Public Health Risks Associated with Chronic, Low-Level Domoic Acid Exposure: A Review of the Evidence

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Abstract

Domoic acid (DA), the causative agent for the human syndrome Amnesic Shellfish Poisoning (ASP), is a potent, naturally occurring neurotoxin produced by common marine algae. DA accumulates in seafood, and humans and wildlife alike can subsequently be exposed when consuming DA-contaminated shellfish or finfish. While strong regulatory limits protect people from the acute effects associated with ASP, DA is an increasingly significant public health concern, particularly for coastal dwelling populations, and there is a growing body of evidence suggesting that there are significant health consequences following repeated exposures to levels of the toxin below current safety guidelines. However, gaps in scientific knowledge make it difficult to precisely determine the risks of contemporary low-level exposure scenarios. The present review characterizes the toxicokinetics and neurotoxicology of DA, discussing results from clinical and preclinical studies after both adult and developmental DA exposure. The review also highlights crucial areas for future DA research and makes the case that DA safety limits need to be reassessed to best protect public health from deleterious effects of this widespread marine toxin.

Keywords

domoic acid; amnesic shellfish poisoning; neurotoxicity; development; chronic exposure; public health

Abbreviations

AMPA – α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; ASP – Amnesic Shellfish Poisoning; BBB – blood-brain barrier; bw – body weight; CL – total body clearance; CL/F – total body clearance after oral administration; CL_r – renal clearance; CSL – California Sea Lions; CoASTAL – Communities Advancing the Studies of Tribal Nations Across the Lifespan; D – dopamine; DA – domoic acid; DG – dentate gyrus; ECoG – electrocorticography; EEG –

electroencephalography; EFSA – European Food Safety Authority; fe – fraction excreted unchanged in urine; GABA – γ-aminobutyric acid; GAD – glutamic acid decarboxylase; GD – gestational day; GFAP – glial fibrillary acidic protein; ic – intracoelomic; ip – intraperitoneal; iv – intravenous; KA – kainic acid; LOAEL – lowest-observed-adverse-effect-level; MFS – mossy fiber sprouting; MRI – magnetic resonance imaging; MRT – mean residence time; MWM – Morris water maze; ND – neuronal degeneration; NMDA – N-methyl-D-aspartic acid; NOS – nitric oxide synthase; PND – postnatal day; PPI – pre-pulse inhibition; ROS – reactive oxygen species; sc – subcutaneous; TH – tyrosine hydroxylase; TK – toxicokinetic; V_{ss} - volume of distribution at steady-state; WA – Washington; WHO – World Health Organization

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1. Introduction

Domoic acid (DA), the excitotoxic glutamate receptor agonist known to cause an acute neurotoxic syndrome called Amnesic Shellfish Poisoning (ASP), is produced by marine algae in the genus *Pseudo-nitzschia*, found worldwide (Bates, 2000; Bates et al., 1989; Bates, Hubbard, Lundholm, Montresor, & Leaw, 2018; Bates & Trainer, 2006; Perl, Bedard, Kosatsky, Hockin, & Todd, 1990; Todd, 1993). When these toxigenic algae divide rapidly, high-density toxic “blooms” emerge in marine waters, where they can persist for months (McCabe et al., 2016; Trainer et al., 2012). Production of DA, however, is variable, and, while some environmental conditions seem to enhance production, it remains unclear as to why these algae produce the toxin (Brunson et al., 2018). When DA is present in the environment, filter feeding marine life, such as clams, oysters, mussels, crabs, and anchovies, can accumulate DA and pass the toxin to humans and wildlife (D’Agostino et al., 2017; Fire et al., 2010; Kvitek, Goldberg, Smith, Doucette, & Silver, 2008; Lefebvre, Bargu, Kieckhefer, & Silver, 2002; Lefebvre, Silver, Coale, & Tjeerdema, 2002).

While regulations developed in the late 1980s have prevented acute human DA poisonings (i.e. ASP), other exposure scenarios have been of increasing concern (Lefebvre & Robertson, 2010; Wekell, Jurst, & Lefebvre, 2004). With the intensification of algal bloom conditions due to climate change (McKibben et al., 2017; Trainer et al., 2020; Wells et al., 2020, 2015) and recent consumption surveys identifying that many shellfish harvesters may be regularly exposed to low levels of DA (Andjelkovic, Vandevijvere, Van Klaveren, Van Oyen, & Van Loco, 2012; Ferriss, Marcinek, Ayres, Borchert, & Lefebvre, 2017), there is an urgent need to comprehensively understand the health impacts associated with chronic, low-level exposure to this prevalent neurotoxin. The following review synthesizes the evidence from epidemiological and *in vivo* laboratory studies on DA toxicity, while identifying persistent data gaps that hinder our understanding of the present-day public health risk of DA.

2. A Human Domoic Acid Poisoning Event

DA is a small amino acid, structurally similar to the neurotoxin, kainic acid (KA), and the endogenous neurotransmitter, glutamate (Fig. 1) (Wright et al., 1989). DA was first identified in the 1950s in Japan, when it was characterized as an anti-parasitic treatment, administered in doses of 20 mg (Takemoto & Daigo, 1958). It was not until nearly thirty years later, in 1987, when the potent neurotoxicity of the compound was revealed. In early December of that year, a national health bulletin was posted on Prince Edward Island, Canada, warning of a new mussel-associated intoxication, after three people were hospitalized with symptoms of confusion, disorientation, and memory loss after consuming mussels contaminated with 310-1280 ppm DA. In total, over 150 people were sickened and four people died after very high levels of DA exposure (estimated up to 290 mg/patient) (Perl, Bedard, Kosatsky, Hockin, & Todd, 1990; Perl, Bedard, Kosatsky, Hockin, Todd, et al., 1990). DA was not detected in blood or cerebral spinal fluid; instead, cases were considered positive if respondents experienced symptoms within 48 h of consuming shellfish (Perl, Bedard, Kosatsky, Hockin, & Todd, 1990). Of those who met this case definition, most reported upset stomachs, vomiting and diarrhea that developed within 4-5 h of exposure. Nearly a fifth of the poisoning cases were admitted to the hospital with seizures and a host of other neurological symptoms, which ranged from uncontrollable emotionality to coma. The term “ASP” is now widely used to refer to the clinical symptoms associated with acute DA toxicity (Perl, Bedard, Kosatsky, Hockin, Todd, et al., 1990). Neuropsychological examinations of some of the most severely affected ASP patients revealed a unique pattern of functional losses consistent with anterograde amnesia (Todd, 1993), which is characterized by the lack of ability to form new memories (Tulving, 1983). In extreme DA poisoning cases, patients with amnesia had persistent and long-term memory deficits (Zatorre, 1990).

Several individuals sickened by DA underwent magnetic resonance imaging (MRI), positron emission tomography (PET) scans, and electroencephalography (EEG) assessments. MRI and other imaging results from patients indicated that those affected had acute neuronal death in the amygdala and parahippocampal gyrus, as well as moderate to severe disturbances in electrophysiology, as observed by spikes and seizure-like activity on EEG exams (Gjedde & Evans, 1990; Teitelbaum, Zatorre, Carpenter, Gendron, & Cashman, 1990). In addition to the three patients that died shortly after initial DA exposure, one patient survived the poisoning, but later developed temporal lobe epilepsy and died within a year (Cendes, Andermann, Carpenter, Zatorre, & Cashman, 1995). Histopathological follow-up in deceased patients revealed extensive neurotoxic injury in the amygdala and hippocampus, with neuronal death and astrocyte reactivity noted in the amygdala, hippocampus, olfactory cortex, and thalamus (Carpenter, 1990), reflecting the potent neurotoxic nature of DA.

Following the Prince Edward Island poisoning, public health officials implemented DA monitoring programs for seafood and instituted a 20 ppm DA action level for closing beaches to shellfish harvesting (see Section 8 for details). There have been no episodes of ASP since the 1987 poisoning episode.

3. Sea Lions as Sentinels for Health Impacts of Domoic Acid

While humans have been protected by this action level, multiple DA poisoning events have occurred in naturally exposed marine mammals over the past three decades. In May and June of 1998, California sea lions (CSLs) along the Pacific coast of California were observed exhibiting seizures, ataxia, abnormal scratching, and related neurological symptoms (Gulland, 2000; Scholin et al., 2000). Analysis of blood, urine, and feces from subsets of the estimated hundreds of impacted animals identified the presence of DA (Lefebvre et al., 1999; Scholin et al., 2000). This, in conjunction with a simultaneous *Pseudo-nitzschia* algal bloom, as well as the detection of both DA and the DA-producing algae in sea lion feces and the anchovy prey of CSLs, collectively led to the first documentation of DA poisoning in a marine mammal species (Lefebvre et al., 1999; Scholin et al., 2000). Since then, dozens to hundreds of CSLs off the coast of California are diagnosed with DA poisoning each year (Bargu, Goldstein, Roberts, Li, &

Gulland, 2012; Bargu, Silver, Goldstein, Roberts, & Gulland, 2010; Greig, Gulland, & Kreuder, 2005).

In a 1998 poisoning event and subsequent follow-up, CSLs with acute DA toxicosis consistently exhibited excitotoxic cell death in the hippocampus (Gulland et al., 2002; Scholin et al., 2000; Silvagni, Lowenstine, Spraker, Lipscomb, & Gulland, 2005). Researchers have also identified an additional, long-lasting, DA-associated clinical syndrome in CSLs, characterized by reoccurring seizures following sublethal exposure (Goldstein et al., 2008; Ramsdell & Gulland, 2014). Persistent seizures are often accompanied with other lingering, adverse effects of DA. These include poor performance on spatial memory challenges, MRI changes in hippocampal structure and connectivity (Cook, Berns, Colegrove, Johnson, & Gulland, 2018; Cook et al., 2015), and aberrant behavior, including impaired spatial navigation, repetitive behaviors, and unusual aggression (Cook, Reichmuth, & Gulland, 2011; Cook et al., 2016; Goldstein et al., 2008). In instances of chronic seizures and related effects after DA exposure, researchers often observe unilateral hippocampal atrophy that is distinct from direct DA-associated atrophy (Buckmaster, Wen, Toyoda, Gulland, & Van Bonn, 2014; Goldstein et al., 2008).

CSLs have been an invaluable sentinel species in DA research, as marine mammal exposures are similar to the human oral exposure route, and the symptoms of acute CSL toxicosis syndrome are analogous to ASP (Goldstein et al., 2008). Given the expanding reports detailing the prolonged effects related to sub-lethal DA exposure described above, researchers should consider expanding investigations of CSLs to examine the effects of chronic, low-level DA exposures in both adult and developing marine mammals.

4. Toxicokinetic Properties of Domoic Acid

An important factor in cross-species comparisons of chemical exposures and effects is the toxicokinetics (TK) in humans and animal models. DA is a water soluble (logP = -0.23), small molecule compound (molecular weight: 331.33 g/mol) that is ionized with 3 negative charges and 1 positive charge at physiological pH of 7.4 (Walter, Leek, & Falk, 1992). Consistent with its hydrophilicity and ionization state at physiological pH, the plasma protein binding of DA is negligible (fraction unbound (f_u) = 1), as measured in monkey and human plasma (Jing et al., 2018), and the transcellular permeability of DA is low, as shown in Caco-2 cells (Kimura, Kotaki, Hamaue, Haraguchi, & Endo, 2011). Based on these physicochemical properties, DA is not expected to distribute widely in the body and is mainly eliminated unchanged in the urine through glomerular filtration. Unfortunately, the TK of DA in humans is not known. During the 1987 Prince Edward Island DA poisoning, clinical specimens of blood and cerebral spinal fluid were collected from patients, but DA was not detected in any of these samples, likely due to the delayed sampling time (1-2 weeks after hospital admission) and inadequate sensitivity of the detection method (Todd, 1993). The TK of DA in laboratory animal models, however, have been described.

