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Published in:
Environmental Science & Technology Letters

Link to article, DOI:
[10.1021/acs.estlett.7b00159](https://doi.org/10.1021/acs.estlett.7b00159)

Publication date:
2017

Document Version
Peer reviewed version

[Link back to DTU Orbit](#)

Citation (APA):
Rolle, M., & Jin, B. (2017). Normal and Inverse Diffusive Isotope Fractionation of Deuterated Toluene and Benzene in Aqueous Systems. *Environmental Science & Technology Letters*, 4(7), 298-304.
<https://doi.org/10.1021/acs.estlett.7b00159>

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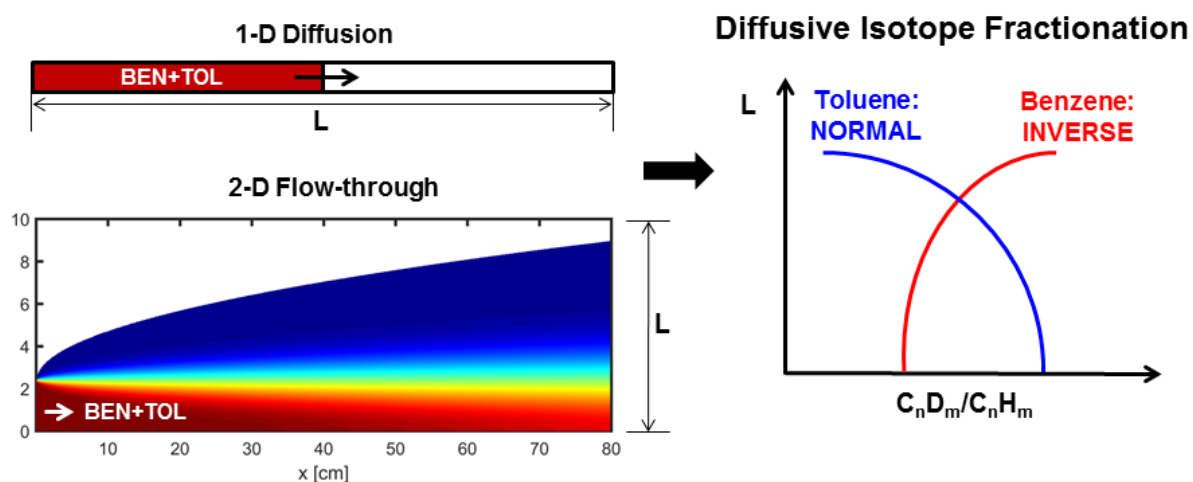
Normal and Inverse Diffusive Isotope Fractionation of Deuterated Toluene and Benzene in Aqueous Systems

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1 **Abstract**

2 Diffusive isotope fractionation of organic contaminants in aqueous solution is difficult to quantify
3 and only a few experimental datasets are available for compounds of environmental interest. In this
4 study, we investigate diffusive fractionation of perdeuterated and non-deuterated benzene and
5 toluene. Multitracer experiments were carried out in 1-D gel dissection tubes and in a quasi 2-D
6 flow-through porous medium. The experiments allowed us to simultaneously and directly compare
7 the diffusive and dispersive behavior of benzene and toluene. We observed an unexpected, opposite
8 behavior of the two monoaromatic hydrocarbons. Toluene showed a normal diffusive isotope effect
9 ($D_{C_7D_8}/D_{C_7H_8}=0.96$) with enrichment of the non-deuterated isotopologue in the direction of the
10 diffusive and transverse dispersive fluxes. Conversely, the measured trends for benzene indicate
11 inverse diffusive fractionation ($D_{C_6D_6}/D_{C_6H_6}=1.02$), with a remarkably faster diffusion rate of the
12 perdeuterated isotopologue that was enriched in the downgradient portion of the diffusion tubes and
13 at the fringes of the contaminant plumes in the flow-through setup. These outcomes can neither be
14 interpreted as mass-dependent fractionation nor be described as purely hydrodynamic (i.e., mass
15 independent) effects. The results of this study are relevant for the use of labeled/non-labeled
16 mixtures of organic compounds as conservative and (bio)reactive tracers in environmental
17 applications.

