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Sallach, J. Brett, Crawford, Robert, Li, Hui et al. (2019) Activated carbons of varying pore structure eliminate the bioavailability of 2,3,7,8-tetrachlorodibenzo-p-dioxin to a mammalian (mouse) model. Science of the Total Environment. pp. 2231-2238. ISSN: 0048-9697

https://doi.org/10.1016/j.scitotenv.2018.09.270

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Activated carbons of varying pore structure eliminate the bioavailability of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin to a mammalian (mouse) model

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Abstract

The use of activated carbon (AC) as an *in situ* sorbent amendment to sequester polychlorinated-dibenzo-*p*-dioxins and furans (PCDD/Fs) present in contaminated soils and sediments has recently gained attention as a novel remedial approach. This remedy could be implemented at much lower cost while minimizing habitat destruction as compared to traditional remediation technologies that rely on dredging/excavation and landfilling. Several prior studies have demonstrated the ability of AC amendments to reduce pore water concentrations and hence bioaccumulation of PCDD/Fs in invertebrate species. However, our recent study was the first to show that AC had the ability to sequester 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in a form that eliminated bioavailability to a mammalian (mouse) model. Here we show that three commercially available ACs, representing a wide range of pore size distributions, were equally effective in eliminating the bioavailability of TCDD based upon two sensitive bioassays, hepatic induction of *cyp1A1* mRNA and immunoglobulin M antibody-forming cell response. These results provide direct evidence that a wide range of structurally diverse commercially available ACs may be suitable for use as *in situ* sorbent amendments to provide a cost-effective remedy for PCDD/F contaminated soils and sediments. Potentially, adaption of this technology would minimize habitat destruction and be protective of ecosystem and human health.

Key Words

TCDD, immune response, remediation, sorbent amendments

1. Introduction

The ubiquitous occurrence of polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans (PCDD/Fs) in the environment results from their formation as unintentional byproducts of chemical manufacturing, including pesticide production and the historic chlor-alkali process, and from both anthropogenic (incineration) and natural (forest fires and volcanic activities) combustion [1,2]. The natural *in situ* formation of predioxins and octachlorodibenzo-*p*-dioxin may also occur on the surfaces of ball clays [3]. In recent decades, significant technological and regulatory improvements have limited the anthropogenic release of these compounds to the environment. However, their widespread distribution and recalcitrance in soils and sediments, coupled with their high toxicity at low levels of exposure, contributes to their high priority for remediation throughout the world [4,5].

Human exposure of PCDD/Fs is potentially associated with many adverse health effects including cardiovascular disease, diabetes, cancer, porphyria, endometriosis, altered hormone levels and reproductive health, skin, tooth, and nail abnormalities amongst others [5, 6]. Perhaps most alarming, exposure to PCDDs at levels only a single order of magnitude greater that current mean background levels for the general population (viz. 15 ppt serum lipid basis) manifests negative health outcomes [7]. Exposure to PCDD/Fs has been linked to prenatal mortality in a number of mammalian species including mice, rabbits and mink [8]. Interestingly, the proliferation of antibiotic resistance genes in the gut microbiota of mice has been associated with the immune response induced by TCDD exposure [9].

Remediation of PCDD/F contaminated soils and sediments often involves removal by excavation or dredging and disposal in hazardous waste landfills, with varied degrees of

effectiveness [10,11]. This traditional remedy is associated with high cost and substantial habitat destruction, for example detrimental effects on benthic ecosystems, and can result in re-distribution of contaminated sediments [12]. Therefore, efforts have been made to develop new remediation technologies that are less expensive and destructive while being protective of ecosystem and human health. The use of activated carbon (AC) sorbent amendments has emerged as a particularly promising treatment alternative [13].

