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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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29   **Abstract**

30   The use of activated carbon (AC) as an *in situ* sorbent amendment to sequester

31   polychlorinated-dibenzo-*p*-dioxins and furans (PCDD/Fs) present in contaminated soils and

32   sediments has recently gained attention as a novel remedial approach. This remedy could be

33   implemented at much lower cost while minimizing habitat destruction as compared to traditional

34   remediation technologies that rely on dredging/excavation and landfilling. Several prior studies

35   have demonstrated the ability of AC amendments to reduce pore water concentrations and hence

36   bioaccumulation of PCDD/Fs in invertebrate species. However, our recent study was the first to

37   show that AC had the ability to sequester 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in a form

38   that eliminated bioavailability to a mammalian (mouse) model. Here we show that three

39   commercially available ACs, representing a wide range of pore size distributions, were equally

40   effective in eliminating the bioavailability of TCDD based upon two sensitive bioassays, hepatic

41   induction of *cyp1A1* mRNA and immunoglobulin M antibody-forming cell response. These

42   results provide direct evidence that a wide range of structurally diverse commercially available

43   ACs may be suitable for use as *in situ* sorbent amendments to provide a cost-effective remedy for

44   PCDD/F contaminated soils and sediments. Potentially, adaption of this technology would

45   minimize habitat destruction and be protective of ecosystem and human health.

46   **Key Words**
47   TCDD, immune response, remediation, sorbent amendments
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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

76   effectiveness [10,11]. This traditional remedy is associated with high cost and substantial habitat

77   destruction, for example detrimental effects on benthic ecosystems, and can result in re-

78   distribution of contaminated sediments [12]. Therefore, efforts have been made to develop new

79   remediation technologies that are less expensive and destructive while being protective of

80   ecosystem and human health. The use of activated carbon (AC) sorbent amendments has

81   emerged as a particularly promising treatment alternative [13].

82   A select number of studies showing reductions in pore water concentrations of PCDDs

83   and subsequent reductions in bioaccumulation amongst benthic organisms and soil invertebrates

84   has provided the impetus for further scientific investigation of this technology [13–17].

85   However, from a public policy standpoint, mammalian exposure and bioavailability has been

86   considered in order to make decisions protective of human health. In 2012, based on evidence

87   that 16-28% of measured PCDD/Fs in Midland bulk soils were orally bioavailable to mammals,

88   Dow Chemical (Midland, Michigan, USA) was granted a site-specific variance in soil

89   remediation targets (from 90 to 250 ppt TEQ) by the Michigan Department of Environmental

90   Quality (MDEQ) [18,19]. The significance of the direct oral exposure pathway has been

91   established through studies on the advertent and inadvertent ingestion of soil documented in

92   humans, especially children, and wild animals [20-22].

93   We recently showed that a commercial AC has the ability to sequester PCDD/Fs in a

94   form that eliminates mammalian bioavailability. When TCDD was sequestered by AC it failed to

95   elicit a hallmark of TCDD exposure, i.e. suppression of immune system response; other sorbent

96   materials including silica and smectite (e.g. montmorillonite) clays did not reduce TCDD

97   bioavailability to the mammalian (mouse) model [23–25]. In addition, TCDD sequestered by AC

98   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

122 environment would plausibly be energetically favorable for hydrophobic compounds such as

123 TCDD [29].

124   The goal of the current study was to investigate the effectiveness of AC materials

125 representing a wide range of pore structure distributions, as well as specific surface areas, in

126 reducing the bioavailability of TCDD using a mammalian (mouse) model. The mouse has been

127 extensively characterized with respect to its biological and toxicologic responses to PCDD and

128 dioxin-like compounds with induction of hepatic enzyme, cytochrome P-4501A1 (cyp1A1), and

129 suppression of the primary IgM antibody response being among the most sensitive to PCDD/F

130 exposure. For this reason the mouse and these specific responses were assayed when

131 determining bioavailability of PCDDs. In addition to the WPC AC used in our previous study,

132 two additional AC materials were selected for study (Table 1; FM1 and G60). The three ACs

133 were loaded with TCDD via the incipient wetness method, and delivered to mice via oral gavage.

