu.ca

Published online 17 November 2016 in Wiley Online Library (wileyonlinelibrary.com).
DOI: 10.1002/etc.3689

Protection Act [22]; and similar regulatory programs throughout the world. It is the objective of the present 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 field studies, in vitro experiments, and in vivo experiments in terms of a common quantity (i.e., chemical activity), so that they can be compared using statistical methods. This technique 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 chemical dynamics in both 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]

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

For substances that are liquid at environmental temperatures, including DEHP, 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 in which the chemical activities of the chemical in 2 or more media (e.g., media i and j) are equal [39]

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

This is useful because it provides a method for relating and comparing activities in multimedia systems such as natural environments and for testing hypotheses of equilibrium partitioning in the environment. In the environment, however, physical and biological processes often interfere with the chemical's natural tendency to achieve 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 with 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 strategy 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 artifacts, often because of 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 of 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 nonpolar 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 the 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 technique, but one that requires further study, is that for chemicals with certain modes of toxic action (e.g., nonpolar and polar narcosis), chemical activities appear to be additive [33,34]. This is likely relevant to the risk assessment for DEHP because 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; however, it 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. Furthermore, 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 nonlinearity between chemical activity and concentration is expected [39].

When comparing chemical activities among different media in a risk assessment, it is important to avoid making erroneous assumptions regarding the occurrence of chemical equilibrium of DEHP among environmental media. This is especially important when comparing chemical activities in abiotic media (e.g., water, sediment, or soil) with 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 a chemical that is biotransformed, for example, 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, concentration of suspended solids, 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 the present study in the *Methods* section and in the Supplemental Data) 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 Gobas et al. [40]. Solubilities (mol/m^3) of DEHP in pure water (S_W), pure air (S_A), and pure lipids (S_L) were determined from the reported aqueous solubility, Henry's law constants, and octanol–water partition coefficient (K_{OW}), which have been compiled and reviewed [3,6]. The authors' recommended values for the chemical properties (Supplemental Data, Table S1) were used in the calculations. Henry's law constants and octanol–air partition coefficient values were adjusted for temperature as described in Gobas et al. [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 K_{OW} 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 Gobas et al. [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 (Supplemental Data, Table S1). Sorptive capacities of DEHP and MEHP in proteins and organic carbon were calculated as $0.05\,S_L$ [41] and $0.35\,S_L$ [42], respectively. For heterogeneous environmental media consisting of a combination of media (e.g., surface water samples containing water and suspended solids, and biological media containing water, protein, and lipids), the combined solubility (S_T) in the environmental matrix (Equation 3) was determined as

$$S_T = \sum_{j=1}^{m} \phi_j \times S_j \quad (3)$$

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 Supplemental Data, Table S2.

Environmental concentrations

Reported concentrations of DEHP ($n = 23\,651$ measurements) and MEHP ($n = 1232$ measurements) in 16 environmental media (i.e., outdoor air, sediment, surface water, soil, sludge, wastewater treatment plant effluent, algae, plankton, invertebrates, fish, amphibians, birds, seals, meat for human consumption, milk, and blood) from locations in the United States, Canada, Europe, and Asia over the period between 1995 and July 2010 were compiled from 1131 studies for DEHP and 43 studies for MEHP reported by Clark [7] and summarized in the Appendix to the Supplemental Data. Clark [7] used the Klimisch et al. 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, 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 quality assurance/quality control measures, high concentrations noted in blanks, studies including data that may not be representative of ambient conditions [e.g., studies that included samples from a known source of DEHP]); and 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 1131 DEHP exposure studies used in this analysis. In the Supplemental Data, 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] nor in the present study because this would result in removal of some potentially high-quality data. Nondetectable concentrations were set to one-half of the reported detection limit [7]. The mean DEHP and MEHP concentrations from each study in categories 1, 2, and 4 were 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 studies 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 Supplemental Data.

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 2 reports. First, as part of a DEHP risk assessment in 2008, the European Union [8] reviewed acute and chronic toxicity studies of DEHP in aquatic and terrestrial organisms. From a total of 148 studies, 7 high-quality no-observed-effect concentrations (NOECs) were selected by the European Union 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 Supplemental Data, Table S3.