A select number of studies showing reductions in pore water concentrations of PCDDs and subsequent reductions in bioaccumulation amongst benthic organisms and soil invertebrates has provided the impetus for further scientific investigation of this technology [13–17]. However, from a public policy standpoint, mammalian exposure and bioavailability has been considered in order to make decisions protective of human health. In 2012, based on evidence that 16-28% of measured PCDD/Fs in Midland bulk soils were orally bioavailable to mammals, Dow Chemical (Midland, Michigan, USA) was granted a site-specific variance in soil remediation targets (from 90 to 250 ppt TEQ) by the Michigan Department of Environmental Quality (MDEQ) [18,19]. The significance of the direct oral exposure pathway has been established through studies on the advertent and inadvertent ingestion of soil documented in humans, especially children, and wild animals [20-22].

We recently showed that a commercial AC has the ability to sequester PCDD/Fs in a form that eliminates mammalian bioavailability. When TCDD was sequestered by AC it failed to elicit a hallmark of TCDD exposure, i.e. suppression of immune system response; other sorbent materials including silica and smectite (e.g. montmorillonite) clays did not reduce TCDD bioavailability to the mammalian (mouse) model [23–25]. In addition, TCDD sequestered by AC was shown to eliminate characteristic TCDD influences on the gut microbiome [26]. The

99 beneficial effect of AC in reducing mammalian toxicant exposure in the gut has motivated its
100 recommended use for livestock and humans following acute exposures [27-29].

101 The efficacy of AC as a sorbent for organic contaminants is well established accounting
102 for its use in many treatment processes including both water and gas flu treatment [30,31]. This
103 is especially true in the case of planar hydrophobic compounds which are intrinsically suitable
104 for hydrophobic pore-filling processes coupled with van der Waals attraction characteristic of
105 contaminant sorption by AC [32]. The sorption capacity of ACs have been shown to be orders of
106 magnitude greater than the primary native soil/sediment sorptive component for hydrophobic
107 contaminants, namely amorphous organic matter [33]. Pore structure is known to play an
108 important role in the sorption potential of all porous media across a variety of scales [34]. The
109 pore structure of ACs is dependent on the source material as well as physical and chemical
110 processes utilized in their formation. Because of this, the pore structure of ACs varies
111 significantly among different commercially available products, likely affecting their suitability
112 for specific applications [35].

113 Pore characteristics play a significant role in determining the irreversibility of
114 contaminant sorption, or the kinetic release, by ACs. For example, a common assumption is that
115 a pore size of 1.3-1.7 times the molecular (kinetic) diameter of a particular compound manifests
116 the greatest sorption energy and preferential sorption [36]. This concept has been utilized in the
117 pharmaceutical industry to help modulate drug delivery via a porous silica media [37]. Dynamic
118 molecular simulations show that the energetics of sorption are most favorable with pores slightly
119 larger than TCDD molecules [38]. As the pore size increases, the mean potential energy of
120 sorption for the TCDD molecule decreases. Molecular simulations also suggest that the water
121 density within individual pores decrease as pore size decreases. The resulting sub-aqueous

environment would plausibly be energetically favorable for hydrophobic compounds such as TCDD [29].

The goal of the current study was to investigate the effectiveness of AC materials representing a wide range of pore structure distributions, as well as specific surface areas, in reducing the bioavailability of TCDD using a mammalian (mouse) model. The mouse has been extensively characterized with respect to its biological and toxicologic responses to PCDD and dioxin-like compounds with induction of hepatic enzyme, cytochrome P-4501A1 (cyp1A1), and suppression of the primary IgM antibody response being among the most sensitive to PCDD/F exposure. For this reason the mouse and these specific responses were assayed when determining bioavailability of PCDDs. In addition to the WPC AC used in our previous study, two additional AC materials were selected for study (Table 1; FM1 and G60). The three ACs were loaded with TCDD via the incipient wetness method, and delivered to mice via oral gavage. Bioavailability in mice was determined through enumeration of the anti-sheep erythrocyte (sRBC) IgM antibody forming cells (AFC) and induction of *cyp1A1* mRNA, two hallmark responses of TCDD exposure in mammals.

