Supporting Information: Comprehensive Assessment of Hormones, Phytoestrogens, and Estrogenic Activity in an Anaerobic Swine Waste Lagoon

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**Field Site Description:** The field site for this project is a commercial swine farrowing AFO in southeastern North Carolina, centrally located in the major swine production region of the state. As a dedicated farrowing facility, this operation houses exclusively female swine, all of which are breeding, gestating, or lactating. All sampling focused on a single lagoon, which receives waste from barns housing approximately 2500 sows. The lagoon is approximately 3 meters deep with 30 cm of freeboard, and was constructed with 3% side slopes, with dimensions measuring roughly 166 x 94 meters. Maximum capacity of the lagoon is estimated at approximately 50 million liters. At the time of each lagoon sampling, total slurry and sludge depths in the lagoon were measured using a Lowrance LCX-18C Sonar/GPS Chartplotter (Lowrance, USA). Average slurry depth over all sampling periods was found to be 1.7 meters (estimated total slurry volume = 26.3 million liters), and average sludge depth was found to be 1.3 meters (estimated total sludge volume = 19.7 million liters).

**Table SI-1:** Physiochemical characteristics of the lagoon slurry and sludge. Values listed are mean +/- standard deviation.

|       | Slurry temp. (°C) | Slurry dissolved oxygen (mgO/L) | pH    | TSS (mg/L) in slurry | %OC in slurry solids | DOC (mgC/L) in slurry liquids | %OC in sludge solids | DOC (mgC/L) in sludge liquids |
|-------|------------------|---------------------------------|-------|----------------------|----------------------|-------------------------------|---------------------|-------------------------------|
| Jun-09 | 27.3 ± 0.7       | 0.62 ± 0.23                     | 7.4 ± 0.1 | 1101 ± 79            | 42 ± 0.8             | 243 ± 59                      | 23 ± 4               | Not measured                   |
| Apr-10 | 20.0 ± 0.2       | 0.55 ± 0.39                     | 7.6 ± 0.03 | 828 ± 57             | 38 ± 1.1             | 250 ± 24                      | 25 ± 4               | 219 ± 51                      |
| Feb-11 | 8.0 ± 0.2        | 0.93 ± 0.75                     | 7.9 ± 0.2 | 772 ± 74             | 43 ± 0.4             | 511 ± 7                       | 24 ± 4               | 725 ± 22                      |
Supporting Information: Sample Extraction and Processing

Solid-Phase Extraction (SPE) of Liquid Samples: SPE of lagoon liquids proceeded immediately after the centrifugation and filtration of these samples. 500-mg Supelclean™ LC-18 SPE cartridges (Supelco) were pre-conditioned with 5 ml of pico-pure water followed by 5 ml of a solution of 5% methanol and 95% pico-pure water. 500-ml aliquots of each filtered lagoon liquid sample were then passed through Visiprep™ Large Volume Samplers (Supelco) and loaded onto individual cartridges at a rate of 10-15 ml per minute. After samples were loaded, the cartridges were washed with 1 ml of pico-pure water, and then dried by running the vacuum pump for 5-10 minutes. Extracts were eluted by passing two 4-ml aliquots of methanol through the cartridges. The eluents were evaporated to near dryness using a gentle stream of N₂ and warming (40°C) in a water bath, and 10 µl of picopure water was added to each extract to prevent evaporation to complete dryness. Extracts were resuspended in two washings of ethanol to a final volume of 1 ml, and were stored at -20°C. For LC/MS-MS analysis, 200-µl aliquots of each sample extract were shipped overnight on ice to the US Geological Survey Organic Geochemical Research Laboratory (OGRL) in Lawrence, KS. At OGRL, sample aliquots then were stored at -20°C until processed for analysis.

Extraction of Solid Samples: Freeze-dried solids and aliquots of the liquid ethanol SPE extracts were shipped on ice to OGRL for LC/MS-MS analysis. Accelerated solvent extraction (ASE) and SPE of all solids was performed. 0.2-1 g aliquots of each freeze-dried sample was weighed, mixed in a mortar and pestle with 1 g of diatomaceous earth, and packed into 22-ml stainless steel cells preloaded with a glass fiber filter at the bottom of the cell approximately 15 mm of sand and another glass fiber filter. The stainless steel
cells then were filled with Ottawa sand, capped, and loaded onto the ASE. The samples were extracted at 50°C and 1500 psi with two 2-minute static cycles, 120-second purge cycle, and 150% flush volume resulting in the use of approximately 45 ml of extraction solvent consisting of 50% methanol and 50% ethylacetate in solution. Samples were then re-extracted into separate vials with a solution of 50% methanol and 50% water. The methanol/ethylacetate and methanol/water extracts for each sample were evaporated to 5 ml and 20 ml, respectively, and then combined and evaporated to less than 5 ml. The sample eluates were transferred to 50-ml polyethylene centrifuge tubes, and 45 ml of acetonitrile was added to precipitate proteins. Samples were centrifuged at 4°C at 13,000xg for 30 min. The liquid eluate then was decanted into a 60-ml amber glass vial and evaporated to a volume of 1 ml. Each sample aliquot was diluted with 50 ml of water and 1 ml of a 5% EDTA solution followed by extraction on a 200-mg HLB cartridge (Waters Corp., Milford MA) using a method modified from Matejicek et al. The sample eluates then were evaporated to 10 µl and brought to a final volume of 1 ml with a solution of 90% water and 10% ethanol, and transferred to a 2-ml silanized, glass chromatography vial. An 800-µl aliquot of each sample extract was shipped overnight on ice to the NCSU Toxicology Laboratory for YES analysis. Extracts were stored at -20°C prior to processing for analysis.

