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eprints@whiterose.ac.uk https://eprints.whiterose.ac.uk/ 1 Natural Organic Matter Does Not Diminish the Mammalian Bioavailability of 2,3,7,8-

- 2 tetrachlorodibenzo-p-dioxin
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19 Core ideas:

- 20 Aquatic natural organic matter (NOM) was used to study the bioavailability of TCDD.
- 21 NOM-sorbed TCDD induced hepatic *cyp1A1* mRNA expression in mice.
- 22 NOM-sorbed TCDD suppressed humoral immune function in mice.
- 23 NOM-sorbed TCDD manifested no reduction in bioavailability compared to the control.

24 Abstract:

25 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is a toxic and persistent organic pollutant found in 26 soils and sediments. It has been linked to several adverse health outcomes in humans and 27 wildlife, including suppression of the immune system. TCDD is strongly sorbed to 28 soils/sediments due to its extremely low water solubility. Presently, the bioavailability of 29 soil/sediment-sorbed TCDD to mammals is not completely understood. Our previous studies 30 demonstrated that TCDD adsorbed to representative inorganic geosorbents (i.e. porous silica 31 and smectite clay) exhibited the same bioavailability to mice as TCDD dissolved in corn oil, 32 whereas sequestration by activated carbons eliminated TCDD bioavailability. In this study, we 33 evaluated the effects of amorphous natural organic matter (NOM), primarily in the form of 34 aquatic humic and fulvic acids, on the mouse bioavailability of TCDD. An aqueous suspension 35 of TCDD mixed with NOM was administered to mice via oral gavage. The relative bioavailability 36 of TCDD was assessed by two sensitive aryl hydrocarbon receptor-mediated responses in mice: 37 1) hepatic induction of *cyp1A1* mRNA; and 2) suppression of immunoglobulin M (IgM) antibody-38 forming cell (AFC) response which is an indicator of immunotoxicity. Hepatic induction of 39 *cyp1A1* mRNA and suppression of IgM AFC induced by TCDD were similar in the NOM-sorbed 40 form and dissolved in corn oil, revealing no loss of bioavailability when associated with NOM. 41 Hence, NOM-associated TCDD is as capable of suppressing humoral immunity in mice as 42 TCDD dissolved in corn oil, indicating that NOM-sorbed TCDD is likely to fully retain its 43 bioavailability to mammals and, by inference, humans.

44 Keywords: TCDD, amorphous natural organic matter, bioavailability

45 Introduction

46 Polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) are groups of highly toxic 47 chemicals with exceptionally low aqueous solubility. They were listed as persistent organic 48 pollutants (POPs) in the 2001 Stockholm Convention with an estimated plasma half-life in 49 humans of approximately 7 years (Pirkle et al., 1989). Substantial studies have been performed 50 on the relationship between exposure to PCDD/Fs and ecological and human health problems, 51 especially with TCDD. Cohort studies and animal experiments strongly indicated that unsafe 52 and/or long-term exposure to dioxins can cause cancer, damage the immune system, cause 53 reproductive and developmental problems, and skin conditions such as chloracne (Hinsdill et 54 al., 1980; Assennato et al., 1989; Bertazzi et al., 1993; Li et al., 1995; Alaluusua et al., 1996; 55 Avlward et al., 1996; Bertazzi et al., 2001; Luong et al., 2018). Concerns surrounding PCDD/Fs toxicity has caused the US EPA to consider lowering the cleanup criterion for residential soils 56 57 from its current value of 1 ppb TEQ to 0.07 ppb TEQ (EPA, 2009); the State of Michigan

58 criterion is currently 0.09 ppb TEQ (MDEQ, 2012).

59 PCDD/Fs are generated as byproducts from both anthropogenic activities and natural events. 60 Although new regulatory controls and improved technologies have contributed to significantly 61 reduced industrial emissions of dioxin-like compounds (DLCs) (approximately 90% reduction 62 from 1987 to 2000 in the US (EPA, 2006)), their production will never cease. The majority of 63 dioxins and DLCs are produced from waste incineration, poorly- or un-controlled combustion 64 such as backyard barrel burning of refuse, forest and landfill fires, volcanic activities, and from 65 industrial processes including metal operations, chemical manufacturing, and chlorine bleaching 66 in pulp and paper mills (EPA, 2006; Kulkarni et al., 2008). Formation of dioxins through 67 microbial activities during composting (Malloy et al., 1993), additions of CI to phenols in soils 68 (Hoekstra et al., 1999), photolysis (Lamparski et al., 1980) or clay-catalyzed dimerization (Gu et 69 al., 2011) of highly chlorinated phenols, exposure of pesticides to sunlight (Holt et al., 2012), 70 and *in situ* on ball clays (Gu et al., 2008) are also known but (likely) less significant than that 71 from combustion and/or industrial processes. It remains unclear why there are 5000 kg/year 72 more octachlorodibenzo-p-dioxin (OCDD) deposited onto world soils than that can be accounted 73 for by known emissions (Baker and Hites, 2000).

Dioxins and furans are ubiquitous in the environment, with background totals in soils averaging
~1 ppb (ng/g), most of which is OCDD (EPA, 2007; Demond et al., 2008). Global atmospheric
emissions of PCDD/Fs have been estimated at 2000-3000 kg per year (Baker and Hites, 2000).

77 Soils and sediments serve as the most significant reservoirs of PCDD/Fs due to their deposition 78 from the atmosphere (Brzuzy and Hites, 1995; DuarteDavidson et al., 1997) and extremely low 79 water solubility, which are estimated at 19 parts per trillion for TCDD and 0.23 part per trillion for 80 OCDD (Marple et al., 1986; Oleszek-Kudlak et al., 2007). PCDD/Fs persist in soils and 81 sediments due to their resistance and/or limited access to biodegradation and 82 photodegradation, and low vapor pressure leading to extremely slow volatilization (Hagenmaier 83 et al., 1992; Orazio et al., 1992; Li et al., 2012). Both laboratory and field studies indicated long-84 term persistence of PCDD/Fs in soils (Orazio et al., 1992; Hagenmaier et al., 1992) with half-85 lives ranging from 10 to 100 years (Seike et al., 2007; Young, 1983; Nauman and Schaum,

86 1987).

87 Due to their toxicity and persistence, PCDD/Fs in soils pose health risks to humans and wildlife 88 from soil and dust ingestion, with greater concern for vulnerable groups like young children and 89 pregnant women. Daily soil ingestion for young children was reported to be greater than 100 mg 90 soil/d (Stanek and Calabrese, 1995). Deliberate soil ingestion, pica, has been documented 91 during pregnancy in some poverty-stricken populations (>31% in low-income Mexican women 92 and 65% in low-income black women) (Cooksey, 1995; Simpson et al., 2000) further increasing 93 the risk of exposure to dioxins and DLCs through contaminated soils. General daily ingestion of 94 contaminated soils/dusts can also expose infants as demonstrated by elevated levels of 95 PCDD/Fs in breast milk and the umbilical cord in women residing near a highly contaminated 96 site in Vietnam (Nghi et al., 2015; Boda et al., 2018). Wildlife animals such as zebra can ingest 97 soils up to 3 g/kg body mass/day (Turner et al., 2013) while the Colorado mule deer has an 98 estimated soil ingestion of approximately 30 g/d (Arthur and Alldredge, 1979). Michigan deer 99 hunters are advised to minimize their consumption of fat from deer taken along the 100 Tittabawassee or Saginaw rivers where contamination has been identified (MDHHS, Accessed 101 on February 6, 2019).