2. Materials and Methods

2.1 Selection of ACs

In a previous study, five activated carbons were characterized using nitrogen absorption to determine specific surface area and pore size distribution [23]. Of the five ACs, three were selected for use in the current study (Table 1). WPC, used in the previous feeding study, represented a microporous dominant AC while Darco FM-1 (Cabot Corp) represented a mesoporous dominant AC. Specific surface area was also considered for selection of AC materials. The specific surface area of FM-1 was smaller than WPC while Darco G60 (Cabot

145 Corp) had increased specific surface area compared to WPC and with a more even distribution of
146 mesopores and micropores.

147 < Table 1. Structural properties of activated carbon >

148

149

150 *2.2 Preparation of ACs*

151 Loading the three ACs with the required amount of 2,3,7,8-TCDD via aqueous sorption
152 was impractical due to TCDD's extremely low solubility in water (0.2-0.3 µg/L) [39]. Therefore,
153 the incipient wetness method was employed to load TCDD into the pore structures of the three
154 AC sorbents as conducted and validated in previous studies [23-25].

155 Two 500 mg portions of each of the three ACs were measured in Corex glass centrifuge
156 tubes (30 mL). TCDD spike solutions were prepared in DMSO at concentrations (47.7, 63.37, &
157 100 µg/mL) necessary to deliver equivalent masses of TCDD in DMSO volumes (336, 253, 160
158 µL) equivalent to the pore volume of the respective ACs (G60, FM-1, WPC) listed in Table 1.
159 Removal of DMSO followed the method described in our previous study [23]. The procedure
160 resulted in TCDD-AC of either 0 or 32 µg/g for each of the three ACs. Thermogravimetric
161 analysis (TGA) was used to quantify mass loss during heating. Approximately 30 mg of AC was
162 placed in a ceramic crucible and placed in the TGA (model TGA/SDTA851e, Mettler Toledo,
163 OH). The samples were heated from 25 to 1000 °C at an increment rate of 20 °C per minute in a
164 N_2 atmosphere. The TGA data are reported as percent mass loss of the initial mass and the 1^{st}
165 derivative of the mass loss curves which shows the temperature ranges where mass-loss events
166 occur.

167 Aqueous suspensions, necessary to administer the samples to the mice via oral gavage,
168 were prepared by combining 156.25 mg of each TCDD-AC material with 5 mL of deionized
169 water in 20 mL glass scintillation vials.

170 *2.3 TCDD Analysis*

171 Samples of each of the three AC materials, loaded with TCDD following the incipient
172 wetness method described above, were analyzed by Pacific Rim Laboratories INC (Surrey, BC,
173 Canada) following the EPA 1613b standard reference method [40]. Briefly, 0.5 g samples of
174 each AC underwent 64 hours of Soxhlet extraction using toluene. Resulting extracts were
175 brought up to 100 mL with toluene. A 10 µL aliquote of sample extract was combined with 0.5
176 ng of $^{13}C_{12}$-2,3,7,8-TCDD and 1.0 ng of $^{13}C_{12}$-1,2,3,4-TCDD and made up to a final volume of
177 50 µL prior to HRGC/HRMS analysis. Results from the analyses of all three AC materials are
178 provided in Table 2.

179 < Table 2. Analytical detection and recoveries of TCDD from activated carbon materials >

180 *2.4 Animals*

181 Eight to twelve week old pathogen-free B6C3F1 female mice, purchased from Charles
182 River Breeding Laboratories (Portage, MI, USA), were randomly divided into 9 experimental
183 groups (5 mice per group). Each group was placed into its own plastic cage containing sawdust
184 bedding. Prior to the start of the experiment, the mice were acclimated for two weeks to allow
185 their body weights to reach approximately 20 g. Animal holding rooms were operated with 12-
186 hour light/dark cycles at temperatures of 21-24°C and 40-60 % relative humidity. Water and
187 food (Purina Certified Laboratory Chow) were provided without restraint and all procedures

188 involving mice were in accordance with the Michigan State University Institutional Animal Care
189 and Use Committee.

190 *2.5 Seven Day Feeding Trials*

191 Following the feeding protocol previously established [17–19], a 7-day feeding study
192 comprising 4 TCDD treatments was performed. Treatment groups included those receiving 1.0
193 µg/mL TCDD in either corn oil (TCDD-CO) or in the three different AC solutions (TCDD-AC).
194 In addition, control groups consisting of the vehicles (corn oil or AC) only were prepared. The
195 final group was kept naïve, receiving no treatment regimen. Details of the treatment matrix are
196 provided in Table 3.