Second, more than 750 studies reporting median lethal concentration (LC50) and lowest-observed-adverse-effect level (LOAEL) values for DEHP effects in laboratory animals were reviewed by the US Consumer Product Safety Commission in 2010 [9]. From these, 6 studies with LOAEL values for effects on liver, reproduction, and development in rats were chosen by the Consumer Product Safety Commission assessors as representative toxicological endpoints and used in the calculation of acute, subchronic, and chronic acceptable daily intakes for the general population, children, men, and women of childbearing age. The selected LOAEL values are listed in Supplemental Data, Table S3. The 2008 European Union risk assessment-selected NOECs [8] and the US Consumer Product Safety Commission–selected LOAELs [9] were expressed in terms of chemical activities using the same methods as those described for the exposure concentrations.

DEHP toxicity in vitro

The ToxCast screening program included DEHP in 1080 high-throughput screening assays (as of March 2016) for effects in vitro using intact cells, membrane incubations, and reporter gene assays, for example. 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 the nominal concentration, in μM, at which 50% of maximal biological activity was observed (AC50). Di-ethylhexyl phthalate was stated to be 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 Armitage et al. [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, lipids, and water. The in vitro incubation conditions used to calculate the activities are the same as those used in Armitage et al. [44] and are assumed to be identical across all assays. The details of the calculations are described in the Supplemental Data, and the values are listed in Supplemental Data, Table S4.

The chemical activity values obtained from ToxCast high-throughput screening technology were compared with 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 Shen et al. [45]. In total, 5 endpoints representative of DEHP effects observed in in vitro bioassays were expressed in terms of chemical activity (using the reported incubation parameters) and are presented in Supplemental Data, Table S3.

MEHP toxicity in vivo

To date, only 5 studies report on biological responses of MEHP in whole organisms: 1 LC50 value in carp [46] and 4 median effective concentration (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 Supplemental Data, and the values are given in Supplemental Data, Table S5.

MEHP toxicity in vitro

Mono-ethylhexyl phthalate has been examined using the ToxCast technology utilizing the same 1080 tests as those used for DEHP. Mono-ethylhexyl phthalate 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 Supplemental Data. The ToxCast AC50 values expressed as chemical activity are given in Supplemental Data, Table S6. Mono-ethylhexyl phthalate in vitro effects on steroidogenesis or cytotoxicity were compiled from the primary literature, and results from 10 studies are given in Supplemental Data, Table S5. These EC50 values and median inhibitory concentration (IC50) values were converted to chemical activity using reported incubation parameters as described in the Supplemental Data.

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). These variations reflect 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 because differences in DEHP's affinity for different media are 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's t test ($p < 0.05$). 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. The 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, as well as associated low bioavailability for microbial degradation, may