102 In soil- and sediment-water systems, PCDD/Fs are present predominately in the sorbed state 103 owing to their exceedingly low water solubilities. Sorption occurs via interactions with one or 104 more of the major component geosorbents comprising these natural materials, with each 105 potentially influencing PCDD/F bioavailability differently. Soils can be viewed as dual phase 106 sorbents consisting broadly of organic matter and mineral matter (Chiou, 2002). Soil organic 107 matter itself can be viewed as a dual phase sorbent consisting of both amorphous organic 108 matter (AOM) which functions as a partition phase, and pyrogenic carbonaceous matter (PCM) 109 which is an adsorbent (Chiou et al., 2000, 2015). As detailed below, the adsorptive affinity of

110 PCM for PCDD/Fs is considerably greater than that associated with partitioning into AOM 111 (Chiou et al., 2015; Cornelissen et al., 2005). However adsorption of neutral organic 112 contaminants (NOCs) like PCDD/Fs by PCM can be limited by its available surface area and/or 113 pore volume. The adsorption of NOCs by PCM is a competitive process among coexisting 114 solutes whereas partitioning into AOM is not (Chiou et al., 1979, 2015; Chiou 2002). Among 115 mineral phases in soils, smectite clays can effectively adsorb certain classes of NOCs including 116 dioxins (Boyd et al., 2001; Liu et al, 2009). The effective adsorption domains in the clay 117 interlayers consist of planar hydrophobic siloxane surfaces made available by the presence of 118 weakly hydrating exchangeable cations such as K+ and Cs+ (Boyd et al., 2001; Liu et al., 2009; 119 Rana et al., 2009; Boyd et al., 2011a). The abundance of such sites in soils can be limited by 120 the absence of smectite clays in certain locations and the fact that K+ and Cs+ are not typically 121 dominant exchangeable cations. The role of clay minerals in sorption of PCDD/Fs in soils is 122 probably not dominant unless the soil organic carbon content is very low (<0.1%) (Cheng et al., 123 2012). Soil organic matter (SOM), although less than 10% of the total mass in most soils, is 124 generally considered the dominant geosorbent for NOCs including PCDD/Fs (Luthy et al., 125 1997). Support for the importance of SOM in controlling dioxin sorption comes from the strong 126 correlation (r² ranged from 0.88 to 0.99) between PCDD/F sorption and SOM contents of soils 127 (Brzuzy and Hites, 1995).

128 Bioavailability of a soil-sorbed contaminant is critical to evaluating organismal exposure, 129 understanding risk, and predicting the feasibility of biodegradation (Council, 2003; Ren et al., 130 2018). While association with soils decreases the oral bioavailability of PCDD/Fs to mammals 131 (Budinsky et al., 2008; Kimbrough et al., 2010), the mechanism for this bioavailability reduction 132 remains unknown. Prior studies have measured bioavailability from 1 to 80% (relative to the 133 liquid vehicle used to administer PCDD/Fs without soil) using 15 whole soils and five species of 134 mammals (Budinsky et al., 2008; Kimbrough et al., 2010). However, the soils were only 135 minimally characterized so it is difficult to extrapolate these results to predict PCDD/Fs 136 bioavailability in other soils. Our contention is that PCDD/Fs bioavailability from soils would be 137 better understood and perhaps predicted by analyzing the bioavailability of PCDD/Fs sorbed to 138 the individual geosorbents that comprise soils. To test this hypothesis, we have measured the 139 relative oral bioavailability in mice of the most important congener, TCDD, when adsorbed to 140 porous silica (Kaplan et al., 2011) and to both synthetic and natural smectite clays (Boyd et al., 141 2011b). The TCDD adsorbed to these minerals caused similar toxicity responses as observed 142 without the minerals, indicating that mineral-sorbed TCDD was 100% bioavailable to mice

relative to TCDD in a corn oil vehicle (Boyd et al., 2011b; Kaplan et al., 2011). By contrast, in
another study the relative oral bioavailability of TCDD to mice was completely eliminated (~0%
bioavailable) through adsorption to activated carbon (Boyd et al., 2017).

146 Activated carbon (AC) is an anthropogenic form of pyrogenic carbonaceous matter (PCM) 147 prepared at high temperatures (Fig. 1) (Pignatello et al., 2017). AC has high surface area 148 associated with a large internal porosity consisting of structured, polyaromatic (graphitic) 149 surfaces (Marsh and Rodriguez-Reinoso, 2006) at which hydrophobic molecules like PCDD/Fs 150 readily accumulate. Most PCMs form at much lower temperatures than AC, and therefore have 151 smaller amounts of polyaromatic surface area (Pignatello et al., 2017). At the other extreme is 152 AOM which forms naturally in soils at lower ambient temperatures. This sorptive component is 153 viewed as a low surface area (Pennell et al., 1995; Chiou et al., 2000) bulk phase organic 154 partition medium of intermediate polarity. Contaminant (e.g. TCDD) retention results from the 155 solubilization of solutes into the interior network of the partition phase (Chiou et al., 1979; Chiou 156 2002). The extent of sorption is dependent on the solubility of the solute in this phase versus its 157 solubility in water. Hence the retention mechanisms by AOM versus PCM are fundamentally 158 different with the former involving contaminant dissolution in an organic partition phase and the 159 latter solute condensation on graphitic surfaces. Sorption measurements for PCDDs show that 160 adsorption to more aromatic PCM, such as AC, is 10-1000 times stronger than PCDD 161 partitioning into AOM (Barring et al., 2002; Persson et al., 2002; Cornelissen et al., 2005). We 162 hypothesize that PCDD/F sorption and bioavailability follow trends indicated in Fig. 1, with 163 PCDD/F sorption increasing and bioavailability of sorbed PCDD/Fs decreasing along the 164 continuum from AOM (minimum) to AC (maximum). This hypothesis indicates the possibility of 165 PCDD/F bioavailability reduction by natural PCM, since it possesses, albeit to a lesser extent,

166 many structural characteristics of AC (Pignatello et al., 2017).



Figure 1. Schematic representation of the properties of natural soil organic matter (SOM)
 and pyrogenic carbonaceous matter (PCM). SOM comprised of amorphous organic matter
 (AOM) and naturally formed PCM is compared with anthropogenic PCM such as biochar and
 activated carbon (AC) regarding properties, sorption mechanisms, and bioavailability. These
 complex materials form a continuum as indicated.

173 Based on previous studies, the median PCM content (as a fraction of the total organic C

174 content) for soils is 4% and 9% for sediments (Cornelissen et al., 2005; Pignatello et al., 2017).

- 175 Additionally, only a fraction of any natural PCM will be composed of very strongly sorbing
- 176 graphitic domains like those found in AC. PCDD/F sorption by SOM therefore involves, to
- 177 varying extents, partitioning into AOM and adsorption to graphitic domains of PCM. We have
- shown that TCDD sorbed to AC was not bioavailable to mice (Boyd et al., 2017). Thus, we
- 179 hypothesize that TCDD associated with AOM, the portion of SOM that lacks structured and
- 180 adsorptive surfaces, will remain bioavailable to mice. The objective of the present study was to
- 181 quantify that bioavailability.

182 In this study, reference natural organic matter (NOM) was used as a representative form of

- 183 AOM. The bioavailability of NOM-sorbed TCDD to mice was evaluated with two biological
- 184 endpoints that have been used as *in vivo* assays in our previous studies (Boyd et al., 2011b;
- 185 Kaplan et al., 2011; Boyd et al., 2017): 1) Induction of cytochromes signaled by dioxin-ligand
- 186 complexation with the aryl hydrocarbon receptor, and 2) Suppression of humoral immune
- 187 responses to sheep-red-blood-cell (sRBC) antigens. The results, when combined with our
- 188 previous studies on inorganic soil constituents, will provide a better understanding of how
- 189 individual geosorbents contribute to the reduced bioavailability of PCDD/Fs in bulk soil.