**Processing of Liquid and Solid Samples for LC/MS/MS analysis:** For LC/MS-MS analysis, two 100-µl aliquots of solid sample extract were pipetted into 2-ml, silanized glass chromatography vials for each sample. One aliquot (A) was spiked with 25 µl of an internal standard mix containing 1 ng/µl of each analyte as well as 25 µl of methanol. The second aliquot (SA) was spiked with 25 µl each of a 1 ng/µl internal,
surrogate, and analyte mix. Both the A and the SA sample pairs were brought to a final volume of 0.5 ml using a solution of 90% water and 10% methanol, and were stored at -20°C prior to analysis.

For the liquid sample eluates, two 75-µl aliquots were pipetted into 2-ml silanized, glass screw-top chromatography vials containing 400 µl of a 10/90% methanol/water solution. One aliquot (A) was spiked with 25 µl of an internal and surrogate standard mix containing 0.1 ng/µl of each analyte. The second aliquot (SA) was spiked with 25 µl 0.1 ng/µl internal, surrogate, and analyte standard mix containing 0.1 ng/µl of each analyte. The A and the SA sample pairs were stored at -20°C prior to LC/MS-MS analysis.

**Supporting Information: LC/MS-MS Analysis**: All samples were analyzed during the course of this study for 37 compounds (Table SI-2) using high performance liquid chromatography (HPLC) or ultra pressure LC (UPLC)/tandem mass spectrometry (MS-MS) using electrospray ionization (ESI) in negative-ion (NI) or positive-ion (PI) mode with multiple reaction monitoring (MRM). HPLC C18 reverse phase or UPLC reverse phase analytical columns were used to separate the compounds. During the course of this study, HPLC was replaced with UPLC. The chromatography was modified to take advantage of the ultra-pressure systems. MS-MS analyses were performed on AB/SIEX 5000 or 5500 MS-MS or Waters Quattro Micro MS-MS. A subset of samples analyzed using HPLC methods were reanalyzed using the UPLC methods to ensure method equivalence within +/- 20% of the original analysis. Thus modifications to the chromatography did not affect the reported analyte concentrations.
Chromatography methods are summarized in Table SI-3. Twelve compounds including E1, E2β, E2α, E3, EE2, diethylstilbestrol and 6 phytoestrogens were analyzed in NI mode and separated using a water/methanol gradient with a post column infusion of a 10 mM ammonia hydroxide solution to enhance ionization. The sulfate and glucuronide conjugates and α-zearalanol were analyzed in NI mode and the 9 remaining steroidal compounds in PI mode. The majority of the conjugates and steroidal compounds were separated using UPLC with an aqueous/methanol gradient and a preinjection column equilibration of a 0.1% formic acid/methanol (95/5) to improve the chromatography. The round 1 lagoon liquid samples were separated using 0.1% FA aqueous and 0.1% MeOH gradient using NI and PI switching.

Analytes were identified using the compound retention time relative to the retention time of the analyte in each paired standard addition (SA) sample, and the ion ratio of the quantitation and confirming ions of the protonated or deprotonated parent molecule +/- 25% of the average analyte ratio in the standard curve solutions. Analyte responses were normalized using the ratio of the quantitation daughter-ion of the analyte-to-stable-isotope labeled standard. Analytes with matching stable-isotope labeled compounds were quantitated using isotope dilution using a 10-point standard curve with concentrations that range from 0.5 ng/L to 50,000 ng/L. The remaining analytes were quantitated using standard addition to compensate for differential ionization due to matrix effects. Samples extracts with analyte concentrations above the highest standard or that were more than 9 times greater than the standard addition spike concentration were diluted and reanalyzed. Reporting limits were conservatively set in the matrices where the signal-to-noise ratio of the least abundant daughter-ion for each analyte is 5 or greater. For compounds that were
detected in virtually all the samples of a particular matrix, the reporting limit was estimated.

**Supporting Information: Recovery Analysis**

**Recovery Analysis of Liquid Samples:** Liquid lagoon samples were composited and filtered through a 0.7-µm glass fiber filter. Samples were divided into fourteen 500-ml aliquots. Six aliquots were spiked with 200 µl of an analyte standard mix containing 1 ng/µl of each compound, and eight of the aliquots were unspiked. The samples then were extracted using the SPE procedure as described above. Six of the eight unspiked sample eluates then were spiked with the same volume of analyte standard mix as the six samples spiked prior to extraction. All 14 of the sample eluates then were spiked with 100 µl of a 7 compound internal standard mix containing 1 ng/ul of each analyte. The eluates were evaporated to a volume of 100 ul, brought up to a final volume of 1 ml with a solution of 90% water and 10% methanol, and transferred to 2-ml silanized glass chromatography vials. The samples then were analyzed using the methods described in the SI LC/MS/MS analysis section. Samples were quantitated using an external standard curve. The average concentration of analytes detected in the two unspiked samples was subtracted from all the pre- and post-extraction spiked samples. The average concentration for each analyte of the post-extraction spiked samples then was divided into the analyte concentration of each of the pre-extracted spiked analytes. The average percent recovery and relative standard deviation for each analyte then was calculated (Table SI-2).
**Recovery Analysis of Solid Samples:** Solid lagoon samples were composited and divided into twenty-four 1-g aliquots. Each sample aliquot was ground in a mortar and pestle with 1 g of diamateous earth and packed into 22-ml cells using the above describe procedure. Three samples were unspiked, seven samples were spiked at 100 µg/kg (100 µl) with an analyte standard mix containing 1 ng/µl of each analyte prior to ASE, seven after ASE extraction, and seven after the final SPE extraction. The ASE eluates of all the samples was spiked with 100 ul of the seven compound internal standard mix prior to SPE so small aliquots of the sample eluates spiked before and just after ASE extraction could be analyzed so that the analyte recoveries for the ASE extraction prior to SPE could also be calculated (data not shown). The ASE and SPE extraction methods are the same as those described above. The average concentration of each analyte detected in the three unspiked samples then was subtracted from all of the spiked samples. The average concentration of the post-SPE spiked sample aliquots then was divided into the concentration of the pre-ASE spiked sample extracts and the average percent recovery and relative standard deviation for each analyte was then calculated (Table SI-2).

