

Toxicokinetics of domoic acid in a  
nonhuman primate model (*Macaca fascicularis*)

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ABSTRACT

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Domoic acid (DA), a potent neurotoxin produced by algae from the *Pseudo-nitzschia* family, presents an emerging threat to public health. Human consumption of shellfish with high concentrations of DA may result in Amnesiac Shellfish Poisoning, a syndrome with symptoms including short-term memory loss, seizures, and death. As a result, current regulations limit consumption of DA to 0.075 mg/kg/day. However, recent work has demonstrated that DA acts as a neurodevelopmental toxin in fetal and neonatal rodents, even at low doses. Yet how these effects translate to humans is unknown. An essential first step to determining if DA is a human developmental neurotoxin is understanding the basic maternal-fetal disposition of DA. Thus as part of a larger reproductive and neurodevelopmental study, this study aimed to determine the bioavailability, elimination, and distribution kinetics of a low dose of DA in a species closely related to humans, to eventually model and predict human dose-exposure relationships. Three healthy, nonpregnant, adult, female *Macaca fascicularis* were intravenously injected with 0.005 mg/kg of DA in our initial study. In two follow-up studies, the same 3 females were administered oral doses of 0.15 mg/kg DA, double the tolerable daily intake (TDI) in humans, and then 0.075 mg/kg DA. Plasma was collected up to 48 hours after dosing, and DA concentrations were measured by LC-MS/MS. The i.v. study produced data consistent with findings from previous work, with no visible observed side effects. The i.v. disposition of DA

showed two-compartmental kinetics with a terminal half-life of one hour and levels below the limit of quantification (0.622  $\mu\text{g/mL}$ ) at four hours. Following oral administration, the exposure of DA was prolonged due to rate-limiting slow absorption, with a terminal half-life greater than 15 hours. The oral bioavailability of DA was 9-12%. From the pattern of DA disposition observed in these studies, we hypothesize that even low doses of DA will accumulate in plasma when administered chronically. Current studies using chronic exposures are underway to test this hypothesis. The first of its kind, this study not only demonstrated a higher oral bioavailability in primates than previously hypothesized, but also revealed that DA displays oral flip-flop kinetics characterized by a rate limiting absorption phase, resulting in the plasma accumulation of DA during chronic dosing but lower peak concentrations than would be expected if rapidly absorbed. Because current regulations for human consumption are based on the hypothesis that the exposure from DA is short, these results may indicate a higher risk for neurodevelopmental effects following in utero DA exposure.

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# **CHAPTER 1**

## **Introduction and Background**

## 1.1: Domoic Acid

### 1.1.1: General Background

Domoic acid (DA) is a small, water soluble, excitatory amino acid biotoxin (Fig. 1), often produced by members of the algal genus *Pseudo-nitzschia*.<sup>1</sup> DA is found in coastal habitats around the world.<sup>2</sup> If present in the water column, DA may accumulate in the digestive tissues of some bivalves, finfish, and crustaceans.<sup>3</sup> When ingested by higher trophic level organisms, including humans, sea lions, and some sea birds, DA acts a glutamate agonist, producing potent neurotoxic effects.<sup>4</sup> The environmental conditions that promote the production of DA are not entirely understood, but decreased nutrient concentrations seem to play a large role in determining the presence of DA.<sup>5</sup>

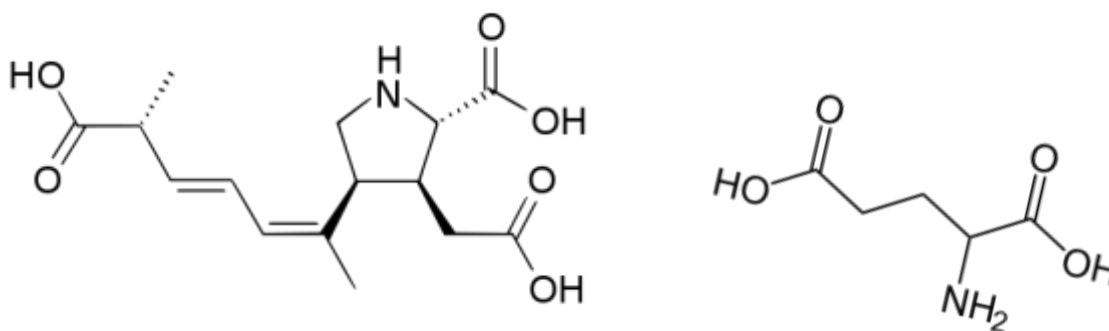


Figure 1: Shows the structures of domoic acid (L) and glutamic acid (R).

DA was first isolated in the macroalgal species *Chondria armata* in 1958 by Japanese scientists.<sup>6,7</sup> In historic Japan, seaweed containing DA was used as a home remedy to treat intestinal pinworms.<sup>7</sup> People consumed doses up to approximately 0.33 mg/kg without any observed negative side effects.<sup>6</sup> After a retrospective analysis of water samples, 1961 was identified as the first year in which widespread DA toxicity was recorded. Sea birds on the Pacific Coast were reported to be flying askew, aggressively attacking people, and dropping dead out of the air.<sup>8</sup> In 1987, a DA related mass poisoning on Prince Edward Island in Canada affected approximately 100 – 250 people who consumed contaminated shellfish.<sup>9,10</sup> The first identified cases were hospitalized with symptoms that included a sudden onset of confusion, disorientation, and memory loss within 48 hours of ingesting DA estimated to be between 0.9 – 2.0 mg/kg.<sup>9</sup> This illness was later named Amnesiac Shellfish Poisoning (ASP), as a tribute to its

hallmark symptom of memory loss. Postmortem examination of affected brain tissue revealed that most of the damage was concentrated in the hippocampus, a region of the brain essential for memory and balance. Morphological changes were described by lesions and atrophy in the hippocampus, some complete neuronal loss in the pyramidal neurons of the hippocampus, and decreased glucose metabolism in the medial temporal lobes.<sup>11</sup> Though no additional human poisonings have been recorded since 1987, sea animals, including many species of mammals and birds, continue to be affected by DA algal blooms.<sup>3</sup> Additionally, algal blooms have been steadily increasing worldwide in both frequency and duration.<sup>12</sup> In 2015, scientists documented the largest recorded DA HAB along the western coast of the United States, stretching from the southern reaches of California into Alaska.<sup>13</sup>

### 1.1.2: Human Health and Risk

Investigations into the details of the 1987 poisoning have revealed the potent neurotoxic properties of DA in humans. A regulatory standard of 20 ppm DA in shellfish or 0.075 mg/kg maximum daily intake have been adapted by many countries worldwide, but several assessments estimate that this limit may not be conservative enough to protect chronic consumers of shellfish.<sup>14-17</sup> Further, no studies have attempted to understand possible health effects of the low, chronic consumption of DA in humans, especially high risk populations. Outlined below is the limited history of DA toxicity in humans.

#### *Human Health Effects*

In 1987, between 100 and 250 people who consumed shellfish contaminated with DA reported strange symptoms after eating mussels from Prince Edward Island, Canada.<sup>9</sup> Patients typically began developing symptoms from 1 to 10 hours post-exposure, with most initially experiencing gastrointestinal related discomfort and illness, usually in the form of vomiting.<sup>10</sup> The mean hospital admitting time was between four and five and a half hours post-exposure.<sup>9,10</sup> No DA was detected in urine samples from these patients at the time of admitting, possibly due to rapid elimination of DA or poorly sensitive HPLC detection methods.<sup>10</sup>

The most severe outcomes were seen in those who consumed the higher estimated DA doses, up to 800 ppm in mussels or approximately 2 mg/kg.<sup>10</sup> Symptoms in these patients were marked by a wide variety of altered behavior, ranging from abnormal aggression, to seizures and comas. Most patients recovered and returned to normal behavior and general good health within 48 hours to 12 weeks post-exposure.<sup>18</sup> A subset of fourteen patients admitted to the hospital at the time of poisoning agreed to participate in a neurological study of long term toxic effects as a result of DA exposure.<sup>18</sup> Neurological testing 4 to 6 months post-exposure revealed that patients had developed an anterograde memory disorder, or an inability to create new short term memories or recall short-term memories from before the poisoning. Some patients also experienced severe retrograde amnesia affecting memories over 20 years old.<sup>18</sup> This study indicates that though ASP is usually a temporary syndrome, high doses of DA may cause long term problems, well after DA has been excreted.<sup>10,11,18</sup> Motor tests 4 to 6 months post-exposure revealed that 11 of the patients had also developed a distal atrophy and generalized muscle weakness, symptoms indicative of a motor neuronopathy or axonopathy.<sup>18</sup>

Four patients died from exposure to DA.<sup>10</sup> These four patients were between 71 and 84 years old, and died within the three months following exposure. All four experienced symptoms typical of acute DA poisoning directly following exposure, including tremors, shakes, seizures, and memory loss.<sup>11</sup> Postmortem histological analysis of the brain tissue from these patients showed shrunken eosinophilic neurons, vacuolization of neutrophils, enlarged astrocytes and astrogliosis concentrated in the H3 and H4 regions of hippocampus.<sup>11</sup> The H1 region showed some damage, yet had no changes in astrocyte number, size, or activity. Additionally, pathological analysis of the deceased showed significant necrosis in both the olfactory tubercle and the dentate gyrus regions of the brains.<sup>11</sup> Recent analyses hypothesized that decreased renal function may have made these people more sensitive to DA toxicity, possibly resulting in a high mortality rate in older adults.<sup>19,20</sup>

### *Current Regulations*

To minimize the risk of ASP and acute DA toxicity in humans, the FDA currently sets the tolerable daily limit (TDI) of DA at 0.075 mg/kg per person per day.<sup>17</sup> This regulatory estimate was based on the following equation:

$$TDI = \frac{NOAEL}{10}$$

The current TDI is based on an oral study in nonhuman primates. A no observed adverse effects level (NOAEL) of 0.75 mg/kg from an acute oral (p.o.) DA exposure was reported. A 10-fold safety factor to protect sensitive populations was used.<sup>21</sup> The calculation did not include a species conversion safety factor, as the lowest observed adverse effects levels (LOAEL) were similar in both humans and nonhuman primates (~1 mg/kg).<sup>21</sup> Thus the final equation is as follows:

$$TDI = \frac{0.75 \text{ mg/kg}}{10}$$

To estimate how this TDI translates into tolerable limits (TL) in Dungeness crab and razor clam tissue, shellfish consumers on the coast of Washington were surveyed to estimate consumption rates. In particular, the study focused on DA concentrations in the whole tissue of razor clams and Dungeness crab hepatopancreas and viscera, as these organisms are most frequently consumed on the Washington coast and often have the highest levels of DA concentrations.<sup>21-23</sup> The TL of DA in these organisms were calculated from the following formula, adjusted for bodyweight (BW) and consumption level ( $CL_{0.84}$ ):

$$TL = \frac{TDI \cdot BW}{CL_{0.84}}$$

To set a TL in razor clam tissue, the calculated TDI for an average adult (bodyweight of 70 kg), and the 84<sup>th</sup> percentile of surveyed clam consumption (270 g clam meat per meal) were included. This estimation established that razor clam tissues should not exceed 20 ppm DA.<sup>21</sup> The survey data detailing Dungeness crab consumption habits indicated that the upper 84<sup>th</sup> percentile of consumers consumed an average of 114 g of crab viscera and hepatopancreas in one meal. Thus the calculated TL of DA in crab organs was set at 31.5 ppm.<sup>21</sup> Many health organizations at the state, federal, and international levels have accepted a general tissue allowance of DA in seafood at 20 ppm as a direct result of this calculation.<sup>16,17,24,25</sup>