190 Materials and Methods

191 Natural amorphous organic matter

192 Research grade reference natural organic matter (NOM). isolated from the Okefenokee Swamp 193 region of the Suwannee River was obtained from the International Humic Substances Society 194 (IHSS) and used in this study. The aquatic reference NOM was isolated using reverse osmosis 195 (RO) as detailed previously (Serkiz and Perdue, 1990; Green et al., 2015). This avoids the 196 conventional alkaline-extraction method used for organic matter extraction and minimizes 197 artifacts associated with using sodium hydroxide (Lehmann and Kleber, 2015). Such RO of 198 aquatic NOM is one of the few ways to obtain a representative NOM that is not intimately 199 associated with soil minerals so that NOM effects can be studied independently. The IHSS 200 reference NOM sample contains both hydrophobic and hydrophilic acids, and other soluble 201 organic compounds present in Suwannee River. It has a 4% ash content, a 50.7% C content 202 (Table S1) and is composed primarily of fulvic acids and humic acids (80-90% as indicated by 203 the alkaline extraction and resin adsorption method; Paul Bloom, IHSS, personal 204 communication).

205 Animals

206 Five to eight-week old, female pathogen-free B6C3F1 mice were purchased from Charles River 207 Breeding Laboratories. Female mice were used because they are less aggressive, and to be 208 consistent with our previous studies (Boyd et al., 2011b; Kaplan et al., 2011; Boyd et al., 2017; 209 Sallach et al., 2019) (Mice were randomly divided into 9 treatment groups (5 mice/group) and 210 housed in cages with water and feed (Purina Certified Laboratory Chow) for at least a two-week 211 acclimation period, upon which body weights reached approximately 20 g each. Animal housing 212 rooms were maintained on a 12:12-h light:dark cycle with temperatures between 21 to 24 °C 213 and relative humidity between 40 to 60%. All procedures involving mice were in accordance with 214 the Michigan State University Institutional Animal Care and Use Committee.

215 Preparation of treatments

216 A total of nine treatments were used in this study. These included TCDD sorbed to NOM

217 suspended in water (TCDD-NOM) at three exposure concentrations, and their corresponding

- 218 positive controls (TCDD-CO) comprised of equivalent amounts of TCDD dissolved in corn oil.
- 219 Mice were dosed by oral gavage at either a high (10 µg/kg body mass/d), medium (1 µg/kg body

mass/d), or low (0.1 µg/kg body mass/d) TCDD level. These concentrations were selected to be
consistent with the exposures used in our previous studies and are proven to induce a
bioresponse (Boyd et al., 2011b; Kaplan et al., 2011; Boyd et al., 2017). Negative vehicle
controls included both NOM suspension and corn oil without TCDD. In addition, a naïve group
was included in which mice were neither treated (dosed) nor sensitized with sRBC.

225 Specifically, Kimble KIMAX glass vials (Fisher Scientific, Hampton, NH) with PTFE-faced 226 rubber-lined caps were used to prepare TCDD-NOM suspension. To achieve desired exposure 227 dosages, 187.5 mg of NOM was weighed in four separate glass vials. A 60 µL aliquot of TCDD 228 dissolved in dimethyl sulfoxide (DMSO) at concentrations of 100 ppm, 10 ppm, or 1 ppm was 229 directly added to the NOM in each glass vial resulting in TCDD-NOM mixtures at 32 µg/g, 3.2 230 $\mu q/q$, and 0.32 $\mu q/q$, respectively. The stock solution of 100 $\mu q/mL$ TCDD in DMSO 231 (AccuStandard Inc., New Heaven, CT) was used to prepare the high concentration TCDD-232 DMSO solution, and the medium and low TCDD-DMSO solutions were prepared by 10-fold 233 serial dilutions. The TCDD-NOM mixtures were vortex mixed immediately for 10 minutes then 234 suspended in 6 mL of ultrapure water. The final TCDD-NOM suspensions were vortexed again 235 for 10 minutes. Settling of NOM particulates (Fig. S1) indicated that the mixtures were 236 suspensions containing both dissolved and suspended NOM. Correspondingly, TCDD-CO was 237 prepared by spiking 60 µL of TCDD-DMSO (100 ppm, 10 ppm, or 1 ppm) into 6 mL of corn oil. 238 All samples were stored at room temperature for three weeks before administration to mice.

239 Administration of test materials and antigen sensitization of mice

240 The treatment groups, consisting of 5 mice per group, are summarized in Table 1 and this study 241 followed the methods in Boyd et al. (2017). Briefly, mice in each group, except in the naïve 242 control, were administered by oral gavage the test materials suspended in 200 µL of vehicle or 243 vehicle only for four consecutive days. Particular care was taken to mix and resuspend NOM 244 solids prior to each administration. On day three, each mouse (excluding naïve) received an 245 intraperitoneal injection of 1×10⁹ sheep red blood cells (sRBCs, Colorado Serum Co, Denver, 246 CO) to initiate a humoral immune response. On the seventh day, mice were euthanized by 247 cervical dislocation. Resected livers and spleens were collected, weighed, and homogenized. 248 Liver tissues were stored at -70°C in TRI Reagent (Sigma-Aldrich, St. Louis, MO). Spleens were 249 immediately processed for quantification of the anti-sRBC immunoglobulin M (IgM) antibody-250 forming cells (AFCs) response.

251

Table 1. Experimental treatment groups. Mice in each treatment group, except for the Naïve,
 were administered corresponding samples listed in the table below by oral gavage. Each group
 contained five mice.

Group	Treatment
1	Corn oil vehicle
2	Corn oil + TCDD Low (0.1 µg/kg/d)
3	Corn oil + TCDD Medium (1 µg/kg/d)
4	Corn oil + TCDD High (10 μg/kg/d)
5	NOM vehicle
6	NOM + TCDD Low (0.1 µg/kg/d)
7	NOM + TCDD Medium (1 µg/kg/d)
8	NOM + TCDD High (10 μg/kg/d)
9	Naïve

255 TCDD: 2,3,7,8-tetrachlorodibenzo-p-dioxin; NOM: amorphous natural organic matter.

256 Antibody forming cell response

257 Enumeration of anti-sRBC IgM secreting AFCs in the spleen was performed following the Jerne 258 plaque assay (Jerne and Nordin, 1963) as detailed previously. Briefly, diluted mouse 259 splenocytes were mixed with 0.5% melted agar (Difco/BD), guinea pig complement 260 (Gibco/Invitrogen), and sRBCs from the same lot used for sensitization. Each mixture was 261 vortex mixed then poured onto a Petri dish then covered with a 24×50 mm microscope glass 262 slide. Following overnight incubation at 37°C, the AFCs, specifically antibody-secreting plasma 263 cells, were quantified using a Bellco plaque viewer at ×6.5 magnification. Total splenocytes from 264 diluted samples were determined employing a ZI Coulter particle counter (Beckman Coulter, 265 Brea, CA) and used to normalize anti-sRBC IgM AFCs/1×10⁶ splenocytes.