The TK of DA following intravenous (iv) dosing has been reported in multiple preclinical animal models (Table 1). As expected, based on the physicochemical properties, DA was rapidly eliminated in urine following an iv dose and has a short plasma half-life (1-2 h) in both monkeys and rats (Jing et al., 2018; Suzuki & Hierlihy, 1993; Truelove & Iverson, 1994). The volume of distribution (V_{ss}) of DA was reported as less than the total body water content (60-70%) in both monkeys and rats over a wide range of doses, suggesting that DA is not widely distributed in the body and the distribution is independent of dose. Consistent with the low V_{ss}, the brain-to-blood ratio of DA was low (0.04-0.06) in rats following a single iv and intraperitoneal (ip) dose. The same ratio following repeated dosing has not been reported.

Species differences have been observed in the pathways of elimination of DA. In monkeys, 30-70% of the iv dosed DA was excreted through the urine, suggesting extrarenal elimination (Jing et al., 2018; Truelove & Iverson, 1994). The remaining fraction of the dose was attributed to be eliminated through biliary excretion, as DA was detected in primate feces following an iv dose (Jing et al., 2018). The renal clearance of DA in monkeys was reported to be about 60% of the creatinine clearance (Jing et al., 2018), indicating tubular reabsorption of DA. In contrast, in rats, DA was predominantly (~100%) eliminated through urine following an iv dose (Suzuki & Hierlihy, 1993; Truelove & Iverson, 1994). Moreover, the renal clearance of DA has been reported to be similar to inulin clearance in rats, suggesting minimal tubular reabsorption of DA (Suzuki & Hierlihy, 1993).

Although the onset and duration of the toxicological effects have been shown to be significantly different following iv and oral dose (Tryphonas, Truelove, & Iverson, 1990; Tryphonas, Truelove, Todd, Nera, & Iverson, 1990), the oral TK of DA has not been reported until recently in cynomolgus monkeys (Jing et al., 2018; Shum et al., 2020). In this species, DA was absorbed slowly in the gut, limiting its oral bioavailability to less than 10% (Jing et al., 2018; Truelove et al., 1997). This observation is consistent with previous observation in rats that ~100% of orally dosed DA was recovered in feces (Iverson et al., 1989). This slow absorption significantly increased the apparent half-life of DA (10 h) in monkeys, indicating that DA follows flip-flop kinetics (when the absorption rate constant (k_a) is less than the elimination rate constant (k)) after an oral dose (Jing et al., 2018; Shum et al., 2020). In cynomolgus monkeys, the slow absorption following an oral dose may also explain the slow onset and longer duration of toxicological effects following an oral dose compared to iv dose, which was supported by the predicted brain concentration-time profile using a physiologically-based pharmacokinetic (PBPK) model of DA. Furthermore, a more-than-dose-proportional increase in AUC has been observed in cynomolgus monkeys following oral doses of DA suggesting potential saturation kinetics in either the absorption processes, elimination processes, or both (Shum et al., 2020). This observation suggests that drug transporters may play an important role in the disposition of DA and may contribute to species differences in the TK of DA.

Another major concern of DA toxicity is its toxicological effect on the developing fetal brain, as DA has been shown to distribute to the fetus following maternal exposure in CSLs (Brodie et al., 2006; Lefebvre et al., 2018), monkeys (Shum et al., 2020), and rodents (Maucher

Fuquay, Muha, Wang, & Ramsdell, 2012). Maternal-fetal disposition of DA has been reported in monkeys following repeated oral doses and in rats following a single iv dose. The TK of DA is not significantly altered during pregnancy suggesting that the DA exposure in pregnant animals is similar to that of the nonpregnant animals (Maucher Fuquay et al., 2012; Shum et al., 2020). The fetal/maternal AUC ratio was reported to be less than one (F/M ratio: 0.3) in both monkeys and rats, indicating that placental efflux transport is limiting fetal exposure. On the other hand, DA has been shown to accumulate in the amniotic fluid, acting as a distribution compartment for the fetus (DA recirculates to the fetus through fetal swallowing of amniotic fluid) (Lefebvre et al., 2018; Maucher Fuquay et al., 2012; Maucher & Ramsdell, 2007; Shum et al., 2020). The distribution kinetics between amniotic fluid and the fetus have been shown to increase the apparent fetal plasma half-life in monkeys and rats (Maucher Fuquay et al., 2012; Shum et al., 2020), which may increase the risk of fetal toxicity following repeated dosing to the mom.

Neonatal exposure to DA through breast milk has been estimated following iv exposure in lactating rats (Maucher & Ramsdell, 2005) and oral exposure in lactating CSLs (Rust, Gulland, Frame, & Lefebvre, 2014). Unequivocally, both studies demonstrated that DA was detected in breast milk, even when DA could no longer be detected in plasma and urine, indicating a long retention time of DA in breast milk. Despite this, DA concentrations in breast milk were quite low, thus minimizing the risk of neonatal exposure through breast milk. With a maternal exposure of 1 mg/kg DA iv, the neonatal rat was exposed to an estimated 60 ng DA/kg through breast milk, or 0.006% of the maternal dose (Maucher & Ramsdell, 2005). Although the relative infant dose is not known following an oral dose, it is likely lower than 0.006%, based on the low oral bioavailability of DA. Therefore, DA exposure through breast milk poses a minor risk to neonates.

These TK concepts, with recent improvements in the sensitivity of bioanalytical methods (Shum et al., 2018), may be useful for the development of a biomarker of exposure for DA. In humans, DA has been detected in urine from those who consumed razor clams containing low levels of DA up to 9 days before urine collection (Lefebvre et al., 2019). These results suggest that urine may be applicable to estimate recent exposure from consuming contaminated seafood. In the same study, a DA-specific antibody was also detected in the serum from subjects who regularly consume razor clams known to contain low levels of DA year-round, suggesting that the antibody may be a viable biomarker for chronic DA exposure. These new methods to estimate recent and chronic DA exposure will facilitate the understanding of the dose-response relationship of DA in humans.

Summary and Future Directions

DA is not widely distributed in the body and is mainly eliminated unchanged in the urine. Most relevant for humans, the TK of DA following oral dose in cynomolgus monkeys follow flip-flop kinetics, which are a result of slow intestinal absorption. Other kinetic data suggest that drug transporters may play an important role in the TK of DA and may contribute to species difference of DA disposition. The maternal-fetal kinetics suggests that the placenta acts as a partial barrier, thus limiting fetal exposure to DA, but DA can accumulate in amniotic fluid, which prolongs the exposure to the fetus. The neonatal exposure to DA through breast milk is expected to be minimal based on the low estimated relative infant dose.

Future mechanistic studies are warranted to further elucidate the role of drug transporters in the TK and maternal-fetal kinetics of DA. New advances have pioneered two potential biomarkers (e.g. urine DA levels, DA-specific antibody) to aid in understanding the human dose-response relationship, but additional data are necessary to confirm these results in broader human populations. Additionally, breast milk concentration in lactating women chronically exposed to DA should be measured to confirm that this route poses a low risk of neonatal exposure in humans. Building off these data, standardized biomarkers, could facilitate the diagnosis of health effects associated with chronic, low-level DA exposure.

5. Neurological Effects of Domoic Acid in Adults

Uncovering Adult Neurological Responses to Domoic Acid

Since the 1987 human DA poisoning, ample laboratory research has been conducted to identify the effects of DA in the adult nervous system. Early traditional toxicology experiments were aimed at disseminating information on the acute toxicity of this poison and revealed several key findings related to overt toxicity. Over the past 30 years, research has moved away from acute toxicity testing and towards assessing DA effects following chronic, low-level exposures, such as those observed in coastal populations. Results of studies reporting neurotoxic effects of DA in adult subjects are summarized below and in Table 2, including parts A, B, C, and D. Summaries of human epidemiological studies, as well as experimental studies using nonhuman primates, rodents, and fish laboratory models are presented in the following sections: overt neurotoxicity; functional effects on cognition, emotionality, motor responses, and neuroimaging; neuropathology; and neurochemical and molecular responses.

Overt Neurotoxicity

Similar to the human syndrome ASP, laboratory mammals exposed to acute, high doses of DA exhibit a common pattern of symptoms. Macaques administered >0.25 mg/kg iv (Scallet et al., 1993; Schmued, Scallet, & Slikker, 1995; Tryphonas, Truelove, & Iverson, 1990), 4 mg/kg ip (Tryphonas, Truelove, & Iverson, 1990), or oral doses of >5 mg/kg (Tryphonas, Truelove, Todd, et al., 1990), exhibited an explicit progression of toxicity, beginning with increased vocal expression (chirping), quickly moving to gastrointestinal distress (excessive salivation, gagging, vomiting), unusual motor activity (so called “wet-dog shakes”), postural positioning somewhat indicative of praying, and ending with tremors, seizures, and death.

In rodent models, this pattern of symptomology is repeated, with some slight discrepancies. Acute toxicity in both rats and mice is dose-dependent and has been well documented in the literature. The median ip toxic dose in mice is approximately 3-4 mg/kg and in rats is near 1 mg/kg (Fujita et al., 1996; Iverson et al., 1989; Sobotka et al., 1996; Tasker, Connell, & Strain, 1991). After DA administration, rodents demonstrate a short period of hypoactivity, which is quickly followed by a deeper sedative state. Advancing symptoms have been described as a sudden increase in activity, with signs of stereotypic behaviors, a loss of postural control and tremors and convulsions. Reported stereotypic behaviors include head-bobbing or weaving, circling, and hindlimb scratching near the ear. The appearance of the hindlimb scratching is so distinctive of this toxin, that it has been used as the primary assay for acute DA toxicity (Iverson & Truelove, 1994). Vomiting, one of the primary symptoms associated with DA in primates, is noticeably lacking in the progression, as rodents cannot vomit. Rats are more sensitive than mice to overt signs of toxicity, which may be due to differences in physiological parameters or pharmacological response (Iverson et al., 1989).

There is some evidence that DA effects vary depending on the sex and age of the subject, with male (Baron et al., 2013; Wetmore & Nance, 1991) and older (Hesp, Clarkson, Sawant, & Kerr, 2007) mice and rats responding more severely to the toxin. This apparent sensitivity in older males was also noted in the 1987 human poisoning (Perl, Bedard, Kosatsky, Hockin, & Todd, 1990). At the time, researchers postulated that sex-based differences in seafood consumption and age-related changes in kidney function may have contributed to variations in toxic responses.

Summary. Following acute DA exposure, laboratory models exhibit progressive symptoms similar to those of ASP in humans, with effects that include activity level changes, gastrointestinal distress, stereotypic behaviors, seizures, and death. The potential for both sex-specific and age-related susceptibilities to DA exposure effects is notable and under active, ongoing investigation (Personal Communication, Dr. David Marcinek).

Functional Effects

Studies of adult humans, as well as nonhuman primates and rodents have also investigated the association between low-level DA exposure and more subtle neurological effects, such as changes in cognition, emotionality, or motor responses.

Effects on Cognition. Cognitive effects have been the focus of both clinical and preclinical research, as memory loss was the hallmark symptom of acute DA poisoning in human episodes of ASP (Perl, Bedard, Kosatsky, Hockin, Todd, et al., 1990). The only human cohort study dedicated to understanding the health effects of DA is the Communities Advancing the Studies of Tribal Nations Across the Lifespan (CoASTAL) cohort. The CoASTAL cohort is comprised of volunteer Native American adults who live on the coast of Washington (WA) State and regularly consume shellfish that contain DA (Tracy, Boushey, Roberts, Morris, & Grattan, 2016). In this group, 97% of adults frequently consume fish or shellfish, and many of these adults eat more than one meal/month of razor clams (Tracy et al., 2016), a filter feeder known to have persistent DA concentrations up to a year after a toxic marine event ends (Wekell, Gauglitz, Barnett, Hatfield, & Eklund, 1994). Preliminary studies using the verbal cognitive CVLT-II Standard test in 513 adults suggested a subtle decrease in cognitive performance in those who consumed more than 15 clams/month (Grattan et al., 2016). A follow-up study of a subset of CoASTAL adults used additional surveys to assess everyday memory, a measure of the frequency of memory “failures” in day-to-day life (Grattan et al., 2018). Adults who consumed above the group median amount of razor clams in the past week, but not the past year, were nearly 4x more likely to report problems with everyday memory. While the median level of consumption was not reported, DA levels in clams were between 4-14 ppm. Most recently, results from a study in over 100 CoASTAL adults suggests that low-level DA exposure (~324 ng DA/kg/day over one month) was linked to decreased verbal memory recall, but not to measures of intelligence (Stuchal et al., 2020). The authors postulated that this memory deficit was an attenuated form of ASP in adults.

