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TCDD Adsorbed on Silica as a Model for TCDD Contaminated Soils: Evidence for Suppression of Humoral Immunity in Mice

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Abstract

2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), the prototypical aryl hydrocarbon receptor (AhR) ligand, exhibits immune suppression *in vivo* and *in vitro*. Suppression of primary humoral immune responses in particular has been well characterized as one of the most sensitive functional immune endpoints in animals treated with TCDD. Previous studies have used purified TCDD to elucidate the mechanisms by which TCDD and dioxin-like compounds (DLC) impair IgM production by B cells, but did not represent the route by which animals and humans are likely to be exposed environmentally. In the studies reported here, mice were treated with TCDD adsorbed onto a well-defined synthetic silica phase of known purity and physical properties, followed by sensitization with sheep erythrocytes to initiate a humoral immune. We found that surfactant-templated mesoporous forms of amorphous silica provided an ideal combination of purity, dispersibility and textural properties for immobilizing TCDD. TCDD-adsorbed silica distributed to the spleen and liver after oral administration as assessed by induction of *cyp1a1* gene expression. Most notably, TCDD delivered in the adsorbed state on amorphous silica and as a solute in corn oil (CO) produced similar suppression of the anti-sheep red blood cell immunoglobulin M antibody forming cell response (sRBC IgM AFC) response at equivalent doses of TCDD. These results suggest that TCDD immobilized on silicate particles found in soils distributes to the spleen and suppresses humoral immunity.

Keywords

TCDD; dioxin; immunotoxicity

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1. Introduction

Soils and sediments are complex, heterogeneous mixtures consisting of inorganic mineral matter, and smaller amounts of organic matter, usually <10%. Clays and other silicate phases, both crystalline and amorphous, are among the most common naturally occurring inorganic mineral phases in soils and sediments. Organic matter is a structurally complex mixture of known biochemicals (e.g., carbohydrates, proteins, lipids), humic substances (e.g., humic and fulvic acids), and high surface-area carbonaceous materials (e.g., chars). In soils and sediments, organic matter has often been viewed as playing a disproportionately large role, relative to its abundance, in the sequestration of sparingly soluble neutral organic contaminants. However recent investigations have shown that these fine-grained minerals function as important sorptive phases for the immobilization of several prominent classes of organic contaminants including dioxins under environmentally relevant conditions (Liu et al., 2009; Nolan et al., 1989). Dioxins and many other similar AhR ligands (e.g. PCBs, PAHs) have exceptionally low water solubility and hence exist predominantly as sorbed species in soils and sediments due to their interactions with inorganic and organic geosorbents (Ferrario et al., 2000; Green et al., 2004; Hoekstra et al., 1999).

There is good evidence that silicate minerals function as both an environmental sinks and as subsequent sources for polychlorinated-dibenzo-*p*-dioxins (PCDDs) and -dibenzofurans (PCDFs), and may even play a role in their *in situ* formation (Gu et al., 2008). Perhaps the best-known and well-documented association of PCDDs/PCDFs with naturally occurring silicate minerals occurs in a material commonly referred to as “ball clay”. This is a commercial term used in reference to earthen materials comprised of mixtures of clays and other silicate minerals. They occur as prehistoric geologic deposits in nature, and are mined in several locations and used to make ceramics, tiles, bricks, etc. Ball clays have also been used widely as livestock feed additives. They impart certain favorable properties to feeds (e.g. anti-caking), and claims have been made that they may even promote animal health, for instance by adsorbing fungal toxins. However, it has now been realized that ball clays can be reservoirs for high levels of PCDDs, with concentrations as high as 15,000 pg WHO-TEQ/g (Gadomski et al., 2004). These clays have caused livestock contamination in several instances when they were used as feed additives (Hayward and Bolger, 2005). It was reported that 5% of national poultry production, and at least 35% of farm-raised catfish in the USA, was contaminated by PCDDs originating from clay added to animal feed (Ferrario et al., 2000; Hayward and Bolger, 2005; Hayward et al., 1999).

Although association of PCDDs with mineral phases does not render them biologically unavailable, the extent to which immobilized AhR ligands are available for metabolic uptake by microorganisms and mammals, and how this is dependent on the exact nature of the adsorbent phase (e.g. clays, silica, etc.), is not clear. Naturally occurring clays and related fine-grained inorganic minerals are complex materials of variable composition and purity and, as such, they are not ideally suited for quantitative studies of the biodistribution of adsorbed toxicants (Ehlers and Luthy, 2003; Semple et al., 2004). In order to better understand the toxicology of chemical environmental contaminants that function as AhR ligands, the objective of the present study was to investigate whether, and to what extent, TCDD immobilized on a well-defined synthetic silicate phase of known purity and physical properties, impairs immune competence as assessed by the anti-sRBC IgM AFC response. Administration of TCDD adsorbed on silica delivered in an aqueous solution by oral gavage mimics ingestion of TCDD-contaminated soils. Mesoporous amorphous silica with a surface area and pore size specifically engineered for sequestering TCDD was selected as the sorptive medium for the study.

2. Materials and Methods

2.1 Reagents

Sodium silicate containing 27 wt.% SiO_2 and 14 wt.% NaOH, 1.3.5-trimethylbenzene, acetic acid and reagent grade dimethylsulfoxide (DMSO) were purchased from Aldrich Chemical Co (Milwaukee, WI). TCDD in DMSO solution (100 μg/ml) was purchased from Accustandard (New Haven, CT). CO vehicle for delivery of nonadsorbed TCDD was purchased from Sigma (St. Louis, MO). Pluronic P123 surfactant was obtained from BASF (Wyandotte, MI). All reagents were used as received without further purification.

2.2 Synthesis of Mesocellular Foam Silica, MSU-F

Amorphous silica in mesocellular foam form (Schmidt-Winkel et al., 1999) was assembled from aqueous sodium silicate as the SiO_2 source, Pluronic P123 as the structure-directing surfactant porogen, 1, 3, 5-trimethylbenzene (TMB) as a micelle expanding co-surfactant, and acetic acid as the requisite acid according to previously described methods (Kim et al., 2000). The overall molar composition of the reaction mixture was 1.0 SiO_2 : 0.78 NaOH : 0.017 P123 : 0.83 CH_3COOH : 0.69 TMB : 230 H_2O. The general method of synthesis has been described previously (Kim et al., 2000). Briefly, a micellar solution of P123 porogen was mixed with an amount of aqueous acetic acid solution equivalent to the NaOH content of the silicate source and stirred for 2 h. TMB was added to the porogen solution and the resulting mixture was stirred for an additional hour. Sodium silicate then was added to the solution of P123 porogen solution and TMB pore expander under vigorous stirring at ambient temperature and the mixture was allowed to age at 25°C for 24 h. The mixture was then heated at 100°C for 24 h under static conditions. The porogen-intercalated mesostructured product was recovered by filtration and dried in air at ambient temperature. The final porogen-free mesostructure, denoted MSU-F, was obtained by ethanol extraction of the as-made product under reflux for 2 h. The ethanol-extracted product was allowed to dry in air at room temperature for several days prior to use.

