Domoic Acid in California Sea Lion Fetal Fluids Indicates Continuous Exposure to a Neuroteratogen Poses Risks to Mammals.

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Keywords:

Abstract:

Domoic acid (DA) is a neuroexcitotoxic amino acid that is naturally produced by some species of marine diatoms during harmful algal blooms (HABs). The toxin is transferred through the food web from plantivorous fish and shellfish to marine mammals resulting in significant morbidity and mortality. Due to the timing and location of DA producing HABs, it is well documented that pregnant female California sea lions (CSL) are regularly exposed to DA through their diet thereby posing exposure risks to a neuroteratogen in developing fetuses. In the present study, fluids from 36 fetuses sampled from naturally exposed pregnant CSLs were examined for DA. Domoic acid was detected in 79% of amniotic fluid (n=24), 67% of allantoic fluid (n=9), 75% of urine (n=4), 41% of meconium (n=17) and 29% of stomach content (n=21) samples opportunistically collected from CSL fetuses. The distribution of DA in fetal samples indicates an increased prenatal exposure risk due to recirculation of DA in fetal fluids and continuous exposure to the developing brain.

Introduction:

Domoic acid (DA) is a potent glutaminergic excitatory neurotoxin that is naturally produced by some species of marine diatoms in the genus *Pseudonitzschia* (Bates, 2000; Berman and Murray, 1997). The toxin accumulates in planktivorous finfish and shellfish such as anchovies, sardines, clams, mussels and scallops resulting in trophic transfer to marine mammals and humans (Andjelkovic et al., 2012; Bejarano et al., 2008b; James et al., 2005; Lefebvre et al., 2002). Domoic acid toxicosis was first characterized in humans in 1987 when over 100 people became ill after consuming DA-contaminated mussels

47 (Perl et al., 1990; Wright et al., 1989). Since then, increasingly frequent *Pseudonitzschia*
48 blooms along the west coast of the United States have resulted in repeated exposure of
49 marine mammals to DA, causing widespread mortality, especially of California sea lions
50 (CSL, *Zalophus californianus*) (Bejarano et al., 2008a; Scholin et al., 2000). These
51 blooms have resulted in closures of many seafood fisheries to protect human health, with
52 levels of 20 ppm set as the regulatory limit in seafood destined for human consumption
53 (McCabe et al., 2016).
54 Domoic acid has also been implicated in intrauterine fetal death, abortions, and
55 neonatal death in sea lions when DA exposure occurred during pregnancy (Brodie et al.,
56 2006). In these cases, DA was detected in fetal sea lion fluids including amniotic fluid
57 (Brodie et al., 2006). Domoic acid in amniotic fluid raises concern that fetal sea lions
58 could be exposed during development by ingestion of contaminated amniotic fluid. Such
59 exposure is of concern as *in utero* DA exposure in rodent models has been shown to
60 reduce the number of live fetuses brought to term and is associated with post natal
61 hippocampal damage, and lasting behavioral effects (Dakshinamurti et al., 1993;
62 Doucette and Tasker, 2016). Domoic acid toxicity models in rodents have been
63 extrapolated to predict similar toxicity from *in utero* exposure in CSLs (Ramsdell and
64 Zabka, 2008). In a rat exposure model, it was suggested that recirculation of DA from the
65 amniotic fluid may result in greater fetal sensitivity to low doses of DA as a result of
66 repetitive exposure during gestation (Maucher Fuquay et al., 2012).
67 In human seafood consumers, low dose exposures are more likely to occur than
68 high exposures due to regulatory measures that protect against high-level exposures that
69 are known to induce excitotoxic symptoms such as seizures (Lefebvre and Robertson,

2010). A recent shellfish consumption study reports that some razor clam consumers (including women of childbearing age) in the Pacific Northwest (USA) are chronically exposed to low levels of DA for multiple consecutive months and/or sporadically exposed to levels above the currently established tolerable daily intake limit (0.075 mg/kg) due to high razor clam consumption and DA levels retained in clams (Ferriss et al., 2017; Marien, 1996). Additionally, a recent long term exposure study in mice revealed significant cognitive deficits after six months of weekly exposures to low levels of DA (doses below those that elicit visible signs of excitotoxicity) (Lefebvre et al., 2017). Accordingly, public health advisories have recently included warnings for pregnant women consuming legally harvested shellfish at a rate of more than 15 clams per month per year (Washington State Department of Health October 7, 2016 https://www.doh.wa.gov/Portals/1/Documents/1600/NewsReleases/2016/16-116-RazorClamsAdvisoryNewsRelease.pdf).