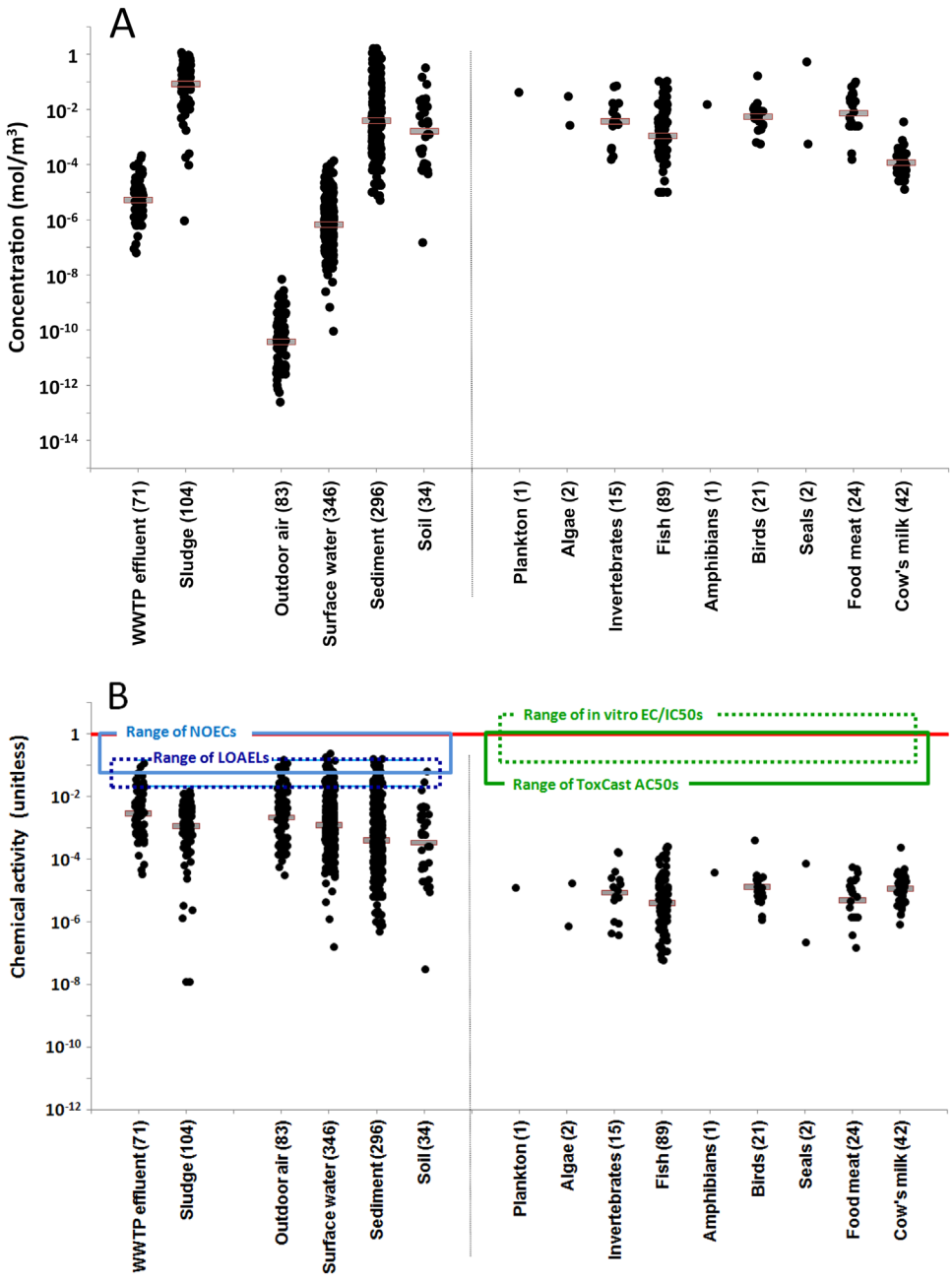

Figure 1. (**A**) Concentrations (mol/m^3) and (**B**) corresponding chemical activities (unitless) of DEHP in various environmental, abiotic, and biotic samples from locations around the world [7]. Each datapoint is the mean or median value of concentration observations in 1 study. The number of studies is given in parentheses. Geometric means of chemical activities of DEHP in the various media are indicated by the horizontal bars. The red line signifies the maximum possible chemical activity ($a = 1$). The ranges of chemical activities associated with selected biological–response endpoints are illustrated by the rectangles. Biological responses included are: the NOECs for DEHP effects in fish, amphibians, invertebrates, bacteria, and plants exposed via diet, sludge, sediment, or soil, as reviewed and recommended in Pakalin et al. [8]; the LOAELs for effects of DEHP on the liver, reproduction, and development in rats after oral exposure, as reviewed and recommended in Carlson [9]; in vitro bioassay responses of estrogenic, androgenic, and thyroid activities [10,45]; and AC50s from the ToxCast screening program. DEHP = di-ethylhexyl phthalate; NOEC = no-observed-effect concentration; LOAELs = lowest-observed-adverse-effect level; EC/IC50s = median effect or inhibitory concentrations; AC50s = median active concentrations; ToxCast = USEPA's Toxicity Forecaster; WWTP = wastewater treatment plant.

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 with 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 1 particular location shows that lipid normalized concentrations of DEHP in biota (which is a proxy for chemical activity) follow a statistically significant decline in concentrations with increasing trophic levels [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 5 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], as a result of the high hydrophobicity and sorption affinity to the particulate matter of its precursor (DEHP), and MEHP's high apparent microbial biodegradability, caused by 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