2.3 Physical Characterization of Silica

N_2 adsorption-desorption isotherms for MSU-F mesocellular foam silica were measured at −196°C on a Micromeritics ASAP 2010 sorptometer. The sample was degassed at 150°C under vacuum ($<10^{-6}$ Torr) overnight prior to analysis. The intra-particle pore volume of the silica was taken to be equal to the volume of nitrogen adsorbed at a partial pressure of 0.99. Pore size and window size distributions of the mesocellular foam structure were determined by fitting to the Barret-Joyner-Hallender (BJH) equation to the adsorption and desorption legs of the isotherm, respectively. Transmission electron microscopy (TEM) images were obtained on a JEOL 2200FS field emission microscope with a ZrO/W Schottky electron gun and an accelerating voltage of 200 kV. Sonification was used to disperse the powdered samples in ethanol, and the resulting suspension was dripped onto 300 mesh copper grids 126 for imaging analysis.

2.4 Immobilization of TCDD on MSU-F Silica

TCDD was immobilized on MSU-F silica by the incipient wetness method. In this procedure, an aliquot of TCDD solution equal to the intraparticle pore volume of the silica sample is dropped onto to the surface of dry silica powder in a glass vial with the use of a hypodermic syringe. The vial is sealed with an aluminum-lined screw cap and the mixture is vigorously agitated on a vortex mixer until the liquid is uniformly dispersed in the powder and the powder is uniformly dry and returned to a free flowing state. The resulting mixture is then equilibrated overnight at 60°C to ensure uniform distribution of the TCDD solution within the intra-particle pores of the silica. After each impregnation, the DMSO solvent is

removed in a vacuum oven at 60°C overnight in order to obtain a uniform distribution of TCDD molecules on the walls of the pores. The procedure is repeated for TCDD loadings requiring sequential impregnations to achieve the desired loading. The TCDD-impregnated silica is then used to prepare aqueous suspensions for use in the mouse dosing experiments.

2.5 Animals

Pathogen-free female B6C3F1 mice, 6 weeks of age, were purchased from Charles River Breeding Laboratories (Portage, MI). On arrival, mice were randomized, transferred to plastic cages containing sawdust bedding (5 animals/cage), and quarantined for 1 week. Mice were given food (Purina Certified Laboratory Chow) and water *ad libitum* and were not used for experimentation until their body weight was 17–20 g. Animal holding rooms were kept at 21–24°C and 40–60% relative humidity with a 12-hr light/dark cycle. All procedures involving mice were approved by the Michigan State University Institutional Animal Care and Use Committee.

2.6 In vivo antibody forming cell response (AFC)

Mice (5 per treatment group) were administered corn oil vehicle (CO VH), TCDD, silica alone, DMSO-adsorbed silica or TCDD-adsorbed silica (doses provided in figure legends) by oral gavage once per day for 5 consecutive days. The silica or TCDD-adsorbed silica was delivered in 200 µl water. On day 3, mice were sensitized with 5×10^8 sRBC per mouse by i.p. injection, which allowed for TCDD exposure surrounding antigen sensitization. Four days after sRBC sensitization, mice were sacrificed and total body and spleen weights were recorded. Enumeration of the antibody forming cells was based on the Jerne plaque assay (Jerne and Nordin, 1963). Briefly, 100 µl aliquots of the recovered splenocytes were combined with 0.5% melted agar (Difco/BD, Franklin Lakes, NJ), guinea pig complement (Gibco/Invitrogen, Carlsbad, CA) and sheep erythrocytes. The mixture was vortex mixed, poured onto a petri dish, and overlaid with a 24mm × 50mm glass cover slip, and allowed to solidify. The petri dishes were incubated for at least 3 h at 37°C, after which AFCs were enumerated at 6.5× magnification using a Bellco plaque viewer (Bellco Glass Co., Vineland, NJ). Cell number was determined using a Z1 Coulter particle counter (Beckman Coulter, Brea, CA).

2.7 Real time polymerase chain reaction (PCR)

Mice (5 per treatment group) were administered CO, TCDD (5 µg/kg/day), DMSO-adsorbed silica or TCDD-adsorbed silica (5 µg/kg/day) by oral gavage once per day for 5 consecutive days. Twenty-four hr after the last dose, spleens and livers were placed in TRI Reagent (Sigma) and stored at −70°C. On the day of RNA extraction, spleens and livers were homogenized. Following phase separation with bromochlorophenol, RNA was precipitated from the aqueous phase with isopropanol. The remainder of the extraction, purification and DNase treatment was done using the Promega SV Total RNA Isolation System (Promega, Madison, WI). Total RNA was reverse transcribed using random primers with the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA). cDNA was amplified with Taqman a primer/probe set for mouse *cyp1a1* purchased from Applied Biosystems and analyzed using a 7900 HT Fast Real-Time PCR System (Foster City, CA). Fold-change values were calculated using the ΔΔCt method (Livak and Schmittgen, 2001).

2.8 Statistical analysis

The mean ± S.E. was determined for each treatment group. Differences between means were determined with a parametric analysis of variance. When significant differences were detected, treatment groups were compared to the appropriate control using Dunnett's two-tailed *t* test. For real time PCR, statistical analysis was performed on the ΔCt values.

Statistical analyses were performed using GraphPad Prism version 4.0a for Macintosh OS X, GraphPad Software (San Diego, CA).