266 Cyp1A1 gene expression

267 *Cyp1A1* (Cytochrome P450 Family 1 Subfamily A Member 1), encoded by the *cyp1A1* gene, is

a protein in the drug metabolizing cytochrome P450 family of enzymes. Expression of this gene

is induced by AhR agonists, including TCDD, resulting in elevated levels of *cyp1A1* mRNA in

270 the liver corresponding to increased exposure with the agonist and can be quantified by

271 polymerase chain reaction (PCR). Homogenized livers were phase-separated with

bromochlorophenol and RNA precipitation facilitated by isopropanol. Extraction, purification, and

- 273 deoxyribonuclease treatment was carried out using the Promega SV total RNA isolation system.
- 274 Random primers were employed for reverse-transcription of total RNA using a high-capacity
- 275 complementary deoxyribonucleic acid (cDNA) reverse-transcription kit (Applied Biosystems,
- 276 Foster City, CA). A TaqMan primer/probe set for mouse *cyp1A1* (Applied Biosystems, Foster
- 277 City, CA) was used to amplify the cDNA. A 7900 HT fast real-time PCR system (Applied
- 278 Biosystems, Foster City, CA) was used for amplification analysis. The results were expressed
- as fold change and calculated using the $\Delta\Delta C_{T}$ method (Livak and Schmittgen, 2001).

280 Statistical analysis

- 281 The mean ± SEM (standard error of mean) was determined for each treatment group. Statistical
- analysis on the difference of means was determined with a parametric analysis of variance.
- 283 When significant differences were detected, Dunnett's two-tailed *t* test was then used to
- 284 determine the difference between treatment groups and corresponding controls. For real-time
- 285 PCR, statistical analysis was performed on ΔC_T values. All analyses were performed using
- 286 GraphPad Prism Version 4.0a.

287 **Results and Discussion**

288 Natural organic matter (NOM)

289 This study is acontinuation of our investigation into the mammalian bioavailability of TCDD 290 associated with the major component geosorbents in soils and sediments. The long-term goal is 291 to determine which component(s) could account for observed reductions in PCDD/F 292 bioavailability to a variety of organisms in contaminated field soils (Budinsky et al., 2008; 293 Kimbrough et al., 2010). Our prior studies with component geosorbents have demonstrated that 294 TCDD adsorbed to porous silica (Kaplan et al., 2011) or intercalated in smectite clays (Boyd et 295 al., 2011b) fully retained its bioavailability (relative to TCDD in corn oil) when administered orally 296 to a mammalian (mouse) model. In companion studies, activated carbon, which can be viewed 297 as an anthropogenic end-member of pyrogenic carbonaceous matter (Fig. 1), has been shown 298 to completely eliminate TCDD bioavailability to mice (Boyd et al., 2017; Sallach et al., 2019) and 299 is being considered as a sorbent amendment for remediation of soils contaminated with 300 PCDD/Fs. One remaining geosorbent type that has not been evaluated is amorphous soil 301 organic matter, which is generally recognized as a major sorptive component for the retention of 302 nonionic organic contaminants (NOCs) in soils and sediments (Chiou et al., 1979; Chiou et al., 303 2002). High surface area pyrogenic carbonaceous matter is considered the primary geosorbent 304 for NOCs only at very low relative (aqueous) concentrations (concentration in water/water 305 solubility) (Chiou et al., 2000).

306 To conduct this study, a representative sample of natural AOM was needed. Most soil organic 307 matter presents at least two challenges in isolating AOM. First, AOM and PCM form a 308 continuum (Fig. 1), and it is difficult to entirely separate them (Pignatello et al., 2017). Secondly, 309 SOM also comprises a continuum of organic fragments of different molecular sizes. Smaller 310 fragments show higher oxygen contents (lower c/o ratio) which manifests increasing polarity and 311 stronger reactivity toward mineral surfaces (Lehmann and Kleber, 2015). As a result, pure AOM 312 is very difficult to obtain from soils, since it may contain PCM and/or mineral-sorbed SOM. Thus, 313 we used a natural organic matter (NOM) (IHSS reference material) isolated from the Suwannee 314 River to represent AOM. Prior studies have demonstrated that such dissolved organic matter 315 functions similarly to amorphous bulk phase soil organic matter as a partition phase for NOCs 316 (Chiou et al., 1983; Chiou et al., 1986). Hence, this study advances our understanding of the 317 bioavailability of TCDD associated with natural amorphous organic matter.

318 NOM-sorbed TCDD-induced hepatic cyp1A1 mRNA expression

319 *Cvp1A1* gene expression is a mammalian biomarker for exposure of organisms to aryl 320 hydrocarbon receptor (AhR) agonists including TCDD, which was used in this study. The AhR, 321 which functions as a ligand activated transcription factor induces expression of the cyp1A1 gene 322 (Denison and Nagy, 2003). Therefore, increased exposure to TCDD manifests increased AhR-323 mediated cyp1A1 gene transcription. Evaluation of cyp1A1 mRNA in the liver serves as a 324 particularly sensitive bioassay for measuring exposure of the mouse to TCDD as it possesses 325 multiple dioxin response elements within its promoter. The hepatic level of cyp1A1 mRNA in 326 mice fed both TCDD dissolved in corn oil (TCDD-CO) and TCDD associated with NOM (TCDD-327 NOM) varied with treatment dosages (Fig. 2). In the TCDD-CO groups, a measurable but not 328 statistically significant increase in cvp1A1 expression was observed at the low (0.1 $\mu q/kq/d$) 329 dose compared to the vehicle, whereas a substantially increased (p<0.05) cyp1A1 expression 330 was determined at the medium $(1 \mu g/kg/d)$ and high $(10 \mu g/kg/d)$ doses. However, no statistically significant difference in cyp1A1 expression was observed between the medium and 331 332 high doses, suggesting that maximum cyp1A1 was achieved. The TCDD-NOM treatment 333 groups showed a similar (p>0.05 at each TCDD dosage level) response to TCDD exposure 334 compared to the TCDD-CO control groups. That is, compared to respective vehicles, the low 335 TCDD dose induced a measurable but not statistically significant elevation in cyp1A1 336 expression, whereas the medium and high TCDD doses induced significantly increased cyp1A1 337 expression. Moreover, induction of cyp1A1 mRNA in the mice receiving the NOM-TCDD 338 demonstrated successful delivery of TCDD to the liver, the first target organ after 339 gastrointestinal (GI) absorption. Hence, NOM-associated TCDD was equally bioavailable as 340 TCDD in the corn oil vehicle, to the mammalian (mouse) model. That no other AhR agonists 341 were included in the experimental setting is confirmed by the lack of cyp1A1 induction in either 342 of the vehicle control groups.



343

Figure 2. Liver *cyp1A1* mRNA expression induced by TCDD. TCDD dissolved in corn oil
(CO) or sorbed to natural organic matter (NOM) was administered to mice at 0 (VH), 0.1 (low), 1
(medium), and 10 (high) μg/kg/d, respectively. Levels of *cyp1A1* mRNA in mice administered
with each TCDD dose were compared to corresponding vehicles (VH) in terms of fold change.
Expression of *cyp1A1* mRNA in mice administered NOM-sorbed TCDD were compared to that
in mice receiving TCDD dissolved in corn oil for each TCDD dose. * indicates statistically
significant difference (p<0.05) between the treated group and the corresponding VH.