In this study, DA levels were quantified in fetal fluids collected opportunistically from naturally exposed pregnant sea lions to characterize fetal DA exposure risks in environmentally relevant conditions. Pathways for DA recirculation and fetal exposure are proposed based on these findings of DA distribution in fetal fluids.

Methods:

Sample Collection

A combination of amniotic fluid, allantoic fluid, urine, meconium and/or stomach content samples were opportunistically collected from 36 California sea lion fetuses. The fetus with intact placenta and fetal membranes was removed from the gravid uterine horn

of adult female sea lions that stranded along the central California coast and either died or were euthanized due to poor prognosis based on their clinical signs of DA toxicosis as defined in Goldstein et al (Goldstein et al., 2008). In some cases, fetuses were also collected when adult females aborted. Amniotic and allantoic fluids were collected by sterile hypodermic syringe from the appropriate fetal membrane compartment while urine was collected from the fetal urinary bladder, and meconium from the rectum upon dissection of the fetus.

Quantification of Domoic Acid

Domoic acid was extracted from amniotic fluid, allantoic fluid, urine, meconium and/or stomach content samples in 50% methanol in a 1:4 wt:wt ratio, followed by homogenization for 1 minute using a LabGEN 700 110V homogenizer with a stainless steel 10 mm rotor-stator generator probe tip. Homogenized samples were then centrifuged at 5,000 rpm for 20 minutes in a DuPont Sorvall RC-5C Plus, and filtered through a 0.22 membrane microcentrifuge tube filter (Millipore Ultra-Free MC-GV centrifugal filters). Samples were then diluted in 10% methanol in PBS-T at ratios of 1:10 for amniotic fluid, allantoic fluid and urine samples, and at ratios of 1:100 and 1:50 for meconium and stomach content samples, respectively. Domoic acid levels were quantified using commercially available Biosense (ASP) enzyme-linked immunosorbent assay (ELISA) kits (www.abraxiskits.com).

Selected fetal samples (n = 1 urine; n = 5 allantoic fluid; n = 5 amniotic fluid) were also analyzed via LC/MS-MS methods for confirmation. For LC/MS-MS quantification, the above samples were extracted with 100% methanol at 1:1 v/v ratio. Samples were centrifuged at 16,100 g for 15 minutes to precipitate protein. Clear

supernatant was collected for analysis. Standard curves were prepared in naïve human urine with spiked domoic acid concentration ranging between 0.3 – 40 ng/mL. Domoic acid was quantified in extracted samples using AB Sciex 6500 qTrap Q-LIT mass spectrometer (AB Sciex, Foster City, CA) in line with a Shimadzu UFLC XR DGU-20A5 (Shimadzu Scientific Instruments, Columbia, MD). Analytes were separated using Synergi™ Hydro-RP 100 Å LC column (2.5 µm, 50 x 2 mm; Phenomenex) with a guard cartridge (2 x 2.1mm, sub 2µm; Phenomenex). A gradient elution at 0.5 mL/min initiated with 95% A (A = water with 0.1 formic acid) and 5% B (B= 95:1 (v:v) acetonitrile and water with 0.1% formic acid) for 1 minute, increased to 100% B by 4 minutes, returned to 5% B by 5 minutes and continued to run at initial conditions until 9 minutes. Domoic acid was detected using positive ion electrospray ionization at the mass transitions (m/z): 312 → 266. The method was validated according to the FDA guidance for bioanalytical method validation and had <20% CV% at all concentrations quantified. The Limit of quantification for the method was 2.5ng/mL.

Results:

Table 1 summarizes the DA levels quantified in all fetal samples analyzed. As samples were obtained opportunistically not all matrices were available for each fetus (amniotic fluid n = 24; allantoic fluid n = 9; urine n = 4; meconium n = 17; and stomach contents n = 21). Domoic acid was detected in 79% of amniotic fluid samples, 67% of allantoic fluid samples, 75% of urine samples, 41% of meconium samples, and 29% of stomach content samples examined (Figure 1). In six fetuses from which at least four matrix types were available for testing, the highest levels of DA were detected in

allantoic fluid (Table 1). A comparison of DA levels quantified in four fetuses that had detectable levels of DA in both allantoic and corresponding amniotic fluids revealed consistently higher toxin levels in allantoic fluids (Figure 2).