134 Bioavailability in mice was determined through enumeration of the anti-sheep erythrocyte

135 (sRBC) IgM antibody forming cells (AFC) and induction of *cyp1A1* mRNA, two hallmark

136 responses of TCDD exposure in mammals.

137 **2. Materials and Methods**

138 *2.1 Selection of ACs*

139 In a previous study, five activated carbons were characterized using nitrogen absorption to

140 determine specific surface area and pore size distribution [23]. Of the five ACs, three were

141 selected for use in the current study (Table 1). WPC, used in the previous feeding study,

142 represented a microporous dominant AC while Darco FM-1 (Cabot Corp) represented a

143 mesoporous dominant AC. Specific surface area was also considered for selection of AC

144 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 1st

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 1×10^9 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

210   prepared for each mouse sample (5 mice per treatment) resulting in 10 assays per treatment

211   group. AFC counts were normalized with total cell counts enumerated with a ZI Coulter particle

212   counter (Beckman Coulter, Pasadena, CA, USA) and figures are presented as anti-sRBC IgM

213   AFC/1×10^6 splenocytes.

214   *2.6 Cyp1A1 gene expression*

215   Induction of *cyp1A1* mRNA was quantified by real time polymerase chain reaction

216   (PCR). Sacrificed mouse livers, stored at -70C in TRI Reagent (Sigma-Aldrich, St. Louis, MO,

217   USA), were homogenized then phase separated using bromochlorophenol. RNA was precipitated

218   from the aqueous phase using isopropanol. Extraction, purification, and DNase treatment

219   followed using a Promega SV total RNA isolation system. A high capacity cDNA reverse

220   transcription kit (Applied Biosystems, Foster City, CA, USA) was employed for reverse

221   transcription of total RNA using random primers. Amplification of the of the cDNA using a

222   Taqman primer/probe set for mouse *cyp1A1* (Applied Biosystems) preceded analysis with a 7900

223   HT fast real-time polymerase chain reaction (PCR) system (Applied Biosystems). Fold change

224   values were calculated using the $\Delta\Delta C_T$ method [42].

225   *2.7 Statistical Analysis*

226   Real-time PCR statistical analysis was performed on ΔC_T values using Prism version 4.0a

227   (Graphpad, La Jolla, CA, USA). Statistically significant differences between treatment groups

228   and controls were determined by Dunnett's two tailed *t* test.

229   **3. Results and Discussion**

230   *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 %)

276   < G60 (67.1 %). The percent removals (Figure 1) confirm that the masses of DMSO removed

277   (WPC<FM1<G60) correlates with the masses of DMSO added and the AC pore volumes. The

278   derivatives of the mass loss thermograms (DTG) (Figure 1) provide evidence of pore filling for

279   both micro and meso pores in the three ACs. The large negative peak in the DTG curves at 150

280   °C for both G60 and FM1 corresponds to rapid removal of DMSO from larger mesopores (2-50

281   nm). In addition, the two ACs with significant micropore volumes, WPC and G60, both showed

282   significant tailing in the DTG curve at higher temperatures extending from 150 to 300 °C. This

283   tailing is characteristic of the removal of DMSO from micropores (<2 nm), which requires more

284   time and energy.

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

288

289   When TGA analysis was performed on the AC materials after DMSO removal at 200°C

290   for 2 hours, an insignificant mass loss was observed demonstrating the effectiveness of the

291   heating protocol for DMSO removal. The early peaks in the derivative curves for all three ACs is

292   likely associated with a small amount of moisture that condensed on the sample during the

293   cooling process after the material has been heated. Taken together, data from TGA analysis

294   provides good evidence that the incipient wetness method was effective in pore-filling of both

295   meso- and micro- pores. In addition, these data also confirmed that the heating protocol, 200°C

296   for 2 hours, was effective in removing DMSO from AC materials.

