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Domoic acid in milk of free living California marine mammals indicates lactational exposure occurs

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Domoic acid (DA) is a marine biotoxin produced by some toxicogenic species of the diatom, *Pseudo-nitzschia*, that appears to be blooming more frequently along the west coast of the United States (Fryxell *et al*. 1997, Van Dolah 2000). This increase is a concern for marine mammal health because DA is an excitatory amino acid that has a high affinity for the α-amino-5-hydroxy-3-methyl-4-isoxazole propionic acid (AMPA) and kainate subclasses of glutamate receptors that are present in the mammalian central nervous system and heart. The interaction of DA and these glutamate receptors causes cell depolarization, dysfunction, and death, resulting in seizures, epilepsy, cardiomyopathy, and death depending upon the ingested dose (Olney *et al*. 1979, Jeffery *et al.* 2004). In humans, DA is the cause of a neurotoxic illness termed amnesic shellfish poisoning (ASP), first recognized in Canada in 1987 following consumption of contaminated shellfish (Perl *et al*. 1990). In marine mammals, DA toxicosis is best documented in California sea lions (*Zalophus californianus*) that develop severe neurological signs associated with acute and long term effects of DA exposure (Scholin *et al*. 2000, Gulland *et al.* 2002, Goldstein *et al*. 2008). Lesions in affected sea lions include neuronal necrosis in the hippocampus, which may progress to hippocampal atrophy (Silvagni *et al*. 2005, Goldstein *et al*. 2008) and degenerative cardiomyopathy (Zabka *et al.* 2009). More recently DA toxicosis was reported in northern fur seals (*Callorhinus ursinus*; Lefebvre *et al*. 2010), harbor seals (*Phoca vitulina*; McHuron *et al*. 2013) and southern sea otters (*Enhydra lutris nereis*; Kreuder *et al*. 2003) off the California coast. The toxin has been detected in feces of live blue (*Balaenoptera musculus*) and humpback (*Megaptera novaeangliae*) whales (Lefebvre *et al*. 2002), stranded pygmy and dwarf sperm whales (*Kogia* spp.; Fire *et al.* 2009), and cetacean carcasses. In 2002 common dolphin (*Delphinus* spp.) strandings in southern California

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were temporally associated with DA producing *Pseudo-nitzschia* blooms (Torres de la Riva *et al*. 2009). To date, the lesions associated with DA exposure in cetaceans have not been characterized.

California sea lions (CSL) have stranded along the California coastline over the last 15 yr due to DA toxicosis (Scholin *et al*. 2000, Gulland *et al*. 2002). Domoic acid exposure also causes reproductive failure in CSL, and DA intoxicated females admitted to The Marine Mammal Center (TMMC) during two algal blooms in 1998 and 2002 showed improved clinical signs after abortion or premature birth of their pups (Brodie *et al*. 2006, Goldstein *et al.* 2009). Domoic acid was detected in sea lion maternal urine, fetal gastrointestinal contents and urine, and amniotic fluid, demonstrating that DA can be transferred to developing fetuses *in utero* (Brodie *et al*. 2006). *Pseudo-nitzschia* blooms occur during the summer months off the central California coast which coincides with the breeding season of the CSL (Ramsdell and Zabka 2008). On San Miguel Island, California, 64 premature CSL pups were sampled in 2005 and 2006 and DA was detected in 17% of them. In three cases, the most significant lesions were brain edema and placental abruption, as seen in DA-exposed adults that aborted fetuses in rehabilitation, suggesting DA can cause spontaneous abortion (Brodie *et al*. 2006, Goldstein *et al*. 2009). Controlled studies with laboratory mice have demonstrated that DA can cross the placenta, causing progressive hippocampal damage 14 d postnatally (Dakshinamurti *et al*. 1993). Mice studies have also shown oral dosing results in DA in milk, causing developmental effects in young rodents (Maucher and Ramsdell 2005, 2007). Although *in utero* exposure of marine mammals has been demonstrated (see above), lactational transfer of DA in marine mammals has not been examined to date.

To determine the potential for lactational transfer of DA in naturally exposed marine mammals, and hence for effects of exposure on developing young animals, a range of samples was collected opportunistically from 54 stranded marine mammals admitted to TMMC in California. Eleven of those animals were either pregnant or the uterus showed signs of recent parturition at necropsy. Between 2005 and 2013, samples of milk, urine, feces, bile, stomach contents, serum, aqueous humor, and amniotic fluid were collected at necropsy and stored at −80°C until processing from CSL (n = 42), harbor seals (n = 1), northern fur seals (n = 2), and harbor porpoises (*Phocoena phocoena*) (n = 9). The majority of samples were collected within one week of stranding, but some as late as 52 d after live stranding and rehabilitation.