Domoic acid levels in ten fetal fluid samples selected for validation via LC/MS-MS analyses are shown in parenthesis in Table 1. Limits of detection were lower for ELISA than LC/MS-MS, but linear regression for ELISA and LC/MS-MS values confirmed a significant relationship ($p = 0.003$; R square = 0.69; Figure 3) and provided confirmation of DA positive samples. Five of the seven fluid samples positive for DA via ELISA were also positive via LC/MS-MS although two of those were below the limit of quantification (BLQ) by LC/MS-MS (Table 1). In all but one case, ELISA values were higher than corresponding LC/MS-MS values and the fold-change for DA positive samples by ELISA and LC/MS-MS was 2.3 ± 1.9 (mean ± sd). Two samples (12510-F and 10832-F) with the lowest DA values reported via ELISA, were below detection limits (ND=not detected) of LC/MS-MS. All samples positive via LC/MS-MS were also quantifiable via ELISA.

Discussion:

Initial exposure of fetal sea lions to DA presumably results from placental transfer of DA in blood from mother (post ingestion) to fetus. Once in the fetal blood stream, the dynamics of DA metabolism are unknown but recirculation of DA can be inferred from the levels reported here and the anatomy of fetal structures. The sea lion has a zonary endotheliochorial placenta, with an allantoic sac surrounding the amnion (Talent and Talent, 1975). The detection of DA in fetal meconium and urine indicate that fetal sea

lions are excreting DA through the kidneys and gastrointestinal tract, as is expected from distribution studies in laboratory rodents and primates (Iverson et al., 1989; Suzuki and Hierlihy, 1993). When excreted in fetal urine, DA accumulates in the allantois, and when excreted in meconium during fetal stress, DA could reach the amniotic fluid (Figure 4). The presence of DA in allantoic and amniotic fluids suggest there is transfer of DA between these two compartments. Once in amniotic fluid, sea lion fetuses can be re-exposed to DA following swallowing of amniotic fluid during gestation. Thus DA can be recirculated through the gastrointestinal tract, allowing for continuous exposure of the developing fetus (Figure 4).

These data indicating recirculation of DA in fetal fluid compartments suggest that any ingestion of even low levels of DA by pregnant females poses a risk of DA exposure to the developing fetal brain (Figure 4). This is consistent with previous laboratory studies in rodent models that have documented maternal transfer of DA to the developing fetus and postnatal neurologic effects of intrauterine exposure including progressive hippocampal injury (Dakshinamurti et al., 1993), impairments in locomotor and cognitive domains (Levin et al., 2005), severe learning and memory impairment (Tanemura et al., 2009), and diminished social investigation and altered sensorimotor gating (Zuloaga et al., 2016). These effects of intrauterine exposure occurred after intravenous (IV), intraperitoneal (IP) or subcutaneous (SC) injection of DA in pregnant females at doses that did not elicit visible signs of toxicosis in the dams, emphasizing the likelihood that fetal sea lions are affected by DA even when acute excitotoxic DA symptoms are not obvious in adult pregnant sea lions.

184 Further evidence supporting the hypothesis that low level DA exposure in
185 pregnant females can have persistent effects in offspring was observed in a recent oral
186 gavage study by Shiotani et al (2017). Pregnant mice were orally exposed to DA at doses
187 that did not induce visible signs of excitotoxicity in the dams (1 and 3 mg/kg). The
188 offspring were then tested at multiple time points (early development, adolescence, and
189 adulthood) for lasting neurobehavioral consequences after exposure during gestation.
190 Both dose and sex related differences where observed in motor coordination, circadian
191 activity patterns, and exploratory behavior (Shiotani et al., 2017). This study was
192 particularly valuable in that the oral exposure doses in pregnant mice were very low at 1
193 and 3 mg/kg and comparable to predicted human exposure levels of 0.045 (Costa et al.,
194 2010)and 0.075 mg/kg (Ferriss et al., 2017) especially in light of the fact that humans
195 exhibit excitotoxic symptoms at oral DA doses at least ten times lower than rodents
196 (Iverson et al., 1989; Lefebvre and Robertson, 2010; Perl et al., 1990; Teitelbaum et al.,
197 1990; Todd, 1993). Persistent neurobehavioral effects observed at these low maternal
198 doses not only emphasizes that sea lion offspring are likely impacted due to the
199 prevalence of DA in sea lion prey, but also suggests risks of fetal exposure in human
200 seafood consumers (Ferriss et al., 2017; Grattan et al., 2016).