3. Results

3.1 Mesoporous Silica as the Sorptive Medium

In order to administer adsorbed TCDD in doses likely to suppress an immune response in mice over a period of days, the TCDD binding capacity of the sorbent phase needs to be sufficient to allow the formation of a stable suspension in 200 μl of water. This is the maximum volume tolerated by a mouse in a single oral gavage feeding. Surfactant-templated mesoporous forms of amorphous silica (Ciesla and Schuth, 1999) provide an ideal combination of textural properties and water dispersibility for delivery of adsorbed TCDD by oral gavage in 200 μl aliquots. It is noteworthy that naturally occurring clays and related silicate minerals are not ideally suited for quantitative TCDD biodistribution studies, in part, because they lack the textural properties (i.e., surface areas, pore sizes, and pore volumes) needed to form a suspension suitable for administration by oral gavage.

Accordingly, mesoporous silica with a mesocellular foam structure, denoted MSU-F silica (Kim et al., 2000), was assembled at 100°C from sodium silicate solution in the presence of Pluronic 123, a polyethylene oxide-polypropylene oxide-polyethylene oxide block co-polymers with the molecular formula $HO(CH_2CH_2O)_{20}(CH_2CH(CH_3)O)_{70}(CH_2CH_2O)_{20}H$ as the surfactant porogen and 1,3,5-trimethylbenzene as a co-surfactant. Ethanol extraction of the surfactant afforded the three-dimensional mesocellular foam structure shown by the transmission electron micrograph in Figure 1A. This structure is comprised of nanometric silica struts linked to define pore openings (“windows”) and pores (‘cages”). The size of the windows and cages are determined by fitting the Barret-Joyner-Hallender (BJH) pore model to the desorption and adsorption legs of the nitrogen adsorption isotherms shown in Figure 1B. The size distribution curves provided in the inset to Figure 1B shows the respective average window size and cage size to be 15 and 24 nm, or approximately, an order of magnitude larger than the TCDD molecule. Thus, the porosity of the silica is sufficient to accommodate TCDD in all of the pores of the foam structure. Also, the nitrogen isotherms indicate the BET surface area to be 385 m^2g^{-1} and the framework pore volume to be 2.0 cm^3g^{-1}, as measured at a nitrogen partial pressure of 0.99.

3.2 Sequestration of TCDD on Mesoporous Silica

The adsorption of TCDD from aqueous solution onto a sorptive phase is not practical due to the limited solubility of the dioxin. However, incipient wet impregnation of the pore structure of MSU-F silica with a solution of TCDD in dimethylsulfoxide is effective in delivering precise quantities of TCDD into the pore structure. In this method, the mesoporous silica is mixed with an aliquot of TCDD in DMSO solution equal in volume to the pore volume of the silica. Equilibration of the mixture causes the liquid to completely fill the pores of the solid, resulting in a *dry* powder in which the TCDD solution completely fills the pore structure of the solid host. This ensures encapsulation of all the TCDD solution in the pore network of the silica. The impregnated powder is then heated under vacuum to remove the DMSO solvent leaving behind the non-volatile TCDD immobilized on the pore walls of the silica at the desired concentration level. The impregnated silica is suspended in distilled water at a concentration suitable for administration to mice by oral gavage in a volume of 200 μL.

3.3 TCDD-adsorbed silica suppresses humoral immunity

The experimental design for this and all subsequent studies utilized treatment groups that included corn oil vehicle (CO VH) only, TCDD dissolved in CO, or TCDD-adsorbed silica.

In addition, silica alone was used as a control for TCDD-adsorbed silica (and in the direct comparison studies in Figures 5 and 6, DMSO was adsorbed on silica to verify the absence of a DMSO effect when adsorbed on silica). These treatments were administered once per day for 5 consecutive days at the indicated doses by oral gavage. Thus, cumulative doses for an experiment are 5 times higher than those indicated in the graphs. On day 3, the mice received an i.p. injection of sRBC and the anti-sRBC IgM AFC response was enumerated 4 days after sRBC sensitization.

As a proof of principle experiment, we first examined the effect of a high dose of TCDD adsorbed on a high dose of silica in order to maximize TCDD adsorption and to determine the effects on humoral immune function. It is important to emphasize that this initial experiment was performed to establish several technical principles, including the feasibility of adsorbing TCDD on silica, delivering silica and/or TCDD-adsorbed silica by oral gavage, and finally, using the high TCDD dose allowed for a confirmation that a biological effect would be observed. The TCDD dose of 6 μg/kg/day delivered in CO was used as a control to demonstrate that the IgM antibody response was suppressed by TCDD as previously reported (Dooley and Holsapple, 1988; North et al., 2009). Silica alone (concentration 66.5 mg/ml in water administered at 0.2 ml per 0.02 kg mouse = 665 mg/kg/day) greatly enhanced the anti-sRBC IgM AFC response, but this was still strongly suppressed by TCDD-adsorbed silica (Figure 2). It is noteworthy that despite the very high dose of TCDD administered in the TCDD-adsorbed silica group (113 μg TCDD/kg/day), the mice survived the treatment with no signs of overt toxicity, and it was only in this initial study that the high dose of TCDD was used to determine if additional studies would be warranted. These results did indeed suggest that TCDD adsorbed on silica was capable of distributing to the spleen to suppress humoral immune function.

3.4 Silica dose response

Since the high dose of silica used in the proof of principle experiment enhanced the sRBC-induced AFC response, titration experiments were performed to identify a concentration of silica that would allow maximal adsorption of TCDD while alone not producing an effect on the AFC response. Concentrations of silica (10 mg/ml in water) that resulted in doses lower than 100 μg/kg/day produced no enhancement of the sRBC-induced AFC response (Figure 3) and therefore, a dose of 50 mg/kg/day silica was selected for all subsequent studies for TCDD adsorption.

3.5 TCDD-adsorbed silica dose response

Using a 50 mg/kg/day silica dose (5 mg/ml silica in water), TCDD was adsorbed onto silica in various loading amounts. In the first study, TCDD-adsorbed silica at doses between 5 and 50 μg/kg/day suppressed the anti-sRBC IgM AFC response, but a dose-response relationship was not observed over this range suggesting that maximal suppression of the anti-sRBC IgM AFC response was achieved at all of the doses tested (Figure 4A). In a subsequent study, TCDD-adsorbed silica at lower doses (0.1–5 μg TCDD/kg/day) was evaluated. These studies showed a dose-related suppression of the anti-sRBC IgM AFC response with a no-effect level at approximately 0.5 μg/kg/day (Figure 4B). Interestingly, the magnitude of the suppression produced by TCDD in CO (6 μg TCDD/kg/day) is similar to that produced by TCDD-adsorbed silica at 5 μg TCDD/kg/day. Thus, we conducted an additional dose-response study directly comparing TCDD delivered in CO to TCDD adsorbed onto silica using equivalent TCDD doses (Figure 5). In this study, we also confirmed that DMSO adsorbed on silica was a more appropriate VH control for the TCDD-adsorbed silica since DMSO-adsorbed silica was not significantly different from CO VH (compare TCDD in CO to TCDD-adsorbed silica at the “0” dose). Regardless of the vehicle used in these studies to deliver TCDD, TCDD suppressed the anti-sRBC IgM AFC response to a similar magnitude.