18

19 **Introduction**

20 Aromatic hydrocarbons are widespread contaminants, frequently found in soils and aquatic
21 environments. Among these chemicals BTEX (i.e., benzene, toluene, ethylbenzene and xylene)
22 compounds are of particular concern due to their relatively high solubility, mobility and toxicity.¹
23 Mixtures of labeled and nonlabeled compounds have been often applied as a diagnostic tool to
24 understand and quantify contaminant transport and transformation mechanisms. For instance, in the
25 field of groundwater contamination, deuterium-labeled BTEX compounds have been used in tracer
26 tests, push-pull tests and in situ microcosms to evaluate contaminant retardation and in situ rates of
27 transformation during groundwater bioremediation and natural attenuation.²⁻⁶ One important aspect
28 that has not (yet) been evaluated in detail is the extent of diffusive isotope fractionation of BTEX
29 compounds in aqueous solution and its implications in environmental systems. The evaluation of
30 such effect is important since recent investigation of subsurface solute transport has highlighted the
31 key controlling role of aqueous diffusion for groundwater contaminant transport at different
32 scales.⁷⁻¹⁰ Despite the increased recognition of the quantitative importance and of the macroscopic
33 impact of small scale diffusive processes on large scale transport of organic contaminants, only a
34 few experimental and modeling studies have attempted to quantify diffusive isotope fractionation
35 for organic compounds.¹¹⁻¹⁶ The lack of data and mechanistic understanding of organic chemicals'
36 diffusive isotope fractionation becomes apparent when compared with the advances in the related
37 field of inorganic isotope geochemistry, in which numerous studies have been carried out to
38 investigate diffusive isotope effects of major cations, anions and dissolved gases in both aqueous
39 solutions¹⁷⁻²⁹ and non-aqueous systems.³⁰⁻³³

40 In this work we focus on diffusive transport of perdeuterated and non-deuterated benzene and
41 toluene. The diffusive behavior of these chemicals (particularly of benzene) was investigated in
42 early studies,³⁴⁻³⁶ mostly as self-diffusion or as tracer diffusion in organic solvents. However, to the

43 best of our knowledge, no study has reported a comparison of diffusive isotope fractionation of
44 these contaminants in aqueous solution. The purpose of this Letter is to report the unexpected,
45 contrasting fractionation behavior of toluene and benzene that we have observed in a series of
46 experiments performed in different setups under purely diffusive conditions (1-D gel dissection
47 tubes), as well as in flow-through systems (2-D flow-through chamber). A key feature of the
48 experiments was the simultaneous presence of non-deuterated and perdeuterated mixtures of
49 toluene and benzene undergoing diffusion and lateral dispersion. In this way, in each experiment the
50 4 tracers (C_6H_6 , C_6D_6 , C_7H_8 , C_7D_8) were all transported under the same conditions, which facilitates
51 a direct comparison of the effects of diffusion and transverse dispersion on the concentration of
52 non-deuterated and perdeuterated benzene and toluene and, more importantly, on their diffusive
53 isotope fractionation.

54

55 **Materials and Methods**

56 **Chemicals and Analytical Methods.** High-purity organic compounds (99.5%, Sigma-Aldrich,
57 Germany) were used in the experiments. Measurements were carried out with a 7890A gas
58 chromatograph (GC) with a capillary column (30m×250 μ m, 1.0 μ m film thickness; Agilent, USA)
59 coupled to a 5975C tri-axis mass selective detector (MSD) (Agilent, USA). Headspace samples
60 were injected for analysis using a COMBIPAL multi-purpose autosampler system.