In rodents, learning and memory effects have been described after sub-lethal doses of DA. Rats given 0.04 mg/kg DA iv performed poorly on a radial maze, with a longer time to achieve success (Nakajima & Potvin, 1992), while mice administered a single of dose of 2 mg/kg ip had prolonged latencies and difficulties in finding the platform on the Morris Water Maze (MWM) test (Petrie, Pinsky, Standish, Bose, & Glavin, 1992). In a series of research studies designed to assess potential compounds that ameliorate the decrements of DA, mice given 2 mg/kg/day ip for 21-28 d also demonstrated decreased spatial memory, as noted by decreased object recognition as well as increased latencies both in the ability to find the platform on the MWM and in the step-through passive avoidance task (Lu et al., 2013; D. Wang, Zhao, Li, Shen, & Hu, 2018; Wu et al., 2013, 2012). A single dose of 1.32 mg/kg ip in rats did not, however, result in changes in passive or active avoidance tasks in other research (Sobotka et al., 1996). Working memory on a match-to-sample task was decreased in animals given single doses of 1 and 2 mg/kg ip (Clayton, Peng, Means, & Ramsdell, 1999). In the same study, memory effects were replicated with repeated exposure to doses of 1 mg/kg ip, but not doses of 2 mg/kg ip, delivered every other day for 7 d (total of 4 doses). This suggests that there may be a potential resistance to the effects of multiple, higher doses. In all of these rodent studies, however, animals displayed some signs of overt toxicity (e.g. changes in locomotion, stereotypic behaviors, hindlimb scratching) in addition to learning and memory deficits.

Only one laboratory study has been designed to assess learning and memory at doses below those that produce overt toxicities. Lefebvre and colleagues conducted a long-term study using low-level exposure (~0.75 mg/kg ip), where mice were exposed to DA once a week for up to 25 weeks (Lefebvre et al., 2017). After 25 weeks, animals had fewer successful trials on the radial water tread maze, but this deficit in learning and memory was reversed after a 9-week wash-out period. Authors additionally noted that recovered mice maintained their ability to navigate the maze throughout old age, suggesting that a chronic, low-level exposure in mice may produce subtle changes in memory that are recoverable after cessation of exposure.

In studies with adult humans and animal models, DA-related effects on cognition are evident. Importantly, deficits in learning and memory occurred in both humans and animals in absence of signs of overt toxicity.

Effects on Emotionality. DA effects on emotionality have been studied in a small set of research projects using adult animal models, mostly using observations of behavior in an open field. Rats exposed to 1.8 mg/kg ip demonstrated more grooming behaviors and other stereotypic actions in an open field test, in absence of overt DA toxicity (Baron et al., 2013). Authors suggested that this was indicative of heighted emotionality or distress. Similar results of longer habituation times and increases in grooming behaviors in open field test were reported in another study with rats exposed to 1 mg/kg ip DA (Schwarz et al., 2014). In a separate rodent study designed to create a model for epilepsy, doses of 1 mg/kg ip given at least 2x (once per h, for up to 5 h) produced increases in defensive aggression in epileptic rats (Tiedeken & Ramsdell, 2013). While emotional effects of DA in adult models are inadequately documented, the findings presented here encourage additional investigation.

Effects on Motor Responses. Laboratory studies using adult animal models exposed to DA have also investigated effects on the motor system and associated reflexes. A recent seminal study used macaque monkeys exposed to daily, oral doses of 0.075 and 0.15 mg/kg/day for up to 11 months to study the maternal reproductive and offspring neurodevelopmental effects of DA (Burbacher et al., 2019). Findings from this cohort documented an increased incidence in subtle upper limb tremors in adult females, when performing a reaching task. In rats given daily doses of 0.2-1.6 mg/kg ip for 30 days, motor coordination was also decreased after just 10 days of exposure (Xu et al., 2008). Another study documented an exaggerated auditory startle response in rats exposed to 1.32 mg/kg ip, which was paired with signs of overt locomotive toxicity (Sobotka et al., 1996). In these studies, however, few researchers have sought to clarify whether changes in motor measures are driven by damage directly to the motor neurons or other neurotoxic effects. This point may be more salient when considering that DA has been shown to directly damage the spinal cord in rodents (Xu et al., 2008). At present, there is evidence that lower levels of DA can cause motor effects, but these effects are subtle, and the origin of motor changes is unknown.

Effects from Neuroimaging Studies. Seizures and electrophysiological changes are known to occur after DA exposure in humans and animals (Cendes et al., 1995; Perl, Bedard, Kosatsky, Hockin, & Todd, 1990; Tasker et al., 1991), but only a few studies have employed neuroimaging to investigate these changes. Adult monkeys in the aforementioned reproductive study, who were orally exposed to 0.075 and 0.15 mg/kg/day for 1-2 years, underwent structural MRI, magnetic resonance spectroscopy (MRS) and EEG assessments (Petroff et al., 2020, 2019). Structural MRI scans in a subset of these macaques suggested that DA-related tremors observed during a reaching task were connected to decreased white matter integrity in key white matter motor tracts and increased lactate in the thalamus (Petroff et al., 2019). DA-exposed animals, on average, also had decreased delta power and increased theta, alpha, and beta power on resting, sedated EEG exams (Petroff et al., 2020).

EEG imaging has been used to examine the effects of DA exposure in rats. Doses of 1-10 mg/kg ip (Binienda, Beaudoin, Thorn, & Ali, 2011; Fujita et al., 1996; Sawant, Tyndall, et al., 2010; Scallet, Kowalke, Rountree, Thorn, & Binienda, 2004) and intrahippocampal exposure to 10-300 pmol of DA (Dakshinamurti, Sharma, & Sundaram, 1991; Sawant, Mountfort, & Kerr, 2010) led to extensive activation in the hippocampus, increased electrographic spiking and seizures, and increased delta, theta, alpha, and beta power. Changes like these are indicative of subtle neuroelectric variations that have been linked to deficits in learning and memory and the diagnosis of neurodegenerative disorders (Harmony, 2013; Newson & Thiagarajan, 2019). Further analyses of these similarities may reveal more about the underlying functional and cellular effects of the DA-induced neuroimaging changes reported here.

Summary. Due to its prominent role in ASP, memory has been the focus of the majority of DA research. Both symptomatic and asymptomatic DA doses are known to cause adverse learning and memory outcomes, which were reversible in asymptomatic rodents. Effects on other functional domains have not been studied well, but results from a few recent studies suggest that anxiety-related behaviors and motor function are impacted after low-level, asymptomatic exposure. Subtle electrophysiological, neurochemical, or structural changes in the brain may underlie these functional changes.

Neuropathological Effects

Effects on Neurons. After acute, high-dose DA exposures, neuronal degeneration and gross lesions have been documented in several mammalian brain regions. DA most notably causes damage in the hippocampus, the memory center of the brain. In monkeys given single doses of DA >0.2 mg/kg iv (Tryphonas, Truelove, & Iverson, 1990) or >6 mg/kg oral (Tryphonas, Truelove, Todd, et al., 1990), large neuropathic lesions are evident in the hippocampus (including CA1, 3 and 4), hypothalamus, and medulla, but not other regions in the brain. Lower amounts of neuronal degeneration have also been documented in the hippocampus, subiculum, thalamus, and lateral septum, as well as the entorhinal and piriform cortices after doses >0.5 mg/kg iv (Schmued et al., 1995). In rats given >2 mg/kg ip (Appel, Rapoport, O'Callaghan, Bell, & Freed, 1997) or mice given > 4 mg/kg ip (Peng, Taylor, Finch, Switzer, & Ramsdell, 1994; J. C. Ryan, Cross, & Van Dolah, 2011; Strain & Tasker, 1991), similar persistent lesions and neuronal damage occur in the hippocampus, hypothalamus, thalamus, amygdala, olfactory and piriform cortices, and septal area. A comprehensive brain survey of DA damage in rodents largely confirmed these results, while also suggesting that individual regions in target areas, such as the dentate gyrus of the hippocampus, are largely unaffected by acute DA exposures at 4 mg/kg ip (Colman, Nowocin, Switzer, Trusk, & Ramsdell, 2005).

Results from studies that examine pathology at multiple time points after the initial exposure suggest that the complete picture of neuronal degeneration in the brain may only be visible sometime after sub-lethal doses (2-7 mg/kg ip and 0.75 mg/kg iv), with rats not expressing any neuronal damage until at least 2 days after the initial exposure (Ananth, Thameem Dheen, Gopalakrishnakone, & Kaur, 2001; Bruni, Bose, Pinsky, & Glavin, 1991; Vieira et al., 2015). Thus, histopathology conducted less than 24 h after sub-lethal, but symptomatic, DA exposure may not be the most useful way of assessing neuropathological changes. Histopathology after asymptomatic exposures in rodents has not revealed any gross neuronal effects (Lefebvre et al., 2017; Moyer et al., 2018).

Effects on Axons. Limited evidence suggests that axonal damage is typically less extensive than damage to the neuronal body. In adult monkeys, axon terminal degeneration was reported after exposure to 1 and 1.25 mg/kg DA iv (Scallet et al., 1993; Schmued et al., 1995). Authors suggested that the injury may have been caused by the death of the cell body and not by damage directly to the axon. In rats, a single exposure of 2.25 mg/kg led to axonal damage in the hippocampus (Appel et al., 1997), whereas repeated exposure to 1 mg/kg ip was connected with axonal injury in both the olfactory bulb and thalamus (Tiedeken, Muha, & Ramsdell, 2013). Results from another study with mice given 4 mg/kg ip indicated axonal damage in the same regions, as well as in the septal area, but not the amygdala (Colman et al., 2005). However, other studies have not reported axonal damage after similar exposures in rodents (Clayton et al., 1999; Peng et al., 1994). Further, lower exposures (2 mg/kg ip) do not appear to impact axons or the associated myelination (Scallet, Schmued, & Johannessen, 2005).

Effects on Glia. Important glial responses to DA have been documented in early studies using animal models. Most commonly, in acute, high-dose DA exposure, a marked astrocytic reaction, detected typically with glial fibrillary acidic protein (GFAP) immunohistochemistry, has been observed in symptomatic monkeys (>0.2-0.5 mg/kg iv) (Scallet et al., 1993; Tryphonas, Truelove, & Iverson, 1990) and rodents (rats: >1 mg/kg ip (Sobotka et al., 1996; Vieira et al., 2015); mice: >2 mg/kg ip (Lu et al., 2013)). In zebrafish, however, asymptomatic exposure to DA for up to 6 weeks did not alter whole brain GFAP expression (Hiolski et al., 2014), suggesting that either GFAP-positive cell responses are highly regional, species-dependent, or do not change after asymptomatic toxic exposures. Several studies in rodents have also documented a potential microglial reaction and suggest that microglial pathology may only be observable at least 2-7 days after initial DA exposure (Ananth, Gopalakrishnakone, & Kaur, 2003a, 2003b; Ananth et al., 2001; Appel et al., 1997; Vieira et al., 2015). This finding is contrary to early studies, which did not observe microglia differences, but examined histopathology immediately after overt behavioral signs of toxicity.