3.6 TCDD-adsorbed silica induction of cyp1a1 gene expression in liver and spleen

The observation that TCDD suppressed the anti-sRBC IgM AFC response regardless whether corn oil or silica served as the vehicle suggests that TCDD distributed to various target organs, including the spleen. Distribution of TCDD delivered in CO or on silica was verified using *cyp1a1* gene expression, a hallmark of TCDD exposure. As seen in Figure 6A, TCDD induced *cyp1a1* gene expression in the spleen, the target organ for the anti-sRBC IgM AFC response. In addition, we confirmed TCDD distribution to the liver, through which TCDD would initially pass following oral administration (Figure 6B). Interestingly, the DMSO-adsorbed silica also induced *cyp1a1* gene expression in the liver, but it was not significant as compared to TCDD in CO or TCDD-adsorbed silica. Consistent with the suppression of the anti-sRBC IgM AFC response, TCDD delivered in CO or on silica induced *cyp1a1* gene expression to similar levels in both the liver and spleen. These results suggest that the distribution of TCDD delivered in CO or on silica is similar.

4. Discussion

These model studies of TCDD-contaminated soil were undertaken in order to determine if TCDD, when administered under conditions in which it was adsorbed onto amorphous silica, was distributed to the spleen and, if so, would it produce a similar magnitude of immune suppression as observed with the soluble TCDD in CO. The results show that TCDD-adsorbed silica did distribute to the spleen and liver as assessed by *cyp1a1* induction and/or suppression of the anti-sRBC IgM AFC response. Moreover, administration of TCDD-adsorbed silica produced suppression of the anti-sRBC IgM AFC response that was comparable to TCDD administered in CO.

Initial animal studies were designed in order to maximize loading of TCDD onto silica and to determine whether a relatively high dose of TCDD would produce a biological effect. Several important observations resulted from the initial study. First, it was determined that high doses of silica alone enhanced the anti-sRBC IgM AFC response. Again, a major goal of the studies was to evaluate immunotoxic effects of TCDD adsorbed onto a constant amount of silica delivered in an aqueous solution by gavage to mimic ingestion of TCDD-contaminated soils. Thus, it was critical to determine a no-effect level of silica alone so that the only variable in the study design was the dose of TCDD delivered on silica. In the initial proof of principle study, the observation that high doses of silica exacerbated the sRBC-induced AFC response was not surprising, given that mesoporous silica is being exploited in vaccine strategies as an adjuvant (Ho et al., 2010), and that silicosis following crystalline silica exposure induces, among other things, increased antibody production in the bronchoalveolar lavage fluid (Misson et al., 2007). Thus, the 50 mg/kg/day silica dose was used because alone it did not enhance the anti-sRBC IgM AFC response, and it was capable of delivering doses of TCDD up to 50 μg/kg.

The second interesting observation from the initial proof of principle study was that TCDD potently suppressed the anti sRBC-induced IgM AFC response in the absence of overt toxicity. Evaluation of the effects of lower TCDD doses resulted in a dose-response relationship with a no effect level of approximately 0.1–0.5 μg/kg/day. These findings are consistent with previous reports that oral administration of TCDD given 2 days prior to sRBC sensitization in C57BL/6 mice resulted in a steep dose-response curve with an ED_{50} of 0.74 μg/kg/day (Kerkvliet and Brauner, 1990).

The suppression of the anti-sRBC IgM AFC response suggests that TCDD distributes to the spleen. Absorption of TCDD from the GI tract is further confirmed by induction of *cyp1a1* expression in the spleen and liver. These studies demonstrate biodistribution of TCDD to the spleen and liver regardless of whether administered in CO or adsorbed on silica. Taken

together, these results suggest several possibilities concerning how TCDD-adsorbed silica moves from the GI to distribute to the spleen and liver. First, TCDD could be displaced from the silica particles and taken up from the GI, most likely associated with lipids and/or proteins. Second, the silica could dissolve in the GI tract allowing release of TCDD from silica followed by absorption in the stomach and further along in the GI. Third, the TCDD-adsorbed silica particles could be taken up from the GI and potentially engulfed by antigen presenting cells in various organs, including Kupffer cells in the liver or macrophages and dendritic cells in the spleen, where the TCDD is subsequently released intracellularly. The final, and most likely, scenario is that all of these mechanisms occur simultaneously to varying degrees, and contribute to the uptake of TCDD from the GI and biodistribution to the spleen and liver.

Being an amorphous phase, MSU-F silica can be expected to have a solubility in water similar to other forms of amorphous silica. Indeed, it recently has been shown that a 0.50 wt % suspension of mesoporous MCM -41 silica with a hexagonal framework structure comes into equilibrium with 128 ppm of dissolved silica at pH =7.0, a temperature of 298°C and an equilibration time of 7 days (Guthrie and Reardon, 2008). Conventional forms of amorphous silica that lack a mesoporous structure are somewhat less soluble (120 ppm). Mesocellular MSU-F silica is compositionally and structurally analogous to MCM-41 silica. Because a 0.5 wt% aqueous suspension the MSU-F suspension of MSU-F silica also was used to deliver the adsorbed TCDD in the animal studies, it is reasonable to expect the initial concentration of soluble silica in the 200μl gavage aliquots to be approximately 120–130 ppm. This would mean that only about 2.5 wt % of the total silica phase is initially in solution form. Thus, the vast majority of the delivered TCDD is initially in silica-immobilized form. Admittedly, the mouse body temperature (~36°C) will favor an increase in silica solubility, as will the presence of salts in the gastric fluid (Hanton-Fong, 1992). However, the pH of the gastric fluid (pH ~ 1.0 – 2.0) will shift the solution equilibrium in the direction of lower solubility, as expected for the formation of silicic acid in the dissolution process: $2H_2O(l) + SiO_2(s) \leftrightarrows H_4SiO_4(aq) \leftrightarrows H^+(aq) + [H_3SiO_4]^-(aq)$. Thus, several factors can affect the concentration of soluble silicic acid as the MSU-F particles move through the digestive tract, but the soluble form may represent only a small fraction of the total silica administered.