### *Regulatory Implications for Public Health*

Despite this accepted limit, several reports have suggested that a tolerable limit of 20 ppm may not fully protect human health (Table 1). The initial approach defining the current regulatory guidelines was limited by a number of assumptions.<sup>14</sup> These include the lack of dose-response data, as well as missing exposure data that may be gleaned from a pharmacokinetic study or a physiological based pharmacokinetic model. Further, these limitations reduce current regulations to only pragmatic guidelines, not strict risk analysis or toxicological based safety limits.<sup>14</sup>

In 1998, Slikker et al. used a nonhuman primate model to recalculate a TDI based on biological dose-response data, instead of data from single dose acute studies. He used a design with 24 *Macaca fascicularis*, also known as cynomolgus monkeys or crab-eating macaques, intravenously (i.v.) injected with DA (0.25 – 4 mg/kg).<sup>26</sup> They found that the LOAEL in this species was 0.5 mg/kg i.v. instead of the previously reported 0.75 mg/kg.<sup>26</sup> Additionally, using one tenfold safety factor to translate the LOAEL to the NOAEL, another tenfold safety factor for sensitive individuals, and a three-fold safety factor for nonhuman primate to human translation as shown in the equation below, they calculated that the safe limit of DA related toxicity in humans should be as low as 0.034 mg/kg.<sup>26</sup> This estimation also assumes a 5% bioavailability of DA when orally consumed by humans. Further calculations equated this TDI to a 12 ppm maximum allowed concentration of DA in 200 g of shellfish for a 70 kg adult.<sup>26</sup>

$$TDI = \frac{LOAEL_{nonhuman\ primates}}{10 \times 10 \times 3}$$

A benchmark dose (BMD) is also often used to limit risk of toxicity to the upper 10% of the population at risk. Many risk assessments now encourage a BMD calculation to maximize human safety.<sup>27</sup> Using this approach with the same safety factors as above, Slikker et al. calculated the dose that would limit excess risk to only 10% of the population. This calculated dose (ED<sub>10</sub>) was 0.26 mg/kg i.v. in macaques. In humans, assuming a 5% bioavailability, this

would translate into doses no higher than 0.018 mg/kg or 6.4 ppm in shellfish.<sup>26</sup> The formula is as follows:

$$BMD_{humans} = \frac{ED_{10,nonhuman\ primates}}{10 \times 10 \times 3}$$

Table 1: Shows estimations of allowable limits of DA. \* denotes current regulatory limits.

Maximum Dose	Shellfish Concentration	Method	Source
0.075 mg/kg	20 ppm*	LOAEL	Marien, 1996.
0.034 mg/kg	12 ppm	LOAEL	Slikker et al., 1998.
0.018 mg/kg	6.4 ppm	BMD	Slikker et al., 1998.

### High Risk Populations

The limited data on which current regulatory limits are based may be particularly harmful to select populations. Although the majority of people may not eat much shellfish or finfish from DA contaminated areas, there are many populations, like those of the First Nation or those from Korean or Japanese backgrounds, who consume large amounts of seafood as a result of cultural norms.<sup>1,3</sup> In addition to these cultures, people living on coasts or those who harvest shellfish out of economic necessity often chronically consume large amounts of shellfish, putting them at risk for exceeding regulatory limits of DA consumption.<sup>3,15</sup> A recent survey of Belgian shellfish consumption patterns was accompanied with tissue analysis of DA concentrations in shellfish from 2004-2009. Based on these data, the authors estimated that of all acute shellfish consumers, only 1% consumed DA above the recommended 20 ppm. However, an estimated 3.5-6% of chronic shellfish consumers may have consumed doses above the regulatory limit of 0.075 mg/kg per day.<sup>15,16</sup>

Within these populations, people with reduced renal clearance, such as older adults, may also be more sensitive to DA.<sup>19</sup> In the 1987 poisoning, the odds ratio for hospitalization in 105 patients

was 2.3 (1.7 – 4.0) for age per 10 year increment.<sup>10</sup> Males were also disproportionately affected, with an odds ratio for hospitalization of males to females at 16.9 (3.5 – 80.4).<sup>10</sup> These data suggest that one of the most sensitive adult populations to DA toxicity may be older males who chronically consume DA contaminated shellfish. Of additional concern are young and developing fetus and infants, as the neurodevelopmental processes are highly sensitive to any toxic assault.<sup>28</sup> Because the effects of exposure to DA during development in humans are unknown, developing fetuses and infants should be especially considered in other model species.

### 1.1.3: DA Neurotoxic Effects on Wildlife

In addition to neurotoxic effects in humans, DA affects many free ranging marine animals. DA HABs may lead to DA bioaccumulation in shellfish, finfish, and other filter feeders without any apparent neurotoxic effects in these invertebrates.<sup>5</sup> Once DA has accumulated in the digestive systems of filter feeders or finfish, it may be passed to higher trophic level predators.<sup>29</sup>

California sea lions, seabirds, and pelicans frequently show signs of DA related illness.<sup>30</sup> Other mammals have been documented with DA related illness or death, including whales, seals, otters, and dolphins.<sup>5,31</sup> Marine mammals such as these are important sentinel species in public health, as they share the same coastal environments and food sources as humans; thus their symptomology allows us to examine the effects of DA in naturally exposed living mammals.<sup>32</sup>

California sea lions (*Zalophus californianus*) affected by DA have been well documented since the first episode of widespread toxicity in this species in 1998.<sup>30,33,34</sup> Sea lions stranded on the California coast from 1999 to 2006 were admitted into the Marine Mammal Center, where they were monitored and treated.<sup>30</sup> Of 2963 total stranded animals, 715 were diagnosed with DA toxicosis and underwent extensive neurological testing and were treated for symptoms. DA was detected in the blood (0.004 -0.2 µg/mL), urine (0.01 – 3.72 µg/mL), or feces (1 – 82.02 µg/mL) of 54% of these animals.<sup>33</sup> Researchers at the Marine Mammal Center were able to discern two types of neurological disease associated with DA toxicity; acute DA toxicosis and a chronic epileptic syndrome.<sup>30</sup> Of the 715 admitted sea lions, 551 showed just classic symptoms of acute DA toxicosis, including ataxia, head weaving, vomiting, and seizures.<sup>30</sup> Symptoms in these animals subsided within a week. Additionally, 164 of the admitted sea lions progressed from

acute DA toxicosis to chronic epileptic syndrome determined by MRIs and EEGs.<sup>30</sup> These animals first displayed acute symptoms listed above, followed by a period free from any symptoms. Two or more weeks after admittance, these sea lions began experiencing intermittent seizures, marked by abnormal EEGs, lethargy, muscle spasms, central blindness, and irregular behavior.<sup>30</sup> Sea lions were asymptomatic between seizure episodes. Over time, seizures became more severe and frequent, resulting in a mortality rate of 54.3%, despite veterinary efforts to intervene.<sup>30</sup> Histological examination of deceased sea lions diagnosed with the chronic epileptic syndrome revealed lesions in both the hippocampal and parahippocampal gyrus regions.<sup>30</sup> Further analyses revealed that neuronal loss was followed by typical oligodendrogliosis and astrocytosis. No cell degeneration was seen in the eyes, despite symptoms of blindness during seizures.<sup>30</sup> Because sea lions consume much higher doses of DA with unquantifiable consumption rates and exposures, these data exemplify symptoms that are likely only associated with severe poisonings.

#### 1.1.4: Animal Models of Neurotoxicity

To better understand the neurotoxic effects of DA in humans, several experimental models have been used to determine the mechanisms of neurotoxicity, as well as specific outcomes associated with a predetermined and measured dose of DA. These models range from *in vitro* cell cultures of neuronal cells to *in vivo* studies in mice, rats, and nonhuman primates. *In vitro* models employ cell culture methods to determine the molecular action of DA. To determine the effects of DA *in vivo*, rodents and nonhuman primates have been experimentally exposed to DA via many routes, including direct application onto the brain, subcutaneous (s.c.), intravenous (i.v.), and intraperitoneal (i.p.) injections, and oral gavages (p.o.). To determine neurotoxic effects, researchers have used a combination of observational data to report symptoms, behavioral tests to examine memory, learning, and social changes, and postmortem tissue analysis to determine morphological changes. The following is a review of studies conducted to examine the neurotoxicological action of DA.

## **In Vitro Studies**

Several *in vitro* models have been used to determine the neurotoxic mode of action of DA. The *in vitro* models most commonly used to study DA toxicity are cerebellar granular neuronal cell cultures from mice and full rat hippocampal slices. Cerebellar cell cultures use either neuronal cells or astrocytes extracted from developing neonatal rodent brains to understand the exact mode of action *in vitro*. Hippocampal brain slices allow researchers to measure action potential, connectivity, and voltage created by neuronal action.

Cerebellar granular neuronal cultures dosed with 5 nM – 100 nM DA show that DA acts as a glutamate agonist and interacts with kainic acid (KA) and  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, also known as non-NMDA (N-methyl-D-aspartate) receptors.<sup>35,36</sup> The KA/AMPA receptors comprise a subfamily of neurotransmitter receptors that bind with excitatory amino acids like glutamate.<sup>37</sup> DA notably has a higher affinity for the KA receptors than KA or glutamate,<sup>1</sup> yet binds differently with different members of the glutamate receptor family, with the highest affinity towards “flip” versions of the receptors.<sup>38</sup> Most specifically, DA tends to initiate receptor activation primarily in ionotropic glutamate receptors with the GluK5 subunit.<sup>39</sup> Additionally, the glutamate receptor subfamily with the “flip” exons as well as the GluK5 subunit are located disproportionately in the hippocampus of the rodent brain, where most neurotoxic effects are usually seen.<sup>40</sup>

Pyramidal hippocampus neuronal cultures from neonatal Sprague Dawley rats dosed with concentrations as low as 5 nM activated non-NMDA glutamate receptors, inducing a small influx of sodium and depolarizing the cell.<sup>41</sup> Depolarization results in a similarly small influx of calcium by activating voltage-gated channels.<sup>41</sup> This small amount of calcium does not activate the release of glutamate, but instead lowers the mitochondrial potential, thus inducing mitochondrial oxidative stress.<sup>42</sup> Oxidative stress can trigger the opening of a mitochondrial membrane pore and release Cytochrome C, a protein essential for oxidative phosphorylation.<sup>42</sup> Caspase-3 may then be activated and in turn degrade PARP (poly ADP ribose polymerase), ultimately leading to a signal cascade triggering apoptosis. The P38 and Jun N-terminal kinase (Jnk) signaling cascades are also likely involved in DA-mediated apoptosis, but the exact mechanism is not yet clearly understood.<sup>1</sup> DA concentrations greater than 50 nM did not

produce the same calcium changes, suggesting that two receptors may be involved in DA toxicity, depending on the DA concentration.<sup>41</sup>

High concentrations of DA (greater than 100 nM) administered to cerebellar granular neuronal cultures from neonatal Sprague Dawley rats can similarly bind to KA/AMPA receptors on the neurons, but then initiate a subsequent large and rapid influx of calcium ions which prompts the release of glutamate. Glutamate activates other neurotransmitter receptors, like NMDA receptors, which in turn release more glutamate, causing a self-perpetuating cycle of neuronal excitation.<sup>43</sup> DA toxicity in granular cerebellar neuronal cell cultures is thus primarily controlled by the activation of NMDA receptors, despite DA's inability to directly bind to these receptors.<sup>44</sup> Depolarization from the activation of NMDA receptors may lead to intracellular calcium ion accumulation.<sup>44</sup> This large calcium ion concentration can lead to the production of reactive oxygen species (ROS).<sup>45</sup> Under normal circumstances, GSH is a primary scavenger of ROS. Increased ROS can lead to GSH depletion, thus reducing the normal defense against ROS-induced lipid peroxidation.<sup>42</sup> Thus, rapid lipid peroxidation can lead to cell death and necrosis, and thus reactive gliosis.<sup>46</sup>