Summary. High-exposure DA toxicity leads to neuronal degeneration and the formation of lesions, most recognizably in the hippocampus. Additional brain areas, such as the amygdala, thalamus, and olfactory areas, may be of concern in particular species and exposure scenarios. Axons and myelin do not appear to be impacted in either acute or sub-acute exposure scenarios, but limited findings from studies with glia suggest that there may be astrocyte responses after acute exposures and microglia responses after either acute or low-level exposures, but the timing of histopathological examination is an important factor in these findings.

Neurochemical and Molecular Responses

A combination of *in vivo* and *in vitro* studies has demonstrated that DA binds to α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and KA-type glutamate receptors (Berman & Murray, 1997; Hampson, Huang, Wells, Walter, & Wright, 1992; Hampson & Manalo, 1998; Qiu, Pak, & Currás-Collazo, 2006; Stewart, Zorumski, Price, & Olney, 1990; Watanabe et al., 2011), triggering a series of events typical of glutamate-derived excitotoxicity (Fig. 2) (Y. Wang & Qin, 2010). In acute, high-dose DA exposure scenarios (up to 1000 μM) with *in vitro* cell culture experiments, activated AMPA and KA receptors allow both an influx of Na^+ into the cell and the release of glutamate into the synapse. N-methyl-D-aspartic acid (NMDA) receptors are then indirectly activated via the released glutamate, and Ca^{+2} ions subsequently flood into the cell. This potent activation causes the depolarization of the post-synaptic cell and leads to excessive production of reactive oxygen species (ROS) via the disruption of normal mitochondria function, ultimately activating necrotic cell death pathways.

Consensus on the acute mechanism of toxicity is well established, but the mechanism of action after lower-level DA exposures is still under active investigation (Costa, Giordano, & Faustman, 2010; Lefebvre & Robertson, 2010; Pulido, 2008). Current *in vivo* rodent evidence suggests that sub-lethal and symptomatic or repeat exposures at 0.3-2 mg/kg ip or 0.75 mg/kg iv may not lead to necrotic cell death, but instead produce smaller increases in ROS (Tsunekawa et al., 2013) and related nitric oxide synthase (NOS) products (Ananth et al., 2003a, 2001; Lu et al., 2013; Vieira et al., 2015), which can disrupt normal mitochondrial function (Wu et al., 2013; Xu et al., 2008).

Other sub-cellular effects have increasingly become a focus of investigation, with some studies probing the effects of DA on specific target genes and related products, while additional studies have assessed changes in large-scale gene expression profiles (Hiolski et al., 2014; Lefebvre et al., 2009; J. C. Ryan, Morey, Ramsdell, & Van Dolah, 2005). *Fos* genes and related proteins, a key signal in cell proliferation and apoptotic cell death pathways, were upregulated in the hippocampi of monkeys, mice, and rats, and the brains of fish after both asymptomatic and symptomatic exposure (Lefebvre et al., 2009; Peng & Ramsdell, 1996; Peng et al., 1994; J. C. Ryan et al., 2005; Salierno et al., 2006; Scallet et al., 1993, 2004). To act as a regulatory protein, fos dimerizes with jun proteins, and *Jun*-family gene expressions have been similarly upregulated after DA exposures in rats and zebrafish (Lefebvre et al., 2009; J. C. Ryan et al., 2005; Scallet et al., 2005). Studies have also suggested *in vivo* alterations in important cell signaling and mitochondrial genes and gene products including those in the FOX family (Lefebvre et al., 2009; J. C. Ryan et al., 2005; Wu et al., 2013), MAP-2 (Vieira et al., 2015), MAPK (Lefebvre et al., 2009; J. C. Ryan et al., 2005; Tsunekawa et al., 2013), and Bax/Bcl-2 (Ananth et al., 2001; Hiolski et al., 2014). Gene expression differences in important neuronal health genes like *APOE*, *APP*, *NRXN*, *GABARAP*, and *NPTX* have also been described in whole-brain zebrafish studies (Hiolski et al., 2014; Lefebvre et al., 2009). Notably, gene expression differences are highly dependent on the dose (Lefebvre et al., 2009), exposure duration (Hiolski et al., 2014), and the time between the end of exposure and gene analysis in both mice and fish (J. C. Ryan et al., 2005). These divergent responses are particularly striking when comparing expression differences in symptomatic and asymptomatic animals.

Summary. The mechanism of acute DA toxicity is well established, involving the activation of AMPA and KA-type glutamate receptors, subsequent activation of NMDA receptors, and necrosis processes. Mechanisms of action at lower levels of DA exposure are still under investigation. Future research on DA adult neurotoxicity should work to understand the potential cell death compensatory mechanisms or other means of cell protection that may lead to differences in response, which may include neurogenesis (Pérez-Gómez & Tasker, 2012, 2013), synaptic protein expression changes (Moyer et al., 2018), alterations in the balance of glutamatergic and GABA(γ-aminobutyric acid)ergic neuron functioning (Dakshinamurti et al., 1991; Hiolski et al., 2016; Moyer et al., 2018), and the upregulation of neuroprotection pathways (Giordano, Kavanagh, Faustman, White, & Costa, 2013).

Future Directions

Adult neurotoxicity has been thoroughly described after acute exposure scenarios, but there is only a small body of research on the effects of DA in absence of overt toxicity. Going forward, studies focused on the functional effects of DA should be a priority area of research, especially when considering the new evidence detailing human health consequences from chronic, low-level exposure to DA in the CoASTAL cohort study. Additional avenues of research in the potential sex- and age-variability of responses as well as in molecular and neuroprotective mechanistic pathways should also be pursued. Future studies should include quantitative biomarkers of DA exposure (e.g. blood, urine) to better translate results to public-health risk assessment and policy.

6. Neurodevelopmental Effects of Domoic Acid Exposure

Exploring the Consequences of Prenatal and Neonatal Domoic Acid Exposure

It is a well-established tenet of neurotoxicology that age is an important determinant of exposure-driven outcomes. Frequently, the embryo and fetus exhibit heightened sensitivity to the deleterious effects of chemical exposures. Early exposure to toxic agents has the potential to disrupt brain development in ways that may not be immediately expressed, and some effects may not be manifest until adolescence or adulthood (Kraft et al., 2016). The data from animal laboratory studies on DA collectively suggest that the fetus and neonate have an exaggerated vulnerability to the adverse effects of exposure, and early-life central nervous system injuries can be both progressive and persistent (Costa et al., 2010; Grant, Burbacher, Faustman, & Grattan, 2010). There is compelling evidence that DA is a developmental neurotoxin, causing behavioral and pathological effects, at levels of exposure that do not produce toxicity in adults (Doucette & Tasker, 2016). The adverse consequences of early-life DA exposure are not limited to one developmental system and effects have been found on multiple domains of behavior. To facilitate an understanding of how DA affects developing organisms, the data presented herein are organized as follows: overt neurological toxicities; functional effects on physical development and neurological domains of reflexes, sensory processing, cognition, emotionality, activity/motor function, and social behavior; and neuropathology. To date, no reports of DA-exposed children have been published, but there are studies modeling developmental exposure in macaque monkeys, rodents, and zebrafish. The experimental details of these studies are provided in Tables 3 and 4.

Overt Neurotoxicity

In preclinical animal models with adults, seizures are a hallmark sign of overt DA neurotoxicity. This neurological outcome has also been studied in animals and fish developmentally exposed to DA. No evidence of spontaneous seizures or epilepsy was observed in a rodent study of EEG recordings that used maternal iv doses of 0.6 mg/kg or 1.2 mg/kg DA on gestational day (GD) 13 (Demars, Clark, Wyeth, Abrams, & Buckmaster, 2018). In contrast, a separate study found that a single maternal dose of 0.6 mg/kg DA iv on GD 13 resulted in abnormal basal EEGs (Dakshinamurti, Sharma, Sundaram, & Watanabe, 1993). When challenged with a postnatal dose of DA, animals with a history of prenatal DA exposure exhibited a reduced threshold for seizures. A decreased threshold to chemically-induced seizures has also been observed in zebrafish embryonically exposed to DA (Tiedeken & Ramsdell, 2007). In a recent publication that examined developmental DA exposure over a range of doses in zebrafish, treated larvae displayed pectoral fin flipping and convulsions that were dose and time dependent (Panlilio, Aluru, & Hahn, 2020).

Neonatal exposure to ip doses ranging from 0.05 to 0.4 mg/kg DA on postnatal day (PND) 2, 5 or 10 resulted in hyperactivity, stereotypic scratching, paralysis and tonic/clinic seizures, suggesting a heighten sensitivity of young rat pups to the toxic, even lethal, effects of DA when compared to adult animals (Xi, Peng, & Ramsdell, 1997). An interesting phenomenon referred to as “behavioral seizures” has been replicated in a number of postnatal DA studies with rodent models (Doucette et al., 2004; D. A. Gill, Perry, McGuire, Pérez-Gómez, & Tasker, 2012; Perry, Ryan, & Tasker, 2009). Animals treated with subcutaneous (sc) doses of 5 or 20 µg/kg DA on PND 8-14 displayed low-grade seizure behavior that was not spontaneous, but rather, triggered by the presentation of challenging cognitive tasks. The authors suggest that in rats, neonatal DA exposure may increase susceptibility to stress, which is behaviorally manifested as repetitive squinting, mastication, and head bobbing.

Summary. In mammals, prenatal DA exposure has been linked to abnormalities in electrophysiology and a reduced threshold for chemically induced seizures in some studies. Postnatal DA exposure can induce early spontaneous seizures, but seizure-like behaviors can also be triggered by challenging tests of learning and memory in adulthood. In zebrafish embryonically exposed to DA, repetitive fin-flipping and convulsions have been reported.

Ample studies in different animal models have been conducted to characterize overt neurotoxicity in offspring (seizures, hyperactivity, stereotypic scratching, squinting, mastication, head-bobbing, paralysis, death) following high-dose *in utero* or early postnatal DA exposure. Future studies focused on the high-dose acute toxic effects of DA should seek to characterize the mechanistic aspects of these responses.

Functional Effects

Effects on Physical Development. DA effects on physical development have been studied in several animal models including nonhuman primates. Infant macaques exposed prenatally to maternal oral doses of 0.075 or 0.15 mg/kg/day DA throughout gestation showed no evidence of congenital anomalies or effects on birth size (birthweight, crown-rump length, head width, length and circumference) (Burbacher et al., 2019). DA-exposed offspring also exhibited normal weight gain during their first year of life (Dr. Thomas Burbacher, personal communication).

Studies of physical development in prenatally-exposed rodents using maternal iv or ip doses of 0.6 mg/kg or 0.3-1.2 mg/kg DA on GD 13 did not find significant adverse effects on key variables such as gestation length, litter size, birthweight, and neonatal growth (Dakshinamurti et al., 1993; E. D. Levin, Pizarro, Pang, Harrison, & Ramsdell, 2005). In the only study of prenatal exposure in rodents using maternal oral exposures (1 or 3 mg/kg/day on GD 10-17), early physical development in offspring was regularly assessed by evaluating the timing of hair emergence, incisor eruption, eye opening, descent of testes, and vaginal opening (Shiotani et al., 2017). There were no differences between exposed and control animals in achieving these physical milestones, but weight gain during the preweaning period was greater in DA-exposed pups. Studies of postnatal DA treatment generally do not report changes in physical development as well. Weight gain was unaffected in rat pups exposed to 25-100 µg/kg sc DA on PND 1-2 (E. D. Levin et al., 2006). A series of neonatal exposure studies conducted at the University of Prince Edward Island carefully investigated weight gain and day of eye opening after 20 µg/kg sc DA on PND 8-14 in the rat model. DA did not negatively impact these indices of physical development (Adams, Doucette, James, & Ryan, 2009; Bernard, MacDonald, Gill, Ryan, & Tasker, 2007; Doucette et al., 2004; D. A. Gill et al., 2012; Marriott, Ryan, & Doucette, 2012; Perry et al., 2009; C. L. Ryan et al., 2011; Tasker, Perry, Doucette, & Ryan, 2005), nor did it influence sexual maturation (Burt, Ryan, & Doucette, 2008a). While early postnatal DA exposure does not appear to adversely influence physical development, precocious attainment of eye opening has been documented in exposed pups. In two studies, doses of either 5 or 20 µg/kg sc on PND 8-14 did not affect weight gain, but treated pups, especially females, reached criterion on eye opening before their saline-treated counterparts (Burt et al., 2008a; Doucette, Bernard, Yuill, Tasker, & Ryan, 2003).