It is notable that at the doses used in this study, the TCDD is adsorbed as isolated molecules on the silica surface. For instance, in order to obtain a 1.0 ppb TCCD dose, 68 mg of silica with a surface area of 385 m^2g^{-1} is impregnated with 1.36 μg of TCDD. From the crystal structure of TCDD, the planar surface area of the molecule is estimated to be 0.85 nm^2 (Boer et al., 1972). Thus, at a loading corresponding to a dose of 1 ppb, the TCDD molecules occupy only 8.3×10^{-5} m^2 for every available square meter of silica, which corresponds to a surface coverage of only 0.008 %. In other words, the siting of TCDD on the silica surface corresponds one molecule per 10,300 nm^2. If the TCDD molecules were arranged in a hexagonal pattern on the surface, there would be an average of 114 nm between neighboring molecular centers.

The advantage of these model studies over *in vivo* studies with toxicant-contaminated soils is the control of the toxicant dose and the evaluation of biological effects due to a single contaminant, in this case, TCDD. This model has limitations as well, including the fact that amorphous silica does not represent other important mineral phases in soils; e.g., clay minerals, that can adsorb dioxins, and that humans are often exposed to more than a single toxicant. However, the results demonstrate that TCDD adsorbed to silica and administered orally readily distributes to the liver and spleen. Moreover, TCDD-adsorbed silica can produce immunotoxicity similar to TCDD solubilized in CO, as assessed by *cyp1a1* gene expression and suppression of the humoral immune response. The mechanism(s) by which

TCDD when administered adsorbed to silica distributes to liver and spleen is intriguing, and future studies will examine the mechanisms involved in its uptake and biodistribution.

Abbreviations

AFC	antibody forming cell
IgM	immunoglobulin M
sRBC	sheep red blood cells
TCDD	2,3,7,8-tetrachlorodibenzo-p-dioxin

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References

Boer FP, Van Remoortere FP, North PP, Newman MA. The crystal and molecular structure of 2, 3, 7, 8-tetrachrolordibenzo-p-dioxin. Acta Cryst. 1972:1023–1029.

Ciesla U, Schuth F. Ordered Mesoporous Materials. Microporous and Mesoporous Materials. 1999; 27:131–149.

Dooley RK, Holsapple MP. Elucidation of cellular targets responsible for tetrachlorodibenzo-p-dioxin (TCDD)-induced suppression of antibody responses: I. The role of the B lymphocyte. Immunopharmacology. 1988; 16:167–180. [PubMed: 3267010]

Ehlers LJ, Luthy RG. Contaminant bioavailabilty in soil and sediment. Environ Sci Technol. 2003; 37:295A–302A.

Ferrario JB, Byrne CJ, Cleverly DH. 2,3,7,8-dibenzo-p-dioxins in mined clay products from the United States: evidence for possible natural origin. Environ Sci Technol. 2000; 34:4524–4532.

Gadomski DM, Tysklind M, Irvine RL, Burns PC, Andersson R. Investigations into the verical distribution of PCDDs and mineralogy in three ball clay cores form the United States exhibiting the natural formation pattern. Environ Sci Technol. 2004; 38:715–723. [PubMed: 14968855]

Green NJL, Hassanin A, Johnston AE, Jones KC. Observations on historical, contemporary, and natural PCDD/Fs. Environ Sci Technol. 2004; 38:715–723. [PubMed: 14968855]

Gu C, Li H, Teppen BJ, Boyd SA. Octochlorodibenzodioxin formation on Fe(III)-montmorillonite clay. Environ Sci Technol. 2008; 42:4758–4763. [PubMed: 18678002]

Guthrie CP, Reardon EJ. Metastability of MCM-41 and Al-MCM-41. J Phys Chem. 2008; 112:3386–3390.

Hanton-Fong, CJ. The solubility and dissolution kinetics of amorphous silica in electrolyte solutions at 25°C. Vol. vol.. Waterloo: M. Sc. University of Waterloo; 1992.

Hayward DG, Bolger PM. Tetrachlorodibenzo-p-dioxin in baby food made from chicken produced before and after the termination of ball clay use in chicken feed in the United States. Environ Res. 2005; 99:307–313. [PubMed: 16307972]

Hayward DG, Nortrup D, Gardner A, Clower M. Elevated TCDD in chikcen eggs and farm-raised catfish fed a diet with ball caly from a southern United States mine. Environ Res. 1999; 81:248–256. [PubMed: 10585021]

Ho J, Huang Y, Danquah MK, Wang H, Forde GM. Synthesis of biodegradable polymer-mesoporous silica composite microspheres for DNA prime-protein boost vaccination. Eur J Pharm Sci. 2010; 39:412–420. [PubMed: 20117207]

Hoekstra EJ, DeWeerd H, DeLeer EWB, Brinkman UAT. Natural formation of chlorinated phenols, dibenzo-p-dioxins, and dibenzofurans in soil of a Douglas fir forest. Environ Sci Technol. 1999; 33:243–249.

Jerne NK, Nordin AA. Plaque formation in agar by single antibody-producing cells. Science. 1963; 140:405.

Kerkvliet NI, Brauner JA. Flow cytometric analysis of lymphocyte subpopulations in the spleen and thymus of mice exposed to an acute immunosuppressive dose of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Environ Res. 1990; 52:146–154. [PubMed: 2118453]

Kim SS, Pauly TR, Pinnavaia TJ. Non-ionic surfactant assembly of ordered, very large pore molecular sieve silicas from water soluble silicates. Chem Commun. 2000; 24:1661–1662.

Liu C, Li H, Teppen BJ, Johnston CT, Boyd SA. Mechanisms associated with the high adsorption of dibenzo-p-dioxin from water by smectite clays. Environ Sci Technol. 2009; 43:2777–2783. [PubMed: 19475949]

Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(−Delta Delta C(T)) Method. Methods. 2001; 25:402–408. [PubMed: 11846609]

Misson P, Brombacher F, Delos M, Lison D, Huaux F. Type 2 immune response associated with silicosis is not instrumental in the development of the disease. Am J Physiol Lung Cell Mol Physiol. 2007; 292:L107–L113. [PubMed: 16997884]

Nolan T, Srinivasan KR, Fogler HS. Dioxon (sic) sorption by hydroxyaluminum-treated clays. Clay Clay Min. 1989; 37:487–492.