351 The exact mechanism by which NOM-sorbed TCDD is delivered from the GI tract to the liver is 352 unknown and beyond the scope of this study. Certainly it is plausible that desorption of TCDD 353 from NOM occurred in the GI tract via TCDD association with gastric lipids due to its high 354 lipophilicity (log Kow \approx 7) (Shiu et al., 1988). What is known is that initially the dosed mass of 355 TCDD in the TCDD-NOM treatment groups is predominately presented in the NOM-sorbed 356 form. Prior studies of NOM in the dissolved form demonstrate that it functions as a partition 357 phase with similar effectiveness as bulk phase soil organic matter; the sorptive effectiveness of 358 dissolved humic acids extracted from soils were reduced only by a factor of two on a unit mass 359 basis compared to bulk soil organic matter, i.e. $K_{om}/K_{dom} \approx 2$ (Chiou et al., 1986). In the present 360 study, most of the NOM exists as a solid with a smaller amount presumably dissolved in water. 361 If we assume that the TCDD partition coefficient for the NOM used here is similarly reduced by 362 a factor of two, then estimate a Kom value for TCDD based on its octanol-water partition 363 coefficient (log $K_{ow} \approx 7$) (Chiou et al., 1986), we can calculate the fractional mass of TCDD 364 initially sorbed to NOM. Using the medium TCDD-NOM treatment (0.6 µg TCDD/187.5 mg

NOM), the fractional mass of TCDD sorbed to NOM is ca. 0.9998. Despite the fact that TCDD
has almost completely partitioned into the NOM, these results show that when compared with
freely available TCDD, i.e. TCDD dissolved in corn oil (TCDD-CO), the sorption of TCDD by
NOM (TCDD-NOM) did not reduce the bioavailability of TCDD and hence did not reduce the
exposure of the mouse to TCDD.

370 NOM-sorbed TCDD suppressed humoral immune function

371 The bioavailability of NOM-sorbed TCDD was evaluated by a second independent method, 372 namely its ability to suppress humoral immune function in mice. Whereas induction of cyp1A1 373 represents an indirect measure of TCDD exposure to AhRs in the liver, the Jerne plaque assay 374 provides a measure of TCDD induced suppression of immune function in mice by quantifying 375 the spleen cells (splenocytes) that produce IgM antibodies in response to a specific antigen, i.e. 376 sheep red blood cells (sRBC) in this study. The results were thus expressed as the anti-sRBC 377 IgM antibody-forming cell (AFC) response. In general, the IgM AFC response, using spleen 378 cells, decreased as the dosage of TCDD increased regardless of the vehicle through which 379 TCDD was delivered (Fig. 3). However, in the corn oil control group and the NOM treatment 380 group at the low dose of TCDD (0.1 μ g/kg/d), suppression of the IgM AFC response was either 381 not detected (TCDD-CO group) or measurable but not statistically significant (TCDD-NOM 382 group) (p>0.05). Compared to mice receiving only corn oil (VH), approximately 40% and 60% 383 reductions (p<0.05) in the IgM AFC response were observed for mice administered TCDD in 384 corn oil at the medium $(1 \mu g/kg/d)$ and high $(10 \mu g/kg/d)$ dosages, respectively. The reduction 385 (p<0.05) in the IgM AFC responses for mice administered TCDD associated with NOM were 386 \sim 70% at the medium or high doses compared to that for mice receiving only NOM (VH). 387 Consistent with the results of cyp1A1 expression in the liver, no statistically significant difference 388 (p>0.05) in the IgM AFC response was observed between the corn oil control groups and the 389 NOM treatment groups at each TCDD dosage level. This demonstrated a similar magnitude of 390 suppression of the humoral immune response in mice orally administered NOM-sorbed TCDD 391 compared to those receiving corn oil-dissolved TCDD. Additionally, suppressed humoral 392 immune function further confirms the biodistribution of TCDD in the spleen after oral gavage.



394 Figure 3. Suppression of the anti-sRBC IgM antibody forming cell response by TCDD. Humoral immune function of mice administered TCDD dissolved in corn oil (CO) or natural 395 396 organic matter (NOM) at 0 (VH), 0.1 (low), 1 (medium), and 10 (high) µg/kg/d were evaluated 397 through anti-sRBC IgM antibody-forming cell (AFC) response. The IgM AFC response was 398 expressed by a bar graph. AFC response in mice administered each TCDD dosage were 399 compared to corresponding vehicles (VH). AFC response in mice administered NOM-sorbed TCDD were compared to that in mice receiving TCDD dissolved in corn oil for each TCDD dose. 400 401 * indicates statistically significant difference (p<0.05) between the treated group and the 402 corresponding VH.

- 403 It is noteworthy that the low TCDD dose $(0.1 \,\mu g/kg/d)$ had no statistically significant (p>0.05)
- 404 effects on either *cyp1A1* mRNA expression or the IgM AFC response, which is most likely
- 405 explained by this dose being below the threshold of biological activity; it is lower than the ED₅₀
- 406 (effective dose) of TCDD in mice which was reported at 0.74 μg/kg/d (Kerkvliet and Brauner,
- 407 1990). The small amount of DMSO administered concurrently with NOM did not appear to affect
- 408 the biological responses to NOM-sorbed TCDD, insofar as the same amount of DMSO was
- 409 administered in corn oil at the low TCDD dose and no statistically significant *cyp1A1* mRNA
- 410 induction or IgM AFC suppression in mice was observed.
- 411
- 412 Impact of NOM sorbed TCDD on body and organ weights
- 413 Mouse body, liver and spleen weights were measured at the termination of the study. Body
- 414 weights were monitored in order to determine if TCDD exposure resulted in overt toxicity which
- 415 would be indicated by a significant drop in body weight. No changes in body weight between

416 vehicle and TCDD dosing groups, even at the highest exposure, (Figure 4) indicates no overt 417 toxicity. Changes in liver weight to body weight and spleen weight to body weight ratios resulting 418 from TCDD exposure have been reported in other studies (Bryant et al., 2001; Lamb et al., 419 2016). However, these studies used higher TCDD exposure concentrations and measured 420 these endpoints over a longer period of exposure. There were no significant differences 421 between the liver weight to body weight ratios between any treatment groups (Figure S2). 422 Spleen weight to body weight ratios for each treatment were similar between the TCDD 423 treatments in corn oil and NOM vehicles, respectively. While a significant increase between 424 spleen weight to body weight ratio was observed in TCDD exposed mouse groups compared to 425 their respective vehicle control, the change in ratio (< 0.004) was negligible (Figure S2). That 426 there were no differences between the two vehicles in either organ ratios or bodyweights 427 supports the conclusion that TCDD complexation with NOM does not diminish its oral 428 mammalian bioavailability.

429



430



434 Bioavailability of NOM-sorbed TCDD was not diminished

435 The bioavailability of TCDD sorbed by natural organic matter was evaluated using both cyp1A1 436 induction and the IgM AFC response. The observation of induced hepatic cyp1A1 mRNA 437 expression and suppression of humoral immune function in mice after oral gavage of NOM-438 sorbed TCDD provide two independent biological endpoints for assessing bioavailability of 439 NOM-bound TCDD. These results are similar to those of previous studies showing that TCDD 440 sorption by other component geosorbents, i.e. porous silica Kaplan et al., 2011) and smectite 441 clays (Boyd et al., 2011b), did not reduce oral bioavailability of TCDD in mice. Thus, the major 442 finding of this study is that sorption of TCDD by NOM did not diminish its bioavailability to mice. 443 The consistency of organ impacts between NOM-sorbed TCDD and TCDD dissolved in corn oil 444 further confirmed this finding. We estimated that the fractional mass of TCDD initially sorbed to 445 the NOM administered to mice was ca. 0.9998. Only activated carbon, a high surface area 446 anthropogenic form of PCM, has been shown to sequester TCDD in a form that eliminates its 447 bioavailability to mice (Boyd et al., 2017; Sallach et al., 2019). Taken together, these studies 448 suggest that reductions in the bioavailability of PCDDs present in field soils are likely due to 449 their association with PCM (Fig. 1). Unfortunately, it is difficult to accurately assess the fraction 450 of soil organic matter that exists as PCM, making a priori estimates of site-specific PCDD 451 bioavailability difficult.