61 **1-D Diffusion Experiment.** Gel dissection experiments were performed in cylindrical glass tubes
62 (1.1 cm diameter and 20 cm length) using agarose gel prepared with a minimum amount (1% w/w)
63 of phyto agar (Duchefa, Netherlands). Agar solutions containing mixtures of dissolved toluene,
64 perdeuterated toluene, benzene and perdeuterated benzene (1:1:1:1 volume proportion) were
65 prepared and filled in the first 10 cm of the diffusion tubes. This zone acted as contaminant source
66 during the experiments in which the compounds diffused towards the remaining portion of the tubes

67 that was filled with pure gel medium. The tubes were kept horizontally at a constant temperature of
68 20 °C. After 9 and 15 days, the tubes were sampled by cutting the gel into 1 cm slices with a scalpel.
69 The slices were immediately sealed in 10 ml glass vials with screw caps and sent to GC analysis.

70 **2-D Flow-through Experiment.** Flow-through experiments were performed in a quasi two-
71 dimensional flow-through chamber (inner dimensions: 80cm×18cm×1cm, L×H×W), equipped with
72 10 equally-spaced (1 cm spacing) ports both at the inlet and at the outlet. The flow-through system
73 was filled with homogeneous quartz sand (Euroquarz, Germany) with grain diameter of 1.0-1.5 mm.
74 The sand was washed in an acidic solution and dried for 12 hours in an oven at 120 °C before filling
75 the flow-through chamber. The sand was filled with a wet-packing procedure using ultra-pure Milli-
76 Q water (EvoquaWater, USA) to avoid air entrapment in the porous medium.³⁷ The inlet and outlet
77 ports were connected to two high-precision multi-channel peristaltic pumps (IPC-N24, Ismatec,
78 Switzerland). Sampling was performed with a 10-channel syringe pump (KD Scientific, USA). The
79 system was operated at a seepage velocity of 0.8 m/d. An aqueous solution containing the four
80 isotopologues was continuously injected from the two lowermost ports at the inlet of the flow-
81 through chamber. After establishing a steady-state plume (i.e., exchanging at least two pore
82 volumes), samples were taken at the outlet ports and analyzed for the concentrations of deuterated
83 and non-deuterated compounds.

84 **Modeling Approach.** The governing equations for contaminant transport in the two experimental
85 setups are the 1-D Fick's second law of diffusion³⁸ in the gel dissection tubes, and the 2-D steady
86 state advection dispersion equation in the 2-D flow-through setup.³⁹⁻⁴⁰ The models used to
87 quantitatively interpret the experimental results are based, respectively, on a numerical and an
88 analytical solution of these governing equations. The key parameters controlling transport of the
89 different toluene and benzene isotopologues are their diffusion coefficients and, in the flow-through
90 setup, their transverse hydrodynamic dispersion coefficients. The governing equations and their

