1	Supplemental File
2	Applied Microbiology and Biotechnology
3 4	TCDD administered on activated carbon eliminates bioavailability and subsequent shifts to a key murine gut commensal
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## **30 Additional Experimental Procedure**

- 31 For comparing the influence of corn-oil (CO) vehicle with sesame-oil vehicle, which has been used in
- 32 previous studies (Stedtfeld et al. 2017), the relative abundance of *Enterobacteriaceae* was measured in
- fecal pellets (Fig S6). In all groups, fecal pellets were collected daily and were therefore less than 24
- hours old at the time of collection and freezing. All pellets were stored at -20°C until analyzed. DNA was
- 35 extracted from fecal pellets (100 mg) using the PowerMax Soil DNA Isolation Kit (MOBIO
- 36 Laboratories). DNA samples were quantified on a Nanodrop ND-1000 UV-Vis spectrophotometer
- 37 (Nanodrop Products, Wilmington, DE). Extracted DNA was stored at -20°C. The 16S rRNA gene assay
- targeting *Enterobacteriaceae* (Barman et al. 2008) and universal 16S rRNA gene were run on the
- 39 Chromo4 BioRad using reagents and cycling parameters recommended with the Power SYBR Green PCR
- 40 Master Mix. Relative abundance of ARGs in the total bacterial community was determined by qPCR
- 41 using 16S rRNA gene designed to be universal to bacteria.
- 42

43 Table S1 QPCR primers used to measure SFB specific 16S rRNA gene and putative function	al genes.
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Gene or putative function	Protein ID	Primer	Reference
Universal bacteria 16S		340F: ACTCCTACGGGAGGCAGCAGT	(Barman et al. 2008)
SED specific 16S rDNA		SED 726E: CACCOTCACCATCACACCAT	(Poustre et al. 2008)
gene		SFB_844R: GACGGCACGGATTGTTATTCA	(BOUSKIA et al. 2008)
actin-ADP-ribosylating	BAK56539	TCTACCAATTAACATTTCTTGTTCAAATTC	This study
toxin		GGTTGAAATAAGAGCACCTAAAGGAA	
ADPRT Type-1	BAK55983.1	AAAAGATTATGATGTTGCCGTTTG	This study
		AACTGATGCTGATAAATACGATGCTT	
Arginase	BAK 56991 1	ΔGΔTGCTCΔTGGCGΔTTTGΔΔ	This study
A ginase	B/1K30771.1	GCATGCGAGTGGCATTCC	This study
alpha-N-	BAK55848.1	TGTTAGATACAGTTTATAATCCGGTAACTG	This study
acetylglucosaminidase		AT	2
		AATAGTTCCCCACTTAGACGCACTATT	
aluminum resistance	BAK 56521 1	GATAGCTAATTGCCCATGCTCAA	This study
protein	D/1K50521.1	TCATGTGATGCTCCAATAAGAGAAC	This study
D-ala-D-ala dipeptidase	BAK55824.1	ACATCTACAGCACATGCCCTATTATG	This study
1 1		GGATGCTTATAGACCATTTTACATTCAAA	2
fibronectin binding protein	BAK56752.1	TGGTAGCCATGTTATCCTTGCA	This study

flagellin domain-containing protein FliC2	BAK56419	TGAAGATGATGATGCCTTGCTATAAA TGCTCAAGGTAAGGCAATGGA AATTGCGAAACCTGCTGCAT	This study
flagellin domain-containing protein FliC3	BAK56361.1	GCATTGTCCGAGACTCAAGCA TCTGTATAAGTTCCGTTTGCAGATTG	This study
F-pilin acetylation protein TraX	BAK56001.1	ATTTAGGACGTATTGTTGCACCTACAT GCAGCAACAAACAATCTTATAGTAAATTTC	This study
GMP synthase, large subunit	BAK56417.1	TCTTCCATTAGGTTTTATAGCAAATGC GGTGAAATTGAACCAGATGTATATTCTTAT C	This study
hemolysin A	BAK56433.1	GAGAGAGTTGTAGTGTTTGAAAGGATGA	(Stedtfeld et al.)
histidine kinase	BAK56282.1	CAATIGAIGCAAAATCCCCTITAT CTTGATAAGCAGATTGACATTATTAATTCG ATTCATAGGCCCTTTTATTATACGATCTAA	This study
metallo-beta-lactamase family protein	BAK56334.1	TCCATCGAAAGTTGATGCTATTGTT TCCCATAAACCTTTCCTTT	This study
myosin-cross-reactive	BAK56130.1	ATGAGGCATTTGCAAGACCTATTAA	This study
antigen phospholipase C zinc binding proteins	BAK56527.1	AGATGCAAGTCCTGATCCAATAAGA ACCCAGACATCCAGTTTGATTTGT TTTATGGTCCCTATTAAGGCAAGCT	This study
polysaccharide deacetylase family protein	BAK56650.1	TTTCGTCGTTGGAAGTTTGGTT TGTGTGAAGTCCTATCGAATGGTTATT	This study
bleomycin resistance protein/dioxygenase	BAK56236.1	GTTGATTGTATATCATCGGTAAAGAATGTT CAAATAGAAGGAAAATATACTTGGACAGA AG	This study
RelA/SpoTdomain- containingprotein	BAK57096.1	AGAGATGCTCAGAGATTATTTGATGTTAAA CGCTGTAAGTTGCATTTGATAATTTG	This study
rubrerythrin	BAK56170.1	GGAGAAGTTTTTTATAAGGCTCAAGATG	(Stedtfeld et al.)
Type-3ADPRT	BAK56804.1	CIGGAGCATATICCCCATGTIC CAAAGTAACTTCCGCAGTTGCA AAGGTACTAACCAATTAACTTACACTTTTG AGA	This study