201 **Conclusion:**

202 California sea lion fetuses are exposed to DA during harmful algal bloom events.
203 The varying levels of DA in fetal fluids with highest levels in allantoic fluid suggest that
204 DA is excreted by the fetal kidney to the urine and then to the allantoic sac. Domoic acid
205 is also detected in amniotic fluid after passage through the fetal gastro-intestinal tract. Re-
206 ingestion of amniotic fluid by the fetus results in sustained exposure of the fetus

throughout gestation (Figure 4). Sustained DA exposure *in utero* presents an increased exposure risk to developing fetuses when pregnant animals are exposed to DA in their diet that likely exceeds the risk to post-natal animals. It also indicates that even sub-clinical exposures to pregnant females could have lasting health impacts on offspring. These findings in naturally exposed CSLs have implications for health risks in other marine mammal species as well as for human seafood consumers.

Acknowledgements: This research was supported by the National Institutes of Health (NIH) R01 ES021930 (to DJM and KAL), the National Science Foundation (NSF) OCE-1314088 (to DJM and KAL) and the NIH R01 ES023043 (to NI). California sea lion samples were obtained from the Marine Mammal Center in Sausalito, CA and analyzed in Seattle, WA at the Wildlife Algal Toxins Research and Response Network (WARRN-West/Northwest Fisheries Science Center) and at the Department of Pharmaceutics (University of Washington).

Figure 1: Pie graphs showing the proportion of samples containing quantifiable levels of domoic acid (gray shaded area) for each of the five sample matrices examined; amniotic

fluid (n=24), allantoic fluid (n=9), urine (n=4), meconium (n=17), and stomach contents (n=21).

Figure 2: Domoic acid (DA) levels detected in allantoic and amniotic fluid samples from the four California sea lion (CSL) fetuses that had quantifiable levels of toxin in both corresponding fluids. Domoic acid levels were consistently higher in allantoic fluids.

Figure 3: Linear regression of domoic acid (DA) levels quantified via Enzyme-Linked Immunosorbent Assays (ELISA) and Liquid Chromatography Mass Spectrometry (LC/MS-MS) for ten California sea lion fetal fluid samples ($p = 0.003$; R square = 0.69).

Figure 4: Schematic of domoic acid (DA) transfer from maternal blood to the developing fetus followed by recirculation of DA through fetal fluids and continuous exposure to the fetal brain. Curved arrows represent potential pathways of recirculation. Recirculation via meconium would only occur under fetal stress.

References:

Andjelkovic, M., Vandevijvere, S., Van Klaveren, J., Van Oyen, H., Van Loco, J., 2012. Exposure to domoic acid through shellfish consumption in Belgium. Environment International 49, 115-119.

Bates, S.S., 2000. Domoic acid-producing diatoms: Another genus added! Journal of Phycology 36(6), 978-983.

Bejarano, A.C., Gulland, F.M., Goldstein, T., St Leger, J., Hunter, M., Schwacke, L.H., VanDolah, F.M., Rowles, T.K., 2008a. Demographics and spatio-temporal signature of the biotoxin domoic acid in California sea lion (Zalophus californianus) stranding records. Mar Mammal Sci 24(4), 899-912.

Bejarano, A.C., VanDola, F.M., Gulland, F.M., Rowles, T.K., Schwacke, L.H., 2008b. Production and toxicity of the marine biotoxin domoic acid and its effects on wildlife: A review. Human and Ecological Risk Assessment 14, 544-567.

Berman, F.W., Murray, T.F., 1997. Domoic acid neurotoxicity in cultured cerebellar granule neurons is mediated predominantly by NMDA receptors that are activated as a consequence of excitatory amino acid release. J Neurochem 69(2), 693-703.

Brodie, E.C., Gulland, F.M.D., Greig, D.J., Hunter, M., Jaakola, J., St Leger, J., Leighfield, T.A., Van Dolah, F.M., 2006. Domoic acid causes reproductive failure in california sea lions (Zalophus californianus). Mar Mammal Sci 22(3), 700-707.

Costa, L.G., Giordano, G., Faustman, E.M., 2010. Domoic acid as a developmental neurotoxin. Neurotoxicology 31(5), 409-423.