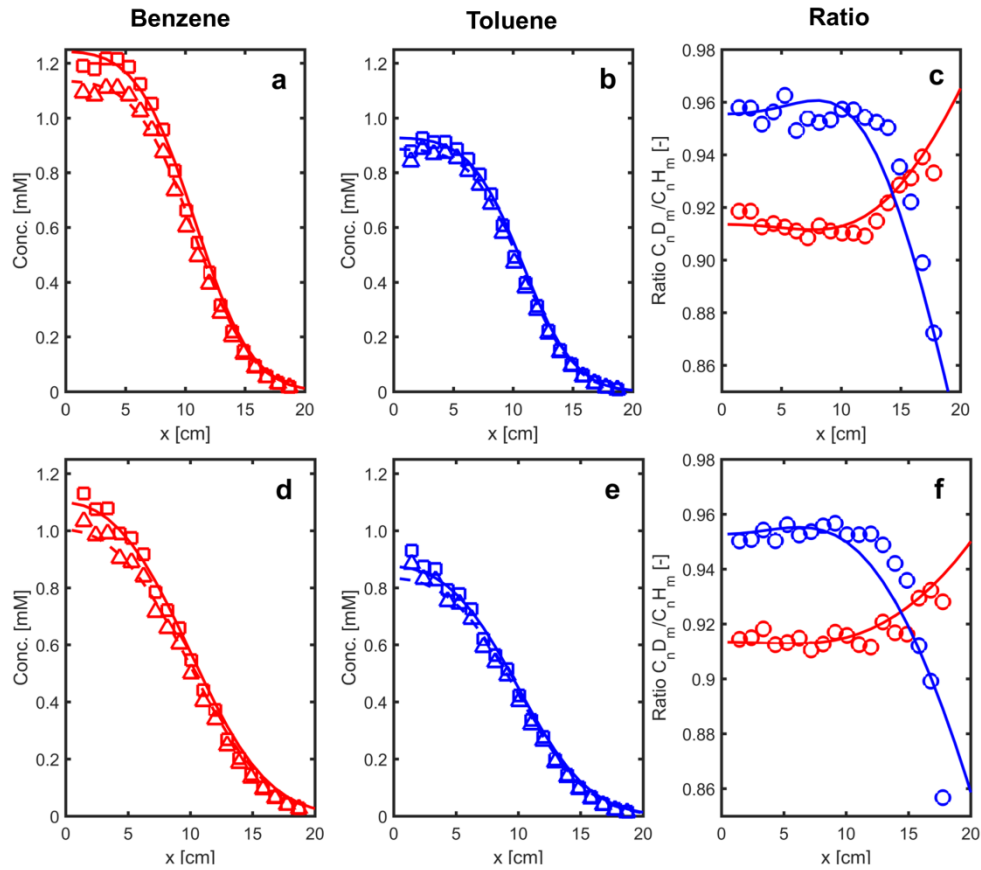
91 boundary conditions are summarized in the Supporting Information together with concentration
92 maps of the steady-state plumes in the 2-D flow-through setup.

93

94 **Results and Discussion**

95 Two examples of benzene and toluene spatial profiles measured in the 1-D setup after 9 and 15 days
96 of diffusion are illustrated in Figure 1. The plots show typical diffusion curves with small
97 differences between deuterated and non-deuterated compounds that are difficult to appreciate in the
98 concentration plots. The differences become apparent by plotting the ratios C_6D_6/C_6H_6 and
99 C_7D_8/C_7H_8 . These ratios surprisingly show an opposite trend with a decreasing pattern for toluene,
100 and a reverse, increasing trend for benzene. To quantify these observations we used a simple
101 inverse power law model,^{21,41} which relates the diffusion coefficients of the deuterated and non-
102 deuterated isotopologues to their molecular masses:

$$\frac{D_{C_nD_m}}{D_{C_nH_m}} = \left(\frac{m_{C_nH_m}}{m_{C_nD_m}} \right)^\beta \quad (1)$$



103

104 Figure 1. Spatial profiles of concentration and isotopologue ratio for benzene and toluene along the
 105 length of the diffusion tubes in the 9-day experiment A (panels a-c) and in the 15-day experiment C
 106 (panels d-f). The symbols (squares for the non-deuterated, triangles for the perdeuterated
 107 isotopologues, circles for the ratios) represent the measured data, whereas the lines are the outcomes
 108 of the simulations.

109

110 Fitting the experimental data allowed estimating the values of the diffusion coefficients for the non-
 111 deuterated and D-labeled isotopologues and, thus, the value of the exponent β expressing the mass
 112 dependence of the isotopologues diffusion coefficients. The fitting procedure was carried out using
 113 the function *lsqnonlin* implemented in MATLAB. Table 1 summarizes the results obtained for the
 114 different tube experiments and reports the observed diffusive isotope fractionation. Both the
 115 graphical representation of the experimental data (Figure 1) and the parameters reported in Table 1