452 Conclusion

453 Knowledge of the bioavailability of POPs including PCDD/Fs in soils/sediments is essential for 454 meaningful risk assessment. Understanding the mechanisms responsible for reduced 455 mammalian bioavailability of soil-sorbed PCDDs are critical for the establishment of site-specific 456 remediation criteria and for conceptualizing new approaches to in situ remediation. In the 457 present study we showed for the first time that sorption of TCDD by amorphous natural organic 458 matter did not manifest reductions of TCDD oral bioavailability to mice. In previous studies, 459 sorption to representative soil minerals, i.e. porous silica (Kaplan et al., 2011) and smectite clay 460 (Boyd et al., 2011b), similarly failed to reduce the oral bioavailability of TCDD to mice. In 461 contrast, activated carbon strongly sequestered TCDD and eliminated its bioavailability (Boyd et 462 al., 2017; Sallach et al., 2019). Collectively, these findings suggest that soils with a higher 463 proportion of PCM should maximize reductions in the bioavailability of soil-sorbed PCDDs. In 464 addition, the use of activated carbon as an in situ sorbent amendment to reduce the 465 bioavailability of PCDD/Fs and similar POPs is a promising new direction in the management 466 and remediation of large areas of contaminated soils. This remedy minimizes costs and ancillary 467 destruction of habitat, but additional studies documenting reductions in the mammalian 468 bioavailability of target contaminants are needed.

469 **References**

- 470
- 471 Alaluusua, S., Lukinmaa, P.L., Vartiainen, T., Partanen, M., Torppa, J., Tuomisto, J., 1996.
- 472 Polychlorinated dibenzo-p-dioxins and dibenzofurans via mother's milk may cause
- 473 developmental defects in the child's teeth. Environ. Toxicol. Pharmacol. 1, 193-197.
- 474 Arthur, W.J., Alldredge, A.W., 1979. Soil ingestion by mule deer in northcentral Colorado. J.
 475 Range Manage. 32, 67-71.
- Assennato, G., Cervino, D., Emmett, E.A., Longo, G., Merlo, F., 1989. Follow-up of subjects
 who developed chloracne following TCDD exposure at Seveso. Am. J. Ind. Med. 16, 119-125.
- Aylward, L.L., Hays, S.M., Karch, N.J., Paustenbach, D.J., 1996. Relative susceptibility of
 animals and humans to the cancer hazard posed by 2,3,7,8-tetrachlorodibenzo-p-dioxin using
 internal measures of dose. Environ. Sci. Technol. 30, 3534-3543.
- Baker, J.I., Hites, R.A., 2000. Is combustion the major source of polychlorinated dibenzo-pdioxins and dibenzofurans to the environment? A mass balance investigation. Environ. Sci.
 Technol. 34, 2879-2886.
- 484 Barring, H., Bucheli, T.D., Broman, D., Gustasson, O., 2002. Soot-water distribution coefficients
- 485 for polychlorinated dibenzo-p-dioxins, polychlorinated dibenzofurans and polybrominated
- 486 diphenylethers determined with the soot cosolvency-column method. Chemosphere 49, 515-487 523.
- Bertazzi, P.A., Consonni, D., Bachetti, S., Rubagotti, M., Baccarelli, A., Zocchetti, C., Pesatori,
 A.C., 2001. Health effects of dioxin exposure: A 20-year mortality study. Am. J. Epidemiol. 153,
 1031-1044.
- 491 Bertazzi, P.A., Pesatori, A.C., Consonni, D., Tironi, A., Landi, M.T., Zocchetti, C., 1993. Cancer
 492 incidence in a population accidentally exposed to 2,3,7,8-tetrachlorodibenzo-para-dioxin.
 493 Epidemiology 4, 398-406.
- Boda, H., Nghi, T.N., Nishijo, M., Thao, P.N., Tai, P.T., Luong, H.V., Anh, T.H., Morikawa, Y.,
 Nishino, Y., Nishijo, H., 2018. Prenatal dioxin exposure estimated from dioxins in breast milk
 and sex hormone levels in umbilical cord blood in Vietnamese newborn infants. Sci. Total
 Environ. 615, 1312-1318.
- Boyd, S.A., Johnston, C.T., Laird, D.A., Teppen, B.J., Li, H., 2011a. Comprehensive Study of
 Organic Contaminant Adsorption by Clays: Methodologies, Mechanisms, and Environmental
 Implications. Biophysico-Chemical Processes of Anthropogenic Organic Compounds in
 Environmental Systems. John Wiley & Sons, Inc., pp. 51-71.
- Boyd, S.A., Johnston, C.T., Pinnavaia, T.J., Kaminski, N.E., Teppen, B.J., Li, H., Khan, B.,
 Crawford, R.B., Kovalova, N., Kim, S.S., Shao, H., Gu, C., Kaplan, B.L.F., 2011b. Suppression
 of humoral immune responses by 2,3,7,8-tetrachlorodibenzo-p-dioxin intercalated in smectite
 clay. Environ. Toxicol. Chem. 30, 2748-2755.
- Boyd, S.A., Sallach, J.B., Zhang, Y.J., Crawford, R., Li, H., Johnston, C.T., Teppen, B.J.,
 Kaminski, N.E., 2017. Sequestration of 2,3,7,8-tetrachlorodibenzo-p-dioxin by activated carbon
 eliminates bioavailability and the suppression of immune functio in mice. Environ. Toxicol.
 Chem. 36, 2671-2678.
- 510 Boyd, S.A., Sheng, G.Y., Teppen, B.J., Johnston, C.J., 2001. Mechanisms for the adsorption of 511 substituted nitrobenzenes by smectite clays. Environ. Sci. Technol. 35, 4227-4234.