N=5 Naive N=5 vehicle corn oil (CO) N=5 CO & 0.1 µg/kg TCDD N=5 CO & 1.0 µg/kg TCDD N=5 CO & 10 µg/kg TCDD N=5 activated carbon (AC) N=5 AC & 0.1 µg/kg TCDD N=5 AC & 1.0 µg/kg TCDD N=5 AC & 10 µg/kg TCDD





46 Fig S1 Experimental design with nine groups, each with five mice. The vehicle of corn oil (CO) or

- 47 activated carbon (AC) included 0, 0.1, 1.0 or 10 µg/kg TCDD. Dosing occurred on first four day of the
- 48 experiment. No dosing was done on days 5, 6, and 7. The takedown and collection of ileal tissue was
- 49 performed on day seven.







Fig S2. Results of the replicate experiment; qPCR analysis of SFB 16S rRNA gene expression from ileal
tissue in response to 0, 0.1, 1.0 or 10 µg/kg TCDD administered with corn oil vehicle (CO\_TCDD) or
activated carbon (AC\_TCDD). ANOVA analysis with a multiple mean comparison Sidak test comparing
naïve, CO with no TCDD (CO), and 10 µg/kg TCDD administered on AC (AC\_10TCDD) versus 10
µg/kg TCDD administered on CO (CO\_10TCDD) was used to calculate p-values. For the replicate
experiment, SFB expression was not measured in groups of mice dosed with 0.1 and 1.0 µk/kg TCDD
administed on AC.



Fig S3. QPCR analysis of SFB gene expression from ileal tissue in response to 10 µg/kg TCDD
administered with corn oil vehicle (CO\_10TCDD) in black; and mean response of all other groups
including naïve, lower doses of TCDD administered on CO and all doses of TCDD administered with AC
in gray. Bars represent mean with standard error of expression per mg ileum. All other groups were
pooled because a significant response was only observed in the group dosed with 10 µg/kg TCDD
administered with corn oil vehicle.





Fig S4. Analysis of bacterial community diversity in response to groups with various dosing, based on
16S rRNA gene sequencing of DNA extracted from ileal tissue. (A) Chao and (B) Shannon diversity
indices in separate groups, and, C) Chao and (D) Shannon diversity indices after grouping all mice dosed
with and without TCDD. A significant difference was not observed with either the Chao or Shannon
indices.



Fig S5. Volcano plots of 16S rRNA gene community structure classified at family level using DNA

extracted from ileum with fold change as a response to TCDD (A) and, fold change in response to corn oil

79 (B).



Fig S6. QPCR analysis of DNA extracted from fecal pellets collected from cages on the fifth day of the experiment shows the relative abundance of *Enterobacteriaceae* within the different groups. Stars indicates a significant difference (p < 0.05) between TCDD and vehicle (V) dosed mice and the naïve control (N). Error bars represent standard deviation of three technical replicates. Fecal pellets were collected daily, and thus all pellets were less than 24 hrs old at the time of collection. Stars indicate significant difference (p<0.05) compared to vehicle and naïve control.



Fig S7. Theoretical analysis of TCDD levels in mice assuming an 11 day half-life. The black arrows point
to the time of analysis, and the black lines shows theoretical amount of TCDD for a previous study
(Bhaduri 2015) with longer duration of exposure. Gray lines indicate the level of TCDD in the mice for
this current experiment.

## 95 Supplemental References

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