197 < Table 3. Treatment groups and experiment timeline >

198 On days one through four, mice received 200 µL aliquots of their respective treatment via
199 oral gavage, with mice in groups 5-8 receiving a mass of TCDD (10 ug/kg bw/day) in their
200 respective vehicles daily. On day 3, Groups 1-8 mice were sensitized with 1 x 10^9 sheep red
201 blood cells (sRBC) by intraperitoneal injection to initiate a T cell dependent humoral immune
202 response. Mice were euthanized by cervical dislocation 4 days post sensitization (day 7). Body
203 weight was immediately determined prior to resection of the liver (for cyp1A1 induction) and
204 spleen (for AFC response). Each liver and spleen were then weighed individually. Mouse
205 feeding trials were repeated approximately 6 months apart to confirm reproducibility. Results are
206 representative of the 2 separate experiments.

207 *Antibody forming cell response*

208 Enumeration of anti-sRBC IgM antibody forming cells (AFCs) was performed using the
209 Jerne plaque assay [41] following the method described previously [23]. Duplicate assays were

prepared for each mouse sample (5 mice per treatment) resulting in 10 assays per treatment group. AFC counts were normalized with total cell counts enumerated with a ZI Coulter particle counter (Beckman Coulter, Pasadena, CA, USA) and figures are presented as anti-sRBC IgM AFC/1x10^6 splenocytes.

2.6 Cyp1A1 gene expression

Induction of *cyp1A1* mRNA was quantified by real time polymerase chain reaction (PCR). Sacrificed mouse livers, stored at -70C in TRI Reagent (Sigma-Aldrich, St. Louis, MO, USA), were homogenized then phase separated using bromochlorophenol. RNA was precipitated from the aqueous phase using isopropanol. Extraction, purification, and DNase treatment followed using a Promega SV total RNA isolation system. A high capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA, USA) was employed for reverse transcription of total RNA using random primers. Amplification of the of the cDNA using a Taqman primer/probe set for mouse *cyp1A1* (Applied Biosystems) preceded analysis with a 7900 HT fast real-time polymerase chain reaction (PCR) system (Applied Biosystems). Fold change values were calculated using the $\Delta\Delta C_T$ method [42].

2.7 Statistical Analysis

Real-time PCR statistical analysis was performed on ΔC_T values using Prism version 4.0a (Graphpad, La Jolla, CA, USA). Statistically significant differences between treatment groups and controls were determined by Dunnett's two tailed *t* test.

3. Results and Discussion

3.1 Analytical assessment of TCDD-AC material

231 Analytical determination of the concentration of PCDD/Fs and similar compounds in
232 carbonaceous materials is complicated by the lack of an established reference method that
233 provides adequate recoveries. Currently, EPA Method 1613 serves as the standard method for
234 the extraction and quantification of tetra- through octa- chlorinated dioxins and furans from
235 numerous matrices including soils and sediments [40]. Following the preparation of ACs by the
236 incipient wetness method, AC samples from each of the three study materials were analyzed by
237 Pacific Rim Laboratories using EPA Method 1613. Total calculated concentrations ranged from
238 7.5 – 8.7 μg/g with corresponding percent recoveries of 23.5-27.1 % (Table 2). Interestingly,
239 extraction efficiency seemed to increase with decreasing percentage of micropore volume. These
240 low results are consistent with extraction efficiencies for PCDD/Fs and similar compounds and
241 from similar carbonaceous materials reported elsewhere, and highlights the irreversibility of
242 TCDD binding and the ineffectiveness of current standard methods for the extraction and
243 analysis of PCDD/Fs from graphitic porous materials [43–45]. Furthermore, the inefficiency of
244 TCDD extraction directly reflects the sequestering ability of ACs and corresponding reduction in
245 bioavailability.