- 512 Bryant, P. L.; Schmid, J. E.; Fenton, S. E.; Buckalew, A. R.; Abbott, B. D. 2001. Teratogenicity
- 513 of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) in Mice Lacking the Expression of EGF and/or
- 514 TGF-α. Toxicol Sci. 62 (1), 103–114.
- 515 Brzuzy, L.P., Hites, R.A., 1995. Estimating the atmospheric deposition of polychlorinated 516 dibenzo-p-dioxins and dibenzofurans from soils. Environ. Sci. Technol. 29, 2090-2098.
- 517 Budinsky, R.A., Rowlands, J.C., Casteel, S., Fent, G., Cushing, C.A., Newsted, J., Giesy, J.P.,
- 518 Ruby, M.V., Aylward, L.L., 2008. A pilot study of oral bioavailability of dioxins and furans from
- contaminated soils: Impact of differential hepatic enzyme activity and species differences.Chemosphere 70, 1774-1786.
- 521 Cheng, H.F., Hu, E.D., Hu, Y.A., 2012. Impact of mineral micropores on transport and fate of 522 organic contaminants: A review. J. Contam. Hydrol. 129, 80-90.
- 523 Chiou, C.T., 2002. Partition and adsorption of organic contaminants in environmental systems. 524 John Wiley & Sons, Inc.
- 525 Chiou, C. T., Cheng, J., Hung, W.-N., Chen, B., Lin, T.-F. 2015. Resolution of Adsorption and
- Partition Components of Organic Compounds on Black Carbons. Environ. Sci. Technol. 49 (15),
 9116–9123.
- 528 Chiou, C.T., Kile, D.E., Rutherford, D.W., Sheng, G.Y., Boyd, S.A., 2000. Sorption of selected 529 organic compounds from water to a peat soil and its humic-acid and humin fractions: Potential 530 sources of the sorption nonlinearity. Environ. Sci. Technol. 34, 1254-1258.
- 531 Chiou, C.T., Malcolm, R.L., Brinton, T.I., Kile, D.E., 1986. Water solubility enhancement of some
 532 organic pollutants and pesticides by dissolved humic and fulvic acids. Environ. Sci. Technol. 20,
 533 502-508.
- 534 Chiou, C. T., Peters, L. J., Freed, V. H. A. 1979. Physical Concept of Soil-Water Equilibria for 535 Nonionic Organic Compounds. Science. 206 (4420), 831–832.
- 536 Chiou, C.T., Porter, P.E., Schmedding, D.W., 1983. Partition equilibria of nonionic organic 537 compounds between soil organic matter and water. Environ. Sci. Technol. 17, 227-231.
- 538 Cooksey, N.R., 1995. Pica and olfactory craving of pregnancy How deep are the secrets. Birth 539 Iss. Perinat. C. 22, 129-137.
- 540 Cornelissen, G., Gustafsson, O., Bucheli, T.D., Jonker, M.T.O., Koelmans, A.A., Van Noort,
- 541 P.C.M., 2005. Extensive sorption of organic compounds to black carbon, coal, and kerogen in
- 542 sediments and soils: Mechanisms and consequences for distribution, bioaccumulation, and
- 543 biodegradation. Environmental Science & Technology 39, 6881-6895.
- 544 Council, N.R., 2003. Bioavailability of contaminants in soils and sediments: processes, tools, 545 and applications. The National Academies Press, Washington, DC.
- 546 Demond, A., Adriaens, P., Towey, T., Chang, S.C., Hong, B., Chen, Q., Chang, C.W.,
- 547 Franzblau, A., Garabrant, D., Gillespie, B., Hedgeman, E., Knutson, K., Lee, C.Y., Lepkowski,
- 548 J., Olson, K., Ward, B., Zwica, L., Luksemburg, W., Maiero, M., 2008. Statistical comparison of
- residential soil concentrations of PCDDs, PCDFs, and PCBs from two communities in Michigan.
- 550 Environ. Sci. Technol. 42, 5441-5448.
- 551 Denison, M. S., Nagy, S. R. 2003. Activation of the Aryl Hydrocarbon Receptor by Structurally
- 552 Diverse Exogenous and Endogenous Chemicals. Annual Review of Pharmacology and 553 Toxicology. 43 (1), 309–334.

- 554 DuarteDavidson, R., Sewart, A., Alcock, R.E., Cousins, I.T., Jones, K.C., 1997. Exploring the
- 555 balance between sources, deposition, and the environmental burden of PCDD/Fs in the UK 556 terrestrial environment: An aid to identifying uncertainties and research needs. Environ. Sci.
- 557 Technol. 31, 1-11.

558 EPA, U., 2006. An inventory of sources and environmental releases of dioxin-like compounds in 559 the United States for the years of 1987, 1995, and 2000. in: National Center for Environmental 560 Assessment, O.o.R.a.D. (Ed.), Washington, DC.

- 561 EPA, U., 2007. Pilot survey of levels of polychlorinated dibenzo-p-dioxins, polychlorinated 562 dibenzofurans, polychlorinated biphenyls and mercury in rural soils of the United States.
- 563 National Center for Environmental Assessment, EPA/600/R-05/048F. Washington, DC.
- EPA, U., 2009. Draft recommended interim preliminary remediation goals for dioxin in soil at
 CERCLA and RCRA sites. U.S. Environmental Protection Agency Office of Superfund
 Remediation and Technology Innovation, Washington, DC.
- Green, N.W., McInnis, D., Hertkorn, N., Maurice, P.A., Perdue, M.E., 2015. Suwannee River
 natural organic matter: isolation of the 2R101N reference sample by reverse osmosis. Environ.
 Eng. Sci. 32, 38-44.
- 570 Gu, C., Li, H., Teppen, B.J., Boyd, S.A., 2008. Octachlorodibenzodioxin formation on Fe(III)-571 montmorillonite clay. Environ. Sci. Technol. 42, 4758-4763.
- 572 Gu, C., Liu, C., Ding, Y.J., Li, H., Teppen, B.J., Johnston, C.T., Boyd, S.A., 2011. Clay mediated 573 route to natural formation of polychlorodibenzo-p-dioxins. Environ. Sci. Technol. 45, 3445-3451.
- Hagenmaier, H., She, J., Lindig, C., 1992. Persistence of polychlorinated dibenzo-p-dioxins and
 polychlorinated dibenzofurans in contaminated soil at Maulach and Rastatt in the southwest
 Germany. Chemosphere 25, 1449-1456.
- Hinsdill, R.D., Couch, D.L., Speirs, R.S., 1980. Immunosuppression in mice induced by dioxin
 (TCDD) in feed. J. Environ. Pathol. Toxicol. 4, 401-425.
- Hoekstra, E.J., De Weerd, H., De Leer, E.W.B., Brinkman, U.A.T., 1999. Natural formation of
 chlorinated phenols, dibenzo-p-dioxins, and dibenzofurans in soil of a Douglas fir forest.
 Environ. Sci. Technol. 33, 2543-2549.
- Holt, E., Weber, R., Stevenson, G., Gaus, C., 2012. Formation of dioxins during exposure of pesticide formulations to sunlight. Chemosphere 88, 364-370.
- 584 Jerne, N.K., Nordin, A.A., 1963. Plaque formation in agar by single antibody-producing cells. 585 Science 140, 405-&.
- 586 Kaplan, B.L.F., Crawford, R.B., Kovalova, N., Arencibia, A., Kim, S.S., Pinnavaia, T.J., Boyd,
- 587 S.Å., Teppen, B.J., Kaminski, N.E., 2011. TCDD adsorbed on silica as a model for TCDD
 588 contaminated soils: Evidence for suppression of humoral immunity in mice. Toxicology 282, 82-
- 589 87.
- 590 Kerkvliet, N.I., Brauner, J.A., 1990. Flow cytometric analysis of lymphocyte subpopulations in
- 591 the spleen and thymus of mice exposed to an acute immunosuppressive dose of 2,3,7,8-592 tetrachlorodibenzo-para-dioxin (TCDD). Environ. Res. 52, 146-154.
- Kimbrough, R.D., Krouskas, C.A., Carson, M.L., Long, T.F., Bevan, C., Tardiff, R.G., 2010.
 Human uptake of persistent chemicals from contaminated soil: PCDD/Fs and PCBs. Regul.
 Toxicol. Pharmacol. 57, 43-54.