Of note, the concentrations of estrone (E1) and equol (EQU) in the lagoon solid samples were too high to differentiate the sample concentrations from the spiked concentration. Thus, quantification of the recovery of these compounds was not possible (Table SI-2). However, based on the recoveries of similar analytes from the solid samples, and because there was essentially no difference between the pre- and post- spiked ASE samples and also no difference between these two sample types
and the post-spiked SPE samples, the recovery of E1 from this phase is estimated to be at least 90%, and the recovery of EQU is estimated to be greater than 70%.
Table SI-2: Summary of compound information, reporting limits (RL), and percent recovery from solid phase extraction.

| Group 1                          | CAS #      | Source    | RL Liquid Samples (ng/l) | RL Solid Samples (ng/kg) | % Recovery from Liquid Samples | % Recovery from Solid Samples |
|----------------------------------|------------|-----------|--------------------------|--------------------------|-------------------------------|------------------------------|
| Estrone                          | 53-16-7    | Steraloids| 0.1-1.0                  | 300-2,000                | 90 ± 2.75                     | *110 ± 5.61                   |
| 17β-Estradiol                    | 50-28-2    | Steraloids| 0.1-1.0                  | 500-3,000                | 88 ± 1.77                     | 91 ± 7.57                    |
| 17α-Estradiol                    | 57-91-0    | Steraloids| 0.1-1.0                  | 500-3,000                | 88 ± 2.51                     | 99 ± 15.8                    |
| Estriol                          | 50-27-1    | Steraloids| 0.1-1.0                  | 15,000                   | 90 ± 1.80                     | 94 ± 8.41                    |
| 17α-Ethynylestradiol             | 77538-56-8 | Steraloids| 0.1-1.0                  | 1,000-5,000              | 82 ± 4.05                     | 84 ± 11.4                    |
| Diethylstilbesterol              | 56-53-1    | Steraloids| 0.1-1.0                  | 500-3,000                | 76 ± 4.02                     | 93 ± 14.6                    |
| Estrone-d4                       | 53866-34-5 | Isotec    |                          |                          |                               |                              |
| 17β-Estradiol-d4                 | 66789-03-5 | Steraloids|                          |                          |                               |                              |
| Estriol-d3                       | 79037-36-8 | Steraloids|                          |                          |                               |                              |
| 17α-Ethynylestradiol-d4          | 350820-06-3| Steraloids|                          |                          |                               |                              |
| 17β-Estradiol-d5                 | 220193-45-4| Steraloids|                          |                          |                               |                              |

| Group 2                          |            |           |                          |                          |                               |                              |
| Genistein                        | 446-72-0   | Steraloids| 0.1-1.0                  | 10-500                   | 89 ± 3.56                     | 110 ± 30.1                   |
| Daidzein                         | 486-66-8   | Steraloids| 0.1-1.0                  | 10-500                   | 99 ± 5.34                     | 45 ± 13.7                    |
| Formonentin                      | 485-72-3   | Steraloids| 0.1-1.0                  | 10-500                   | 97 ± 4.58                     | 94 ± 6.18                    |
| Compound                | CAS Number | Supplier   | Concentration | Range | Group 3A | Group 3B |
|-------------------------|------------|------------|---------------|-------|----------|----------|
| Coumestrol              | 479-13-0   | Steraloids | 0.1-1.0       | 10-500| 94 ± 3.64| 76 ± 20.4|
| Equol                   | 531-95-3   | Steraloids | 0.1-1.0       | 10-500| 91 ± 6.90| *110 ± 12.9|
| Biochanin A             | 491-80-5   | Steraloids | 0.1-1.0       | 10-500| 71 ± 5.74| 81 ± 26.4|
| Genistein-d4            | 187960-08-3| Steraloids |               |       |          |          |
| **Group 3A**            |            |            |               |       |          |          |
| Trenbolone              | 10161-33-8 | Sigma      | 0.1           | 1,000 | 85 ± 5.49| 100 ± 7.11|
| Androstenedione         | 63-05-8    | Sigma      | 0.1           | 500   | 86 ± 7.83| 100 ± 6.35|
| Testosterone            | 58-22-0    | Steraloids | 0.1           | 500   | 92 ± 5.45| 97 ± 6.95 |
| Epitestosterone         | 90-43-7    | Steraloids | 0.1           | 500   | 84 ± 6.51| 110 ± 5.76|
| 19-Norethisterone       | 68-22-4    | Steraloids | 0.1           | 500   | 93 ± 4.38| 110 ± 10.2|
| 11-Ketotestosterone     | 564-35-2   | Sigma      | 0.1           | 500   | 97 ± 6.14| 92 ± 8.04 |
| Progesterone            | 57-83-0    | Sigma      | 0.1           | 500   | 57 ± 7.38| 90 ± 5.86 |
| 6α-methyl-17α-hydroxyprogesterone | 71-58-9 | Sigma | 0.1 | 500 | 75 ± 2.50 | 92 ± 8.57 |
| Norgestimate            | 35189-28-7 | Steraloids | 0.1           | 1,000 | 58 ± 9.85| 47 ± 21.5 |
| Testosterone-d3         | 77546-39-5 | Steraloids |               |       |          |          |
| Progesterone-d8         |            | Steraloids |               |       |          |          |
| **Group 3B**            |            |            |               |       |          |          |
| α-Zearalanol            | 26538-44-3 | Steraloids | 0.1           | 100   | 90 ± 6.11| 36 ± 15.6|