Dakshinamurti, K., Sharma, S.K., Sundaram, M., Watanabe, T., 1993. Hippocampal changes in developing postnatal mice following intrauterine exposure to domoic acid. J Neurosci 13(10), 4486-4495.

Doucette, T.A., Tasker, R.A., 2016. Perinatal domoic acid as a neuroteratogen. Current Topics in Behavorial Neuroscience 29, 87-110.

Ferriss, B.E., Lefebvre, K.A., Ayres, D., Borchert, J., Marcinek, D.J., 2017. Acute and chronic dietary exposure to domoic acid in recreational harvesters: a survey of shellfish consumption behavior. Environment International 101, 70-79.

Goldstein, T., Mazet, J.A., Zabka, T.S., Langlois, G., Colegrove, K.M., Silver, M., Bargu, S., Van Dolah, F., Leighfield, T., Conrad, P.A., Barakos, J., Williams, D.C., Dennison, S., Haulena, M., Gulland, F.M., 2008. Novel symptomatology and changing epidemiology of domoic acid toxicosis in California sea lions (Zalophus californianus): an increasing risk to marine mammal health. Proc Biol Sci 275(1632), 267-276.

Grattan, L.M., Boushey, C., Tracy, K., Trainer, V.L., Roberts, S.M., Schluterman, N., Morris, J.G., Jr., 2016. The association between razor clam consumption and memory in the CoASTAL cohort. Harmful Algae 57(Part B, Sp. Iss. SI), 20-25.

Iverson, F., Truelove, J., Nera, E., Tryphonas, L., Campbell, J., Lok, E., 1989. Domoic acid poisoning and mussel-associated intoxication: preliminary investigations into the response of mice and rats to toxic mussel extract. Food Chem Toxicol 27(6), 377-384.

James, K.J., Gillman, M., Amandi, M.F., Lopez-Rivera, A., Puente, P.F., Lehane, M., Mitrovic, S., Furey, A., 2005. Amnesic shellfish poisoning toxins in bivalve molluscs in Ireland. Toxicon 46(8), 852-858.

Lefebvre, K.A., Bargu, S., Kieckhefer, T., Silver, M.W., 2002. From sanddabs to blue whales: the pervasiveness of domoic acid. Toxicon 40(7), 971-977.

Lefebvre, K.A., Kendrick, P.S., Ladiges, W.C., Hiolski, E.M., Ferriss, B.E., Smith, D.R., Marcinek, D.J., 2017. Chronic low-level exposure to the common seafood toxin domoic acid causes cognitive deficits in mice. Harmful Algae 64, 20-29.

Lefebvre, K.A., Robertson, A., 2010. Domoic acid and human exposure risks: a review. Toxicon 56(2), 218-230.