246 As pyrogenic carbonaceous materials are natural constituents of all soils and sediments,
247 prior environmental assessments using standard methodology may underestimate the actual
248 environmental abundance of these compounds. Our prior published studies on the bioavailability
249 of TCDD sorbed by silica and smectite clay [24,25] followed the incipient wetness method.
250 Results from these studies showed no loss of TCDD associated with the incipient wetness
251 method. Specifically, dose dependent responses in mice were identical when the equivalent doses
252 of TCDD were administered directly in corn-oil or as TCDD–clay and –silica complexes. In the
253 experiments with AC, the extracted concentrations of 7.5 – 8.7 μg/g determined using the

254 standard method and quantified via HR-GC/MS, would be sufficient to elicit a significant
255 bioresponse by both bioassays (i.e. AFC response and *cyp1A1* mRNA induction) in the current
256 and previous studies if that mass of TCDD was bioavailable. In fact, we have shown repeatedly
257 that exposure to TCDD at levels as low as 0.01 μg/mL, which would correspond to TCDD-AC
258 concentrations of 0.32 μg/g, would result in a significant bioresponse in both bioassays assuming
259 the TCDD was bioavailable [23-25]. Therefore, thermal gravimetric analysis was used to verify
260 loading via incipient wetness, and the two sensitive bioassays utilized in our prior published
261 work [23-25] were used to measure bioavailability following oral exposure of the mammalian
262 (mouse) model.

263 *3.2 Confirmation of pore filling*

264 Based on our working hypothesis that smaller micropores sorb TCDD more strongly than
265 larger mesopores, we hypothesized that bioavailability would increase with increasing proportion
266 of mesoporosity. A benefit inherent in the incipient wetness method is that sorption of TCDD
267 dissolved in DMSO is directly related to pore filling, since the volume of DMSO solvent
268 (containing dissolved TCDD) added corresponds to the pore volume of each AC. When added to
269 the AC, the material is mixed rigorously until all the solvent has been internalized within the AC
270 pore structure. Thus, the TCDD-DMSO fills both mesopores and micropores.

271 To quantify the pore filling process, thermogravimetric analysis (TGA) was performed on
272 AC samples loaded with DMSO via the incipient wetness method, both before the 2-hour 200°C
273 solvent removal and after. Thermograms of the mass removal curves (TG) and their derivatives
274 (DTG) are shown in Figure 1. The amount of DMSO added was equal to the pore volume of the
275 mass of AC used based on the following measured pore volumes: WPC (32 %) < FM1 (50.5 %)

< G60 (67.1 %). The percent removals (Figure 1) confirm that the masses of DMSO removed (WPC<FM1<G60) correlates with the masses of DMSO added and the AC pore volumes. The derivatives of the mass loss thermograms (DTG) (Figure 1) provide evidence of pore filling for both micro and meso pores in the three ACs. The large negative peak in the DTG curves at 150 °C for both G60 and FM1 corresponds to rapid removal of DMSO from larger mesopores (2-50 nm). In addition, the two ACs with significant micropore volumes, WPC and G60, both showed significant tailing in the DTG curve at higher temperatures extending from 150 to 300 °C. This tailing is characteristic of the removal of DMSO from micropores (<2 nm), which requires more time and energy.

< Figure 1. Thermogravimetric analysis (TGA) of DMSO infused activated carbons via the incipient wetness method. Thermograms (TG) of mass loss loss (top) and their derivatives (DTG) (bottom) following a heating ramp to 500°C. >

When TGA analysis was performed on the AC materials after DMSO removal at 200°C for 2 hours, an insignificant mass loss was observed demonstrating the effectiveness of the heating protocol for DMSO removal. The early peaks in the derivative curves for all three ACs is likely associated with a small amount of moisture that condensed on the sample during the cooling process after the material has been heated. Taken together, data from TGA analysis provides good evidence that the incipient wetness method was effective in pore-filling of both meso- and micro- pores. In addition, these data also confirmed that the heating protocol, 200°C for 2 hours, was effective in removing DMSO from AC materials.