The greatest effects of DA toxicity are typically in the CA3 and CA1 regions of rat hippocampus slices, where the KA/AMPA receptor density is highest.<sup>1</sup> Specifically, the effects from a single dose of DA were about 20 times more potent in the CA3 region when compared to the CA1 region.<sup>47</sup> Other studies examining effects from DA in hippocampal slices of rat brains showed that neurons dosed with DA were hyperexcited in the CA1 and CA3 regions, producing depolarizations similar to epileptic seizures, likely due to the overstimulation of neurons from excess glutamate.<sup>47</sup>

When comparing DA toxicity in slices from 3 month old rats to slices from 26-29 month old rats, hippocampal slices from young animals showed resiliency against typical DA effects, especially in the CA1 region. Hippocampal slices from older animals did not show any tolerance for DA, indicating that aged brains may not have neuroprotection needed to protect neuronal cells from DA toxicity that younger, but fully developed brains may have.<sup>20</sup>

## **In Vivo Studies**

The effects from DA exposure in adult mouse and rat models have been well studied. Most studies administer a single high dose of DA to adult rodents via an injection (subcutaneous, intraperitoneal, or intravenous), and examine observable symptoms of toxicity via behavioral observations or task performance and brain tissue morphology. Few rodent studies have examined *in vivo* effects of DA when orally administered at a low, environmentally relevant dose.<sup>3,48</sup>

Nonhuman primates can be similarly used as a model organism in which to study DA toxicity. However, only six studies have examined DA toxicity in nonhuman primates, despite this model sharing many physiological characteristics with humans.<sup>49-54</sup> Only one study examined how a chronic oral dose of DA may cause toxicity.<sup>53</sup> However, this study used a dose of 0.5 mg/kg, far above the TDI. Thus despite a general understanding of effects from an acute, high dose of DA, effects from low, oral, environmentally relevant doses remain principally unknown.<sup>3,48</sup> Specific details of *in vivo* studies of DA toxicity are outlined below.

### *Mice*

Single i.p. doses of DA ranging from 0.5 mg/kg to 2.0 mg/kg in adult CF1 mice resulted in dose-dependent increases in serum concentrations of DA and behavioral aberrations, with a maximum serum concentration of 0.124 µg DA/ml, measured 120 minutes after dosing.<sup>55,56</sup> Behavioral symptoms reported included hindlimb scratching, hypoactivity or sedation, forelimb or body rigidity, tremors or convulsions, and death.<sup>57</sup> Decreased onset times of hindlimb scratching, one of the first visible symptoms associated with toxicity, is highly correlated with increasing doses of DA.

At 2 mg/kg, a single i.p. injection of DA results in long lasting behavioral changes in adult DBA mice. Mice performed poorly in spatial learning tasks, particularly the Morris water maze, up to two weeks post-dose administration when compared to their control counterparts.<sup>58</sup>

Injections of DA in CD1 mice resulted in an LD<sub>50</sub> of 3.6 – 6.0 mg/kg.<sup>57,59</sup> 4 mg DA/kg i.p. injections in CD1 mice were associated with seizures and lesions in the hippocampus.<sup>60</sup> Post-

mortem analysis revealed that hippocampal CA3 pyramidal neurons were most damaged, with over 80% of these neurons dead or degenerating.<sup>60</sup> Further damages were reported, marked by swollen astrocytes, shrunken pyramidal neurons, and vacuolization. These damages were largely limited to the hippocampus.<sup>60</sup>

Oral gavage administration of DA in CF 1 mice resulted in hindlimb scratching, tremors, and death at doses above 47 mg/kg.<sup>56</sup> Some neuronal degeneration in the CA3 and CA4 regions was present. No symptoms were observed below 35 mg/kg DA and no information on DA concentrations in blood was collected.<sup>56</sup> No DA was found in urine and 100% of the dose was found in feces.<sup>56</sup> How more relevant, low, oral doses affect mice remains unknown.

### *Rats*

I.v. injection of DA at 0.5 and 1 mg/kg in Sprague-Dawley rats caused symptomology similar to mice; toxicity was visually observed with symptoms that included salivation, scratching, and seizures (Table 2). Yet just twenty-five minutes post-dosing, no obvious signs of toxicity persisted, signifying that DA is likely rapidly eliminated from the body.<sup>56</sup> No data were collected on blood concentrations of DA.

Lower DA doses (1.32 mg/kg) administered to Sprague Dawley rats via i.p. injections were associated with slight hindlimb scratching, in addition to long lasting behavioral effects marked by an exaggerated response to auditory startle tests.<sup>61</sup> Histopathological examination of rodent brain tissue showed some lesions, primarily in the olfactory and CA3 hippocampus regions, as a direct result of neuronal degeneration and gliosis (Table 2).<sup>61</sup>

Female Sprague Dawley rats were also administered 2.5 mg/kg DA i.p. and studied for 54 days post-dosing. Typical symptoms, including hyperactivity, scratching, and tremors were pronounced at 5-10 minutes after administration, but quickly subsided thereafter.<sup>62</sup> Notably, lesions did not develop in the brains until five days after the initial exposure, suggesting that other studies reporting negative results from brain tissue analysis before five days post-dose administration may have observed falsely negative results.<sup>62</sup> At five days post-exposure, neuronal degeneration and necrosis in the CA3 and CA4 regions of the hippocampus were reported. Additional areas of necrosis in the amygdala and piriform cortex were reported.<sup>62</sup>

Other Sprague Dawley rats administered higher doses of DA (4 -7.5 mg/kg i.p.) showed neuronal loss in the CA3, CA4, and CA1 regions of the hippocampus within 24 hours of exposure.<sup>56</sup>

In Sprague Dawley rats, oral administration of 60, 70, and 80 mg/kg of DA resulted in symptoms described above; primarily hypoactivity, chewing, and seizures (Table 2).<sup>51</sup> One rodent in the highest dose group died. Brain tissues of rodents dosed with 80 mg/kg had substantial astrocyte swelling, neutrophil vacuolization, and neuronal degeneration in the CA3 region of the hippocampus and olfactory bulb.<sup>51</sup>

Chronic administration of 0.1 and 5 mg DA/kg/day via oral gavage in adult Sprague Dawley rats resulted in no observable behavioral, gross histology, or serum chemistry changes.<sup>63</sup>

Table 2: Shows typical effects of DA exposure in rats.

Route	Dose (mg/kg)	Symptoms	Brain Histopathology
<b>i.v.</b> <sup>56</sup>	1.0	Seizures, scratching, chewing	Not examined
	0.5	Minor seizure	Not examined
<b>i.p.</b> <sup>54,56</sup>	7.5	Seizures, shakes, death	Hippocampal and retinal lesions
	4	Seizures, shakes, death	Hippocampal and retinal lesions
	2.5	No reaction	None
	1.32	Scratching, seizures	Gliosis and neural degeneration
<b>p.o.</b> <sup>51</sup>	80	Hypoactivity; seizures; death	Vacuolization/neural degeneration

### *Nonhuman Primates*

Nonhuman primates (*Macaca fascicularis*) were given a single i.v. injection of DA between 0.025 mg/kg and 4 mg/kg (1-3 animals per group). The first symptoms were reported as an increase in chirping and excessive lip smacking (Table 3).<sup>49,50</sup> The latency period between dosing and symptoms was dose dependent, with larger doses producing a shorter latency to visible symptoms. Symptoms progressed to include signs of nausea, marked by chewing, salivation, gagging, and vomiting.<sup>50</sup> 0.5 mg/kg DA administered intravenously to one animal also caused a period of discomfort, displayed by prostration and exhaustion, hindlimb scratching, and difficulty respiring.<sup>50</sup> Four animals which received the higher doses (1.0 – 4.0 mg/kg i.v.)

died. One primate dosed with 4 mg/kg DA i.p. showed strong evidence of excitotoxicity, marked by violent vomiting starting just 2 minutes after dosing (Table 3).<sup>52</sup> Symptoms in this animal progressed to include spasms and shaking, with unsteadiness and a general loss of balance. After two hours, vomiting ceased, but tremors and seizures increased. Ultimately, the individual perished from hypothermia and an acute pulmonary edema.<sup>52</sup> Postmortem analysis of brain tissue samples showed damage in areas other than the hippocampus, but no damage in the hippocampus. The area postrema, hypothalamus, and retina showed vacuolization in the pyramidal layers of neurons and astrocytic swelling as result of DA exposure.<sup>52</sup> In the survivors, all symptoms subsided within six hours after dosing.<sup>50</sup> Only doses at 0.5 mg/kg i.v. and above produced morphological changes in the brain, as a result of vacuolization in the solitary tract of the medulla oblongata and pyramidal neuron body and axon death in the CA2 and CA1 regions of the hippocampus.<sup>50</sup> No behavioral or morphological side effects were observed at doses below 0.025 mg/kg i.v.<sup>49,50</sup>

Table 3: Shows typical effects of DA exposure in nonhuman primates

Route	Dose (mg/kg)	Symptoms	Brain Histopathology
<b>i.v.</b> <sup>50</sup>	4	Nausea; scratching	Neuron/axon death in hippocampus
	2	Nausea; scratching	Neuron/axon death in hippocampus
	0.5	Nausea; lethargy	Lesions/vacuolization in solitary tract
	0.2	Nausea	None
	0.025	Nausea	None
<b>i.p.</b> <sup>52</sup>	4.0	Scratching, mastication, vomiting, and salivation; Tremors and convulsions Death	Lesions and vacuolization in the area postrema, solitary tract, and retina
<b>p.o.</b> <sup>51</sup>	10	Nausea; exhaustion	Vacuolization and neuronal degeneration
	5	Nausea	Vacuolization and neuronal degeneration
	0.5	None	None

Only two studies have examined the oral toxicity of DA in nonhuman primates, despite their biological similarities to humans. Tryphonas et al. first experimentally studied oral toxicity by administering doses of DA from 0.50 mg/kg to 10 mg/kg to nonhuman primates and observing them for up to four days (Table 3).<sup>51</sup> Symptoms of nausea, including mastication, gagging and vomiting, were associated with doses above 5 mg/kg and typically persisted from 2 hours post-dosing up to 96 hours. Animals administered doses above 6 mg/kg also showed signs of exhaustion. Doses above 5 mg/kg resulted in lesions in CA1, CA3, and CA4 regions of the hippocampus, marked by neuronal shrinkage and degeneration and neutrophil vacuolization. Animals showed no symptoms or visual morphological changes four days after administration of doses at or below 0.5 mg/kg.<sup>51</sup> No data were collected on DA concentrations in blood, urine, or feces.