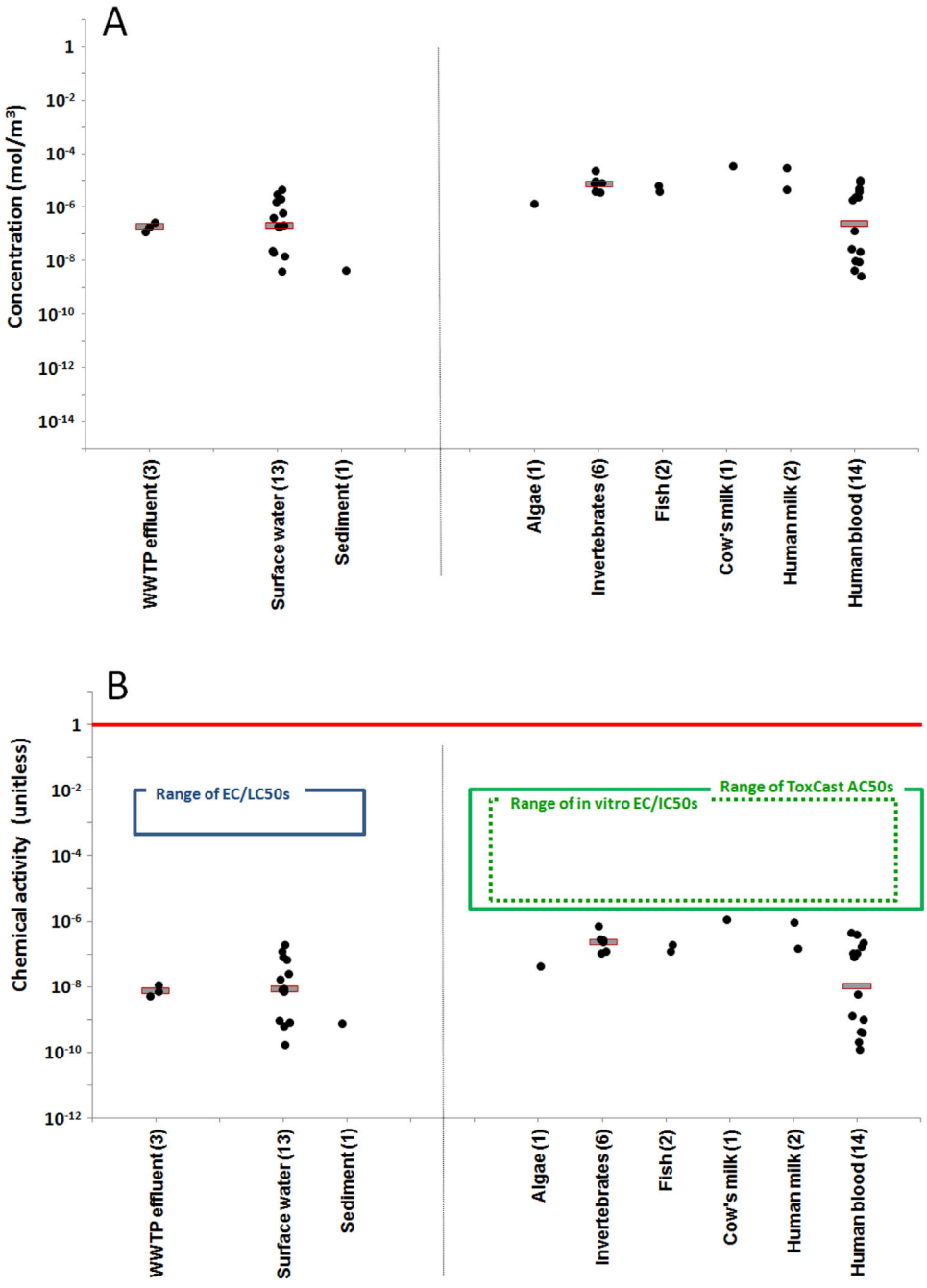

Figure 2. (**A**) Concentrations (mol/m^3) and (**B**) corresponding chemical activities (unitless) of MEHP in various environmental, abiotic, and biotic samples from locations around the world [7]. Each datapoint is the mean or median value of concentration observations in 1 study. The number of studies is given in parentheses. Geometric means of chemical activities of MEHP in the various media are shown by the horizontal bars. The red line represents the maximum possible chemical activity ($a = 1$). The ranges of chemical activities associated with selected biological–response endpoints are indicated by the rectangles. Biological responses included are LC50s and EC50s in aquatic organisms [46,47], in vitro bioassay effects of MEHP on steroidogenesis or cytotoxicity, and AC50s from the ToxCast screening program. MEHP = mono-ethylhexyl phthalate; EC/LC50s = median effect or lethal concentrations; EC/IC50s = median effect or inhibitory concentrations; AC50s = median active concentrations; ToxCast = USEPA's Toxicity Forecaster; WWTP = wastewater treatment plant.