3.3 Activated carbon effect on reducing bioavailability via cyp1A1 induction and the Jerne plaque Assay

The induction of *cyp1A1* is a hallmark response of AhR agonists including PCDDs and was measured using liver tissue of mice. When exposed to TCDD via the corn oil vehicle, *cyp1A1* mRNA expression increased by more than 4000 fold compared to that of mice exposed to the corn oil vehicle with no TCDD (Figure 2). This response is in excellent agreement with the *cyp1A1* expression in mice exposed to the same levels of TCDD in corn oil from previous work [23]. However, when the same mass of TCDD was delivered to mice in each of the three AC vehicles, no significant response was detected (Figure 2). Likewise, compared to the corn oil control, no significant difference in *cyp1A1* expression resulted from administrating any of the three AC vehicles without TCDD. Importantly, sequestration of TCDD by any of the three ACs eliminated TCDD bioavailability to bind the AhR, whereas TCDD freely available in corn oil was clearly bioavailable and resulted in increased expression of the *cyp1A1* gene.

< Figure 2. Cyp1a1 mRNA fold expression in mouse liver after treatment with corn oil or activated carbon of various pore structures with and without 2,3,7,8 - tetrachlorodibenzo-*p*-dioxin. * indicates a significant difference at $p<0.05$ level compared to the respective vehicle control group. >

Suppression of immune function is another hallmark of TCDD toxicity in mammals. Hence, in addition to *cyp1A1* induction in the liver, the Jerne Plaque Assay was employed to evaluate TCDD-induced suppression of humoral immune function in mice, and its elimination via reduction in bioavailability, through quantification of antigen-specific T cell dependent IgM AFC response. Mice were sensitized to the antigen, sRBC, on day three of the seven day feeding trial (Table 3). Our work has demonstrated that AC materials, alone, do not interfere with the IgM responses in mice [23]. Therefore, in experiments where the mammalian (mouse) model was exposed to corn oil, TCDD and AC, alone and in various combinations, suppression of the anti-sRBC IgM AFC response results from exposure to TCDD, and establishes the

bioavailability of TCDD. As expected, the AFC response was significantly suppressed in mice following exposure to TCDD in the corn oil vehicle (Figure 3). However, the AFC response in mice exposed to TCDD-AC showed no evidence of suppression compared to the corresponding groups exposed to each AC with no TCDD. This was true for all three TCDD-AC materials despite substantial differences in pore size distribution, i.e. the relative percentages of micro- and meso-pores. These results confirm and expand our prior findings [23].

< Figure 3. Suppression of humoral immunity observed in response to 2,3,7,8 - tetrachlorodibenzo-*p*-dioxin administered by oral gavage in either corn oil or sorbed on to activated carbon. * indicates a significant difference at $p<0.05$ level compared to the respective vehicle control group.>

Previous studies have shown that TCDD exposure in mice can impact organ mass relative to body weight [23-25]. In agreement with these prior observations, TCDD exposure in corn oil resulted in an increased liver weight ratio (to body mass) and decreased spleen weight ratio compared to the corn oil vehicle (Figure 4). Mice fed TCDD sequestered by the AC materials did not manifest this characteristic response, again indicating the elimination of TCDD bioavailability. In fact, TCDD-G60 resulted in a significant reduction in liver weight ratio compared to G60 alone.

< Figure 4. Organ to total body weight ratios for the liver (top) and spleen (bottom) of mice after treatment with corn oil or activated carbon with and without 2,3,7,8 - tetrachlorodibenzo-*p*-dioxin. >

3.4 Environmental implications

These results demonstrate that TCDD sequestration by structurally diverse ACs eliminate its oral bioavailability to a mammalian (mouse) model. This result was not evident *a priori* since