A separate examination of chronic oral exposure to DA found that doses of 0.5 mg/kg and 0.75 mg/kg orally administered for 15 days had no evident effects on primates.<sup>53</sup> These primates showed no changes in weight, behavior, and food or water consumption throughout the study. Pathological examination of brain tissue post-dosing showed no discernable effects from control animals.<sup>53</sup>

### *Summary*

In general, DA toxicity primarily acts in the central nervous system in mammals, crossing the rat blood brain barrier very slowly, indicating that transport to the brain is likely mediated by a carrier via active transport.<sup>64</sup> High dose acute effects produce similar diagnosable neurotoxic effects in most animal models tested (Table 4). If administered a high dose of DA, both rodents and primates may begin to tremor, with notable shaking particularly visible in extremities. As time progresses, animals may begin to masticate and salivate excessively. These symptoms may be accompanied with a period of general lethargy or hypoactivity, or followed by a period of excitation. If administered a fatally high dose, animals will progress to experience seizures and may ultimately die from cardiac arrest.<sup>48,62</sup> Morphological changes are typically in the hippocampus, a region of the brain associated with memory and learning.<sup>46,52,65,66</sup>

Table 4: Shows general neurotoxic effects from intraperitoneal\* and intravenous DA administration across animal models.

Species	Dose	Symptoms	Brain Histopathology
<b>Mice</b> <sup>60</sup>	4.0 mg/kg*	Reduced mobility Scratching Tremors and convulsions Loss of balance	Neuronal death, particularly in the CA3 region of hippocampus Vacuolated cytoplasm Shrunken basophilic cells Misshapen nucleus of pyramidal neurons
<b>Rats</b> <sup>67,62</sup>	1.5-3.5 mg/kg*	Scratching Tremors and convulsions Death	Activated microglia Astrogliosis Necrosis and shrunken neurons Vacuolization of neutrophils Increased NO levels
<b>Nonhuman Primate</b> <sup>51,54</sup>	0.50-4.0 mg/kg	Tremors and shakes Disorientation	Vacuolization Brain lesions Retina lesions

### 1.1.5: Models of Developmental Toxicity

Processes involved in neurodevelopment are highly sensitive to toxic assaults like DA.<sup>1,48</sup> Synaptogenesis and connectivity is regulated, at least in part, by NMDA and KA/AMPA receptors; thus signal disruption by excitatory amino acids may lead to improper development, poor connectivity, and improper formation of synapses.<sup>68</sup> Though it is unclear whether DA is a human developmental toxin, current evidence shows that it is a developmental neurotoxin in some model species. Importantly, DA can accumulate in fetal fluids and fetal rat brains, suggesting that in utero exposure to DA may occur if a mother was to ingest DA.<sup>69,70</sup> Additionally, DA can be excreted into milk in rats, suggesting that neonates may be exposed to DA following maternal ingestion.<sup>71</sup> Subcutaneous injections of DA during mouse and rat development and microinjections in zebrafish eggs alters hippocampal functioning and

connectivity into adulthood.<sup>1,72</sup> DA interestingly displays a wide variety of effects on neurodevelopment in these animal models, depending on the developmental stage of the animal when exposed to DA.<sup>1</sup> DA also is reported to have sex specific effects in rodent models.<sup>1</sup> However, no research to date has investigated DA effects on development in nonhuman primates.

### *In Utero Exposure Studies*

Sprague Dawley dams administered 0.6 mg/kg DA i.v. on gestational day (GD) 13, the peak of hippocampal neurogenesis in the fetus, resulted in significant changes in brain chemistry and morphology in offspring.<sup>73</sup> Offspring showed reduced GABA receptors and increased glutamate, calcium, and kainite receptors in the hippocampus. These alterations were correlated with neuronal degeneration in the CA3, CA4, and dentate gyrus regions of the hippocampus at postnatal day (PND) 14.<sup>73</sup> The DA exposed mice had abnormal EEGs at PND 30, reported as continuous burst discharges, activity similar to epilepsy.<sup>73</sup> Exposed offspring also had decreased tolerance for a second DA exposure, beginning to seize and die at i.v. injections of 0.6 mg/kg, unlike their control counterparts.<sup>73</sup>

Maternal subcutaneous injection of DA (0.3 - 1.2 mg/kg) in Sprague Dawley dams on GD 13 caused behavioral aberrations in offspring persisting into adolescence and adulthood.<sup>74</sup> These offspring displayed no overt symptoms of DA toxicity. However, offspring exposed in utero showed extended response latency during T-maze testing in adolescence, as well as increased locomotor activity and decreased accuracy on radial arm mazes in adulthood, indicating long-lasting deficits of working memory.<sup>74</sup> Interestingly, male offspring were more affected than their female counterparts, with the normal male-female performance difference almost entirely attenuated.<sup>74</sup>

Additional research of in utero DA exposure examined social behavioral changes in C57BL/6J (B6) mice.<sup>75</sup> Dams were subcutaneously injected with 1.5 mg/kg of DA on GD 16, the period immediately following peak hippocampal neurogenesis, and juvenile offspring were tested for social behavior on PND 21, 25, and 35. Female offspring did not display social behavior different from the controls. However, male mice displayed significantly less social behavior

than their control counterparts on PND 25.<sup>75</sup> Researchers also analyzed ultrasonic vocalizations (USVs), as another measure of social behavior, and found that PND 25 males trended towards fewer vocalizations than control mice.<sup>75</sup> MRIs from these DA administered mice revealed that DA treated mice had increased connectivity in the infralimbic and orbital areas of the brain, but decreased connectivity in the CA3 region of the hippocampus.<sup>75</sup>

### *Postnatal Exposure Studies*

Sprague Dawley neonates subcutaneously (s.c.) administered 0.02 mg/kg daily from PND8 to PND14 displayed long-lasting differences in behavior, memory performance, and brain structure when compared to untreated rats. During adolescence, DA administered rats performed better than their control counterparts when challenged with a radial 8-arm maze, indicating enhanced working memory. In adulthood, DA rats performed slightly better on the Morris Water Maze, suggesting sustained improvements in working memory.<sup>76</sup> No pathological or morphological analyses were completed in this study.

However, in another study with the same dosing parameters, rats displayed seizure like behaviors on PND 75 when tested in the Morris Water Maze, confronted with novel environments, and subjected to stress.<sup>77,78</sup> Other rats treated with the same dosing regimen of DA showed deficiencies in pre-pulse inhibition and latent inhibition, indicating long-lasting changes in the dopaminergic or glutamic neuronal functions.<sup>79</sup> Examination of social behavior of adolescent rats dosed with 0.02 mg/kg DA s.c. from PND 8 to PND 14, revealed increased social avoidance and increased avoidance to social interactions in male DA rats.<sup>80</sup> Furthermore, neonatal rats with the same treatment as above spent less time REM cycling during sleep than untreated rats, despite sleeping for the same amount of time.<sup>81</sup> Pathological analysis of adult brain tissue of rats neonatally exposed to 0.02 mg/kg DA s.c. from PND 8 to PND 14 had fewer hippocampal cells, with increased mossy fiber sprouting.<sup>77</sup> Expression of the BDNF gene, often correlated with temporal lobe epilepsy in humans, was elevated in the CA1 region of the hippocampus of DA treated rats.<sup>77</sup>

As rats age, the sensitivity to DA is diminished. Sprague Dawley rats dosed subcutaneously with 0.02 mg/kg DA at PND 0, 5, 14, and 22, showed decreasing behavioral sensitivity to DA with

increasing age. Younger animals administered 0.02 mg/kg DA had more severe symptoms than their older counterparts; PND 14 rats needed double the dose as PND 5 rats to produce the same symptoms typical of DA toxicity, including tremors, shakes, scratching, and seizures.<sup>82</sup>

#### 1.1.6: Models of Other DA Toxicities

In addition to neurotoxic and developmental effects from DA, there is some limited evidence that DA is associated with reproductive, cardiovascular, and renal toxicity in mammals.<sup>83-85</sup>

Stranded pregnant California sea lions diagnosed with DA toxicosis from 1998 to 2002 were monitored by the Marine Mammal Center. Of the 209 stranded pregnant mothers, all fetal pups died from abortions, premature live births, still births, or maternal death while in utero.<sup>83</sup> 109 of these mothers also perished. These effects may be a direct result of DA toxicity or a possible side effect from typical neurotoxic effects of DA diminishing the overall health of a dam.<sup>83</sup> In the laboratory, Sprague Dawley dams also showed decreased litter sizes when administered i.p. injections of DA 0.5 mg/kg on GD 7-16.<sup>86</sup> No changes were seen in fetal weight, litter size, or percent of fetuses with abnormal morphology when compared to the control group.<sup>86</sup>

Out of 715 nonpregnant stranded sea lions admitted to the Marine Mammal Center in California diagnosed with DA toxicity, 102 animals displayed symptoms of cardiomyopathy.<sup>30</sup> Detailed postmortem tissue analysis of a subset of 8 animals showed vacuolization of cardiomyocytes, as well as necrosis and apoptosis.<sup>87</sup> In adult Sprague Dawley rats, injections of 2 mg/kg DA i.p. resulted in cardiomyocyte vacuolization, muscle cell degeneration, and myofiber loss 7 days after exposure.<sup>88</sup> Further, *in vitro* examination of rat mitochondria and cardiomyocytes exposed to DA found decreases in mitochondrial flavin adenine dinucleotide (FAD) and nicotinamide adenine, indicating disruption to the respiratory function of mitochondria, but no changes in whole cardiomyocyte function.<sup>84</sup>

Based on the 1987 DA poisoning in humans, decreased renal function may be connected with increased severity of DA toxicity.<sup>12,89</sup> Experimental data indicates that DA can accumulate in the kidneys, and possibly extended the duration of exposure or increase the severity of toxicity, of some animals exposed to DA, like Coho salmon.<sup>90</sup> In adult Sv128/Black Swiss mice, 0.1 - 2.5 mg/kg i.p. injections of DA for 3 days yielded stark evidence of renal toxicity.<sup>85</sup> DA largely

accumulated in the kidneys of animals, despite minimal detection in other target organs. Even the lowest doses of DA showed preferential toxicity in the proximal tubules, as well as elevated biomarkers (urinary AKI) of nephrotoxicity.<sup>85</sup>

### 1.1.7: Summary

Though most algal species play an essential role in aquatic ecosystems, some species may produce biotoxins dangerous to humans. DA is an excitatory neurotoxin produced by some members of the algal genus *Pseudo-nitzschia* during HABs. In humans, Amnesiac Shellfish Poisoning (ASP) is the direct result of neuroexcitotoxicity in the hippocampus and long-lasting short term memory loss. ASP is associated with oral consumption of shellfish harvested from DA HAB areas.<sup>10</sup> Though few human deaths from acute DA toxicity have been recorded, many populations are frequently exposed to lower doses of DA.<sup>3,15</sup> The neurotoxic effects of DA in wildlife and human populations have been of utmost concern, but low-level effects are still not well understood.<sup>3</sup>

Though current limits have a 10-fold safety factor to protect sensitive populations from acute toxicity, it is possible that elderly, those with poor renal function, or the developing fetus may still be subject to low-level effects at exposure levels currently considered safe.<sup>14,15</sup> DA likely causes more severe symptoms in elderly because aged brains typically have reduced antioxidants, and thus a higher likelihood for ROS damage. Moreover, reduced renal clearance may extend the duration of exposure of DA in the body.<sup>19,50</sup> Consumption of shellfish during pregnancy is of high concern, as low doses of DA may accumulate in amniotic fluid, potentially acting as a neurotoxin for the fetus.<sup>69,83</sup> Investigation into the subtleties of DA toxicity in model species have revealed significant developmental toxicity, leaving afflicted animals with life-long neurological changes.<sup>1,91</sup> Prenatal and postnatal rats and mice are more sensitive to the neurotoxic effects of DA than adults, likely as a result of a disruption to the sensitive processes essential to proper neurodevelopment.<sup>48</sup> Even still, humans and nonhuman primates are more sensitive to DA than rodent models, but the exact cause and estimation of the difference in sensitivities are principally unknown.<sup>3,52</sup> These gaps of information and the results from

scientific discovery in the last thirty years suggest that additional studies need to be completed to establish a TDI based on the most sensitive toxicological outcomes.