North CM, Crawford RB, Lu H, Kaminski NE. Simultaneous in vivo time course and dose response evaluation for TCDD-induced impairment of the LPS-stimulated primary IgM response. Toxicol Sci. 2009; 112:123–132. [PubMed: 19675145]

Schmidt-Winkel P, Lukens WW Jr, Zhao DY, Yang PD, Chmelka BF, Stucky GD. Mesocellular siliceous foams with uniformly sized cells and windows. J Am Chem Soc. 1999; 121:254–255.

Semple KT, Doick KJ, Jones KC, Burauel P, Craven A, Harms H. Defining bioavailability and bioaccessibility of contaminated soil and sediment is complicated. Environ Sci Technol. 2004; 38:228A–231A.

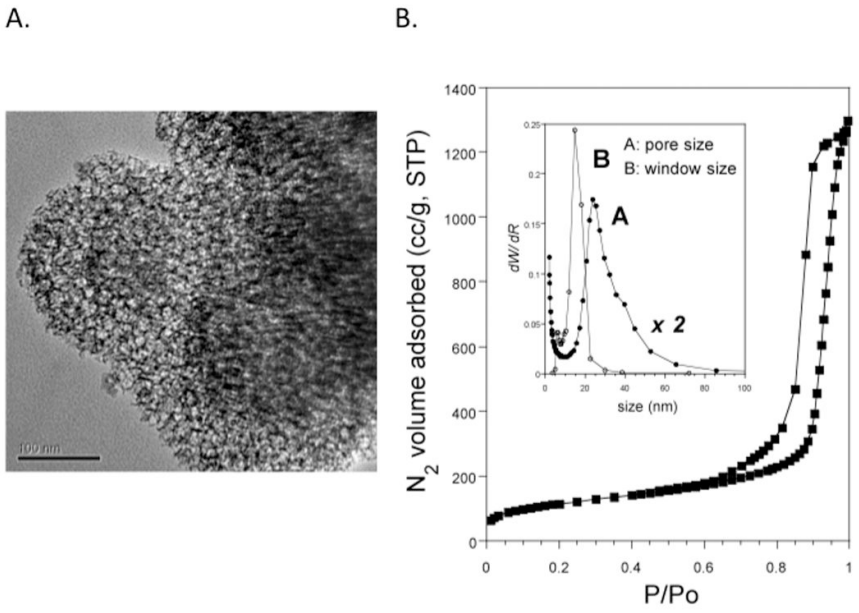

Figure 1. Characterization of silica

(A.) Transmission electron image of MSU-F silica with a mesocellular foam structure. (B.) Nitrogen adsorption – desorption isotherms for MSU-F silica. The insert provides the window and cage size distribution for the mesocellular foam structure.

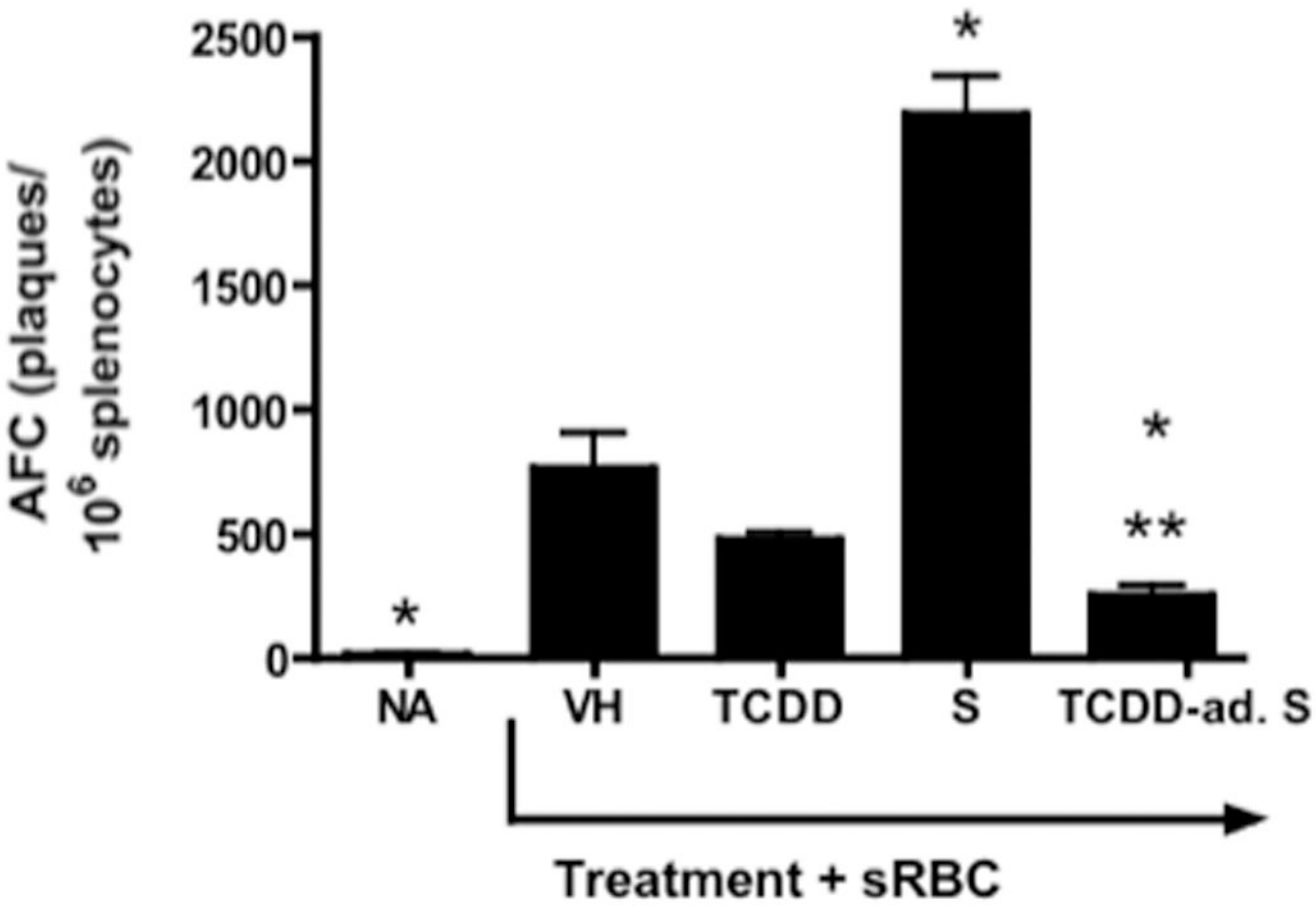

Figure 2. TCDD-adsorbed silica suppresses the anti-sRBC IgM AFC response

Mice (N = 5) were treated with TCDD (6 μg/kg/day), silica (665 mg/kg/day), or TCDD-adsorbed silica (113 μg TCDD/kg/day loaded onto 665 mg silica/kg) for 5 days by oral gavage. On day 3, mice were sensitized with 5×10^8 sRBC per mouse i.p. Four days after sensitization, the number of anti-sRBC IgM AFC was determined from SPLC by plaque assay. * $p < 0.05$ as compared to VH (VH, vehicle; CO); ** $p < 0.05$, difference between silica and TCDD-adsorbed silica. S = silica; NA = naïve, untreated.