because of 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 European Union [8] and the US Consumer Product Safety Commission [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 the studies reviewed by the European Union [8] and the US Consumer Product Safety Commission [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 to or less than 1. This issue was also recognized by the European Union risk assessment [8] and the US Consumer Product Safety Commission [9], resulting in the selection, respectively, of 7 NOECs and 6 LOAELs that are below solubility and representative of DEHP in vivo toxicity. The selected studies indicate that chemical activity ranges between 0.05 to 1 for NOECs and from 0.02 to 0.15 for LOAELs (Figure 1B). The range of abiotic chemical activities of 0.02 to 1 associated with in vivo NOECs and LOAELs is similar to the biotic activity range of 0.01 to 1 associated with nonpolar narcosis [33,34]. At first glance, this may indicate that DEHP causes biological responses through a nonspecific mode of action similar to nonpolar 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

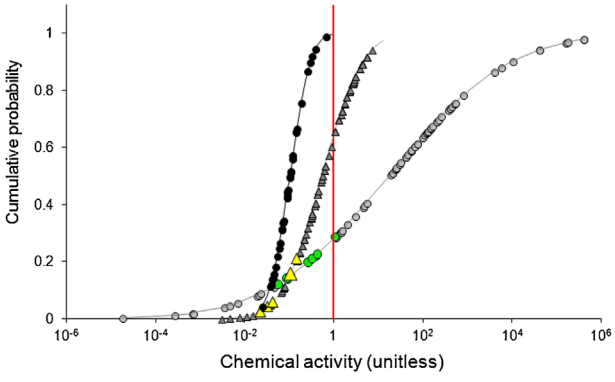

Figure 3. Cumulative probability distributions of apparent chemical activities corresponding with biological–response endpoints of DEHP in various studies. Gray circles represent NOEC values reported in 148 studies, from which European Union risk assessors [8] selected 7 NOEC values for fish, amphibians, invertebrates, microorganisms, and plants exposed via diet, sediment, soil, or sludge (green circles) to illustrate the toxicity of DEHP. Gray 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 Product Safety Commission [9] selected 6 values representative of biological effects in rats (yellow triangles). Black circles signify AC50s of DEHP from the ToxCast database. The red line indicates a maximum possible chemical activity value of 1. DEHP = di-ethylhexyl phthalate; NOEC = no-observed-effect concentration; LOAEL = lowest-observed-adverse-effect level; AC50s = median active concentrations; ToxCast = USEPA's Toxicity Forecaster.

associated with nonpolar narcosis. This suggests that the observed biological responses of DEHP in the in vivo tests may not be associated with nonpolar narcosis.

Under the ToxCast program, in vitro testing showed biological activity of DEHP in 40 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 from 0.025 to 1.06. In vitro toxicities in non-ToxCast studies range from 0.13 to 0.45, with the exception of 1 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 NOECs and LOAELs in the 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 method may be a useful tool for in vitro to in vivo extrapolation of biological responses. The range of chemical activities associated with biological activities in the in vitro tests also overlaps with the activity range associated with nonpolar narcosis (0.01–1). This suggests that biological activities in these bioassays may represent a mode of action described by nonpolar 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 nonpolar, 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 and 0.005, which are lower than those observed for DEHP in the in vitro tests. Chemical activities corresponding with acute mortality of MEHP in the in vivo studies ranged between 0.0005 and 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 the in vivo and the in vitro tests overlap (Figure 2B), establishing that biological activity of MEHP in the in vivo and the 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. Nevertheless, the fact that only 2.9% of the ToxCast in vitro bioassays show biological activity conveys the importance of choosing appropriate receptors in the in vitro testing protocols.

Risk

Risk is defined in the present study 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 NOECs