## 1.2: Toxicokinetics

An essential part of understanding the relationship between DA consumption and toxicological outcomes begins with determining how a specific ingested amount of DA compares to the actual exposure that a person may experience.<sup>92</sup> Biological processes, like active or facilitated transport, renal or biliary excretion, or metabolism can alter how much of a toxin is absorbed into the body or how long the toxin circulates.<sup>93</sup> Toxicokinetic or pharmacokinetic parameters describing the absorption, distribution, metabolism, and excretion of DA can help describe the actual exposure and allow researchers to understand how dose and exposure relate to toxic endpoints, thus estimate risk.<sup>92,93</sup>

These parameters are typically measured from analyses of plasma concentrations over time. Some of the most common parameters include half-life ( $t_{1/2}$ ), area under the curve (AUC), clearance (CL), volume of distribution (V), bioavailability, maximum concentration ( $C_{max}$ ), and time until maximum concentration ( $T_{max}$ ). Half-life is the time in which it takes for half of the concentration to be removed from plasma. The total dose is typically eliminated in approximately 4-5 half-lives.<sup>94</sup> AUC is an estimation of the total exposure based on plasma concentrations.<sup>94</sup> Clearance is the rate of elimination from the body. Volume of distribution is marked by the total body burden of a toxin.<sup>94</sup> This may include plasma, extracellular fluids, lipids, organs, or bones. Bioavailability is the percent of total dose that is absorbed into the body when administered orally.<sup>94</sup>

The experimental intravenous values of toxicokinetic parameters disposition of DA have been well documented in rats.<sup>50,70,95,96</sup> Yet these parameters in nonhuman primates have only been studied with extremely high doses, generally much larger than typical human exposure.<sup>50,53</sup> Further, only few studies have examined the excretion pathways of DA in these models, yielding limited evidence to model the relationship between the dose of DA, absorption of bioavailable DA, and the excretion pathways.<sup>53,56,85,97</sup> No published data have documented the kinetic parameters of a low, environmentally relevant, oral dose of DA in humans or any other species. Understanding these parameters in a model species closely related to humans would help further the biological understanding of DA toxicity, essential to calculating a toxicologically based TDI. The following is a summary of studies focused on the toxicokinetics of DA in mammalian species.

### 1.2.1: Rodent Toxicokinetics

In Sprague Dawley rats, a single i.v. injection of 0.5 mg/kg and 1.0 mg/kg DA showed that DA displays two-compartmental kinetics, marked by two distinct phases; an alpha phase describing the distribution from plasma into other compartments of the body and a beta phase describing the terminal elimination from the body after pseudo-equilibrium is reached. The volume of distribution at steady state ( $V_{ss}$ ) was 311.4 mL/kg for the 0.5 mg/kg dose and 228.9 mL/kg for the 1.0 mg/kg dose, similar to the 265 mL/kg of extracellular fluid volume in rats.<sup>98</sup> Thus, these data suggest that the distribution of DA in rats is primarily limited to the extracellular fluid. I.v. injection of DA also has a very short  $\beta$  half-life, just over 20 minutes and the volume of distribution ( $V_{d\beta}$ ) was 333.6 ml/kg for the low dose and 236.9 ml/kg for the high dose, supporting the hypothesis that DA is rapidly eliminated from the body due to a small volume of distribution (Table 5).<sup>50</sup> The  $\alpha$  half-life was unreported. Additionally, Iverson and Truelove documented that the AUC was approximately 50  $\mu\text{g/mL}/\text{min}$  for the 0.5 mg/kg dose and 140  $\mu\text{g/mL}/\text{min}$  for the 1 mg/kg dose.<sup>50</sup>

The clearance of DA in Sprague Dawley rats following a single i.v. injection of 0.5 mg/kg and 1.0 mg/kg DA was 7-10 mL/min/kg.<sup>50</sup> Another study investigating the renal clearance of i.v. administered DA in Sprague Dawley rats produced a similar clearance.<sup>97</sup> The systemic clearance matches the renal glomerular filtration clearance in rats, indicating that renal excretion via glomerular filtration is likely the primary elimination route.<sup>97</sup> Further, all DA administered intravenously was collected in urine samples within 160 minutes of dosing, suggesting that DA is only cleared via renal excretion.<sup>97</sup> If all absorbed DA can be accounted for in urine, bioavailability can be estimated after oral exposure. Based on this assumption, urine analysis after a single dose of 5 mg/kg DA administered to rats estimated that bioavailability is likely between 1.4-2%.<sup>63</sup>

No data exist on oral bioavailability of DA in mice. However, oral studies after a 14.3 mg/kg DA dose showed that no DA was detected in the urine, and all was detected in the feces.<sup>56</sup> These data suggest that bioavailability may be even lower in mice than in rats, or excretion is primarily through biliary pathways. Additional oral gavage data based on a 7 day chronic exposure of 5 mg/kg/day and 15 mg/kg/day DA in CB1 mice suggest that there is no plasma accumulation of DA in seven days.<sup>96</sup> A lack of plasma accumulation suggest that the bioavailability of DA is

minimal or elimination of DA in mice is extremely rapid. No additional data exist on the parameters of DA kinetics in rodents.

### 1.2.3: Nonhuman Primate Toxicokinetics

In nonhuman primates (*Macaca fascicularis*), a single i.v. injection of 0.05 mg/kg DA shows that primate species share the two-compartmental kinetics and a short half-life of intravenously administered DA with rodents. Iverson and Truelove found that a 0.05 mg/kg i.v. dose of DA had a half-life at approximately  $114.5 \pm 59.0$  minutes, an AUC at  $46 \pm 18.7$   $\mu\text{g/mL}/\text{min}$ , and a total volume distribution at  $159.3 \pm 28.8$  mL/kg.<sup>50</sup> Approximately 65 – 90% of the total dose was found as parent compound in the urine after i.v. dosing, indicating little to no metabolism and that renal excretion is the primary route of excretion. However, because the full dose was not present in the urine, it is possible that renal excretion is not the only excretion route.<sup>50</sup> They found large interindividual variability in their small sample size, leading to a large range for every variable.<sup>50</sup> Two animals with the shortest durations of vomiting (9-11 minutes) were the same animals with the shortest half-lives (65-66 minutes).<sup>50</sup> Episodes of vomiting greater than 20 minutes were matched with terminal half-lives of 140 and 185 minutes.<sup>50</sup> These data suggest that DA toxicity may be related to an individual's exact distribution or elimination of DA.

A 30-day study of three animals gavaged daily with 0.5 mg/kg and 0.75 mg/kg DA examined serum and urine concentrations of DA once a day to understand chronic effects of DA administration. Little to no change in plasma concentration after 30 days was reported, with mean concentrations of 12.7, 13.2, and 28.9 ng/ml in each animal. The serum concentrations at the end or the beginning did not statistically differ from the mean, indicating that there were likely no changes in either absorption or elimination with repeated exposures. Analysis of the 24 hour urine samples showed that 4-7% of the total dose was present, suggesting a similar oral bioavailability of approximately 4-7%.<sup>53</sup> No other data were reported.

#### 1.2.4: Sea Lion and Seal Toxicokinetics

DA was also detected in fluid samples collected from sea lions naturally exposed to DA and diagnosed with toxicosis. DA was found in serum, urine, and fecal samples, with the highest detectable limits seen in fecal samples.<sup>33,34</sup> Because dose information is unavailable, these data can only suggest that some portion of ingested DA is renally excreted, but most is cleared by fecal excretion, likely as a result of poor gastrointestinal (GI) absorption.

Free range Pacific harbor seals also showed evidence of DA in urine, serum, and fecal samples. DA was found in 65% of the urine samples and the majority of the fecal samples collected from animals diagnosed with DA toxicosis, after admittance to the Marine Mammal Center.<sup>99</sup> This indicates that chronic consumption of high doses of DA may result in prolonged DA exposures. Like the sea lions, most of the DA was found in fecal samples, with significantly less in the urine. DA was also detected in some amniotic fluid, milk, and stomach fluids, with none in the bile.<sup>99</sup> These data suggest that DA can accumulate in the amniotic fluids in seals.

#### 1.2.5: Summary

Below is a summary of current data from studies examining blood, urine, and fecal samples of DA in model species.

*Table 5: Shows a sampling of plasma disposition characteristics from model species.*

<b>Species</b>	<b>Dose (mg/kg)</b>	<b>t<sub>1/2</sub> (min)</b>	<b>AUC (ug/mL/min)</b>	<b>Bioavailability</b>
<b>Adult Sprague Dawley Rats<sup>50</sup></b>	<b>i.v. – 1.0</b>	21	140.7	1.4-2% <sup>56</sup>
<b>Cynomolgus Monkey<sup>50</sup></b>	<b>i.v. 0.05</b>	114.5	46.1	
	<b>p.o. 0.5</b>	Not measured	Not measured	4-7%

Because many coastal populations rely on shellfish as a primary food source and the number of DA-producing algal blooms are increasing, we need to understand the kinetic characteristics and associated health effects of DA exposure in humans. Based on the data described above, as well as hypotheses from the 1987 Canadian outbreak of Amnesiac Shellfish Poisoning, the following predictions can be made regarding DA toxicokinetics in humans:

1. Absorption: DA is likely poorly absorbed.<sup>52,53</sup>
2. Distribution: It does not distribute to much of the body, mostly limited to extracellular fluids.<sup>50</sup>
3. Metabolism: DA likely undergoes little to no metabolism.<sup>50,56,97</sup>
4. Excretion: Oral exposures of DA will mostly be unabsorbed, thus excreted in the feces. The small absorbed portion of DA will likely be excreted via renal filtration.<sup>50,97</sup>

Still, the kinetics of species closely modeling humans are not well understood, despite drastically different sensitivities to DA in rodents and humans.<sup>3</sup> Additionally, no studies have examined the disposition characteristics of DA levels that model real world exposure, near the TDI of 0.075 mg/kg. Establishing these parameters is essential to better characterize the risk of exposure to DA in human populations.

### 1.3: Specific Aims

Despite significant consequences to human health at high doses, the health effects of low, chronic doses of DA in humans remain principally unknown. As part of larger study focused on understanding the effects of a low, chronic dose of DA on reproduction and neurodevelopment, we sought to first map out the basic disposition and clearance of DA in a model species closely related to humans. To thoroughly understand the health outcomes related to DA exposure, the kinetic disposition in a model for humans is essential. The first step, and primary aim of this study, was to describe the toxicokinetics of a low, nontoxic i.v. dose of DA in *Macaca fascicularis*. To date, the only kinetic study in nonhuman primates examined a high, toxic dose (0.05 mg/kg i.v.). A tenfold lower dose would allow us to quantify low dose disposition characteristics. When these data are combined with low dose oral data, absolute oral bioavailability can be determined. No study to date has calculated absolute bioavailability. Further, few studies have examined the effects of a low, environmentally relevant dose of DA, and none have quantified the oral kinetics in nonhuman primates. This study sets forth to produce kinetic models with data gathered from a cohort of female, adult macaque monkeys, *Macaca fascicularis*, intravenously exposed to a low dose of DA and orally exposed to two additional low doses of DA. With these data, we will quantify the oral disposition of DA in nonhuman primates. Thus, the following are the specific aims of this study:

1. To replicate a single dose i.v. kinetics of DA disposition, studying toxicokinetic characteristics including half-life, volume of distribution, and AUC of DA in *Macaca fascicularis*, when administered in an i.v. bolus.
2. To determine and quantify a low dose kinetics model of DA disposition, studying characteristics including  $C_{\max}$ , half-life, volume of distribution, and AUC of DA in *Macaca fascicularis*, when orally administered at 0.15 mg/kg and 0.075 mg/kg.
3. To determine and quantify renal clearance of orally administered DA in *Macaca fascicularis*.