299 Levin, E.D., Pizarro, K., Pang, W.G., Harrison, J., Ramsdell, J.S., 2005. Persisting
300 behavioral consequences of prenatal domoic acid exposure in rats. Neurotoxicology
301 and teratology 27(5), 719-725.
302 Marien, K., 1996. Establishing tolerable dungeness crab (Cancer magister) and razor
303 clam (Siliqua patula) domoic acid contaminant levels. Environmental Health
304 Perspectives 104(11), 1230-1236.
305 Maucher Fuquay, J., Muha, N., Wang, Z., Ramsdell, J.S., 2012. Toxicokinetics of domoic
306 acid in the fetal rat. Toxicology 294(1), 36-41.
307 McCabe, R.M., Hickey, B.M., Kudela, R.M., Lefebvre, K.A., Adams, N.G., Bill, B.D.,
308 Gulland, F.D.M., Thomson, R.E., Cochlan, W.P., Trainer, V.L., 2016. An
309 unprecedented coastwide toxic algal bloom linked to anomalous ocean conditions.
310 Geophysical Research Letters 43.
311 Perl, T.M., Bedard, L., Kosatsky, T., Hockin, J.C., Todd, E.C., Remis, R.S., 1990. An
312 outbreak of toxic encephalopathy caused by eating mussels contaminated with
313 domoic acid. N Engl J Med 322(25), 1775-1780.
314 Ramsdell, J.S., Zabka, T.S., 2008. In utero domoic acid toxicity: A fetal basis to adult
315 disease in the california sea lion (Zalophus californianus). Marine Drugs 6(2), 262-
316 290.
317 Scholin, C.A., Gulland, F., Doucette, G.J., Benson, S., Busman, M., Chavez, F.P.,
318 Cordaro, J., DeLong, R., De Vogelaere, A., Harvey, J., Haulena, M., Lefebvre, K.,
319 Lipscomb, T., Loscutoff, S., Lowenstine, L.J., Marin, R., 3rd, Miller, P.E., McLellan,
320 W.A., Moeller, P.D., Powell, C.L., Rowles, T., Silvagni, P., Silver, M., Spraker, T.,
321 Trainer, V., Van Dolah, F.M., 2000. Mortality of sea lions along the central
322 California coast linked to a toxic diatom bloom. Nature 403(6765), 80-84.
323 Shiotani, M., Cole, T.B., Hong, S., Park, J.J.Y., Griffith, W.C., Burbacher, T.M.,
324 Workman, T., Costa, L.G., Faustman, E.M., 2017. Neurobehavioral assessment of
325 mice following repeated oral exposures to domoic acid during prenatal development.
326 Neurotoxicology and teratology.
327 Suzuki, C.A., Hierlihy, S.L., 1993. Renal clearance of domoic acid in the rat. Food Chem
328 Toxicol 31(10), 701-706.
329 Talent, L.G., Talent, C.L., 1975. An extrauterine fetus in a Steller sea lion, *Eumetopias*
330 *jubata*. California Fish and Game 61, 233-234.
331 Tanemura, K., Igarashi, K., Matsugami, T.R., Aisaki, K., Kitajima, S., Kanno, J., 2009.
332 Intrauterine environment-genome interaction and children's development (2): Brain
333 structure impairment and behavioral disturbance induced in male mice offspring by a
334 single intraperitoneal administration of domoic acid (DA) to their dams. The Journal
335 of toxicological sciences 34 Suppl 2, Sp279-286.
336 Teitelbaum, J.S., Zatorre, R.J., Carpenter, S., Gendron, D., Evans, A.C., Gjedde, A.,
337 Cashman, N.R., 1990. Neurologic sequelae of domoic acid intoxication due to the
338 ingestion of contaminated mussels. N Engl J Med 322(25), 1781-1787.
339 Todd, E.C.D., 1993. Domoic acid and amnesic shellfish poisoning: A review. Journal of
340 Food Protection 56(1), 69-83.
341 Wright, J.L.C., Boyd, R.K., Freitas, A., Falk, M., Foxall, R.A., Jamieson, W.D., Laycock,
342 M.V., McCulloch, A.W., McInnes, A.G., et al., 1989. Identification of domoic acid, a
343 neuroexcitatory amino acid, in toxic mussels from eastern Prince Edward Island. Can.
344 J. Chem 67(3), 481-490.

345 Zuloaga, D.G., Lahvis, G.P., Mills, B., Pearce, H.L., Turner, J., Raber, J., 2016. Fetal
346 domoic acid exposure affects lateral amygdala neurons, diminishes social
347 investigation and alters sensory-motor gating. Neurotoxicology 53, 132-140.
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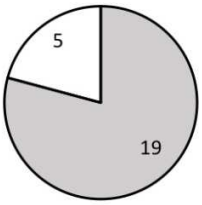
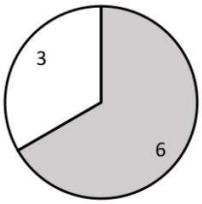


Amniotic fluid



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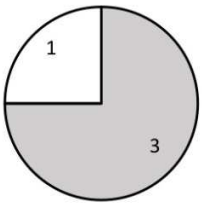
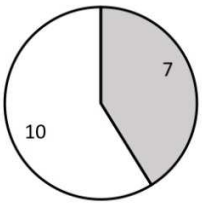
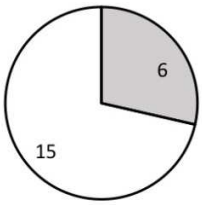