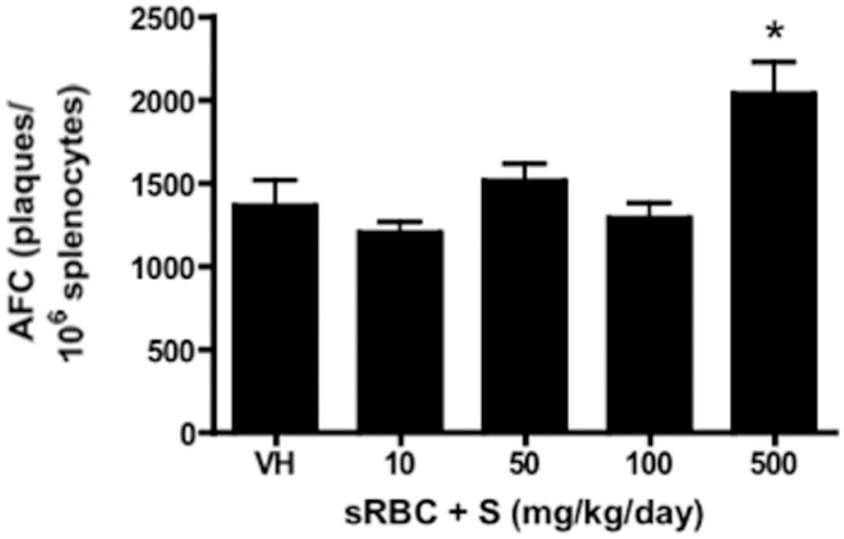

Figure 3. Silica alone does not induce an anti-sRBC IgM AFC response at low doses

Mice (N = 5) were treated with silica (10–500 mg/kg/day) for 5 days by oral gavage. On day 3, mice were sensitized with 5×10^8 sRBC per mouse i.p. Four days after sensitization, the number of anti-sRBC IgM AFC was determined from SPLC by plaque assay. * $p < 0.05$ as compared to VH (VH, vehicle, water). S = silica.

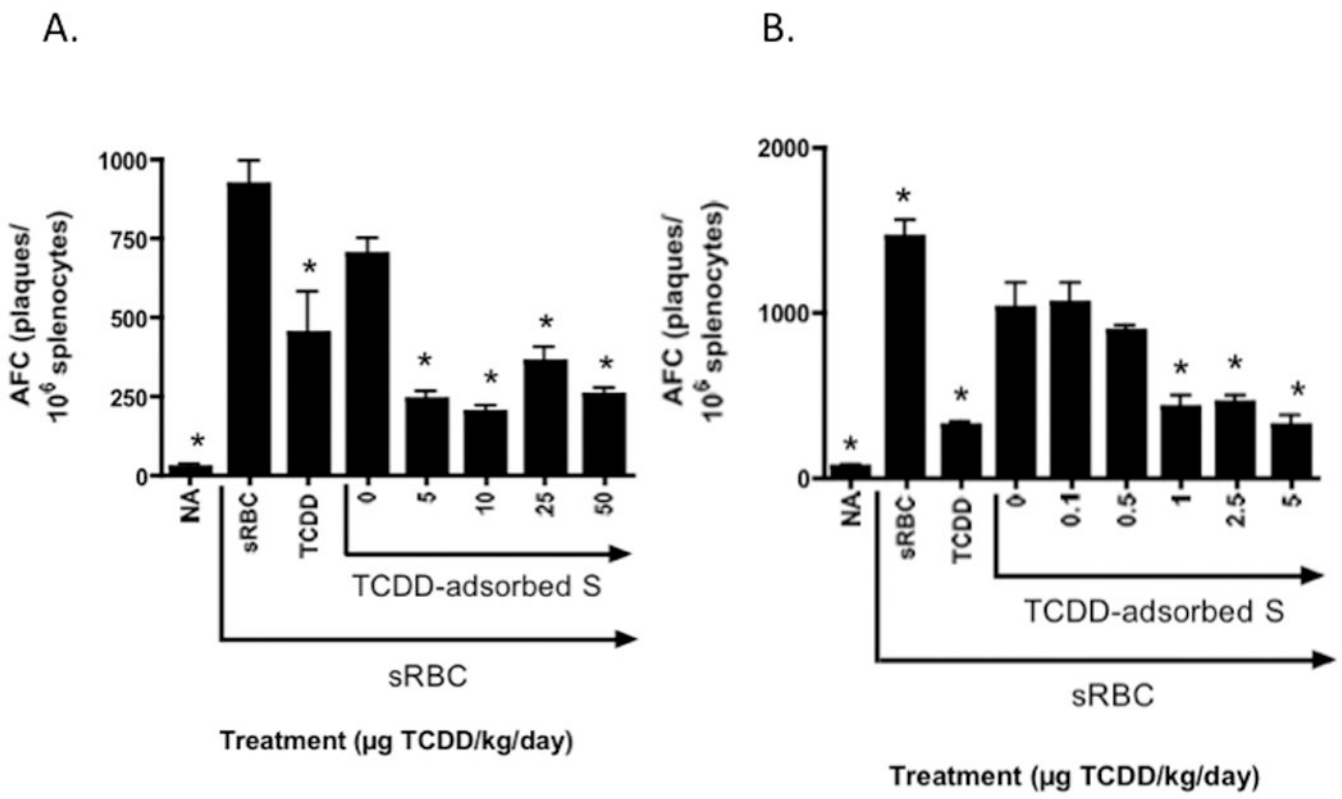

Figure 4. TCDD-adsorbed silica dose response relationships

Mice (N = 5) were treated with TCDD (6 µg/kg/day), silica (50 mg/kg/day), or TCDD-adsorbed silica for 5 days by oral gavage. (A.) 0–50 µg TCDD/kg/day loaded onto 50 mg silica/kg or (B.) 0–5 µg TCDD/kg/day loaded onto 50 mg silica/kg. On day 3, mice were sensitized with 5×10^8 sRBC per mouse i.p. Four days after sensitization, the number of anti-sRBC IgM AFC was determined from SPLC by plaque assay. *$p<0.05$ as compared to 0.