## **CHAPTER 2**

### **Toxicokinetics**

## 2.1: Introduction

To best understand the oral disposition of DA in *Macaca fascicularis* at doses relevant to real world human exposures, i.v. studies in a model species closely related to humans are needed. To date, only one study examined the toxicokinetic parameters of a large, toxic, i.v. dose of DA at 0.05 mg/kg in *Macaca fascicularis*.<sup>50</sup> This study revealed large interindividual variation, presenting some difficulties in using these data to determine DA disposition characteristics in future oral studies. Thus, we set forth to first confirm previously published i.v. data by examining plasma concentrations of DA in *Macaca fascicularis* after administered a low, nontoxic i.v. dose of DA. Data detailing the i.v. disposition of a low dose of DA also allows us to compute oral bioavailability and absorption when combined with oral kinetic data. These two measures are essential for translating oral kinetic data to eventually determine human risk.<sup>92,93</sup>

Expanding on data from our i.v. studies, we also designed studies to determine the oral disposition of DA in nonhuman primates at doses relevant to real-life human exposures. Animals were given two low oral doses of DA, and urine was collected during the study. If single dose oral exposure data support the common hypothesis that DA is rapidly cleared from the body,<sup>100</sup> then DA should not accumulate in the plasma when chronically administered and will not be detected in the plasma past 24 hours post-dosing. Alternatively, if a single dose exposure is not rapidly cleared from the body and DA is still detected in the plasma past 24 hours, chronic administration of DA may result in plasma accumulation.

Nonhuman primates are the best model to determine the disposition and health effects of low, oral doses of DA for several reasons. Despite normal physiological differences across species, both nonhuman primates and humans are similarly sensitive to orally ingested DA, both showing visible signs of toxicity around 1 mg/kg.<sup>21</sup> Adult rats, the most common model species for DA toxicity, do not display symptoms from oral DA exposures unless given doses above 35 mg/kg.<sup>63</sup> According to FDA calculations that account for cross species differences in metabolism and physiological characteristics, the human equivalent oral LOAEL of 35 mg/kg in rats translates to 5.6 mg/kg in humans, over five times greater than the human LOAEL estimated from the 1987 DA poisoning.<sup>101</sup> Thus, usual physiological differences do not account for the entire difference in sensitivity. This may be due to reduced absorption and increased clearance in rats, or because

the expression of the GluK5 receptor subunit, which DA preferentially binds to and thus initiates neurotoxicity, is highly diminished in adult rats.<sup>39,102</sup>

Additionally, as this study is part of a larger reproductive and neurobehavioral study, nonhuman primates are the best choice to model effects of DA exposure because humans and nonhuman primates share common evolutionary roots.<sup>103</sup> As a result of this shared history, they also share many reproductive and developmental characteristics that other models do not have.<sup>103</sup> Both humans and nonhuman primates have similar ovarian structure and timed menses. When pregnant, both have extended pregnancies that are characterized by protracted central nervous system development, three lobed placentas, and maternal-fetal exchange pathways. Pregnancies for both usually result in one infant, who is dependent on its parents for a lengthy time after birth.<sup>103</sup> Further, both nonhuman primates and humans develop similarly, sharing patterns in learning on perceptual, cognitive, and spatial vision tasks.<sup>103</sup> This development is further shared in social and temperament behaviors. For these reasons, studies using nonhuman primate model produce the best translation of developmental effects of toxicants into human risk<sup>103</sup>

## 2.2: Methods

Three, healthy, adult female *Macaca fascicularis*, 9 to 10 years old and 3.5 to 7.2 kilograms, were enrolled in this study (Table 6). Animals were housed in the Infant Primate Research Laboratory at the Washington National Primate Research Center. In the first portion of this study, females were administered an i.v. bolus of 0.005 mg/kg DA in saline in their saphenous vein. Two mL blood samples were taken from the saphenous vein at eleven time points (baseline, 5 minutes, 10 minutes, 20 minutes, 30 minutes, 45 minutes, 1 hour, 1.5 hours, 2 hours, 3 hours, and 4 hours) into green top, sodium heparin tubes. After each blood draw, animals were rewarded with treats. Animals were unsedated for the duration of the study, with unrestricted access to food and water.

Table 6: Shows the physical characteristics of animals involved in this portion of the study.

Animal Number	Age (years)	Weight (kg)
A14392	9.8	4.6
A14393	9.8	7.2
A14400	10.3	3.5

Following the i.v. study, animals were orally exposed to 0.15 mg/kg DA (Oral Study 1) and 0.075 mg/kg DA (Oral Study 2), with at least two weeks between each exposure. For these studies, lab technicians orally administered each animal DA dissolved in a 5% sucrose solution (1 ml total). Macaques voluntarily drank the solution and were rewarded with a treat. Two mL blood samples were taken from the saphenous vein at eleven time points (baseline, 1 hour, 1.5 hours, 2 hours, 3 hours, 5 hours, 8 hours, 12 hours, 16 hours, 24 hours, 48 hours) into green top, sodium heparin tubes. After each blood draw, animals were rewarded with treats. Animals were unsedated and unfasted for the duration of the study, with unrestricted access to food and water. Animals were fed Lab Diet High Protein Monkey Diet biscuits twice a day, once approximately 2 hours before the dose and once approximately 5 hours after the dose of DA. Additionally, urine was collected from cage pans after every blood draw during Oral Study 2. All animal procedure guidelines followed the Animal Welfare Act and the Guide for Care and Use of

Laboratory Animals of the National Research Council and protocols were approved by the University of Washington Institutional Animal Care and Use Committee.

Blood samples were spun immediately after collection at 3000  $\times$  g for 15 minutes, and plasma was isolated and frozen at -80°C until analyses via LC-MS/MS, with a lower limit of quantification of 0.622 ug/mL. For both blood and urine, LC-MS/MS analyses used an AB Sciex 5500 qTRap Q-LIT mass spectrometer equipped with an Agilent 1290 UHPLC and a Synergi™ Hydro-RP 100 Å LC Column (2.5  $\mu$ m, 50  $\times$ 2 mm). Pharmacokinetic parameters were analyzed using Phoenix WinNonlin software and noncompartmental analysis.

Disposition characteristic parameters were averaged and included in the tables below. The standard error of the mean is also included to examine variance of data.

### 2.3: Results

*IV Study:* The disposition of DA matched a two compartmental kinetic model, with levels below the detection limit at four hours post i.v. administration (Fig. 2, Table 7). Plasma concentrations of DA revealed that DA did not persist in the body for long due to a short alpha phase of distribution, with a mean alpha half-life of  $5.99 \pm 2.49$  minutes, a mean beta half-life of  $59.58 \pm 1.55$  minutes (Table 7). The mean  $AUC_{all}$  of DA was  $28.01 \pm 3.0$  hours\*ng/mL, with a mean calculated volume of distribution at steady state of  $201.7 \pm 46.56$  mL/kg. The mean clearance of DA was  $174.51 \pm 22.06$  mL/hr/kg. Unlike previously published data, the i.v. study did not show large interindividual variability (Table 7).<sup>50</sup> No symptoms of overt DA toxicity were observed during this study.

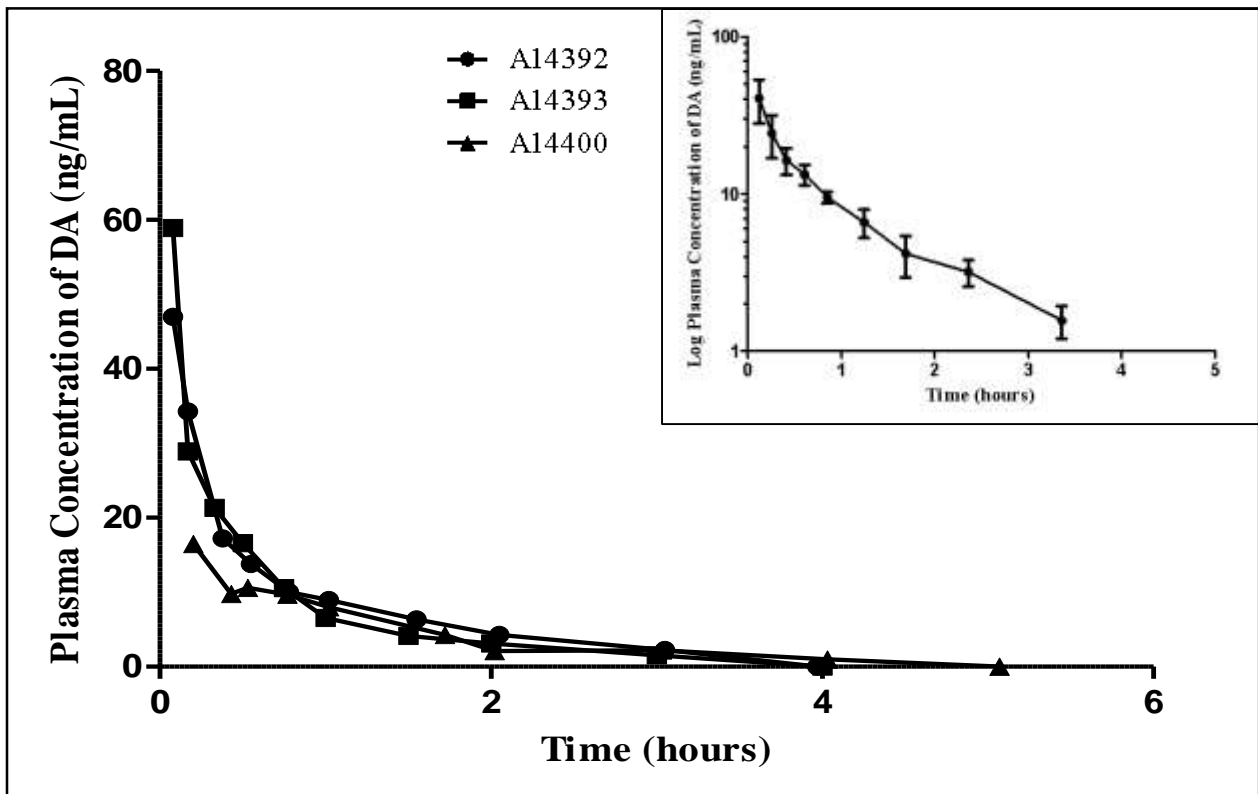


Figure 2: Shows the plasma concentration of DA after an intravenous administration of 0.005 mg/kg DA. Inset shows log transformed data.

Table 7: Shows the disposition characteristics of intravenous administration of 0.005 mg/kg DA in all enrolled animals, with the mean and SEM.