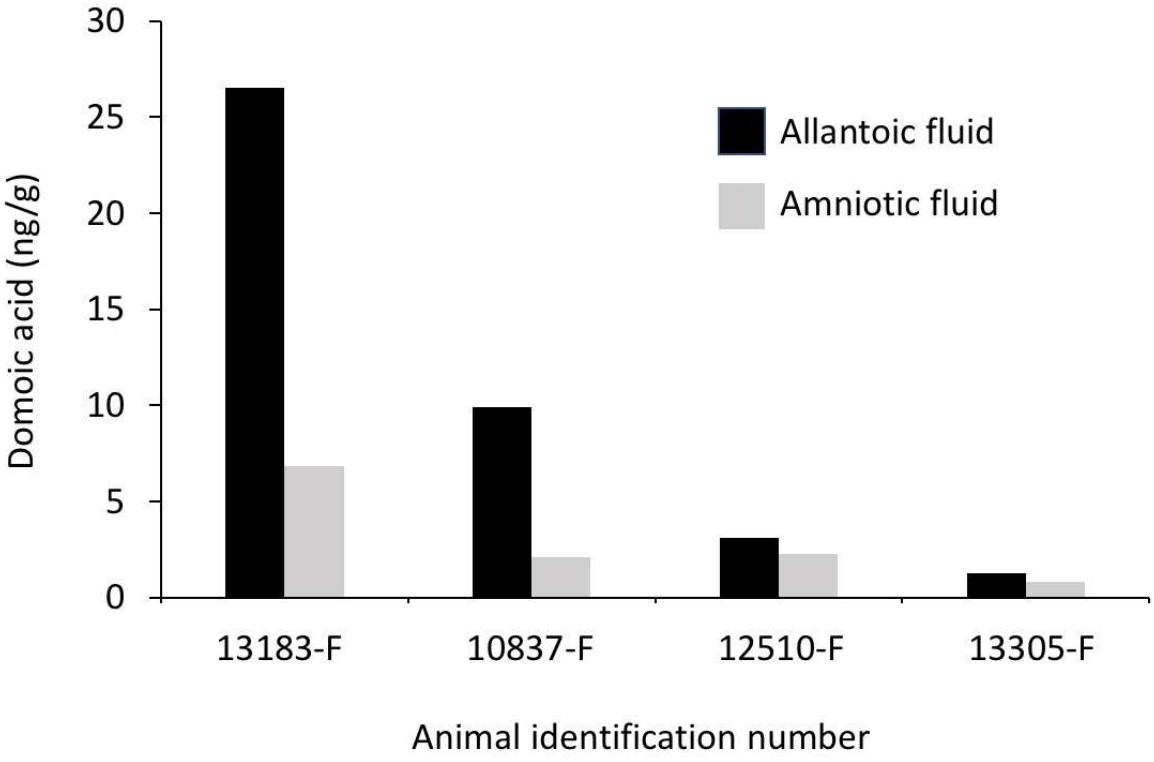
Urine



Meconium



Stomach content


30
25
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15
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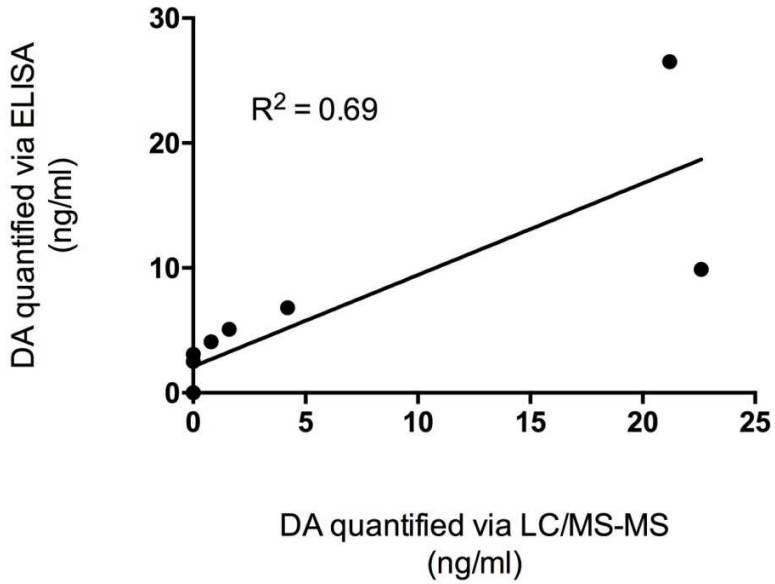
Allantoic fluid
Amniotic fluid
13183-F
10837-F
12510-F
13305-F
Animal identification number