116 show a normal isotope effect for toluene and an inverse isotope effect for benzene. In the case of
 117 toluene, the deuterated isotopologue has a slightly lower diffusion coefficient and results in
 118 decreasing isotope ratios as the toluene species diffuse towards the pure gel medium. For benzene,
 119 instead, the data show an inverse diffusive isotope effect: the molecules of the deuterated
 120 isotopologue diffuse at a slightly faster rate and become enriched in the initially pure gel medium,
 121 as benzene diffuses from the contamination source. This behavior was consistently observed in all
 122 experiments. The β (0.444-0.490) values for toluene are consistent with those of previous
 123 experiments,¹⁵ whereas the negative values for benzene clearly indicate an inverse isotope effect.
 124 Average values characterizing normal and inverse isotope fractionation observed for toluene and
 125 benzene in these diffusion experiments are: $D_{C_7D_8}/D_{C_7H_8}=0.962\pm 0.002$ and
 126 $D_{C_6D_6}/D_{C_6H_6}=1.019\pm 0.002$.

127

128 Table 1. Summary of the results for the multitracer 1-D diffusion experiments.

| Experiment | Time [Days] | $D_{C_nH_m}$ [$\times 10^{-9} m^2 s^{-1}$] | $D_{C_nD_m}$ [$\times 10^{-9} m^2 s^{-1}$] | β [-] | $D_{C_nD_m}/D_{C_nH_m}$ [-] |
|------------|----------------|---|---|----------------|--------------------------------|
| Benzene | | | | | |
| A | 9 | 0.958 \pm 0.040 | 0.976 \pm 0.017 | -0.251 | 1.019 |
| B | 9 | 0.958 \pm 0.008 | 0.978 \pm 0.007 | -0.279 | 1.021 |
| C | 15 | 0.956 \pm 0.021 | 0.972 \pm 0.020 | -0.218 | 1.016 |
| D | 15 | 0.957 \pm 0.025 | 0.974 \pm 0.024 | -0.238 | 1.018 |
| Toluene | | | | | |
| A | 9 | 0.800 \pm 0.010 | 0.768 \pm 0.009 | 0.490 | 0.960 |
| B | 9 | 0.811 \pm 0.015 | 0.780 \pm 0.012 | 0.460 | 0.962 |
| C | 15 | 0.798 \pm 0.012 | 0.769 \pm 0.010 | 0.444 | 0.964 |
| D | 15 | 0.810 \pm 0.019 | 0.780 \pm 0.016 | 0.453 | 0.963 |