Samples were shipped on dry ice to the NOAA NMFS Northwest Fisheries Science Center in Seattle, Washington, where they were extracted and tested for DA using the Biosense enzyme-linked immunosorbent assay (ELISA) that was previously evaluated for matrix effects as described in Lefebvre *et al*. (2010). The limits of detection for DA in each sample type analyzed in this study were: 4.0 ng/g feces, 0.4 ng/mL urine, 1.0 ng/mL milk, 4.0 ng/g intestinal contents, 2.0 ng/g stomach contents, 4.0 ng/mL bile, 0.4 ng/mL amniotic fluid, and 0.4 ng/mL aqueous humor. Only results from animals with positive milk samples are reported here.

Domoic acid was detected in 54 milk samples tested at concentrations ranging from 1.0 to 180.3 ng/mL, and in 72% of these animals DA was also detected in other body fluids (Table 1, 2). A harbor porpoise (C-351) had the highest milk concentration of DA, but no other sample was available for this animal. Harbor porpoise milk sample concentrations ranged from 1.1 to 180.3 ng/mL. Two of these animals (C-307 and C-352) also tested positive in other samples. All of these animals stranded dead so no clinical signs were observed. Both fur seals stranded and died after exhibiting signs of DA toxicosis (Lefebvre *et al*. 2010). The harbor seal (HS 2171) had

Table 1. Samples tested for domoic acid in California sea lions. ns = no sample taken. *bdl* = below detectable limit. Levels in feces are reported in ng/g and levels in milk, urine, and bile are reported in ng/mL.

Animal ID	Milk	Feces	Urine	Bile	Strand date	Sample date
CSL 9218	142.64	56,970	19,340	110.5	11 October 2009	14 October 2009
CSL 10348	103.74	26,480	ns	ns	30 June 2012	2 July 2012
CSL 9096	102.60	71,804	9,635	ns	17 September 2009	21 September 2009
CSL 9770	46.90	134,178	196.7	344.7	14 July 2010	19 July 2010
CSL 9194	36.53	30,109	ns	85.1	7 October 2009	9 October 2009
CSL 9204	35.22	5,945	ns	135.8	8 October 2009	12 October 2009
CSL 9099	26.08	ns	ns	ns	18 September 2009	21 September 2009
CSL 9196	22.96	9,256	ns	341.0	8 October 2009	10 October 2009
CSL 9303	22.24	33,235	50.3	*bdl*	29 November 2009	5 December 2009
CSL 9198	19.73	18,672	77.9	*bdl*	8 October 2009	12 October 2009
CSL 6717	17.6	ns	854.0	ns	7 August 2005	11 August 2005
CSL 10064	15.17	ns	ns	ns	17 August 2011	23 August 2011
CSL 9998	14.90	60.6	ns	ns	14 July 2011	15 July 2011
CSL 9187	14.10	365.4	1,880	*bdl*	4 October 2009	10 October 2009
CSL 9221	10.35	9,950	ns	*bdl*	11 October 2009	17 October 2009
CSL 6857	9.9	1,612	254	ns	19 February 2013	21 February 2013
CSL 9164	9.49	29,473	ns	39.4	29 September 2009	2 October 2009
CSL 9203	8.39	ns	ns	110.4	8 October 2009	10 October 2009
CSL 9274	8.32	ns	ns	23.4	31 October 2009	14 November 2009
CSL 9304	7.98	116.4	8.6	15.8	30 November 2009	3 December 2009
CSL 10241	7.20	ns	10.4	*bdl*	12 November 2011	17 November 2011
CSL 10241	–	ns	406.5	ns	12 November 2011	13 November 2011
CSL 9597	6.21	ns	ns	ns	1 June 2010	26 July 2010
CSL 9299	5.94	11,536	5.2	15.0	27 November 2009	3 December 2009
CSL 6791	5.7	ns	5.9	ns	13 September 2005	23 September 2005
CSL 8681	5.6	5.800	ns	ns	9 July 2009	12 July 2009
CSL 6792	5.2	146.6	296	ns	13 September 2005	20 September 2005
CSL 6687	5.2	4.0	ns	ns	30 July 2005	2 August 2005
CSL 9206	4.92	30.8	2.3	*bdl*	9 October 2009	19 October 2009
CSL 6793	4.7	1,075	ns	ns	13 September 2005	20 September 2005
CSL 9976	4.60	110.8	ns	ns	22 June 2011	24 June 2011
CSL 7448	4.5	ns	ns	ns	1 September 2007	4 September 2007
CSL 9254	3.20	633.7	12.4	*bdl*	20 October 2009	26 October 2009
CSL 9987	3.20	*bdl*	ns	*bdl*	2 July 2011	18 July 2011
CSL 9205	2.84	4,342	10.5	20.4	9 October 2009	12 October 2011

(Continued)

Table 1. (Continued)

Animal ID	Milk	Feces	Urine	Bile	Strand date	Sample date
CSL 8999	2.29	4,388	ns	ns	23 August 2009	4 September 2009
CSL 7438	2.16	35.7	20.9	ns	26 August 2007	30 August 2007
CSL 7326	2.1	ns	ns	ns	7 July 2007	9 July 2007
CSL 7779	2.0	ns	ns	ns	24 July 2008	25 July 2008
CSL 9157	1.94	126.6	14.3	*bdl*	27 September 2009	30 September 2009
CSL 7914	1.7	ns	ns	ns	19 September 2008	23 September 2008
CSL 8784	1.0	ns	ns	ns	24 July 2009	25 July 2009
CSL 6921	1.0	43.0	6.0	–	1 July 2006	2 July 2006

clinical signs of acute DA toxicosis, chronic hippocampal lesions found upon histopathologic examination and milk and urine samples with low concentrations of DA. Domoic acid concentrations in CSL milk samples ranged from 1.0 to 142.6 ng/mL.