In zebrafish models, embryonic DA exposure resulted in defects of the heart and spinal cord (Hong, Zhang, Zuo, Zhu, & Gao, 2015; Tiedeken, Ramsdell, & Ramsdell, 2005). More recently, DA exposure at two days post-fertilization was associated with a high prevalence of uninflated swim bladders (a physical milestone that is essential to survival) when measured with imaging techniques at five days post-fertilization (Panlilio et al., 2020). High dose exposure at four days post-fertilization was related to an abnormal opaque appearance of the brain, suggesting frank neurotoxicity at this dose (0.18 ng DA).

In summary, most investigations conducted with mammals do not report an association between developmental DA exposure and congenital anomalies or deficits in physical growth. Reported effects on day of eye opening, an early physical landmark, are mixed, with reports of either no effect or an accelerated effect. New results with zebrafish indicate structural malformations in exposed larvae and highlight the potential importance of this model for future studies of this toxin.

Effects on Reflexes. The assessment of reflexes in infancy provides a measure of nervous system maturity. In the nonhuman primate study described above, the development of early survival reflexes and responsivity to the environment during the first two weeks of life was unaffected by maternal oral exposure throughout gestation to 0.075 mg/kg or 0.15 mg/kg day DA (Grant et al., 2019). Similarly, righting, cliff avoidance, negative geotaxis, and auditory startle in rodent pups were not adversely impacted by maternal oral exposure to 1 or 3 mg/kg on GD 10-17 (Shiotani et al., 2017). Results of postnatal exposure studies also showcase the resilience of this developmental domain to early-life DA exposure. The auditory startle reflex was not diminished by postnatal DA exposure to doses of either 5 or 20 µg/kg sc DA on PND 8-14 in neonatal rodents (Burt et al., 2008a; Doucette et al., 2004; D. A. Gill et al., 2012; Marriott et al., 2012; Perry et al., 2009).

Two investigations using zebrafish found that developmental exposure to DA abolished the “touch response” reflex (Panlilio et al., 2020; Tiedeken et al., 2005). This survival reflex is elicited when zebrafish are touched, triggering movement to quickly change orientation and swim away. Abnormal startle responses have also been reported in DA-exposed larvae (Panlilio et al., 2020).

The collective results on reflex development in monkeys and rodents suggest that DA does not adversely impact the presence and strength of reflexive behaviors, but, in zebrafish, key survival reflexes are adversely affected and, in some cases, completed eliminated.

Effects on Sensory and Motor Processing. Pre-pulse inhibition (PPI) refers to the phenomenon whereby a weakened pre-stimulus inhibits the subsequent reaction to a stronger reflex-eliciting stimulus. In animal work, PPI is frequently evaluated in the context of auditory startle testing and is used as a measure of both sensory-motor gating and early information processing. Prenatal exposure to maternal sc doses of 1.5 mg/kg DA on GD 16 decreased PPI in exposed male pups, suggesting sex-specific impairments in this outcome (Zuloaga et al., 2016), but this finding was not replicated in a study using maternal oral doses of 1 or 3 mg/kg on GD 10-17 (Shiotani et al., 2017). PPI has also been studied in rodents after postnatal DA exposure, primarily in the context of animal model development for schizophrenia. Using a 20 µg/kg sc dose on PND 8-14, investigators found an association between DA treatment and PPI deficits that was dependent on sex and time of day but the baseline startle response and habituation were not affected (Marriott et al., 2012).

A limited number of studies suggest an association between developmental DA exposure and the presence of sex-dependent shifts in the processing of sensory and motor information. Any effect of DA on PPI appears to be independent from the integrity of reflexive behaviors, like the startle response, that are used to measure this psychological construct.

Effects on Cognition. Memory is considered to be a key outcome that is sensitive to the effects of DA exposure across species. In the only study of DA exposure and cognition in primates (as described in Section 5), visual recognition memory was assessed in prenatally exposed infant macaques (0.075 or 0.15 mg/kg/day maternal oral DA throughout gestation) using a test paradigm based on the Fagan Test of Infant Intelligence (Grant et al., 2019). Scores on this test were not affected by DA exposure when test problems were relatively easy to solve. However, when the problems became more difficult and required processing complex social stimuli (faces), high-dose DA exposed infants performed poorly and failed to provide empirical evidence of memory when compared to their control and low-dose counterparts.

Results from rodent studies of prenatal DA exposure have employed a range of testing paradigms, particularly mazes, to evaluate effects on cognition. Using the radial arm maze to measure spatial cognition, rodents prenatally exposed to maternal sc doses of 0.3, 0.6 or 1.2 mg/kg DA on GD 13 showed no deficits in learning, but normal sex-specific differences in performance were attenuated (E. D. Levin et al., 2005). A chemical challenge with scopolamine, conducted when behavioral testing was complete, indicated greater working memory deficits in the most highly exposed animals. In other rodent studies of prenatal exposure, a cued-fear conditioning test has been used to study the effects of DA on associative learning and memory. Significant decreases in freezing behavior were documented in animals after exposure to maternal ip doses of 1 mg/kg DA on GD 11.5, 14.5 and 17.5 (Tanemura et al., 2009), but these effects were not found in a separate study using maternal oral doses of 1 or 3 mg/kg on GD 10-17 (Shiotani et al., 2017).

The effects of DA on cognition have also been examined after neonatal exposure. A study of rat pups exposed to 25-100 µg/kg sc DA on PND 1-2 found no adverse effects of DA on learning in the radial arm maze (E. D. Levin et al., 2006). However, an investigation using three different types of mazes (elevated plus maze, H-water maze, MWM) found that animals treated with doses of 20 µg DA sc on PND 8-14 solved problems of limited difficulty as adeptly as controls, but significant differences in cognition were revealed when exposed animals were challenged with more complex test environments (D. A. Gill et al., 2012). All DA-treated animals displayed increased perseverative behavior on reversal problems, and, in males, the ability to relearn previously mastered material was impaired relative to controls. In a separate study that also used the MWM, marked learning deficits were identified in females with a history of neonatal exposure (5 or 20 µg/kg sc on PND 8-14) (Doucette, Ryan, & Tasker, 2007).

While these published reports provide evidence of learning impairments after early postnatal DA treatment, other studies using similar dosing paradigms have found accelerated performance on tests of cognition. Young rat pups exposed to 5 or 20 µg/kg sc DA on PND 8-14 demonstrated superior neonatal learning on an olfactory conditioning task (Doucette et al., 2003), while adolescent rats exposed to 20 µg/kg sc DA on PND 8-14 showed improved choice accuracy on the radial arm maze (Adams et al., 2009).

The nicotine-induced condition place preference paradigm is designed to study behavioral responses to appetitive rewards. In studies examining how postnatal treatment to 20 µg/kg sc DA on PND 8-14 affected drug seeking behavior on this task, exposed males did not develop a place preference for nicotine, but exposed females showed an increased sensitivity to the rewarding properties of nicotine in one investigation (Burt et al., 2008a; Burt, Ryan, & Doucette, 2008b).

Finally, suppression of latent inhibition behavior, a measure of attentional processing, has been documented in rodents after 20 µg/kg sc DA on PND 8-14 exposure (Marriott et al., 2012), and males appear to be more adversely impacted than females (Marriott, Tasker, Ryan, & Doucette, 2014).

The effects of DA exposure on cognition are bidirectional, as studies have found both negative and positive effects on performance. There is, however, sound evidence that DA exposure early in life can result in subtle but persistent changes in learning and memory. Treatment effects are often gender-specific, and some study results suggest that deficits are most likely to be revealed when challenging test problems are presented (e.g. complex test stimuli, reversal tasks). Prenatal DA treatment appears to result in more serious effects than postnatal exposure.

Effects on Emotionality. While emotionality is difficult to quantify in animals, ultrasonic vocalizations in neonatal rats and mice can be used to measure early social communications. Ultrasonic vocalizations, also referred to as isolation calling responses, are emitted by pups when separated from their dam or littermates and are used as a proxy to quantify emotionality. While prenatal DA exposure to a maternal dose of 1.5 mg/kg sc on GD 16 did not affect ultrasonic vocalizations at multiple postnatal time points in one study (Mills et al., 2016), the same exposure paradigm resulted in a significant reduction of the number of calls in another (Zuloaga et al., 2016). Prenatal exposure to a maternal ip dose of 1 mg/kg on GD 11.5, 14.5 and 17.5 in rodents was associated with the presence of anxiety-like behaviors on the open field test and elevated plus maze (Tanemura et al., 2009). Changes in anxiety were also documented in a study using maternal oral doses of 1 or 3 mg/kg DA on GD 10-17, but in this case, prenatally exposed male rats displayed reduced anxiety, while treated females displayed increased anxiety on the elevated plus maze (Shiotani et al., 2017). Increased anxiety-related behaviors have also been observed in postnatal rodent studies using doses of 5 or 20 µg/kg sc DA on PND 8-14 with the elevated plus maze, and females appear to be more affected than males (Doucette et al., 2007; D. A. Gill et al., 2012). Finally, treatment with 20 or 60 µg/kg sc DA on PND 8-14 did not increase depression-like behavior on the forced swim assay, but animals appeared more anxious during the open field test (Thomsen et al., 2016).

The body of information on emotionality is limited to the rodent animal model. The primary messages from studies on developmental DA exposure and emotionality point to heightened anxiety and increased susceptibility to stress as sensitive outcome measures. The manifestation of these effects is strongly gender- and dose-dependent.

Effects on Activity. Levels of activity are important indicators of developing neurological function and have been studied in DA research with rodent models. Significant changes in locomotor activity patterns were found on the Figure-8 maze and open field test in prenatal exposure studies using maternal sc or ip doses ranging from 0.3-1.2 mg/kg DA on GD 11.5-17.5 (E. D. Levin et al., 2005; Tanemura et al., 2009). Treatment-related effects on circadian activity levels and motor function (coordination and gait) were also identified in a study using maternal oral doses of 1 or 3 mg/kg DA on GD 10-17 on the open field, Rotarod and CatWalk assessments (Shiotani et al., 2017).

A postnatal exposure study, using sc doses from 25-100 µg/kg DA on PND 1-2, found a significant reduction in locomotor activity on the Figure-8 maze (E. D. Levin et al., 2006). Subcutaneous exposure occurring later in the neonatal period (PND 8-14) has been associated with increased activity in female rats on the elevated plus maze (D. A. Gill et al., 2012), as well as increased activity in females and decreased activity in males on the open field test (Burt et al., 2008a). In other investigations, however, activity levels in open field arenas were not altered after DA treatment with doses ranging from 5-60 µg/kg sc on PND 8-14 (Doucette et al., 2004; J. C. Ryan et al., 2011; Thomsen et al., 2016).

Findings from prenatal and neonatal exposure studies suggest that DA can influence normal activity levels in complex ways and locomotor activity can be increased (particularly in females), decreased, or unaffected.

Effects on Social Behavior. Much of the research focused on social behavior and developmental DA exposure has been conducted in an effort to develop a new rodent model of human psychiatric conditions (e.g. schizophrenia) and neurodevelopmental disorders (e.g. Autism Spectrum Disorder). In rodent offspring exposed *in utero* to maternal doses of 1.5 mg/kg DA sc on GD 16, time spent in social interactions was significantly reduced compared to controls, and this treatment effect was primarily observed in males (Mills et al., 2016; Zuloaga et al., 2016). The results from studies of postnatal exposure using a DA dose of 20 µg/kg sc on PND 8-14 are mixed. In one investigation, exposed males spent more time engaged in social withdrawal behaviors and less time in social contact with conspecifics (C. L. Ryan et al., 2011) while another found no treatment effects on social interactions (Thomsen et al., 2016).