129

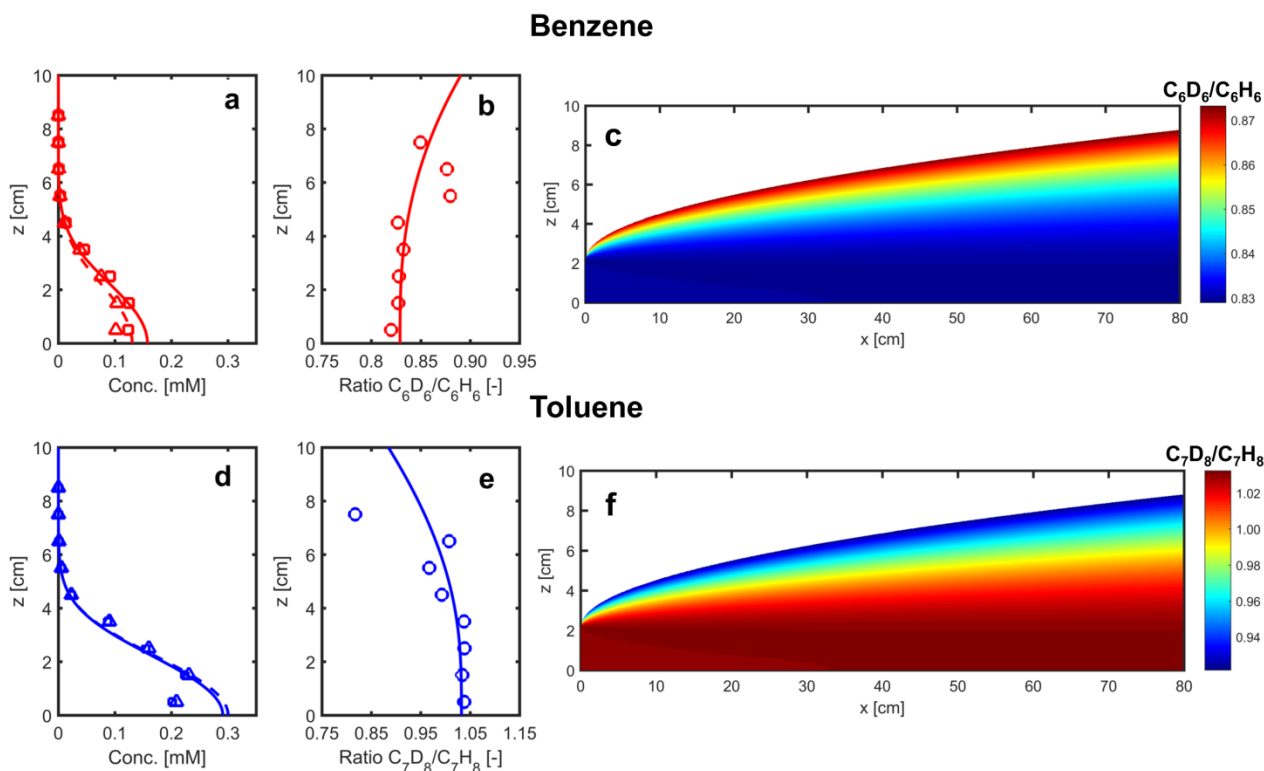
130 Figure 2 shows vertical profiles of concentration and isotope ratio observed for the simultaneous
 131 transport of deuterated and non-deuterated benzene and toluene in the flow-through chamber. The
 132 concentration trends show a typical transverse dispersion profile for plumes continuously injected in
 133 a homogeneous porous medium. The isotope ratio measured at the different ports confirms the

134 normal diffusive fractionation for toluene and the inverse fractionation for benzene. In fact, the ratio
135 C_7D_8/C_7H_8 decreases from the core towards the outer fringe of the plume, whereas the ratio
136 C_6D_6/C_6H_6 increases towards the plume fringe. No fitting procedure was used to evaluate the data
137 from the flow-through experiment. Instead, the experimental results were evaluated with pure
138 forward modeling of solute transport based on the average diffusion coefficients of the 4
139 compounds determined in the tube diffusion setups. The key parameter in this setup is the
140 transverse hydrodynamic dispersion coefficients, which is compound (and isotopologue) specific
141 and was described with the following parameterization:

$$D_T = D_P + D_{aq} \left(\frac{Pe^2}{Pe + 2 + 4\delta^2} \right)^{0.5} \quad (2)$$

142 where D_P [L^2/T] denotes the velocity-independent pore diffusion coefficient; $Pe = vd/D_{aq}$ [-] is the
143 grain Péclet number, in which d [L] is the average grain size diameter and v [L/T] is the seepage
144 velocity; δ [-] is the ratio between the length of a pore channel and its hydraulic radius; a value of
145 $\delta=5.37$ was determined in previous tracer experiments performed in a range of porous media, grain
146 sizes and seepage velocities comprising the conditions of the current flow-through setup.⁴² This
147 parameterization of transverse dispersion has been tested for solute transport in similar quasi 2-D
148 systems⁴³⁻⁴⁵ and verified in pore-scale studies⁴⁶⁻⁴⁷ and fully three-dimensional flow-through
149 experiments.³⁸ The essential feature of Eq. 2 is that it acknowledges the importance of aqueous
150 diffusion also in the non-linear, velocity-dependent mechanical dispersion term. The good
151 agreement of the predictive, purely forward simulations for both measured concentrations and ratios
152 (Figure 2) shows the capability of the model to capture the diffusive fractionation effects in the
153 flow-through system and also the accuracy of the experimentally determined values of aqueous
154 diffusion for the deuterated and non-deuterated tracers obtained from the 1-D tube experiments. The
155 latter compare very well with the values of aqueous diffusion coefficients computed with classical