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| Compound | Concentration | Spike | Sample | Recovery | Recovery |
|----------|---------------|-------|--------|----------|----------|
| Estrone-3-sulfate | 481-97-0 | Sigma | 0.1 | 100 | 93 ± 3.21 | 100 ± 11.0 |
| 17β-Estradiol-17-sulfate | 3233-69-0 | Steraloids | 0.1 | 100 | 91 ± 3.38 | 97 ± 7.66 |
| 17β-Estradiol-3-sulfate | 481-96-9 | Steraloids | 0.1 | 100 | 94 ± 4.04 | 100 ± 5.09 |
| Testosterone sulfate | 57-85-2 | Steraloids | 0.1 | 100 | 95 ± 4.32 | 96 ± 4.44 |
| Estriol-3-sulfate | 481-95-8 | Steraloids | 0.1 | 100 | 80 ± 6.61 | 87 ± 5.59 |
| Estriol-17-sulfate | 42028-21-7 | Sigma | 0.1 | 100 | 89 ± 3.16 | 92 ± 5.18 |
| Androsterone sulfate | 2479-86-9 | Steraloids | 0.1 | 100 | 92 ± 5.99 | 94 ± 3.88 |
| Ethynylestradiol-3-sulfate | 24560-70-1 | Steraloids | 0.1 | 100 | 94 ± 6.48 | 95 ± 11.4 |
| Diethylstilbestrol glucuronide | 2408-40-4 | Steraloids | 1 | 2,000 | 84 ± 6.35 | 84 ± 4.34 |
| Estrone glucuronide | 2479-90-5 | Steraloids | 1 | 2,000 | 91 ± 6.05 | 80 ± 5.20 |
| 17β-Estradiol-17-glucuronide | 1806-98-0 | Steraloids | 1 | 2,000 | 84 ± 6.48 | 90 ± 6.48 |
| Estriol-3-glucuronide | 2479-91-6 | Steraloids | 1 | 2,000 | 30 ± 2.56 | 2.1 ± 1.20 |
| Testosterone glucuronide | 1180-25-2 | Steraloids | 1 | 2,000 | 91 ± 7.17 | 88 ± 9.33 |
| Androsterone glucuronide | 1852-43-3 | Steraloids | 1 | 2,000 | 90 ± 5.22 | 110 ± 6.20 |
| Ethynylestradiol-3-glucuronide | 57-63-6 | Steraloids | 1 | 2,000 | 91 ± 6.12 | 92 ± 4.91 |
| Estrone-3-sulfate-d4 | 285979-80-8 | Isotec | | | | |

*Concentration in prespiked sample too high to calculate recovery. Shown is percent of total concentration pre-ASE spike sample-to-post SPE spike concentration.
Table S1-3: Summary of A) chromatography methods employed for each block of compounds listed in Table SI-2, and B) LC/MS-MS systems.

*Waters Atlantis C18, 150x3mm, 2.1um; *Phenomonex, Kinetex C18, 100x4.6mm, 2.6um; *Waters BEH 50x1.7mm, 2.1um; **Methanol; ***Group 2 compounds only; ****Formic Acid

A:

| Sampling round (2009 – 2011) and sample types (liquid or solid) | Analyte Groups (from Table SI-2) | Analytical Column* | Mobile Phase A | Mobile Phase B | Post column solution | Equilibration mobile phase |
|---|---|---|---|---|---|---|
| 2009 liquids | Group 1 | Atlantis | water | MeOH** | 10 mM NH4OH |
| 2009***, 2010, 2011 liquids; 2009, 2010, 2011 solids | Group 1, 2 | Kinetex | water | MeOH | 10 mM NH4OH |
| 2009 liquid | Group 3a, b | Atlantis | 0.1% FA**** | 0.1% FA | MeOH |
| 2010, 2011 liquids; 2009, 2010, 2011 solids | Group 3a, b | BEH | water | MeOH | 0.1% FA/MeOH (95/5) |

B:

| Sampling round (2009 – 2011) and sample types (liquid or solid) | Analyte Groups from Table 1A | Liquid Chromatograph | Mass Spectrometer |
|---|---|---|---|
| 2009 liquids | Group 1, 3a, b | Shimadzu Prominence | AB/Sciex API 5000 |
| 2009, 2010, and 2011 liquids and solids | Group 1, 2, 3a, b | Waters Acquity H-class Bio | AB/SciexAPI 5500 |
| 2010 liquids; 2009, 2010 solids | Group 1, 2 | Waters Acquity H-class Bio | Waters Quattro Micro |
Supporting Information: Yeast Estrogen Screen (YES)

YES Assay Procedure: Briefly, 200 µL of lagoon sample extract was added to a 96-well plate, and serially diluted 1:2 in a solution of 90% water and 10% ethanol (12 dilutions total). Each sample was run in duplicate on the same plate. E2β served as dose-response standard, and was added to a separate row on the same plate from a standard solution prepared in 10% ethanol. The serially-diluted samples and E2β standard were allowed to incubate with yeast solution for three days. On the third day, an assay buffer containing ortho-nitrophenyl-β-galactoside (Calbiochem, EMD Chemicals, Inc; San Diego, CA) was added, producing a colorimetric response at 405 nm. The reaction was stopped with 200 µL of a 1 M sodium carbonate solution, and the plates were centrifuged at 3,000 rpm for 10 minutes. 100 µL of the resulting supernatant were taken from each well and transferred to a new 96-well microtiter plate to determine the OD at 405 nm and 620 nm.

Data analysis for YES assays was conducted as follows: For each dilution point, OD620 was subtracted from OD405 to account for colorimetric interference from yeast cells. This value was then normalized to the negative control. The sigmoid concentration-response curve of the E2β standard was fitted to a symmetric logistic function using GraphPad Prism software (GraphPad, San Diego, CA). The response of the standard and the sample were expressed as a percentage of the maximum response evoked by E2β, and the concentration of E2β that induced a half-maximal response (EC₅₀) was then fitted using the software. For each sample, the concentration factor of sample extract that induced a half-maximal response (CF₅₀) was also fitted using the software. The
estrogenic activity of each sample, expressed as E2β equivalents (EEQ), was then calculated as:

$$\text{EEQ} = \frac{\text{EC}_{50}}{\text{CF}_{50}} \quad (\text{Equation SI-1})$$