Data from developmental DA studies suggest that exposure is associated with changes in social interactions that include increased withdrawal and avoidance behaviors. Males appear to be more sensitive to this treatment-driven change than females.

Summary. In mammalian models, developmental DA exposure does not result in congenital anomalies or adversely impact physical growth trajectories. Reflex development is likewise, unaffected. Researchers have, however, noted subtle but persistent changes in learning and memory, often observed as animals are presented with increasingly challenging tasks. DA exposure is also associated with deficits in social behavior that are characterized by increased withdrawal and avoidance behaviors. Finally, heightened emotionality and susceptibility to stress have been identified as sensitive outcome measures in animals with a history of early-life DA exposure.

Neuropathological Effects

Several studies using animal models have examined the brains of asymptomatic offspring exposed to DA during gestation using a variety of histological and neuroimaging methods. In a seminal mouse study involving prenatal DA exposure to a maternal dose of 0.6 mg/kg iv on GD 13, Dakshinamurti and colleagues (1993) found evidence of progressive hippocampal injury. No cellular damage was observed on PND 1, but damage to hippocampal CA3 and dentate gyrus regions was detected on PND 10, and decreased regional GABA and increased glutamate levels in the cerebral cortex and hippocampus were documented on PND 30. Treatment-related damage to the hippocampus may contribute to the memory deficits observed in exposed offspring. In a separate study, a single maternal dose of 1.5 mg/kg sc DA administered on GD 16 resulted in a significant increase in the number of parvalbumin-positive cells in the lateral amygdala (both sexes) and in the dentate gyrus (males only) (Zuloaga et al., 2016). These cellular effects, suggesting an increase in GABAergic neurons, were observed in offspring with deficits in social behavior and sensorimotor gating. MRI was used in a study of mice exposed to a maternal dose of 1.5 mg/kg DA sc on GD 16, and investigators found an atypical pattern of connectivity in the anterior cingulate cortex (Mills et al., 2016). Treated animals showed overconnectivity from anterior cingulate cortex to infralimbic and orbital regions and underconnectivity to dorsal retrosplenial cortex and CA3 region of hippocampus. It is possible that changes in anterior cingulate cortex connectivity, known to play an important role in emotional regulation, are related to the heightened emotionality that has been observed after developmental DA exposure. Routine histological examinations of exposed brains were normal in a separate study using a maternal dose of 1 mg/kg ip on GD 11.5, 14.5 and 17.5, but evidence of long-term abnormalities in myelination and the overgrowth of neuronal processes in the cerebral cortex and hippocampus were identified using immunohistochemical methods (Tanemura et al., 2009).

Early postnatal exposure has also been associated with neuropathology. Mossy fiber axon sprouting (MFS), a finding commonly associated with temporal lobe epilepsy, has been studied after neonatal DA exposure, and increased MFS was found in the hippocampus of animals exposed to doses of 5 or 20 µg/kg sc on PND 8-14 (Bernard et al., 2007; Doucette et al., 2004; D. A. Gill, Bastlund, et al., 2010). Despite the presence of increased MFS, the clinical presentation of behaviors that resemble temporal lobe epilepsy have not been documented in neonatally-DA exposed animals (Demars et al., 2018). Doses of 20 or 60 µg/kg sc DA on PND 8-14 in rat pups produced long-term changes in α_2-adrenoceptor binding in limbic brain regions, but the effects were bidirectional and highly dose-dependent (Thomsen et al., 2016). The observed neurochemical effects were detected in the absence of functional alterations in behavior in the low dose animals. Sex-specific variations in protein expression have been described in a study of 20 µg/kg sc DA on PND 8-14 (D. A. Gill et al., 2012). In this investigation, DA-treated male rats showed increased expression of several important stress-related receptors, including the adrenergic receptor subtypes α_2a and α_2c, in hippocampal and non-hippocampal brain areas. Other rodent studies using a similar dosing regimen have found no treatment effects on the expression of important dopamine receptors or enzymes related to tyrosine and glutamate in the prefrontal cortex and hippocampus (Marriott, Tasker, Ryan, & Doucette, 2016) or on glucocorticoid and mineralocorticoid receptors in the hippocampus and hypothalamus (Perry et al., 2009).

Summary. At high exposures, the effects of DA on the developing brain are similar to the neuropathological changes observed in adults, and include neuronal damage and cell loss, particularly in the hippocampus. Lower-level developmental exposures appear to have unique pathological findings with differences in axonal sprouting, connectivity, and more subtle effects in neural protein and receptor expression. Importantly, these effects are dependent on the timing of exposure and may differ based on exposure duing specific windows of developmental susceptibility.

Future Directions

Research findings from animal models have indicated a heightened sensitivity to the adverse effects of DA in the fetus and neonate when compared to adults. Data from the only nonhuman primate study of developmental exposure suggest that subtle changes in early memory are important, but studies of human infants will be required to determine the translational value of the results from animal models. Future investigations in humans and animal models should prioritize the systematic collection of DA biomarkers (e.g. blood, urine) during pregnancy and in exposed offspring to characterize the relationship between increasing body burden of DA and related neurodevelopmental effects.

7. Other Toxicities from Domoic Acid Exposure

While the preponderance of studies examining the effects of DA exposure have focused on the central nervous system, studies of DA impacts on many peripheral organs have also been conducted. Like the nervous system, other organs, including the heart, kidney, spleen, liver, lung, and both male and female reproductive organs, have some level of glutamate receptor expression, which may interact with DA (S. S. Gill, Barker, & Pulido, 2008; S. S. Gill, Mueller, McGuire, & Pulido, 2000; S. S. Gill & Pulido, 2001). Cardiac effects of DA were first noted in the original human poisoning in Canada (Todd, 1993), as well as in wild sea lion populations poisoned by DA (Zabka et al., 2009). Two other research groups used both *in vivo* and *in vitro* models to demonstrate that DA can accumulate in the heart of rats after exposure to a single dose (2 mg/kg ip), intrahippocampal infusion (100 pmol) (Vranyac-Tramoundanas, Harrison, Sawant, Kerr, & Sammut, 2011), and two doses of 2.5 mg/kg ip, spaced 30 days apart (Vieira et al., 2016). In both studies, exposed animals expressed myocardial injuries and damaged cardiac mitochondria, but visible damage was subtle. *In vitro,* DA leads to the uncoupling of rat cardiac mitochondria, but this does not produce ROS, suggesting that the function, but not structure, of cardiomyocytes may be predisposed to DA toxicity (Vranyac-Tramoundanas et al., 2008).

Kidney damage was also noted in the original DA poisoning event, but only one study, using mice exposed to 3 single doses of DA (0.1-2.5 mg/kg ip) over three days, has examined the renal effects of this toxin (Funk et al., 2014). Animals demonstrated signs of kidney damage, with increased urinary biomarkers of KIM-1 and NGAL and evidence of increased cell death in proximal tubules of the kidney. Authors suggested that these effects may be most important in human populations with pre-existing renal disease or compromised renal function, such as aged or diabetic populations, as even low-level DA exposure could exacerbate existing kidney damage. DA may also cause subtle immunomodulatory effects *in vivo*, but results are limited. Infusions of 0.15 µg of DA directly into the lateral septal area of the brain caused neurotoxic lesions and subsequent modulatory effects in the endocrine system of female mice, but not male mice (Wetmore & Nance, 1991). A single dose exposure study conducted with mice given 2.5 mg /kg ip DA reported altered monocyte activity, decreased neutrophil phagocytosis, and decreased T-cell proliferation (M. Levin, Leibrecht, Ryan, Van Dolah, & De Guise, 2008). Immunomodulatory effects have also been reported in sea lions with DA poisoning (M. Levin et al., 2010). Studies that assessed standard serum and urine chemistry, which include biomarkers for both kidney and immune function, found few changes, however, after daily, oral, sub-chronic dosing in both rats exposed to 0.1 and 5 mg DA/kg (Truelove, Mueller, Pulido, & Iverson, 1996) or monkeys given 0.5 mg/kg DA for 15 days and then 0.75 mg/kg for another 15 days (Truelove et al., 1997).

Summary and Future Directions

The limited number of studies on the peripheral organ toxicity of DA collectively suggest that there may be many other, frequently overlooked effects from exposure to this toxin. Future research into the cardiac, renal, and immunomodulatory effects of DA should aim to better characterize these effects, especially considering chronic exposure. Results from these studies will also help reveal the human sub-populations with pre-existing conditions who may be more vulnerable to the toxic effects of this compound.

8. Current Exposures and Public Health Safety

Estimates of Human Exposures

Surveys to estimate real-world DA exposure have been conducted in high-seafood consumption populations in the USA and Europe. One of the first surveys targeted at elucidating DA consumption reported DA levels in commonly caught fish species and mussels consumed by fishers in the state of California (Mazzillo et al., 2010). DA consumption was highly dependent on the type of seafood consumed; mussels collected for the survey had no DA detected, whereas anchovies had levels up to 28.3 mg/kg fish. Researchers reported that those fishers self-reporting whole anchovy consumption may be at highest risk of low-level DA exposure, at up to 1.43 mg DA/meal. Using standardized consumption rates of 50 g fish/meal and a bodyweight (bw) of 60 kg, this equates to 0.024 mg DA/kg bw. Another survey of just 16 fishers in Bulgaria assessed DA exposure via mussels, and found that, while DA was detected in all mussels, the highest exposure in this group was estimated to be 0.27 mg DA/meal, or 0.0024 mg/kg bw (Peteva, Georgieva, Stancheva, & Makedonski, 2017).

A more recent survey of recreational fishers in WA, focused only on the risk of DA exposure after razor clam consumption (Ferriss et al., 2017). This survey aimed to assess the patterns DA exposure throughout the year in different ages and sexes. Data from the survey revealed that the reported number of clams eaten per meal may be much higher than previously estimated, over 7 clams/meal in some age groups. This, in combination with high levels of DA in the shellfish, lead to higher-than-expected exposure levels, ranging from 0.05-0.1 mg DA/kg bw/day. Using models to predict what long-term exposures may look like, researchers further identified that predicted consumption of DA was highest in the springtime and in younger groups

(10-20 years), although they note that some of the highest shellfish consumer groups were underrepresented in their survey.

In Belgium, data from a nationwide dietary survey and samples of mussels, oysters, and scallops to quantify average DA concentrations and data to estimate average exposures (Andjelkovic et al., 2012). DA was detected in 11% of seafood samples and ranged from 0.8-203.4 ppm in shellfish meat. When consumed at the nationally reported levels, these concentrations equated to up to 0.013 mg DA/kg bw/day.

One group at risk for higher exposures includes those of coastal Native American Nations in WA State. In this state, as well as other locations, Indigenous Peoples of coastal Nations share a historical, cultural, and economic connection to the ocean and marine foods, including those contaminated with DA (Crosman, Petrou, Rudd, & Tillotson, 2019). Many coastal Native Americans in WA regularly consume Pacific razor clams (Fialkowski et al., 2010) and are concerned about the health effects of consuming these clams (Roberts et al., 2016). Dietary surveys and measures from 6-month records of DA concentrations in WA clams have estimated that the average monthly DA consumption rates in Native American adults were approximately 0.000218 ng/kg bw/day or 0.00322 ng DA/kg bw/meal (Stuchal et al., 2020). These rates, while below the current regulatory limit, were still connected with adverse health outcomes, demonstrating the necessity of including groups at high risk of DA exposure in regulatory considerations.