156 empirical correlations for organic compounds (see Table S2 in the Supporting Information); this
 157 also indicates that sorption of toluene and benzene was not important in our experiments. The
 158 values of the transverse dispersion coefficients, determined according to Eq. 2, and used in the
 159 profiles and isotope ratio maps shown in Figure 2 are: $D_{T,C7H8}=1.563\times 10^{-9}$, $D_{T,C7D8}=1.547\times 10^{-9}$,
 160 $D_{T,C6H6}=1.641\times 10^{-9}$ and $D_{T,C6D6}=1.649\times 10^{-9}$ m²/s.



161
 162 Figure 2. Observed and simulated vertical profiles of concentrations and ratios at the outlet of the
 163 flow-through setup (a-b, d-e) and spatial maps of the isotopologue ratio for benzene (c) and toluene
 164 (f). The concentration data at the outlet ports (squares for the non-deuterated, triangles for the
 165 perdeuterated isotopologues) are average of triplicate measurements of two sampling events of the
 166 steady-state plumes, carried out after flushing 2 and 4 pore volumes (i.e., 48 and 96 hours,
 167 respectively).

168

169 Diffusive isotope fractionation in condensed systems, such as aqueous solutions, is challenging to
170 investigate and to conceptualize in a solid theoretical framework. Different factors that may affect
171 diffusive isotope fractionation include solute mass, volume, shape, molecular structure, polarity,
172 temperature and solute-solvent interactions. Mass and volume dependencies have dominated the
173 discussion about diffusive fractionation in condensed systems and the interpretation of diffusion
174 experiments. Models that have been proposed to capture the effects of diffusive fractionation
175 include the so-called hydrodynamic description (e.g., Stokes-Einstein relation), as well as the
176 extension to the condensed phase of formulations derived from Chapman-Enskog kinetic theory of
177 gas diffusion.³⁸ The hydrodynamic model does not include mass dependence and the key parameter
178 is the radius and, thus, the molar volume of the solute, whereas the kinetic theory predicts an
179 inverse square root relation with the reduced mass.⁴¹ Most of the experimental studies for different
180 aqueous solutes have reported a mass dependence that is typically weaker than the inverse square
181 root relation from kinetic theory. A simple power law expression (Eq. 1), in which the exponent is
182 derived by fitting the experimental data, is the model that is most often used to relate the diffusive
183 isotope effects to the mass of the diffusing solutes in theoretical, computational and experimental
184 studies.^{21,24-26,41} It is worth pointing out that in this simple model the exponent β is an empirical
185 coefficient which also lumps other factors such as steric effects and solute-solvent interactions that
186 are likely to play an important role on diffusive isotope fractionation. Indeed, the data presented in
187 this study show normal and inverse isotope effects that can neither be interpreted on the basis of
188 mass dependencies nor be ascribed as purely hydrodynamic (i.e., mass independent) effects. In fact,
189 based on the sole mass difference the deuterated benzene should have shown lower diffusion rates
190 than the non-labeled isotopologue. Instead, an inverse effect was observed both in the tube diffusion
191 experiments and in the flow-through setup. Our experiments also allow excluding a purely
192 hydrodynamic interpretation of the results. Molar volumes of deuterated benzene and toluene have