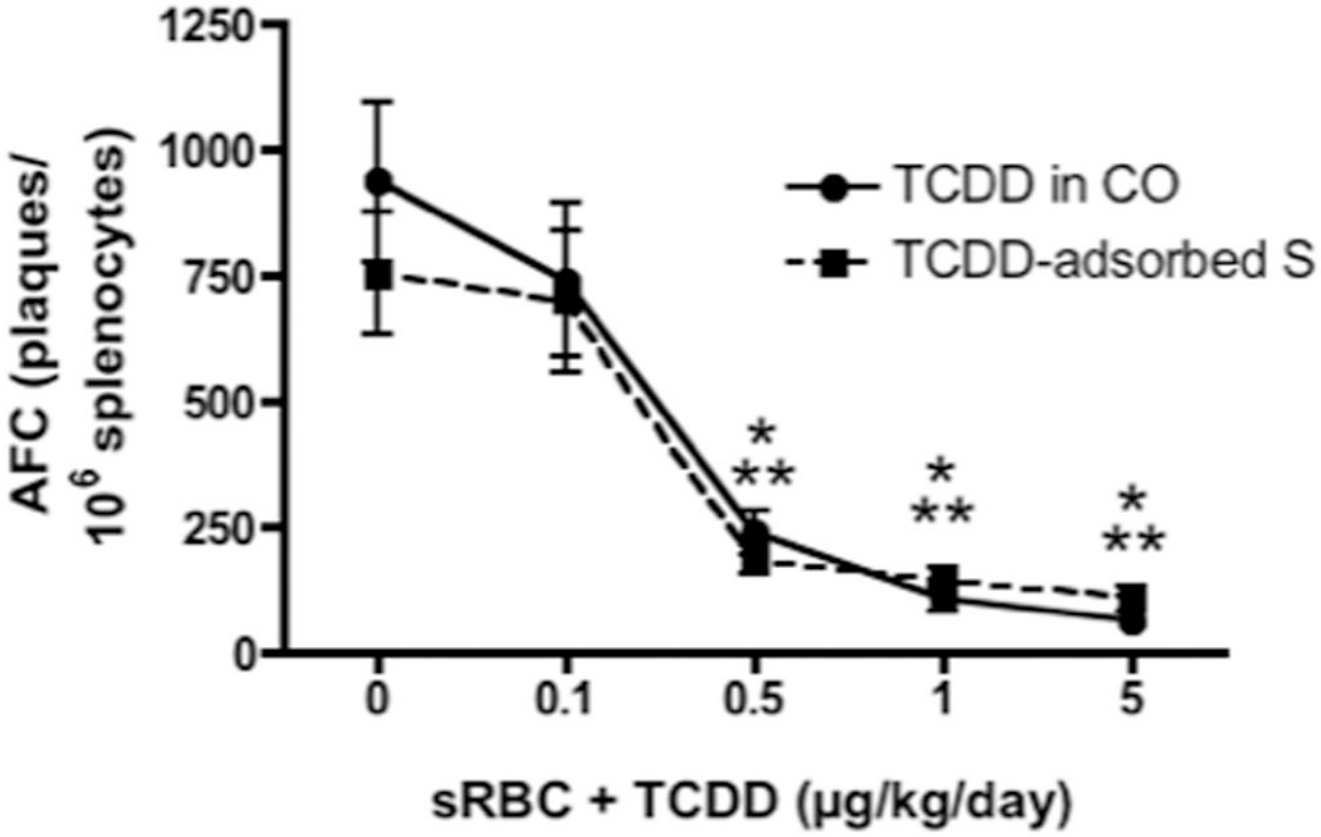

Figure 5. Direct comparison of TCDD delivered in CO and on silica

Mice (N = 5) were treated with CO, TCDD (0–5 µg/kg/day), DMSO-adsorbed silica (100% DMSO loaded onto 50 mg silica/kg) or TCDD-adsorbed silica (0–5 µg/kg/day loaded onto 50 mg silica/kg) for 5 days by oral gavage. On day 3, mice were sensitized with 5×10^8 sRBC per mouse i.p. Four days after sensitization, the number of anti-sRBC IgM AFC was determined from SPLC by plaque assay. *$p<0.05$ as compared to 0 TCDD in CO; **$p< 0.05$ as compared to 0 TCDD-adsorbed S.

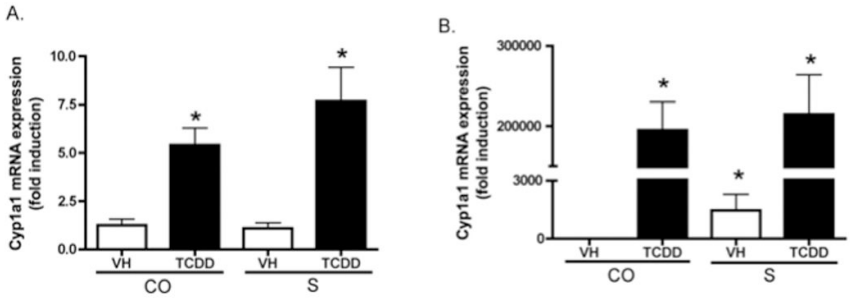

Figure 6. TCDD delivered in CO and on silica induced *cyp1a1* gene expression in spleen and liver

Mice (N = 5) were treated with CO, TCDD (5 μg/kg/day), DMSO-adsorbed silica (100% DMSO loaded onto 50 mg silica/kg) or TCDD-adsorbed silica (5 μg/kg/day loaded onto 50 mg silica/kg) for 5 days by oral gavage. Twenty-four hr after the last dose, spleens (A.) and livers (B.) were harvested and processed for total RNA. After cDNA synthesis, real time PCR was performed for *cyp1a1* and normalized with 18S RNA. $p < 0.01$ as compared to CO VH.