**Estimated Potencies (EP) from Chemical Analysis:** The relative estrogenic potency (REP) for each analyte was calculated as a ratio of the estrogenic activity of E2β to the estrogenic activity of each individual compound tested:

$$\text{REP} = \frac{\text{EC}_{50\text{E2β}}}{\text{EC}_{50i}} \quad (\text{Equation SI-2})$$

in which EC50E2β is the observed half-maximal estrogenic activity of the E2β standard, and EC50i is the observed half-maximal estrogenic activity of analyte in question. Using these REPs, the EP for each lagoon sample was calculated by multiplying each analyte concentration by its relative potency, and summing these potency-adjusted values:

$$\text{EP}_i = \sum \text{REP}_i \text{C}_i \quad (\text{Equation SI-3})$$

where REPi is the REP of a particular analyte in the YES assay and Ci is the concentration of that particular analyte in a sample.
Supporting Information: Partitioning Ratios in the Lagoon

Equilibrium partitioning ratios were calculated for analytes in the lagoon slurry and sludge using the following equations:

**Particle water partitioning ratio** \( (K_D) \): \( K_D = \frac{C_s}{C_W} \)  \hspace{1cm} (Equation SI-4)

in which \( C_s \) is the concentration of an analyte within the solid fraction of a lagoon slurry or sludge sample, and \( C_W \) is the concentration of an analyte within the aqueous fraction of a lagoon slurry or sludge sample.

**Organic carbon-water partitioning ratio** \( (K_{OC}) \): \( K_{OC} = \frac{K_D}{\%OC} \times 100 \)  \hspace{1cm} (Equation SI-5)

in which \( K_D \) is the particle-water partitioning ratio calculated in Equation 4, and \( \%OC \) is the percent organic carbon that was measured in the solid fraction of each slurry or sludge sample.
Table SI-4: Average analyte concentrations +/- coefficient of variation in slurry aqueous and solid phases across the three rounds of sampling (LC/MS-MS results). All concentrations are given in parts-per-trillion (ng/l or ng/kg dry weight). ND = non-detect

|                 | Slurry Aqueous Phase (ng/l) | Slurry Solid Phase (ng/kg) |
|-----------------|-----------------------------|----------------------------|
|                 | 9-Jun          | 10-Apr       | 11-Feb         | 9-Jun         | 10-Apr       | 11-Feb         |
| **E1**          | 4094 ± 32%     | 8880 ± 6%    | 9195 ± 15%     | 1587642 ± 66%| 1569041 ± 31%| 5237500 ± 23% |
| **E3**          | 35 ± 44%       | 174 ± 53%    | 75 ± 23%       | 140785 ± 53%  | 210250 ± 21%  | 350833 ± 42%  |
| **E2β**         | 3 ± 161%       | 225 ± 38%    | 31 ± 64%       | 127214 ± 67%  | 159291 ± 48%  | 153583 ± 31%  |
| **E2α**         | 170 ± 28%      | 1053 ± 20%   | 499 ± 19%      | 85285 ± 83%   | 152500 ± 62%  | 299208 ± 29%  |
| **AN**          | 59 ± 320%      | 51 ± 147%    | 55 ± 23%       | 11785 ± 43%   | 3041 ± 34%    | 1492 ± 281%   |
| **11KT**        | ND             | 15 ± 367%    | ND             | ND            | ND            | ND             |
| **P4**          | 47 ± 165%      | 174 ± 70%    | 523 ± 13%      | 67428 ± 59%   | 28329 ± 59%   | 28741 ± 43%   |
| **E1-3-S**      | 0.01 ± 374%    | ND           | ND             | 800 ± 45%     | 834 ± 59%     | 334 ± 42%     |
| **E2β-17-S**    | ND             | 0.05 ± 282%  | ND             | ND            | ND            | ND             |
| **AN-S**        | 0.07 ± 101%    | 0.1 ± 286%   | 1 ± 20%        | 321 ± 374%    | ND            | ND             |
| **DAI**         | 60 ± 138%      | 118 ± 63%    | 158 ± 74%      | 2745 ± 102%   | 7234 ± 46%    | 93417 ± 69%   |
| **GEN**         | 74 ± 175%      | ND           | ND             | 1088 ± 251%   | 4295 ± 29%    | 2833 ± 211%   |
| **EQU**         | 245 ± 61%      | 2515 ± 88%   | 161250 ± 146%  | 1465428 ± 79% | 9495833 ± 135%| 51241667 ± 27%|
| **COU**         | ND             | ND           | ND             | 1460 ± 133%   | 1304 ± 42%    | 9113 ± 108%   |
| **FOR**         | 1 ± 302%       | ND           | 3 ± 74%        | 154 ± 199%    | 5 ± 489%      | ND             |
Table SI-5: Average analyte concentrations +/- coefficient of variation in sludge aqueous and solid phases across the three rounds of sampling (LC/MS-MS results). All concentrations are given in parts-per-trillion (ng/l or ng/kg dry weight). ND = non-detect