193 been shown to be smaller than those of the corresponding non-labeled isotopologues; for instance,
194 Bartell and Roskos⁴⁸ report the following values at 20 °C: $V_{C_6D_6}$ =88.62 mL/mol, $V_{C_6H_6}$ =88.86
195 mL/mol, $V_{C_7D_8}$ =105.98 mL/mol, and $V_{C_7H_8}$ =106.28 mL/mol. These differences are commonly
196 attributed to the smaller effective radius of the C-D bonds compared to the C-H bonds.⁴⁸⁻⁴⁹ However,
197 a pure hydrodynamic interpretation, based on a simple Stokes-Einstein relation and considering the
198 molar volumes reported above, would predict very weak inverse isotope effects
199 ($D_{C_nD_m}/D_{C_nH_m}$ =1.0009) for both compounds. This was not observed in this study, in which stronger
200 inverse and normal diffusive isotope fractionation effects were observed for benzene and toluene,
201 respectively. We hypothesize that the geometry of the hydration shells surrounding the aromatic
202 molecules and the solvation dynamics play a major role for diffusive isotope fractionation of
203 deuterated and non-deuterated benzene and toluene in aqueous solutions. For instance, a recent
204 computational study of benzene hydration⁵⁰ reports that the hydration shell of a benzene molecule
205 has an average number of 31 water molecules and this number can change and diminish
206 substantially upon increase of temperature and decrease of density. To explain the unexpected
207 inverse fractionation observed for benzene, one could hypothesize a similar effect on benzene
208 hydration due to the deuterium substitutions. Processes connected to the hydrophobic effect of
209 aromatic molecules in solution and associated changes of density and orientation of water
210 molecules surrounding the solute,⁵¹ as well as the structure and patterns of solute hydration⁵² appear
211 to be more important than the effects due to mass and molar volume differences. This hypothesis
212 will require future substantiation that might be provided by molecular dynamic simulations of
213 aqueous diffusion of deuterated and non-deuterated species. Such simulations have been performed
214 for instance for charged species^{22,53-54} and have contributed to elucidate the importance of the
215 hydration shell and of the interaction between solute and water molecules for the observed isotope
216 fractionation of different ions in aqueous solution. The different interaction of deuterated and non-

217 deuterated benzene and toluene molecules with water molecules appears to be an important factor to
218 explain the experimental observations of this study. This can be deduced also by comparing the
219 results of this study with earlier experiments. For instance, benzene self-diffusion⁵⁵ and tracer
220 diffusion in chlorobenzene⁵⁶ showed only very minor or no isotope effects during diffusion of C₆H₆
221 and C₆D₆ in these organic liquids, which contrasts with the rather large fractionation consistently
222 observed in the different aqueous systems considered in our study.

223 We think that the normal and inverse diffusive fractionation effects observed for deuterated and
224 non-deuterated BTEX will stimulate further research to develop a mechanistic understanding of
225 transport and transformation of these contaminants and their labeled mixtures in different
226 environments. Despite fractionation of D/H substituted aromatic compounds due to biodegradation
227 can be large and may be dominant for many transformation pathways,⁵⁷ our study shows that
228 diffusive fractionation is also significant and should be considered when these chemicals are used as
229 tracers and diagnostic tools in environmental systems in which transport and transformation
230 processes are typically coupled. On a broader perspective, the results of this study, showing
231 different diffusive isotope fractionation of very similar compounds, also have implications for the
232 fast developing field of compound specific stable isotope analysis (CSIA) techniques⁵⁸⁻⁵⁹, which
233 will greatly benefit from data for different environmental contaminants. The path towards
234 developing a comprehensive database of organic pollutant diffusive isotope fractionation and a
235 sound theory capturing the key mechanisms causing diffusive fractionation is inherently complex
236 but rich of intriguing future challenges.

237

238 **Supporting Information**

239 Description of the flow-through system and of the modeling approach used to quantitatively
240 interpret the results in the different experimental setups.

241 The authors declare no competing financial interests.

242

243 **Acknowledgments**

244 The authors acknowledge the support of the Deutsche Forschungsgemeinschaft (Grant RO 4169/2-1)

245 and the help of Chrysanthi-Elisabeth Nika, Hanne Bøggild and Mikael Emil Olsson for assistance in

246 the experimental work.

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