- 596 Kulkarni, P.S., Crespo, J.G., Afonso, C.A.M., 2008. Dioxins sources and current remediation 597 technologies - A review. Environ. Int. 34, 139-153.
- 598 Lamb, C. L. Cholico, G. N. Pu, X. Hagler, G. D. Cornell, K. A. Mitchell, K. A. 2016. 2,3,7,8-
- 599 Tetrachlorodibenzo-p-Dioxin (TCDD) Increases Necroinflammation and Hepatic Stellate Cell
- Activation but Does Not Exacerbate Experimental Liver Fibrosis in Mice. Toxicol Appl
 Pharmacol. 311, 42–51.
- Lamparski, L.L., Stehl, R.H., Johnson, R.L., 1980. Photolysis of pentachlorophenol-treated wood - chlorinated dibenzo-p-dioxin formation. Environ. Sci. Technol. 14, 196-200.
- Lehmann, J., Kleber, M., 2015. The contentious nature of soil organic matter. Nature 528, 60-605 68.
- Li, C., Zheng, M.H., Zhang, B., Gao, L.R., Liu, L.D., Zhou, X., Ma, X.D., Xiao, K., 2012. Longterm persistence of polychlorinated dibenzo-p-dioxins and dibenzofurans in air, soil and
 sediment around an abandoned pentachlorophenol factory in China. Environ. Pollut. 162, 138143.
- Li, X.L., Johnson, D.C., Rozman, K.K., 1995. Reproductive effects of 2,3,7,8-tetrachlorodibenzo-
- p-dioxin (TCDD) in female rats ovulation, hormonal-regulation, and possible mechanism(s).
 Toxicol. Appl. Pharmacol. 133, 321-327.
- Liu, C., Li, H., Teppen, B.J., Johnston, C.T., Boyd, S.A., 2009. Mechanisms associated with the
 high adsorption of dibenzo-p-dioxin from water by smectite clays. Environ. Sci. Technol. 43,
 2777-2783.
- 616 Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time 617 quantitative PCR and the 2(T)(-Delta Delta C) method. Methods 25, 402-408.
- Luong, H.V., Tai, P., Nishijo, M., Trung, D., Thao, P., Son, P.V., Long, N.V., Linh, N.T., Nishijo,
 H., 2018. Association of dioxin exposure and reproductive hormone levels in men living near the
 Bien Hoa airbase, Vietnam. Sci. Total Environ. 628-629, 484-489.
- Luthy, R. G.; Aiken, G. R.; Brusseau, M. L.; Cunningham, S. D.; Gschwend, P. M.; Pignatello, J.
 J.; Reinhard, M.; Traina, S. J.; Weber, W. J.; Westall, J. C. 1997. Sequestration of Hydrophobic
 Organic Contaminants by Geosorbents. Environ. Sci. Technol. 31 (12), 3341–3347.
- Malloy, T.A., Goldfarb, T.D., Surico, M.T.J., 1993. PCDDs, PCDFs, PCBs, chlorophenols (CPs)
 and chlorobenzenes (CBzs) in samples from various types of composting facilities in the United
 States. Chemosphere 27, 325-334.
- 627 Marple, L., Brunck, R., Throop, L., 1986. Water solubility of 2,3,7,8-tetrachlorodibenzo-para-628 dioxin. Environ. Sci. Technol. 20, 180-182.
- Marsh, H., Rodriguez-Reinoso, F., 2006. Activated carbon. Elsevier Science, London.
- 630 MDEQ, 2012. DEQ basis of decision and response for a site-specific residential direct contact
- 631 cleanup criterion (SSRDCC) for dioxins/furans (D/F) toxic equivalents (TEQ) for Midland area
- soils. in: Quality, M.D.o.E. (Ed.). Michigan Department of Environmental Quality, Lansing, MI.
- 633 MDHHS, Accessed on February 6, 2019. Dioxins, furans, and your health along the
- 634 Tittabawassee and Saginaw Rivers.
- 635 <u>https://www.michigan.gov/documents/mdch/Dioxin Exposure and Health Final 420292 7.pdf</u>.

- Nghi, T.N., Nishijo, M., Manh, H.D., Tai, P.T., Luong, H.V., Anh, T.H., Thao, P.N., Trung, N.V.,
- Waseda, T., Nakagawa, H., Kido, T., Nishijo, H., 2015. Dioxins and nonortho PCBs in breast
- milk of Vietnamese mothers living in the largest hot spot of dioxin contamination. Environ. Sci.
 Technol. 49, 5732-5742.
- Oleszek-Kudlak, S., Shibata, E., Nakamura, T., 2007. Solubilities of selected PCDDs and
 PCDFs in water and various chloride solutions. J. Chem. Eng. Data 52, 1824-1829.
- 642 Orazio, C.E., Kapila, S., Puri, R.K., Yanders, A.F., 1992. Persistence of chlorinated dioxins and 643 furans in the soil environment. Chemosphere 25, 1469-1474.
- Pennell, K. D.; Abriola, L. M.; Boyd, S. A. 1995. Surface Area of Soil Organic Matter
 Reexamined. Soil Science Society of America Journal. 59 (4), 1012–1018.
- Persson, N.J., Gustafsson, O., Bucheli, T.D., Ishaq, R., Naes, K., Broman, D., 2002. Sootcarbon influenced distribution of PCDD/Fs in the marine environment of the Grenlandsfjords,
 Norway. Environmental Science & Technology 36, 4968-4974.
- Pignatello, J.J., Mitch, W.A., Xu, W.Q., 2017. Activity and reactivity of pyrogenic carbonaceous
 matter toward organic compounds. Environ. Sci. Technol. 51, 8893-8908.
- Pirkle, J.L., Wolfe, W.H., Patterson, D.G., Needham, L.L., Michalek, J.E., Miner, J.C., Peterson,
 M.R., Phillips, D.L., 1989. Estimates of the half-life of 2,3,7,8-tetrachlorodibenzo-para-dioxin in
- Vietnam veterans of operation ranch hand. J. Toxicol. Environ. Health 27, 165-171.
- Rana, K., Boyd, S.A., Teppen, B.J., Li, H., Liu, C., Johnston, C.T., 2009. Probing the
 microscopic hydrophobicity of smectite surfaces. A vibrational spectroscopic study of dibenzo-pdioxin sorption to smectite. Phys. Chem. Chem. Phys. 11, 2976-2985.
- Ren, X.Y., Zeng, G.M., Tang, L., Wang, J.J., Wan, J., Liu, Y.N., Yu, J.F., Yi, H., Ye, S.J., Deng,
 R., 2018. Sorption, transport and biodegradation An insight into bioavailability of persistent
 organic pollutants in soil. Sci. Total Environ. 610, 1154-1163.
- 660 Sallach, J.B., Crawford, R., Li, H., Johnston, C.T., Teppen, B.J., Kaminski, N.E., Boyd, S.A.,
- 661 2019. Activated carbons of varying pore structure eliminate the bioavailability of 2,3,7,8 662 tetrachlorodibenzo-p-dioxin to a mammalian (mouse) model. Sci. Total Environ. 650, 2231-
- 663 2238.
- 664 Serkiz, S.M., Perdue, E.M., 1990. Isolation of dissolved organic matter from the Suwannee 665 River using reverse osmosis. Water Res. 24, 911-916.
- 666 Shiu, W.Y., Doucette, W., Gobas, F., Andren, A., Mackay, D., 1988. Physical-chemical 667 properties of chlorinated dibenzo-para-dioxins. Environ. Sci. Technol. 22, 651-658.
- 668 Simpson, E., Mull, J.D., Longley, E., East, J., 2000. Pica during pregnancy in low-income 669 women born in Mexico. West. J. Med. 173, 20-24.
- 670 Stanek, E.J., Calabrese, E.J., 1995. Daily estimates of soil ingestion in children. Environ. Health 671 Perspect. 103, 276-285.
- Turner, W.C., Imologhome, P., Havarua, Z., Kaaya, G.P., Mfune, J.K.E., Mpofu, I.D.T., Getz,
- 673 W.M., 2013. Soil ingestion, nutrition and the seasonality of anthrax in herbivores of Etosha
- 674 National Park. Ecosphere 4.
- 675
- 676

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