|                         | Sludge Aqueous Phase (ng/l) | Sludge Solid Phase (ng/kg) |
|-------------------------|-----------------------------|---------------------------|
|                         | 9-Jun | 10-Apr | 11-Feb | 9-Jun | 10-Apr | 11-Feb |
| E1                      | 3303 ± 26% | 7932 ± 19% | 8338 ± 41% | 1122285 ± 34% | 1744000 ± 49% | 4850000 ± 85% |
| E3                      | 47 ± 43% | 131 ± 50% | 36 ± 32% | 20714 ± 63% | 142500 ± 28% | 223250 ± 65% |
| E2β                     | 9 ± 68% | 308 ± 37% | 133 ± 43% | 146714 ± 60% | 217500 ± 45% | 130750 ± 83% |
| E2α                     | 153 ± 50% | 523 ± 38% | 325 ± 58% | 116285 ± 60% | 80519 ± 43% | 141625 ± 33% |
| AN                      | 1 ± 282 | ND     | 19 ± 30% | 82 ± 1% | 2350 ± 225% | 1363 ± 33% |
| 11KT                    | ND     | ND     | ND     | ND     | ND     | ND     |
| P4                      | 7 ± 67% | 2 ± 211% | 1 ± 160% | ND     | 3963 ± 23% | 4788 ± 39 |
| E1-3-S                  | 0.08 ± 233% | ND | ND     | ND     | 1309 ± 264% | 513 ± 366 |
| E2β-17-S                | ND     | ND     | ND     | ND     | ND     | ND     |
| AN-S                    | 0.14 ± 254% | 0.2 ± 82% | 0.08 ± 206% | ND | ND | ND |
| DAI                     | 14 ± 133% | 5 ± 60% | 1 ± 282% | 13542 ± 87% | 9200 ± 140% | 1500 ± 283% |
| GEN                     | 7 ± 188% | ND     | ND     | 7557 ± 76% | 7575 ± 85% | ND |
| EQU                     | 76 ± 87% | 145 ± 68% | 2386 ± 69% | 815714 ± 51% | 2806250 ± 120% | 1721625 ± 55% |
| COU                     | ND     | ND     | ND     | 1010 ± 61% | 1645 ± 169% | ND |
| FOR                     | 1 ± 206% | ND     | ND     | 807 ± 54% | 239 ± 185% | ND |
Table SI-6: Average total analyte concentrations +/- coefficient of variation in slurry and sludge across the three rounds of sampling (LC/MS-MS results). All concentrations are given in parts-per-trillion (ng/l whole sample). ND = non-detect

|       | Whole Slurry (ng/l) | Whole Sludge (ng/l) |
|-------|---------------------|---------------------|
|       | Jun-09      | Apr-10      | Feb-11      | Jun-09      | Apr-10      | Feb-11      |
| E1    | 5632 ± 29%  | 10277 ± 9%  | 12858 ± 11% | 96748 ± 38% | 127844 ± 53%| 392664 ± 77%|
| E3    | 182 ± 51%  | 382 ± 48%  | 353 ± 37%  | 1696 ± 76%  | 9963 ± 33%  | 17825 ± 59% |
| E2β   | 136 ± 67%  | 383 ± 49%  | 152 ± 25%  | 11605 ± 64% | 15195 ± 45% | 10527 ± 75% |
| E2α   | 255 ± 33%  | 1191 ± 28% | 709 ± 17%  | 9448 ± 58%  | 5967 ± 46%  | 11406 ± 100%|
| AN    | 70 ± 261%  | 53 ± 138%  | 53 ± 25%   | 8 ± 251%    | 161 ± 31%   | 126 ± 117%  |
| 11KT  | ND         | 15 ± 367%  | ND         | ND          | ND          | ND          |
| P4    | 116 ± 70%  | 200 ± 65%  | 516 ± 12%  | 4 ± 74%     | 273 ± 47%   | 395 ± 73%   |
| E1-3-S| 0.8 ± 48%  | 0.8 ± 64%  | 0.3 ± 41%  | 0.05 ± 255% | 93 ± 59%    | 42 ± 65%    |
| E2β-17-S| ND      | 0.05 ± 283%| ND         | ND          | ND          | ND          |
| AN-S  | 0.4 ± 313% | 0.1 ± 287% | 1 ± 19%    | 0.1 ± 267%  | 0.1 ± 90%   | 0.04 ± 191% |
| DAI   | 61 ± 133%  | 122 ± 61%  | 223 ± 47%  | 1058 ± 105% | 665 ± 149%  | 119 ± 281%  |
| GEN   | 73 ± 171%  | 4 ± 40%    | 2 ± 223%   | 603 ± 92%   | 509 ± 77%   | ND          |
| EQU   | 1750 ± 74% | 11570 ± 101%| 193355 ± 111%| 64615 ± 48%| 178690 ± 114%| 148210 ± 60%|
| COU   | 0.1 ± 210% | 1 ± 42%    | 7 ± 112%   | 84 ± 61%    | 90 ± 179%   | ND          |
| FOR   | 0.5 ± 215% | 0.004 ± 489%| 2 ± 74%    | 64 ± 65%    | 17 ± 190%   | ND          |
Table SI-7: P-values from a paired t-test comparing analyte concentrations in slurry to the corresponding concentrations in sludge within each phase (aqueous or solid) of the lagoon, in each of the three rounds of sampling. Significant differences (p < 0.05) are highlighted.

|                 | Aqueous Phase | Solid Phase |     |
|-----------------|---------------|-------------|-----|
|                 | Slurry vs. Sludge | Slurry vs. Sludge |     |
|                 | Jun-09 | Apr-10 | Feb-11 | Jun-09 | Apr-10 | Feb-11 |
| EEQ             | 0.4610 | <0.001 | 0.0530 | 0.8990 | 0.2750 | 0.9750 |
| E1              | 0.4370 | 0.1630 | 0.5040 | 0.4180 | 0.5380 | 0.7910 |
| E3              | 0.0880 | 0.2290 | <0.001 | 0.0060 | <0.001 | 0.0050 |
| E2B             | 0.1180 | 0.1030 | <0.001 | 0.3120 | 0.0850 | 0.5070 |
| E2a             | 0.7700 | 0.0020 | 0.0280 | 0.0890 | 0.0060 | 0.0310 |
| P4              | 0.0260 | 0.0080 | <0.001 | 0.0040 | <0.001 | <0.001 |
| AN              | 0.2010 | 0.0090 | <0.001 | 0.0020 | 0.0030 | 0.8790 |
| E1-3-S          | 0.2970 | ND | ND | <0.001 | 0.0580 | 0.2030 |
| AN-S            | 0.5420 | 0.7250 | <0.001 | 0.363 | ND | ND |
| DAI             | 0.0470 | 0.0030 | <0.001 | 0.0690 | 0.6420 | <0.001 |
| GEN             | 0.0680 | ND | ND | 0.0290 | 0.1930 | 0.0790 |
| EQU             | <0.001 | <0.001 | 0.0150 | 0.3280 | 0.0630 | <0.001 |
| COU             | ND | ND | ND | 0.0480 | 0.8930 | 0.0110 |
| FOR             | 0.3720 | ND | 0.0020 | 0.0020 | 0.1720 | ND |
Table SI-8: Organic carbon-water partitioning coefficients (log $K_{OC}$) predicted using the EPI Suite software, and the respective log $K_{OC}$ values that were observed in the lagoon slurry and sludge across the three rounds of sampling. Log $K_{OC}$ observed in the lagoon are listed +/- coefficient of variation.