While these reports demonstrate the low-level and persistent exposure to DA in many populations today, DA concentrations vary by seafood species (Andjelkovic et al., 2012; Mazzillo et al., 2010), location (Wekell, Trainer, Ayres, & Simons, 2002), and time of the year (Smith et al., 2018). Further, DA does not degrade with typical cooking and freezing methods (McCarron & Hess, 2006; Vidal, Correa, & Blanco, 2009). Going forward, exposure assessments should include considerations for these variable factors and look towards the use of a biomarker to confirm DA exposures.

Domoic Acid Regulation and Safety Recommendations

Estimates of DA exposure from the Prince Edward Island poisoning were used to establish limits for shellfish harvesting to protect public health (Hynie & Todd, 1990; Todd, 1993; Wekell et al., 2004). Shellfish harvesting is closed when monitoring programs indicate DA concentrations in shellfish of 20 mg/kg or greater. This action level was derived from estimates of DA concentrations in mussels from the Prince Edward Island poisoning (200 mg/kg mussel tissue) and applied with a 12-fold safety factor. This limit was suggested to be well below the approximate no-effect-level in mice, and, therefore, thought to be protective of acute human exposures (Iverson & Truelove, 1994). Continued research after the establishment of this regulation estimated that the limit in seafood is approximately equivalent to 0.075-0.1 mg/kg bw in adults (Alexander et al., 2009; Mariën, 1996; Toyofuku, 2006). This threshold is based solely on information from the single episode of high-dose, catastrophic exposure and does not address the health risks associated with lower dose or chronic exposure.

Since the establishment of the regulatory threshold, several research groups have calculated other consumption limits, by incorporating newly available toxicological data, seafood consumption rates and patterns, and additional protective safety and uncertainty factors (Table 4). The results of these assessments vary significantly from daily consumption limits consistent with the current estimate of 0.075 mg/kg bw (Mariën, 1996; Toyofuku, 2006) to limits approximately 2- to 4-times lower (0.018 to 0.034 mg/kg bw) (Alexander et al., 2009; Slikker, Scallet, & Gaylor, 1998). Most of these assessments, however, indicated that there were not enough data to develop safety limits for chronic consumers of DA (Alexander et al., 2009; Kumar, Kumar, & Nair, 2009; Toyofuku, 2006).

Recently, however, a seafood safety limit considering chronic exposure was developed with data from the CoASTAL cohort study (Stuchal et al., 2020). Using estimates of shellfish intake from questionnaires given to study participants and DA levels in shellfish from the study site, the authors estimated a daily consumption limit of 0.003 mg/kg bw/day would be needed to protect adult consumers from the effects observed in the study (decreased verbal memory recall), a threshold well below the present regulatory action level.

The wide range of results across these studies are the product of different variations and approaches to risk assessment. None of the studies, however, include quantitative data on sensitive groups, such as young, aged, or other biologically compromised populations. These characteristics demand more attention going forward, so that updated regulations can better protect the most vulnerable populations. Future regulatory guidelines should be established with a focus on chronic and low-level effects, particularly in vulnerable and highly exposed populations, to best protect the health of all shellfish consumers. In the promotion of environmental justice, the input and consideration of key stakeholders, including Native American and Indigenous populations as well as other high-risk groups, should be considered vital in the reassessment and establishment of future regulations (Burger & Gochfeld, 2011). Interim guidance limiting chronic seafood consumption, such as that released by the WA State Department of Health, can help promote public health until such regulatory thresholds are adopted (Washington State Department of Health, n.d.).

9. Summary and Conclusion

DA causes overt excitotoxicity in adult mammals, producing striking behavioral symptoms and pathology that primarily manifests in the hippocampus. Since the 1987 human poisoning and subsequent regulation of DA, there have been no documented incidents of acute human ASP, but continued research has deepened our understanding of the perilous nature of this toxin. The compelling body of research collectively detailed in this review illuminates the worrisome effects of DA, even at levels deemed as “safe” under current regulatory limits. The results of preclinical studies indicate that chronic exposure to levels of DA near the human regulatory limit do not cause overt neuroinjury but can cause subtle, neurotoxic effects that impact the function, structure, physiology, and cellular response of the brain. Recent epidemiological studies have also provided new evidence of harm from chronic, low-level DA exposure, highlighting the importance of studies focused on the health effects from repeated exposure to this toxin at levels below the current regulatory limit. Future research efforts should aim to further explore these themes, by designing studies aimed at understanding the underlying mechanisms of toxicity associated with low-level and chronic DA exposure. Potential mechanisms for tolerance should be explored as well. Special considerations for differences in responses based on sex and age should be another focus, to best understand the risk to certain populations.

Health effects have also been documented following DA exposure in vulnerable, developmental laboratory models. If given during development, DA generally does not appear to cause congenital or other physical defects, but perinatal exposure to this toxin has been linked with deficits in measures of learning and memory, as well as aberrant behavior related to social and emotional domains. These changes were observed even in some studies using very low-level DA exposure paradigms administered during early life. Effects such as these may be caused, in part, by irregular mossy fiber sprouting in the hippocampus and altered connectivity in the brain. Other vulnerable populations that have not been well studied may include both those with diminished kidney, cardiac, or immune function, as limited evidence suggests DA may also impact these systems.

Up to now, few studies have included a biomarker of DA exposure. Results from DA studies in adult female nonhuman primates and their offspring (Shum et al., 2018) as well as results from a study of adult humans who chronically consume shellfish (Lefebvre et al., 2012, 2019) have provided evidence indicating that urine or a DA-specific antibody may be useful biomarkers for DA exposure. Future studies in human populations and preclinical models should develop strategies such as these to provide critical data regarding the relationship between DA body burden and related health effects in both the nervous system and other critical off-target organs.

In conclusion, the current literature on the health effects of DA exposure provides strong evidence that the current regulatory limit does not adequately protect populations that are chronic consumers of shellfish, particularly those individuals who may be sensitive to DA effects, such as developing young or aged individuals, as well as those with other comorbidities. New interim guidance in WA suggests limiting the consumption of razor clams to 15 per month for everyone, but particularly for “women who are or might become pregnant, nursing mothers, children, the elderly, and people with compromised renal function” (Washington State Department of Health, n.d.). This is especially pertinent because the health of the highest-exposed groups may already be disproportionately impacted by other environmental contaminants. Current regulatory limits should be reexamined and reestablished, with cooperation from regulators and representation from high-risk communities to best protect the health of populations chronically exposed to this common marine contaminant.

Note to Reader

The authors of the present manuscript have established a tissue repository of adult and juvenile nonhuman primates chronically exposed to low-levels of DA (Supplement 1), from the study described in Burbacher et al., 2019 (NIEHS R01 ES023043). The authors would like to invite researchers interested in utilizing these samples to contact Dr. Burbacher (the corresponding author) for future collaborative investigations on DA toxicity.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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Figure Captions

Figure 1: Reprinted from Petroff, 2020. Chemical structures of domoic acid and analogues. A) domoic acid; B) glutamate; C) kainic acid

Figure 2: Reprinted from Petroff, 2020. Proposed mechanism of action for domoic acid (DA). LEFT: Acute exposures to DA involve the activation of KA- and AMPA-type glutamate receptors, resulting in an influx of Na^+ into the postsynaptic membrane, and the release of glutamate into the synapse. Glutamate activates NMDA receptors, allowing an influx of Ca^{+2} and leading to necrotic cell death. RIGHT: Lower-level exposures do not involve the NMDA receptors, and therefore, lead to mitochondrial distress, the production of ROS, and apoptosis. Abbreviations: AMPA – α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; Ca^{+2} – calcium; DA – domoic acid; K^+ – potassium; KA – kainic acid; Na^+ – sodium; NMDA – N-methyl-D-aspartic acid; ROS – reactive oxygen species

Tables

Table 1: Toxicokinetic Parameters of Domoic Acid

Intravenous (iv) Toxicokinetics of DA

Species	Route of Exposure	Dose (µg/kg)	V_{ss} (mL/kg)	CL (mL/min/kg)	CL_r (mL/min/kg)	MRT (h)	Half-life (h)	Citation
Monkey	Single iv	50	159 ± 29	1.3 ± 0.5	--	2.48 ± 1.31	1.91 ± 0.98	Truelove & Iverson, 1994
Monkey	Single iv	5	131 ± 71	2.1 ± 1.2	0.60 ± 0.50	--	1.2 ± 1.1	Jing et al., 2018
Rat	Single iv	500	272 ± 58	10.8 ± 1.2	12.2 ± 1.73	--	--	Suzuki & Hierlihy, 1993
		2000	244 ± 31	7.8 ± 1.6	8.8 ± 1.6	--	--	
Rat	Single iv	500	311	10.2	--	0.51	0.38	Truelove & Iverson, 1994
		1000	229 ± 88	7.8 ± 2.7	--	0.50 ± 0.09	0.35 ± 0.05	
Rat (pregnant)	Single iv	1000	494.1	6.5	--	1.27	0.49	Maucher Fuquay et al., 2012
Rat (fetus)	Single iv	1000 (maternal)	--	--	--	1.5	9.22	

Oral Toxicokinetics of DA

Species	Route of Exposure	Dose (µg/kg)	CL/F (mL/min/kg)	CL_r (mL/min/kg)	Half-life (h)	Bioavailability (%)	fe (%)	Citation
Monkey	Multiple oral	500, 750	--	--	--	--	5.8 ± 1.7	Truelove et al., 1997
Monkey	Single oral	75	33 ± 12	1.6 ± 0.8	11.3 ± 2.4	6 ± 4	4 ± 2	Jing et al., 2018
		150	27 ± 15	--	9.8 ± 5.9	7 ± 5	--	
Monkey	Single oral	75	--	5.2 (2.8 - 9.9)	--	--	--	Shum et al., 2020
		150	--	6.4 (4.9 - 8.2)	--	--	--	
	Multiple oral	75	61 (49 - 76)	6.2 (5.3 - 7.4)	--	--	2.7 (2.1 - 3.4)	
		150	39 (29-52)	6.2 (4.7 - 8.2)	--	--	4.2 (3.0 - 5.9)	
Monkey (pregnant)	Multiple oral	75	92 (58-146)	11 (7.3 - 17)	--	--	3.2 (2.5 - 4.1)	
		150	54 (38-76)	9.4 (6.6 - 13)	--	--	4.5 (3.2 - 6.3)	

Tissue Distribution

Species	Route of Exposure	Dose (µg/kg)	Brain:Blood Ratio	Citation
Rat	Single ip	500, 1000, 2000	0.04 ± 0.01	Hesp et al., 2007
Rat (pregnant)	Single iv	1000	0.06	Maucher Fuquay et al., 2012
Rat (fetus)	Single iv	1000 (maternal)	0.15	

Maternal-Fetal Disposition

Species	Route of Exposure	Dose (µg/kg)	Fetal:Maternal Ratio	Amniotic Fluid:Blood Ratio	Citation
Monkey	Multiple oral	75	0.3	0.9	Shum et al., 2020
		150	0.3	3.1	
Rat	Single iv	1000	0.3	1.48	Maucher Fuquay et al., 2012

Abbreviations: CL – total body clearance; CL/F – total body clearance after oral administration; CL_r – renal clearance; fe – fraction excreted unchanged in urine; ip – intraperitoneal; iv – intravenous; MRT – mean residence time; V_{ss} - volume of distribution at steady-state