297   *3.3 Activated carbon effect on reducing bioavailability via cyp1A1 induction and the Jerne*

298   *plaque Assay*

299   The induction of *cyp1A1* is a hallmark response of AhR agonists including PCDDs and

300   was measured using liver tissue of mice. When exposed to TCDD via the corn oil vehicle,

301   *cyp1A1* mRNA expression increased by more than 4000 fold compared to that of mice exposed

302   to the corn oil vehicle with no TCDD (Figure 2). This response is in excellent agreement with the

303   *cyp1A1* expression in mice exposed to the same levels of TCDD in corn oil from previous work

304   [23]. However, when the same mass of TCDD was delivered to mice in each of the three AC

305   vehicles, no significant response was detected (Figure 2). Likewise, compared to the corn oil

306   control, no significant difference in *cyp1A1* expression resulted from administrating any of the

307   three AC vehicles without TCDD. Importantly, sequestration of TCDD by any of the three ACs

308   eliminated TCDD bioavailability to bind the AhR, whereas TCDD freely available in corn oil

309   was clearly bioavailable and resulted in increased expression of the *cyp1A1* gene.

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

314

315   Suppression of immune function is another hallmark of TCDD toxicity in mammals.

316   Hence, in addition to *cyp1A1* induction in the liver, the Jerne Plaque Assay was employed to

317   evaluate TCDD-induced suppression of humoral immune function in mice, and its elimination

318   via reduction in bioavailability, through quantification of antigen-specific T cell dependent IgM

319   AFC response. Mice were sensitized to the antigen, sRBC, on day three of the seven day feeding

320   trial (Table 3). Our work has demonstrated that AC materials, alone, do not interfere with the

321   IgM responses in mice [23]. Therefore, in experiments where the mammalian (mouse) model

322   was exposed to corn oil, TCDD and AC, alone and in various combinations, suppression of the

323   anti-sRBC IgM AFC response results from exposure to TCDD, and establishes the

324   bioavailability of TCDD. As expected, the AFC response was significantly suppressed in mice

325   following exposure to TCDD in the corn oil vehicle (Figure 3). However, the AFC response in

326   mice exposed to TCDD-AC showed no evidence of suppression compared to the corresponding

327   groups exposed to each AC with no TCDD. This was true for all three TCDD-AC materials

328   despite substantial differences in pore size distribution, i.e. the relative percentages of micro- and

329   meso-pores. These results confirm and expand our prior findings [23].

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

334

335   Previous studies have shown that TCDD exposure in mice can impact organ mass relative

336   to body weight [23-25]. In agreement with these prior observations, TCDD exposure in corn oil

337   resulted in an increased liver weight ratio (to body mass) and decreased spleen weight ratio

338   compared to the corn oil vehicle (Figure 4). Mice fed TCDD sequestered by the AC materials did

339   not manifest this characteristic response, again indicating the elimination of TCDD

340   bioavailability. In fact, TCDD-G60 resulted in a significant reduction in liver weight ratio

341   compared to G60 alone.

342   < Figure 4. Organ to total body weight ratios for the liver (top) and spleen (bottom) of mice after

343   treatment with corn oil or activated carbon with and without 2,3,7,8 - tetrachlorodibenzo-*p*-

344   dioxin. >

345   *3.4 Environmental implications*

346   These results demonstrate that TCDD sequestration by structurally diverse ACs eliminate

347   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

393   of an extraction method for the efficient removal of PCDD/Fs from carbonaceous materials is

394   urgently needed.

395   In terms of the development of a new remediation technology, this study indicates that

396   AC materials, of various structures, have strong potential for use as *in situ* sorbent amendments

397   for soils and sediments impacted by PCDDs and similar poorly water-soluble organic

398   contaminants. With this understanding, identification of ideal AC materials for use in

399   remediation should focus on other factors including reducing environmental interactions such as

400   biofouling and maximizing sorption kinetics in environmental matrices. Understanding the mass

401   transfer kinetics of PCDD/Fs from contaminated soils and sediments into AC amendments is an

402   essential step in the further development and acceptance of this emerging remediation

403   technology.

404   **Acknowledgements**

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