| EPI Suite Log $K_{OC}$ | Log $K_{OC}$ Observed in Lagoon Slurry | Log $K_{OC}$ Observed in Lagoon Sludge |
|------------------------|----------------------------------------|----------------------------------------|
|                        | Jun-09  | Apr-10  | Feb-11  | Jun-09  | Apr-10  | Feb-11  |
| E1                     | 4.38    | 2.82 ± 17% | 2.64 ± 6% | 3.11 ± 3% | 3.04 ± 15% | 2.87 ± 10% | 3.29 ± 10% |
| E3                     | 3.08    | 3.96 ± 6%  | 3.52 ± 6% | 4.01 ± 3% | 3.21 ± 9%  | 3.66 ± 6%  | 4.36 ± 7%   |
| E2β                    | 4.19    | 4.67 ± 9%  | 3.24 ± 6% | 4.01 ± 6% | 4.74 ± 12% | 3.39 ± 9%  | 3.54 ± 11% |
| E2α                    | 4.19    | 2.96 ± 15% | 2.53 ± 8% | 3.14 ± 8% | 3.46 ± 12% | 2.73 ± 13% | 3.13 ± 10% |
| DAI                    | 3.37    | 1.65 ± 49% | 2.22 ± 16% | 3.20 ± 13% | 3.41 ± 9%  | 3.52 ± 16% | N/A         |
| EQU                    | 4.27    | 3.96 ± 11% | 4.21 ± 32% | 2.99 ± 12% | 4.72 ± 9%  | 4.70 ± 10% | 3.48 ± 11% |
| AN                     | 3.53    | 3.56 ± 29% | 2.40 ± 22% | 2.44 ± 17% | N/A        | N/A        | 2.74 ± 6%   |
| P4                     | 4.00    | 3.82 ± 17% | 2.64 ± 13% | 2.05 ± 11% | N/A        | 3.25 ± 7%  | 3.74 ± 15% |
Table SI-9: Average EEQs, determined using the YES assay; and EPs, estimated based on sample analyte concentrations; in A) aqueous and solid phases of the lagoon slurry and B) aqueous and solid phases of the lagoon sludge. All concentrations (in E2β equivalents) are given in parts-per-trillion (ng/l of aqueous phase, or ng/kg dry mass of solid phase), and coefficient of variation is provided. P-values from paired t-test comparing EEQ to EP are also listed. P-value less than 0.05 are considered significant.

A.

| Slurry Aqueous Phase (ng/l) | Slurry Solid Phase (ng/kg) |
|-----------------------------|-----------------------------|
|                             | Jun-09 | Apr-10 | Feb-11 | Jun-09 | Apr-10 | Feb-11 |
| EEQ                         | 3708 ± 17% | 5477 ± 9% | 3958 ± 7% | 1252942 ± 34% | 824554 ± 29% | 1294303 ± 23% |
| EP                          | 1741 ± 34% | 4428 ± 8% | 4395 ± 15% | 1134779 ± 52% | 904208 ± 32% | 2634273 ± 23% |
| p-value                     | <0.001 | <0.001 | 0.005 | 0.468 | 0.183 | <0.001 |

B.

| Sludge Aqueous Phase (ng/l) | Sludge Solid Phase (ng/kg) |
|-----------------------------|-----------------------------|
|                             | Jun-09 | Apr-10 | Feb-11 | Jun-09 | Apr-10 | Feb-11 |
| EEQ                         | 4034 ± 15% | 4612 ± 11% | 2850 ± 47% | 1584636 ± 35% | 999826 ± 38% | 1302795 ± 54% |
| EP                          | 1565 ± 27% | 4050 ± 20% | 4057 ± 41% | 664454 ± 38% | 1040764 ± 43% | 2414237 ± 85% |
| p-value                     | <0.001 | 0.138 | 0.081 | 0.002 | 0.645 | 0.078 |
**Table SI-10:** Average total EEQs, determined using the YES assay; and total EPs, which are estimated based on sample analyte concentrations in slurry and sludge. All concentrations (in E2β equivalents) are given in parts-per-trillion (ng/l whole sample), and coefficient of variation is provided. P-values from paired t-test comparing EEQ to EP are also listed. P-value less than 0.05 are considered significant.

|         | Whole Slurry (ng/l) | Whole Sludge (ng/l) |
|---------|---------------------|---------------------|
|         | Jun-09   | Apr-10  | Feb-11  | Jun-09   | Apr-10  | Feb-11  |
| EEQ     | 4924 ± 9% | 6182 ± 9% | 4777 ± 8% | 134528 ± 30% | 73236 ± 44% | 105651 ± 48% |
| EP      | 2799 ± 26% | 5250 ± 11% | 6250 ± 11% | 57338 ± 42% | 75537 ± 47% | 195401 ± 77% |
| p-value | <0.001   | <0.001  | <0.001  | 0.001    | 0.681    | 0.059    |
Table SI-11: Average percent contribution of each individual analyte to the calculated EP, in slurry and sludge across the three rounds of sampling.