These findings demonstrate that DA is present in the milk of naturally exposed marine mammals; hence exposure of young suckling mammals occurs. The estimated daily milk intake during the first month for suckling male and female CSL is 723 g and 609 g, respectively (Oftedal *et al*. 1987). Although the oral dose of DA that causes acute symptoms in marine mammals has not been experimentally determined, in humans, a minimum lethal dose is 2.71 mg/kg for a 70 kg individual (Perl *et al*. 1990). In neonatal rats, a low dosage of 0.2 mg/kg induced seizures (Xi *et al*. 1997). Given the highest concentration of DA in a milk sample from a CSL reported here of 142.6 ng/mL for a male pup ingesting approximately 700 mL of milk per day (see above), this would represent a daily dose of 0.126 mg DA, hence a dose of 0.015 mg/kg (based on an average 8.5 kg pup (Oftedal *et al*. 1987). Although well below the lethal dose, if this was ingested more than just once, it is possible that this low dose exposure may have effects on development, as studies in laboratory rodents have shown perinatal animals are sensitive to effects of DA when exposed both pre- and postnatally (Xi *et al*. 1997, Maucher and Ramsdell 2007, Stewart 2010). While many laboratory experiments are based on a one-time dosage (Xi *et al*. 1997, Maucher and Ramsdell 2007), nursing marine mammals may be continually exposed to a low dose of DA for the duration of their nursing period, so the cumulative exposure could be well above the single dose determined to have effects in experimental exposure studies.

Milk may also act as a reservoir of DA as it is not removed from the body without nursing. A fecal sample from a sea lion in this study (CSL 9987) did not have detectable levels of DA, but DA was detected in the milk collected on the same day, suggesting the animal may have cleared the DA from the gastrointestinal tract through excretion but it remained in the milk. A milk sample from one animal (CSL 9597) was collected 52 d after it was admitted into rehabilitation, hence since last potential ingestion, demonstrating persistence of DA in milk. An alternative explanation for this animal is that it was exposed to DA during rehabilitation due to exposure of DA in fish ingested during that time. This is unlikely, as the frozen herring used to feed animals in rehabilitation is sold fit for human consumption, and no animals fed it have shown signs of DA toxicosis when at TMMC. Most animals in this study did not eat fish at TMMC prior to sample collection for DA testing, as 87% ($n = 47$) of the animals were either admitted dead or exhibiting signs of DA toxicosis.

Table 2. Samples tested for domoic acid in other marine mammals. *Phph* = harbor porpoise, HS = harbor seal, and NFS = northern fur seal. ns = no sample taken. *bdl* = below detectable limit. Levels in feces and stomach contents are reported in ng/g and levels in urine, milk, bile, and aqueous humor are reported in ng/mL.

Animal ID	Milk	Feces	Urine	Bile	Aqueous humor	Stomach contents	Strand date	Sample date
C-351 *Phph*	180.3	ns	ns	ns	ns	ns	31 August 2011	2 September 2011
C-350 *Phph*	37.8	ns	ns	ns	ns	1,352	26 August 2011	26 August 2011
C-348 *Phph*	13.3	ns	ns	ns	ns	ns	18 August 2011	19 August 2011
C-352 *Phph*	5.6	ns	ns	11.7	ns	ns	14 September 2011	16 September 2011
C-366 *Phph*	5.2	ns	ns	ns	ns	ns	7 September 2012	7 September 2012
C-236 *Phph*	2.2	ns	ns	ns	ns	ns	6 June 2007	6 June 2007
C-300 *Phph*	1.9	ns	ns	ns	ns	ns	24 July 2009	25 July 2009
C-307 *Phph*	1.9	9.5	ns	ns	ns	ns	18 August 2009	19 August 2009
C-260 *Phph*	1.1	ns	ns	ns	ns	ns	21 June 2008	22 June 2008
NFS 153	154.6	6,730	ns	ns	248.8	64.9	29 July 2005	30 July 2005
NFS 163	1.3	5.0	ns	ns	ns	ns	20 August 2005	28 August 2005
HS 2171	2.2	*bdl*	0.8	*bdl*	ns	ns	15 May 2011	16 May 2011

Note: all cetaceans stranded dead.

In conclusion, this study demonstrates the presence of DA in the milk of several marine mammal species off California, and its persistence in milk for many days after oral ingestion, suggesting exposure of perinatal animals during early development occurs. Understanding the extent of transfer of DA to developing young will help further understanding of the long term effects of DA on development and the health of the newborn pup.

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