30
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DA quantified via ELISA
(ng/ml)
DA quantified via LC/MS-MS
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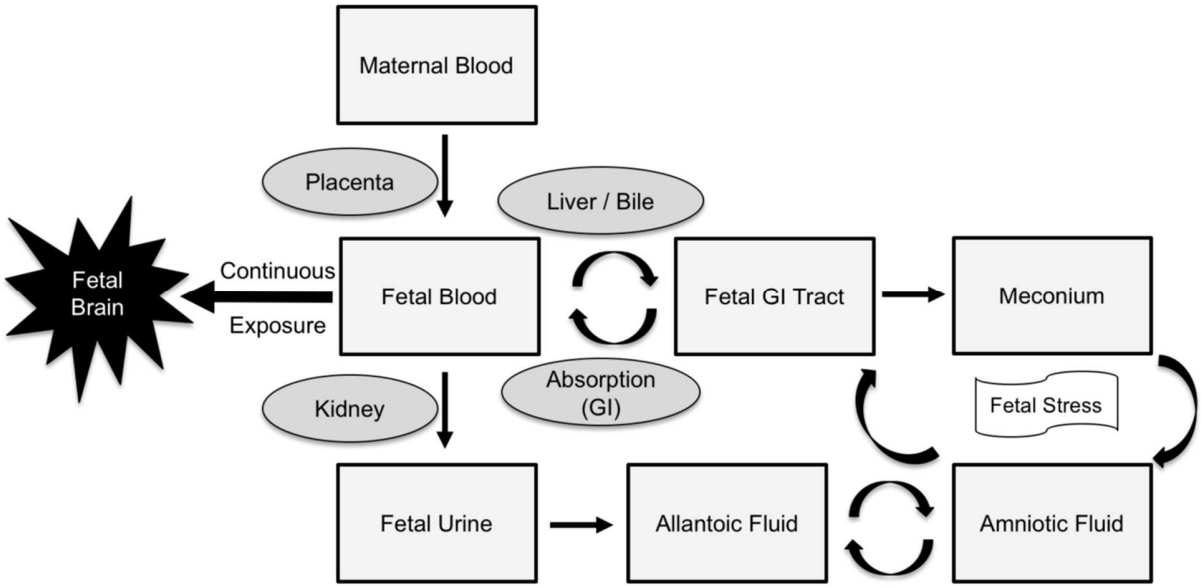
Maternal Blood
Placenta
Liver / Bile
Fetal Brain
Continuous
Exposure
Fetal Blood
Fetal GI Tract
Meconium
Absorption
(GI)
Fetal Stress
Kidney
Fetal Urine
Allantoic Fluid
Amniotic Fluid

Table 1: Domoic acid (DA) levels quantified in samples from 36 California sea lion fetuses analyzed via Enzyme-Linked Immunosorbent Assays (ELISA). Ten samples were also analyzed via Liquid Chromatography Mass Spectrometry (LC/MS). DA concentrations are in ng/ml. LC/MS confirmation values are shown in parentheses. ND = not detected. +BLQ = DA detected but below the level of quantification.

Fetus ID #	Amniotic fluid	Allantoic fluid	Urine	Meconium	Stomach Content
10514-F	**1.2**	*ND (ND)*	**0.5**	*ND*	*ND*
10487-F	*ND (ND)*	**0.4**	**0.4**	**10.9**	
13183-F	**6.8 (4.2)**	**26.5 (21.2)**		**8.8**	**8.9**
13305-F	**0.8**	**1.3**		**4.6**	**3.1**
12006-F	**0.6**	*ND*		*ND*	*ND*
10837-F	**2.1**	**9.9 (22.6)**		*ND*	**2.3**
12817-F	**0.8**	*ND*			*ND*
10787-F	*ND*			*ND*	*ND*
13020-F			*ND (ND)*	*ND*	*ND*
10532-F			**5.9**	**8.6**	*ND*
12510-F	**2.3**	**3.1 (ND)**			
7159-F	*ND*				*ND*
6855-F	**4.1 (+BLQ)**				*ND*
6856-F	**1.9**				**3.3**
11478-F	**0.5**				**2.3**
7167-F	*ND*				*ND*
10840-F				*ND*	*ND*
10889-F				*ND*	*ND*
11114-F				**6.0**	**2.8**
12846-F				*ND*	*ND*
11131-F				**8.9**	*ND*
10909-F				*ND*	*ND*
6451-F	*ND*				
10708	**7.8**				
10879-F	**0.8**				
12793-F	**0.6**				
12806-F	**0.6**				
6857-F	**3.8**				
6860-F	**0.5**				
7147-F	**5.1 (+BLQ)**				
7160-F	**0.5**				
7616-F	**0.4**				
10832-F		**2.5 (ND)**			
11143-F				**57.5**	
11003-F				*ND*	
10503-F					*ND*