	$\alpha$ ( $\text{min}^{-1}$ )	$\alpha t_{1/2}$ ( $\text{min}$ )	$\beta$ ( $\text{min}^{-1}$ )	$\beta t_{1/2}$ ( $\text{min}$ )	$V_{d\beta}$ ( $\text{ml/kg}$ )	$\text{AUC}_{\text{all}}$ ( $\text{hr}*\text{ng/ml}$ )	CL ( $\text{ml/hr/kg}$ )	$V_{\text{ss}}$ ( $\text{ml/kg}$ )
A14392	0.11	6.82	0.011	62.19	187.88	31.59	147.86	171.97
A14393	0.068	10.16	0.012	59.72	222.81	30.39	157.09	140.15
A14400	0.45	1.53	0.012	56.82	280.62	22.04	218.17	292.99
Mean	0.21 $\pm 0.12$	5.99 $\pm 2.49$	0.01 $\pm 0.0003$	59.58 $\pm 1.55$	230.43 $\pm 27.04$	28.01 $\pm 3.00$	174.37 $\pm 22.06$	201.70 $\pm 45.56$

*Oral Study #1:* Oral kinetics of 0.15 mg DA/kg revealed an extended half-life, over a mean of 15 hours, with DA still detected 48 hours post-exposure (Table 8). Plasma concentrations of DA showed maximum concentrations at approximately 6 hours post-dosing. The average AUC was 109 hours\*ng/ml, with a range from 66-146 (Table 8). Unlike the i.v. data, these data showed larger interindividual variability for some parameters (Table 8, Fig. 3). A second peak at the 24 hour sample was noted. No symptoms of overt DA toxicity were observed during this study.

Table 8: Shows the disposition characteristics of an oral dose of DA at 0.15 mg/kg, with the mean and SEM.

	$t_{1/2}$ ( $\text{hours}$ )	$\text{AUC}_{\text{all}}$ ( $\text{hr}*\text{ng/ml}$ )	$\text{AUC}_{0-\infty}$ ( $\text{hr}*\text{ng/ml}$ )	$C_{\text{max}}$ ( $\text{ng/ml}$ )	$T_{\text{max}}$ ( $\text{hours}$ )
A14392	20.97	97.29	115.74	4.56	5
A14393	12.97	137.75	146.36	8.59	8
A14400	11.49	61.6	65.74	2.22	5
Mean	15.14 $\pm 2.94$	98.88 $\pm 22.00$	109.28 $\pm 23.50$	5.12 $\pm 1.86$	6.00 $\pm 1.00$

*Oral Study #2:* Oral kinetics of 0.075 mg DA /kg showed a similar extended half-life as Oral Study 1, over a mean of 11 hours, with DA still detected 48 hours post-exposure (Table 9). Plasma concentrations of DA peaked at  $13.33 \pm 7.70$  hours post-dosing. The average AUC was about half of the AUC seen in the higher dose, at 43.98 hours\*ng/ml (Table 9, Fig. 3). No symptoms of overt DA toxicity were observed during this study.

Table 9: Shows the disposition characteristics of an oral dose of DA at 0.075 mg/kg, with the mean and SEM.

	$t_{1/2}$ (hours)	AUC <sub>all</sub> (hr*ng/ml)	AUC <sub>0-∞</sub> (hr*ng/ml)	C <sub>max</sub> (ng/ml)	T <sub>max</sub> (hours)
A14392	14.25	35.32	40.25	1.03	16
A14393	13.62	55.33	59.65	3.51	8
A14400	5.76	31.8	32.05	1.18	16
Mean	11.21 ± 6.47	40.82 ± 23.57	43.98 ± 25.39	1.91 ± 1.10	13.33 ± 7.70

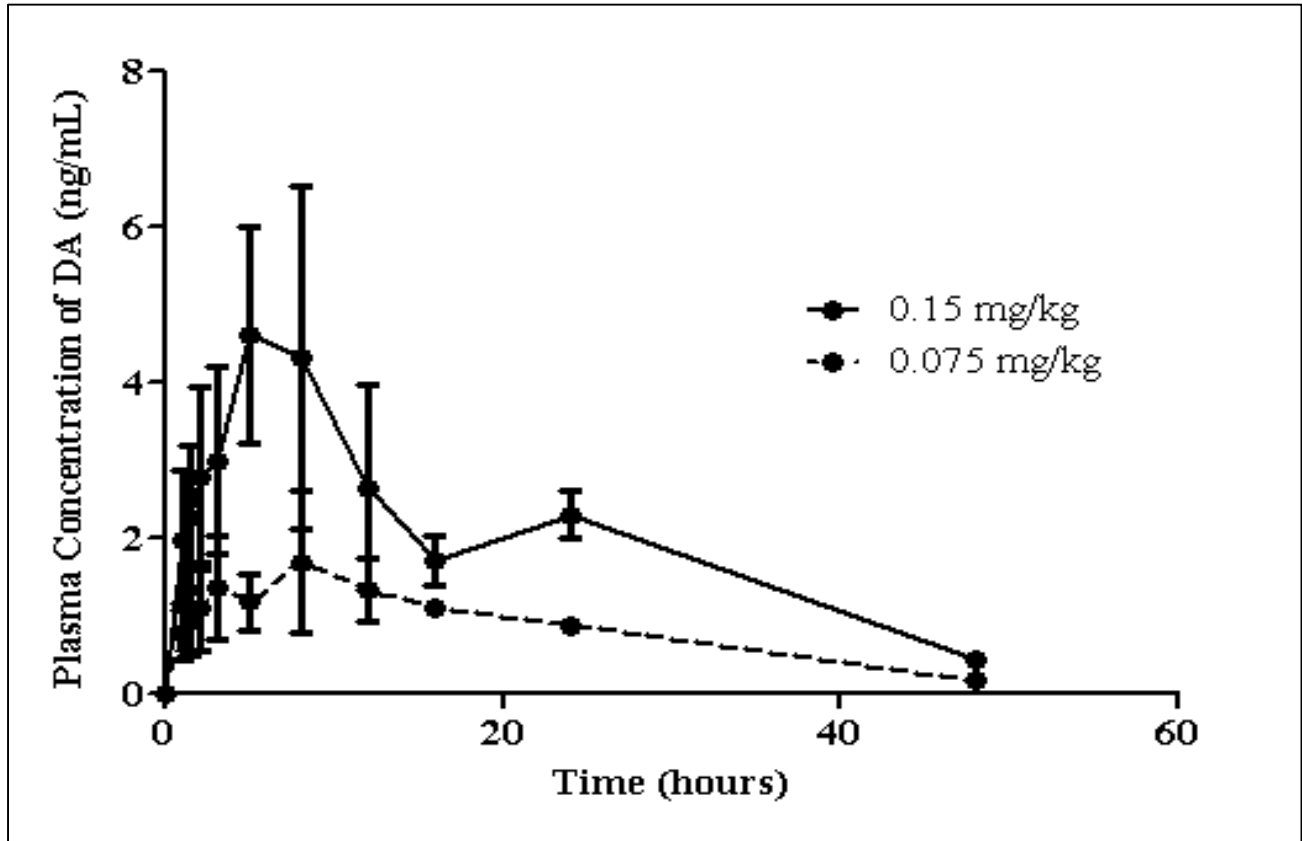


Figure 3: Shows the comparison of plasma concentrations after an oral dose of DA at 0.15 mg/kg and an oral dose of DA at 0.075 mg/kg.

Absolute bioavailability, a measure of the fraction of the total administered dose that is absorbed orally, was calculated from the following formula:

$$\text{Bioavailability} = \frac{\text{Dose}_{i.v.} \times \text{AUC}_{p.o.}}{\text{Dose}_{p.o.} \times \text{AUC}_{i.v.}}$$

Thus, the mean oral bioavailability of DA in this species is between 9.7 and 11.6% (Table 10).

Table 10: Shows the bioavailability calculated from the above equation.

	AUC <sub>i.v.</sub>	AUC <sub>0.15 mg/kg</sub>	Bioavailability of 0.15 mg/kg	AUC <sub>0.075 mg/kg</sub>	Bioavailability of 0.075 mg/kg
A14392	31.59	97.29	0.1036	35.32	0.0745
A14393	30.39	137.75	0.1511	55.33	0.1213
A14400	22.04	61.6	0.0931	31.8	0.0962
Mean	28.01 ± 3.00	98.88 ± 21.00	0.1159 ± 0.018	40.82 ± 7.33	0.0973 ± 0.014

Urine collected after exposure to 0.075mg/kg of DA revealed that a maximum of approximately 2-6% of the total dose was found unmetabolized in urine at 24 hours, notably less than the calculated mean bioavailability of 9.7% (Fig. 4, Table 10). As 24 hours is approximately two half-lives of DA up to 4-8% of DA would be expected to eventually be excreted in urine, still less than the total calculated bioavailability. The mean  $CL_R/CL_{iv}$  was 0.65, indicating that ultimately 65% of the bioavailable dose would be found in the urine when orally administered (Table 11).

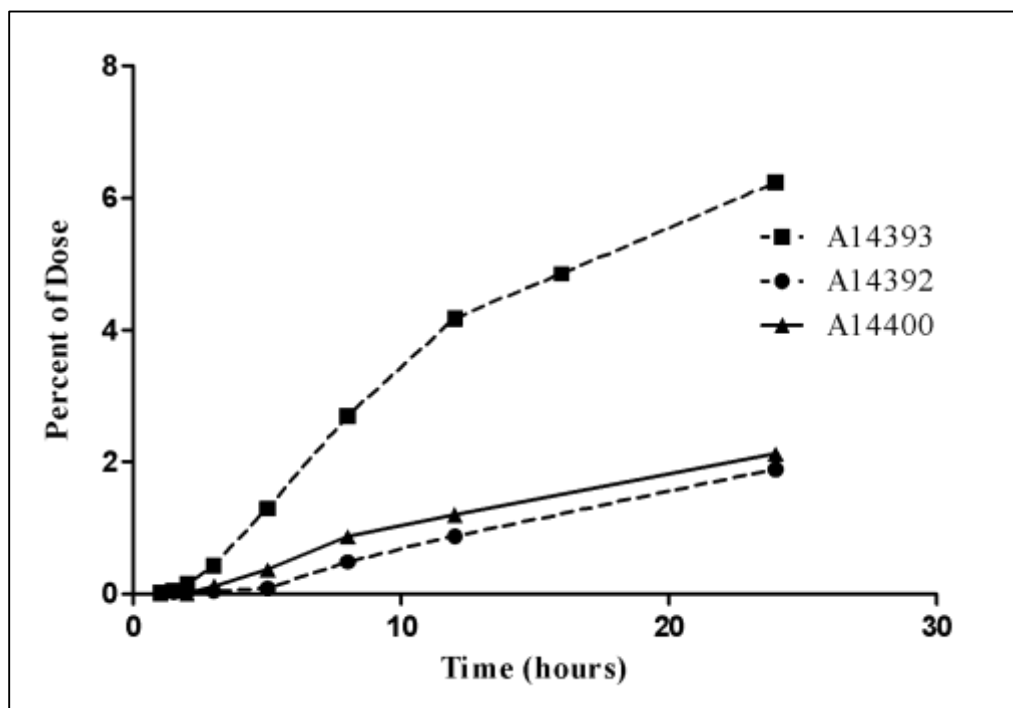


Figure 4: Shows the percent of the original dose of 0.075 mg/kg collected in urine pans after 24 hours.

Table 11: Shows the clearance data from the 0.075 mg/kg DA urine study and i.v. study, with the mean and SEM.

	$CL_R$ (ml/hr/kg)	$CL_{iv}$ (ml/hr/kg)	$CL_R/CL_{iv}$
A14392	74.2	147.9	0.5
A14393	115.5	157.1	0.74
A14400	152.7	218.2	0.7
Mean	$114.13 \pm 22.67$	$174.4 \pm 22.06$	$0.65 \pm 0.074$

## 2.4: Discussion

Data presented here produced a striking pattern of flip-flop kinetics, marked by limited absorption and an extended duration of exposure when orally administered. However, the general patterns of i.v. disposition showed DA does not persist following i.v. administration, due to a short beta phase (Table 7), largely matching previously published data in both macaques and rats.<sup>50</sup> Despite rapid clearance in i.v. studies, the oral half-life of DA was over 15 hours, with DA still detected at 48 hours (Table 8, 9). Oral kinetic data also showed an extended period of exposure (Fig. 3). The oro-caecal transit time of liquids given with food in this species is typically 1.8 hours.<sup>104</sup> The oral half-life seen in this study was over seven times this, suggesting that GI absorption of DA is highly restricted in this species and thus limits the clearance, forcibly extending the duration of exposure.