|          | Percent contribution to Slurry EP | Percent contribution to Sludge EP |
|----------|----------------------------------|----------------------------------|
|          | Jun-09  | Apr-10 | Feb-11 | Jun-09  | Apr-10 | Feb-11 |
| **E1**   |         |        |        |         |        |        |
|          | 95      | 81     | 92     | 78      | 97     | 94     |
| **E2β**  | 4.5     | 19     | 7      | 21      | 2.4    | 5.4    |
| **E3**   | 0.049   | 0.024  | 0.055  | 0.12    | 0.037  | 0.079  |
| **E2α**  | 0.27    | 0.48   | 0.67   | 0.23    | 0.33   | 0.18   |
| **AN**   | 0.0000055 | 0.000000019 | 0.000002 | 0.00000048 | 0.0000015 | 0.00000012 |
| **11KT** | ND      | ND     | 0.00001 | ND      | ND     | ND     |
| **E1-3-S** | 0.000045 | 0.00000014 | 0.000023 | 0.00020 | 0.000052 | 0.00004 |
| **E2β-17-S** | ND | ND | 0.0000000091 | ND | ND | ND |
| **AN-S** | 0.00000024 | 0.0000000024 | 0.000000038 | 0.000000025 | 0.00000019 | 0.000000048 |
| **DAI**  | 0.0000024 | 0.0000014 | 0.0000019 | 0.00000085 | 0.0000033 | 0.00000029 |
| **GEN**  | 0.00042  | 0.00011 | 0.000010 | 0.00011  | 0.000054 | ND     |
| **EQU**  | 0.011    | 0.027   | 0.044   | 0.065    | 0.47    | 0.023  |
| **COU**  | 0.0000024 | 0.000099 | 0.000014 | 0.000096 | 0.000041 | ND     |
| **FOR**  | 0.000000065 | 0.000000042 | ND | 0.00000012 | 0.00000018 | ND |
**Table SI-12:** P-values from a three-way ANOVA indicating the relationship between analyte concentrations, location, depth, and phase (aqueous or solid) in the lagoon slurry. Significant results (p < 0.05) are highlighted.

|       | Jun-09 |     |     | Apr-10 |     |     | Feb-11 |     |     |
|-------|--------|-----|-----|--------|-----|-----|--------|-----|-----|
|       | Location | Depth | Phase | Location | Depth | Phase | Location | Depth | Phase |
| EEQ   | 0.374   | 0.7280 | 0.0010 | 0.8500   | 0.8870 | <0.001 | 0.4970   | 0.6570 | <0.001 |
| E1    | 0.3200  | 0.8710 | 0.0110 | 0.8160   | 0.3410 | <0.001 | 0.8020   | 0.1250 | <0.001 |
| E3    | 0.3940  | 0.7470 | 0.0050 | 0.7827   | 0.2560 | <0.001 | <0.001   | 0.5000 | <0.001 |
| E2B   | 0.3200  | 0.7860 | 0.0090 | 0.751    | 0.4600 | <0.001 | 0.0660   | 0.1150 | <0.001 |
| E2a   | 0.1280  | 0.7430 | 0.0090 | 0.81     | 0.3710 | <0.001 | 0.3840   | 0.0110 | <0.001 |
| P4    | 0.0990  | 0.8160 | 0.0050 | 0.3600   | 0.4220 | <0.001 | 0.8310   | 0.6050 | <0.001 |
| AN    | 0.0100  | 0.1120 | <0.001 | 0.8950   | 0.3900 | <0.001 | 0.5060   | 0.8460 | 0.1310 |
| E1-3-S| 0.0410  | 0.7070 | <0.001 | 0.3560   | 0.9080 | <0.001 | 0.0140   | 0.1900 | <0.001 |
| AN-S  | 0.4440  | 0.4440 | 0.3740 | 0.3280   | 0.2880 | 0.0930 | 0.4710   | 0.3930 | <0.001 |
| DAI   | 0.6600  | 0.5620 | 0.0380 | 0.0860   | 0.8190 | <0.001 | 0.1670   | 0.2370 | <0.001 |
| GEN   | 0.4960  | 0.6130 | 0.2440 | 0.6460   | 0.4080 | <0.001 | 0.3180   | 0.8510 | 0.0340 |
| EQU   | 0.0240  | 0.4880 | 0.0020 | 0.5230   | 0.4380 | 0.0030 | 0.0560   | 0.1990 | <0.001 |
| COU   | 0.0560  | 0.4320 | 0.0520 | 0.2780   | 0.6260 | <0.001 | 0.0860   | 0.7610 | <0.001 |
| FOR   | <0.001  | 0.4470 | <0.001 | 0.4710   | 0.3930 | 0.3340 | 0.0400   | 0.8010 | <0.001 |
Table SI-13: P-values from a two-way ANOVA indicating the relationship between analyte concentrations and location and phase (aqueous or solid) in the lagoon sludge. Significant results (p < 0.05) are highlighted.

|       | Jun-09 Location | Jun-09 Phase | Apr-10 Location | Apr-10 Phase | Feb-12 Location | Feb-12 Phase |
|-------|-----------------|-------------|-----------------|-------------|-----------------|-------------|
| EEQ   | 0.478           | <0.001      | 0.499           | <0.001      | 0.498           | 0.001       |
| E1    | 0.506           | <0.001      | 0.498           | <0.001      | 0.5             | 0.013       |
| E3    | 0.498           | 0.003       | 0.5             | <0.001      | 0.5             | 0.003       |
| E2B   | 0.5             | 0.003       | 0.5             | <0.001      | 0.5             | 0.011       |
| E2a   | 0.501           | 0.002       | 0.494           | <0.001      | 0.499           | 0.03        |
| P4    | 0.5             | 0.004       | 0.498           | <0.001      | 0.5             | 0.005       |
| AN    | 0.505           | 0.359       | 0.5             | <0.001      | 0.498           | 0.057       |
| E1-3-S| 0.5             | 0.265       | 0.5             | 0.001       | 0.5             | 0.004       |
| AN-S  | 0.5             | 0.304       | 0.5             | 0.011       | 0.5             | 0.213       |
| DAI   | 0.501           | 0.027       | 0.5             | 0.083       | 0.5             | 0.351       |
| GEN   | 0.499           | 0.013       | 0.5             | 0.013       | ND              | ND          |
| EQU   | 0.5             | <0.001      | 0.5             | 0.051       | 0.501           | 0.001       |
| COU   | 0.5             | 0.002       | 0.5             | 0.167       | ND              | ND          |
| FOR   | 0.498           | 0.002       | 0.5             | 0.171       | ND              | ND          |
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