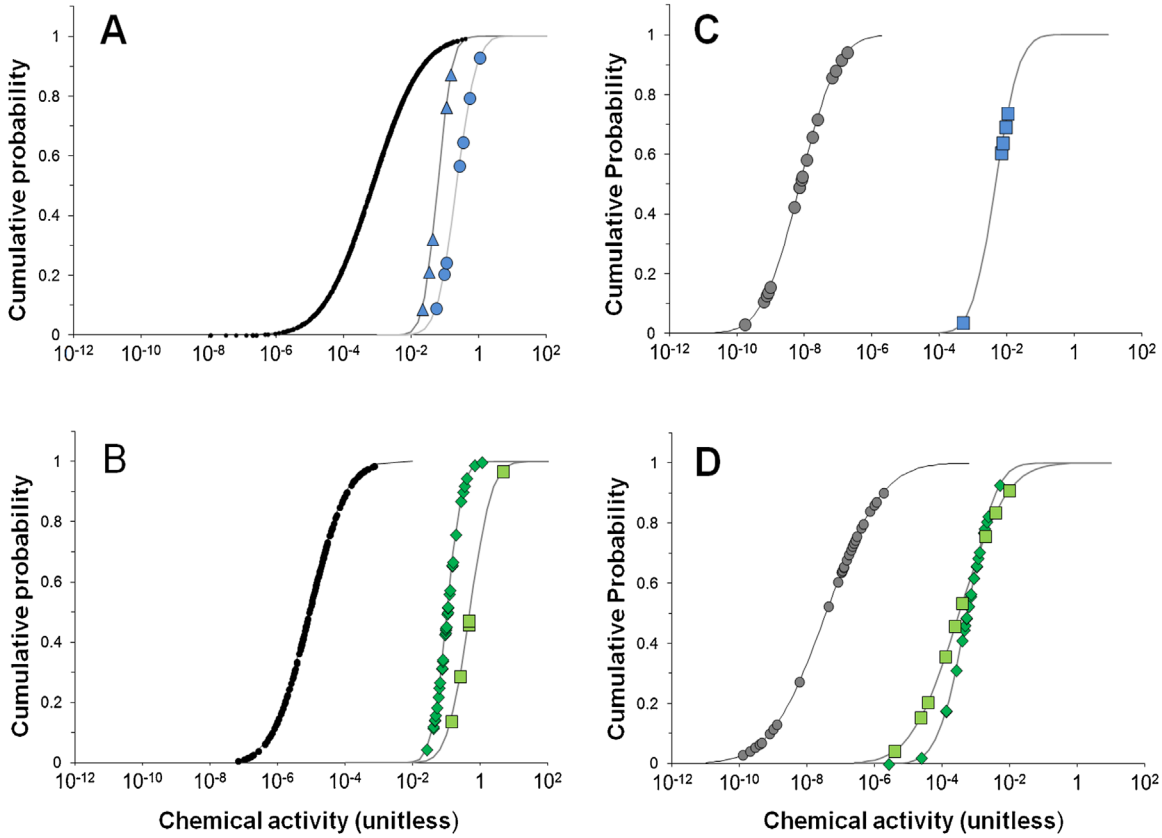

Figure 4. Cumulative probability distributions of chemical activities of DEHP in (**A**) abiotic media and (**B**) biotic media and of MEHP in (**C**) abiotic media and (**D**) biotic media in relation to the range of chemical activities associated with biological responses in the in vivo and in vitro tests. Datapoints represent the following: (**A**) DEHP external exposures (black circles), NOEC values for DEHP from the European Union risk assessment [8] (blue circles), and LOAELs for DEHP from the US Consumer Product Safety Commission [9] (blue triangles); (**B**) DEHP internal exposures (black circles), EC50/IC50 values for in vitro effects of DEHP (green squares), and ToxCast AC50 values for DEHP (green diamonds); (**C**) MEHP external exposures (gray circles), and EC50/LC50 values of toxicological effects of MEHP in *Daphnia* and fish (blue squares); and (**D**) MEHP internal exposures (gray circles), EC50/IC50 values for in vitro effects of MEHP (green squares), and ToxCast AC50 values for MEHP (green diamonds). DEHP = di-ethylhexyl phthalate; MEHP = mono-ethylhexyl phthalate; NOEC = no-observed-effect concentration; LOAELs = lowest observed adverse effect levels; EC50 = median effective concentration; IC50 = median inhibitory concentration; ToxCast = USEPA's Toxicity Forecaster; AC50 = median active concentration; LC50 = median lethal concentration.

established by the European Union risk assessment [8], whereas 76 out of 934 studies (or 8.1%) exhibit mean DEHP activities within the range of LOAELs identified by the US Consumer Product Safety Commission [9]. The mean global DEHP activity in environmental media external to organisms is well below the chemical activity ranges associated with LOAELs and NOECs.

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 and estrogenic, androgenic, and antithyroid hormone activity. These results show that DEHP concentrations in biota at the study locations were not at concentrations that are associated with known biological effects in the in vitro studies.

Mono-ethylhexyl phthalate activities in surface water, sediment, and wastewater 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, (Figure 4D) lower than those associated with in vitro bioassay responses. This implies that MEHP concentrations in the biological samples that were investigated are not at levels that may be of concern.

It should be stressed that 1 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.3689.

Acknowledgment—The authors thank the American Chemistry Council for its grant and other support of this research.

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

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