348 molecular simulations of TCDD interactions with pores suggested more favorable energetics
349 with smaller micropores [38]. Variations in the pore structure of the ACs tested showed no
350 impact on the observed elimination of TCDD bioavailability. However, in actual practice at
351 remediation sites, other confounding interactions must be considered. For instance, soil/sediment
352 constituents have been attributed to the clogging of micropores and reduced contaminant
353 sorption capacity of certain ACs [46]. Likewise, pore clogging by natural organic matter (NOM)
354 in sediments over time has also been shown to attenuate contaminant sorption by AC [47],
355 although other studies including our own have shown that NOM additions enhanced uptake of
356 dioxin by ACs [48]. To be clear, contaminant sorption/sequestration alone is insufficient to
357 ensure concomitant reduction in mammalian (mouse) bioavailability. For example, TCDD
358 intercalated in the smectite clay saponite was equally bioavailable to the mammalian (mouse)
359 model as TCDD dissolved in corn oil [24], i.e. sorption by clay manifested no reduction in the
360 oral bioavailability of TCDD. Also, the solubilization of certain biochar components was
361 implicated as being responsible for increased bioaccessibility of sorbed polychlorinated
362 biphenyls in a simulated gastric fluid [49]. Having confirmed that structurally diverse ACs are
363 equally effective in eliminating the bioavailability of TCDD to a mammalian (mouse) model,
364 selection of AC materials for soil and sediment remediation should be further evaluated based on
365 other environmental processes relevant to *in-situ* application of AC sorbent amendments,
366 including the fouling of ACs by NOM and other materials as well as optimizing the mass transfer
367 of contaminants from environmental geosorbents and media to ACs used in this new remediation
368 technology.

369 **4. Conclusions**

370 The rapid acceptance of remediation strategies that employ sorbent amendments to
371 sequester contaminants in forms that reduce or eliminate bioavailability, specifically involving
372 AC, continues despite a paucity of studies that have evaluated their effectiveness with
373 appropriate mammalian models. Such studies are needed to establish that this remedy effectively
374 reduces PCDD bioavailability to mammals and hence mammalian exposure, and by inference is
375 protective of human health.

376 Recently, we demonstrated one commercial AC material, WPC AC, selected to maximize
377 (viz. higher proportion of micro vs meso pores) the irreversible binding of TCDD, could
378 sequester TCDD in a form that eliminated its bioavailability to an appropriate mammalian
379 (mouse) model. Not only were the results of the prior study using only WPC AC replicated, the
380 ability to eliminate TCDD bioavailability is apparently characteristic of AC materials
381 irrespective of their specific pore structures; three ACs with micropore volume ranging from
382 43.3 to 90.5 percent each eliminated TCDD bioavailability. By measuring cyp1A1 mRNA
383 induction in the liver, anti-sRBC IgM AFC response in the spleen, and organ to body weight
384 ratios, our results showed that ACs comprised of widely differing pore structures were equally
385 effective in the elimination of TCDD bioavailability. This suggests that chemisorption,
386 interactions with the material's specific surface, may be a driving factor, rather than simply pore
387 isolation.

388 This study also highlights the fact that existing standard methods for the extraction and
389 quantification of TCDD in soils and sediments are seemingly ineffective for use with porous
390 high surface area carbonaceous materials, or samples that contain such materials. One
391 implication is that previous surveys of PCDD/F contamination may underestimate their
392 abundance, especially in soils and sediments enriched with chars or black carbon. Development

of an extraction method for the efficient removal of PCDD/Fs from carbonaceous materials is urgently needed.

In terms of the development of a new remediation technology, this study indicates that AC materials, of various structures, have strong potential for use as *in situ* sorbent amendments for soils and sediments impacted by PCDDs and similar poorly water-soluble organic contaminants. With this understanding, identification of ideal AC materials for use in remediation should focus on other factors including reducing environmental interactions such as biofouling and maximizing sorption kinetics in environmental matrices. Understanding the mass transfer kinetics of PCDD/Fs from contaminated soils and sediments into AC amendments is an essential step in the further development and acceptance of this emerging remediation technology.

Acknowledgements

The authors would like to acknowledge Ashwini Phadnis-Moghe, Jingeng Li, Natalia Kovalova, Mike Rizzo, Joseph Henriquez, and Jiajun Zhou for their assistance with mouse handling as well as Premachandra Gnanasiri for assistance with activated carbon preparations. Research reported in this publication was supported by AgBio Research at Michigan State University and the National Institute of Environmental Health Sciences of the National Institutes of Health under Award Number P42ES004911. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

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