The only other study that has documented an extended duration of exposure was done so with relatively high oral doses of DA (13 mg/kg) in Coho salmon.<sup>90</sup> In two salmon, a single oral dose of DA resulted in detectable plasma concentrations of DA up to one week after the dose. Additionally, DA concentrations in plasma did not peak until 24 or 48 hours after exposure.<sup>90</sup> The present data confirm that this phenomenon of a prolonged duration of exposure after the ingestion of DA is conserved across species. No oral data in rodent models currently describe the basic kinetic parameters of DA concentrations in blood more than 2 hours after dose administration.

Possible reasoning for the limited absorption of DA in our model is likely due to several distinct biological mechanisms. First, the physical transport of DA across intestinal cells may be limited due to the poor permeability of DA. If DA is absorbed via active transporters in the GI tract, like those of glutamate, a transporter essential to the uptake of DA may become saturated and contribute to limiting GI absorption.<sup>105</sup> Alternatively, a molecular essential to facilitating diffusion of DA across the GI barrier may be saturated, and limit GI absorption.<sup>64</sup> Other possible contributions to the extended period of exposure may include biliary sequestration or enterohepatic recirculation. Though our i.v. data suggest that DA is primarily excreted through renal filtration, marked by a clearance rate similar to the published glomerular filtration rate,  $186 \pm 30$  ml/hr/kg, urine or fecal samples were not collected during this portion of the study to confirm this clearance path.<sup>106</sup> Urine samples from the low dose, oral study showed that only a

maximum of 74% of bioavailable dose, or 6.5% of the total dose, was excreted in urine at 24 hours post-exposure (Table 10, Fig. 4). Though additional DA may have been excreted after the last collection time, it is possible that DA is excreted through alternative routes, such as biliary excretion. Additional research in other models, including Coho salmon and crabs, show that DA can accumulate in bile and bile organs.<sup>22,90</sup> Therefore, other species may also share biliary accumulation of DA, and thus biliary excretion as well. If DA entered the biliary system, enterohepatic recirculation may have also contributed to extending the half-life of DA. A second peak in plasma concentrations at 24 hours suggests that enterohepatic recirculation may have played a role in DA disposition during this study (Fig. 3).

Contradictory to current evidence in rodent models, these data suggest that chronic ingestion of DA may result in plasma accumulation in humans as a direct result of the prolonged duration of exposure. In *Macaca fascicularis* at these low doses, chronic administration of DA may result in plasma accumulation up to 2 ng/mL (Fig. 5). A baseline level of

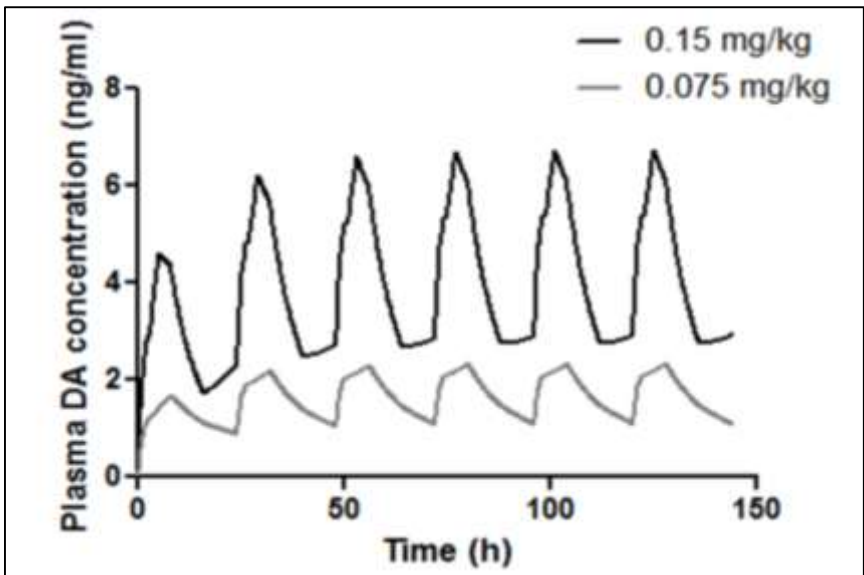


Figure 5: Predicts how DA may accumulate in plasma of *Macaca fascicularis* if administered daily.

DA may then exacerbate particularly high doses of DA if chronically consumed. Additionally, bioavailability calculated from the present data is two-fold greater than previously reported.<sup>53</sup> This suggests that previous estimations of risk and tolerate limits of DA using a lower bioavailability of DA may be overestimating safe consumption levels. Alternatively, if toxicity is associated with a peak concentration of DA, a prolonged duration of exposure may minimize the peak DA concentration of acute exposures, and thus minimize related toxicity. This would suggest that standards may be protective enough for acute shellfish consumers.

These data have significant implications for several populations of chronic shellfish consumers at risk of increased DA exposures. Because sustenance farmers, costal Native populations, and those from Japanese or Korean cultures often disproportionately consume large amounts of shellfish already, it is possible that people in these groups may also consume DA at or even above the TDI.<sup>3</sup> The possible plasma accumulation and increased bioavailability seen in this study indicate that those who chronically ingest DA may be at a much higher risk than current safety levels estimate.

Further, people in these populations with decreased renal or biliary function, such those with kidney or liver disease, may experience an even more pronounced risk. Severe DA toxicity is correlated with increased age and decreased renal function in humans.<sup>10</sup> Because a major excretion pathway for DA is controlled by renal filtration, a decreased ability to excrete DA may lead to a longer duration of exposure, and possibly a heightened peak of DA. Similarly, if DA is excreted via the biliary system, those with decreased biliary or liver function may also experience a longer and higher exposure of DA after ingestion.

Finally, these data may have the most profound effects on developing fetuses. Stranded California sea lions demonstrated that DA can accumulate in fetal fluids, including fetal feces, urine, gastric fluid, and amniotic fluid. Fetal fluid DA concentrations in examined mother-fetal pairs were over 10 times greater than maternal urine concentrations.<sup>83</sup> In Sprague Dawley rats, over 30% of the maternal dose of DA can cross the placenta and accumulate in the fetus, including the fetal brain and plasma.<sup>70</sup> Additionally, fetal rats are subject to amniotic recirculation, showing steady levels of DA in the amniotic fluid and fetal brain even 24 hours after maternal dose administration. Thus, low maternal doses of DA may lead to large and long exposures for the fetus. This may be particularly detrimental to developing fetuses, as excitatory amino acids, such as DA, can disrupt the sensitive signaling processes essential for proper synaptogenesis, neuronal growth and connectivity.<sup>68</sup> Because evidence already shows that rodent and sea lion fetuses are highly sensitive to DA, the increased exposure reported here may present the greatest risk to fetuses in other species as well.<sup>69,83</sup> Future work within this study sets forth to understand how the developing fetus may be affected by low, oral doses of DA ingested by the mother.

## 2.5: Future Studies

Though the data presented here offer many new insights into the disposition of DA under normal circumstances, some questions essential to better characterizing human risk still remain unanswered. Thus, future studies using our *Macaca fascicularis* model will target the following:

1. Understanding the path of elimination of DA in *Macaca fascicularis* by measuring urine and fecal DA concentrations during and after a low dose of intravenously administered DA.

Data from this study will allow us to determine the actual clearance pathways of DA in a model species most closely related to humans. These data will help interpret how dysfunctional kidneys, liver, or gallbladders may affect DA kinetics and increase DA toxicity risk.

2. Quantifying changes of disposition during chronic oral dosing, showing how chronic DA dosing may result in accumulation and possibly affect pharmacokinetic disposition characteristics.

Chronic exposure to DA may result in functional changes in the body. For example, if repeated exposure to DA decreases renal function as a result of DA accumulation in the kidneys, the duration of exposure may be extended. Data from this study will reveal if a body is able to adapt to limit the exposure of DA over time or if chronic administration of DA will impair bodily functions that remove DA from the system to extend the duration of exposure.

3. Quantifying changes of DA oral disposition during pregnancy as a result of typical renal changes and possible amniotic and fetal sequestration of DA by comparing kinetic studies in early and late pregnancy to nonpregnant disposition.

Many disposition characteristics typically change during pregnancy, including renal function. Normal changes as a result of pregnancy may decrease the risk of DA exposure for the mother. Alternatively, fetal processes may also alter the exposure of DA by sequestering it in the fetal fluids. Data from this study will help reveal how pregnancy changes the disposition of DA.

4. Modelling potential reproductive and neurodevelopmental effects of DA in a model species more closely related to humans by studying the possible effects on reproductive success and behavior of young animals exposed in utero to a chronic, low dose of DA

Though some studies have examined how DA affects offspring in rodents, no study has examined how DA orally ingested by the mother may affect offspring in primates. Using a model species closely related to humans and an exposure that mirrors real-world human exposures, data can be collected to provide a better estimate of the potential effects from DA exposure during development. These data can be used to provide an estimate of potential developmental effects in humans.

## 2.6: Conclusions

This study sought to quantify key disposition characteristics of domoic acid (DA) when orally and intravenously administered to a model species closely related to humans. Plasma concentrations of DA were analyzed in three healthy, female *Macaca fascicularis* after i.v. administration of 0.005 mg/kg DA, oral administration of 0.15 mg/kg DA, and oral administration of 0.075 mg/kg DA. Urine samples from the 0.075 mg/kg oral exposure were also analyzed to evaluate oral clearance pathways. From this study, we concluded:

- DA administered intravenously showed two compartmental kinetics with a half-life near 1 hour, confirming already published results
- i.v. clearance closely matches the glomerular filtration rate in primates,  $186 \pm 30$  ml/hr/kg, suggesting renal excretion is a major route of excretion and
- Excretion is slow, but elimination is rapid, due to a low volume of distribution
- Absolute oral bioavailability of DA is between approximately 9 - 12%
- Oral ingestion of DA shows an extended duration of exposure due to slow GI absorption that limits excretion and prolongs half-life, thus exhibiting classic flip-flop kinetics
- Extended duration of DA exposure may lead to plasma accumulation during chronic dosing
- 65% of the oral DA dose is found in urine samples, suggesting that though renal filtration is the primary route of excretion, other routes may exist as well
- A second peak in plasma concentrations suggests that orally administered DA may enter the biliary system

The results from this study suggest that risk from chronic exposure of DA in high risk groups at environmentally relevant doses, near the TDI (0.075 mg/kg) may be elevated compared to current standards. The risk may be greater due to plasma accumulation as a result of flip-flop kinetics detailed in these results. Importantly, this pattern of disposition may disproportionately increase the risk of highly sensitive groups, such as those with poor kidney function.

Particularly, fetal exposure of DA to chronic, oral maternal doses may be increased as a direct result of normal fetal functions. Further, fetuses may be at the most risk from DA toxicity as neurodevelopmental processes are highly sensitive to toxic insults, like excitatory amino acids.

Future studies are set forth to quantify potential kinetic changes as a result of chronic exposure and exposure during pregnancy and determine the nature of DA toxicity on developing fetus in a model species similar to humans.

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