Table 2A: *Adult Clinical and Preclinical Neurotoxic Effects of Domoic Acid*

Route of Exposure	Dose	Duration of Exposure	Biomarker of Exposure	Subject Characteristics	Overt Neurotoxic Effects	Time Onset^	Functional Neurotoxic Effects	Time Neurotoxic Effects Examined^	Neuropathological Effects	Time Pathology Examined^	Citation
Humans											
dietary	up to 290 mg	1x	Blood: none detected CSF: none detected	Prince Edward Island adults	Vomiting, diarrhea, headache, memory loss, seizures, coma, death	<38 h	↓ memory performance	4 mth-1 yr	Hippocampus, amygdala, septal area, olfactory area, frontal cortex: ND and astrocyte reaction	7-98 d	Hynie & Todd, 1990; Perl, Bedard, Kosatsky, Hockin, & Todd, 1990
dietary	>15 razor clams/mth	1+ yr		CoASTAL cohort	None		↓ cognitive and memory performance	1+ yr			Grattan et al., 2016
dietary	Not reported	Either 1 wk or 1 yr		CoASTAL cohort	None		Both 1 wk and 1 yr: ↑ problems in everyday memory	10 d post-start of target week			Grattan et al., 2018
dietary	~324 ng/kg/d	1+ mth		CoASTAL cohort	None		↓ memory performance on verbal recall tests	At least 6 mth after start of exposure			Stuchal et al., 2020
Monkeys											
oral	5.2-10 mg/kg	1x		Females	Salivation, gagging, vomiting, diarrhea	7-96 min			Hippocampus: ND	4-44 d	Tryphonas, Truelove, Todd, et al., 1990
oral	0.5 mg/kg for 15 d, then 0.75 mg/kg for 15 d	Daily, for 30 d	Serum:10-60 ng/ml; Urine: 1-11% of dose	Females	None				Whole brain: no changes in temporal cortex, amygdala, hypothalamus, hippocampus, thalamus, cerebellum	30 d	Truelove et al., 1997
oral	0.075 and 0.15 mg/kg	Daily, for 8-10 mth	Plasma: 0.93 ng/ml and 2.93 ng/ml	Females	Upper limb tremors	1 mth+					Burbacher et al., 2019
oral	0.075 and 0.15 mg/kg	Daily, for 1-2 yr	Plasma: 0.93 ng/ml and 2.93 ng/ml	Females	Upper limb tremors	1 mth+	MRI: ↓ white matter integrity in fornix and internal capsule; MRS: ↑ lactate in thalamus	1+ yr			Petroff et al., 2019
oral	0.075 and 0.15 mg/kg	Daily, for 1-2 yr	Plasma: 0.93 ng/ml and 2.93 ng/ml	Females	Upper limb tremors	1 mth+	EEG power differences; ↓ delta, ↑ alpha, theta, beta	1+ yr			Petroff et al., 2020
iv and ip	0.025-0.5 mg/kg iv; 4 mg/kg ip	1x			Salivation, gagging, vomiting, tremors, death	3-4 min			Hippocampus and hypothalamus: ND and astrocyte reaction at >0.2 mg/kg	3.5-5 h	Tryphonas, Truelove, & Iverson, 1990
iv	0.25-4.0 mg/kg	1x		Adult and Juvenile Males and Females	Gagging, vomiting, tremors, 4 deaths of adults >1 mg/kg	8-75 min			Hippocampus: ND and astrocyte reaction; CA1: ↑ c-Fos at >0.5 mg/kg	2-7 h (for morbidund), otherwise 1 wk	Scallet et al., 1993
iv	0.25-4.0 mg/kg	1x		Adult and Juvenile Males and Females	Gagging, vomiting, tremors, 4 deaths of adults >1 mg/kg	8-75 min			Hippocampus, entorhinal and piriform cortices, subiculum, lateral septum, and thalamus: ND at >0.5 mg/kg	2-7 h (for morbidund), otherwise 1 wk	Schmued et al., 1995
Rats											
oral	60-80 mg/kg	1x			Head on floor, inactivity at 60 mg/kg; rolling, seizures, and death at 80 mg/kg	5 min-5 h			Hippocampus and olfactory bulb: mild ND and astrocyte reaction at 80 mg/kg	1-54 h	Tryphonas, Truelove, Todd, et al., 1990
oral	0.1 and 5 mg/kg	Daily, for 64 d	Serum: Below the limit of detection	Males and Females	None		No changes in urine or blood chemistry	Urinalysis at 55 d, blood chemistry at 64 d	Whole brain: no changes in electron microscopic histopathology or GFAP	64 d	Truelove et al., 1996
ip and oral	1-9.5 mg/kg ip; 60-82 mg/kg oral	1x	Urine: not detected; Feces: 98% of dose		Scratching, seizures, and death at >4 mg/kg ip; chewing at 70 mg/kg oral, death at >80 mg/kg oral	<4 h			Hippocampus and retina: ND at 4-7 mg/kg ip and 80 mg/kg oral	After death (within 4 h)	Iverson et al., 1989
ip	1-10 mg/kg	1x		Males	Wet-dog shakes and seizures in all dose groups, death at >5 mg/kg	2 h	Electrographic spikes and generalized epileptic status	3-24 h, depending on morbidity	CA3: ND at all doses; Other hippocampal areas: damage at >5 mg/kg	3-24 h, depending on morbidity	Fujita et al., 1996
ip	2.25 mg/kg	1x		Males	Hindlimb scratching, convulsions, and death	45 min-3 h			CA1-4, pyramidal tracts, thalamus, amygdala, and olfactory bulb: ND, astrocyte reaction, and potential microglia response	7 d	Appel et al., 1997
ip	2.5 mg/kg	1x	Immunohistochemical DA detected in hippocampal neurons at 6-10 h post exposure	Females	Hyperactivity, scratching, tremors, head jerks	<6 h			No changes at 6-24 h; CA1/3: ND, astrocytosis, microglia reactivity at 5 d; Hippocampus and thalamus: ND, astrocyte reaction, microglia reactivity, and ↑ NOS at 54 d	6 h, 10 h, 24 h, 5 d, and 54 d	Vieira et al., 2015
ip	2.2 and 4.4 mg/kg	1x		Males	Hindlimb scratching, wet-dog shakes, salivation, and seizures at both doses	<150 min	Electrographic seizures and ↑ delta, theta, alpha, and beta power at both doses	Daily, over 7 d	Hippocampus, DG, and olfactory bulb: ↑ c-Fos+ neurons at both doses	2 h	Binienda et al., 2011; Scallet et al., 2004
ip	1 and 1.8 mg/kg	1x		Males and Females	Signs of severe toxicity in 2/7 males	<3 h	↓ activity, then ↑ locomotion, grooming, and stereotypic behaviors	Over 3 h	Hippocampus and olfactory bulb: no changes in GFAP+ astrocytes or pCREB	3 d	Baron et al., 2013

Table 2B*: Adult Clinical and Preclinical Neurotoxic Effects of Domoic Acid, continued*

Route of Exposure	Dose	Duration of Exposure	Biomarker of Exposure	Subject Characteristics	Overt Neurotoxic Effects	Time Onset^	Functional Neurotoxic Effects	Time Neurotoxic Effects Examined^	Neuropathological Effects	Time Pathology Examined^	Citation
Rats, continued											
ip	1 mg/kg	1x		Males	None	N/A	↑ locomotion immediately; ↑ time to habituate in novel field at 8 and 15 d	Immediately, 7 d, and 15 d	Hippocampus: no signs of damage or apoptosis	15 d	Schwarz et al., 2014
ip	0.3-3 mg/kg	1x	Brain: mean 7.2 ng/g	Males					CA1: ND and apoptosis; ↑ WDR35 and p38 MAPK phosphorylation at 1 mg/kg	1 and 5 d	Tsunekawa et al., 2013
ip	0.22-1.32 mg/kg	1x		Males	↓ weight at >0.65 mg/kg; hindlimb scratching, seizures, death at 1.32 mg/kg	<24 h	Motor depression and exaggerated auditory startle at 1.32 mg/kg, no changes in passive or active avoidance	1-8 days	Hippocampus: ND and astrocytosis at 1.32 mg/kg	8 d	Sobotka et al., 1996
ip	0.2 mg/kg	1x		Males					CA4: ND; CA1/4: ↑ c-Fos and c-Jun; CA1/3/4: no changes in myelination, DG: astrocyte reaction; ↓ BBB integrity	3 d	Scallet et al., 2005
ip	0.5-2 mg/kg	1x and preconditioning with 0.125-0.25 mg/kg before	Serum: 24-6133 ng/ml; Brain: 1.2-88.8 ng/g, with aged animals significantly higher	Young adult and aged adult males	Jerks, hindlimb scratching, and wet-dog shakes at 1-2 mg/kg; seizures and death at 2 mg/kg in aged animals	<3 h					Hesp et al., 2007
ip	1 and 5 mg/kg	At least 2x, 1 dose per h, for up to 5 h		Males	Seizures and chronic epilepsy	<3 h					Muha & Ramsdell, 2011
ip	1 mg/kg	At least 2x, 1 dose per h, for up to 5 h		Males	Hindlimb scratching, seizures, and chronic epilepsy	<3 h			Hippocampus, amygdala, thalamus, olfactory, prefrontal, and piriform areas: damaged cell bodies; Thalamus: axonal damage	7 d	Tiedeken et al., 2013
ip	1 mg/kg	At least 2x, 1 dose per h, for up to 3 h		Males	Seizures and chronic epilepsy	<3 h	Motor seizures and dominant/ defensive aggression	Regularly, throughout 12 wk	Hippocampus, amygdala, thalamus, piriform, and olfactory areas: ND	12 wk	Tiedeken & Ramsdell, 2013
sc	0.6-3 mg/kg	1x		Males	Hypoactivity at all doses; hindlimb scratching, wet-dog shakes, seizures at >2 mg/kg	<30 min			Hippocampus, amygdala, thalamus, and cortex: neural and glial swelling	3.5-8.5 h	Stewart et al., 1990
iv	0.75 mg/kg	1x		Males					CA1/3: ND and microglia reactivity; hypertrophic microglia expressing MHC-II; ↑ Bax/BCL-2; no changes in caspase-3	1, 2, 5, 14, and 21 d	Ananth et al., 2001
iv	0.75 mg/kg	1x		Males					Hippocampus: ND and astrogliosis at 5 d; NOS induction and activated microglia at 5 d and 3 mth	5 d and 3 mth	Ananth et al., 2003a
iv	0.75 mg/kg	1x		Males					CA1/3: ND; ↑GFAP+ astrocytes and OX-42+ microglia; ↑ iNOS	5 d	Ananth et al., 2003b
iv and intra-ventricular	0.5-1 mg/kg iv; 0.04-0.08 mg/kg intraventricular	1x, and repeated until EEG observations			Hindlimb scratching, seizures, and death	3-7 min	↓ memory and learning in 0.04 mg/kg intraventricular	<5 min	No changes reported in iv treatments; CA1/3/4: ND in intraventricular treatments	3 d	Nakajima & Potvin, 1992
intra-hippocampal	10-300 pmol	1x		Males	Seizures at >100 pmol	0-7 min	ECoG spikes at 10-50 pmol; bilateral persistent spiking with seizures at >100 pmol	7-45 min	Hippocampus and cortex: ↓ GABA at 100 pmol	150 min	Dakshinamurti et al., 1991
intra-hippocampal	30-1000 pmol	1x		Males	Seizures at >130 pmol	<2.5 h					Sawant, Tyndall, et al., 2010
intra-hippocampal	112.5 pmol	1x at 3 sites		Males	Seizures		↓ learning and memory performance	10 days	Hippocampus and subiculum: ND; Thalamus: astrocyte reaction	10 d	Sutherland et al., 1990
intra-hippocampal	3-8000 pmol	1x and preconditioning with 15 pmol		Males	Freezing, wet-dog shakes, jerking, hindlimb scratching and seizures	<2.5 h					Sawant et al., 2008
intra-hippocampal	100 pmol	1x and preconditioning with 15 pmol		Males	Seizures in all treatments, fewer with preconditioning	<2.5 h	↑ ECoG power with no precondition, normal power with preconditioning	2 h			Sawant, Mountfort, et al., 2010
micro-iontophoretic	0.1 mM	1x		Males			Electrical hippocampal activation in CA1/3	Immediately			Debonnel et al., 1989

Table 2C: Adult Clinical and Preclinical Neurotoxic Effects of Domoic Acid, continued

Route of Exposure	Dose	Duration of Exposure	Biomarker of Exposure	Subject Characteristics	Overt Neurotoxic Effects	Time Onset^	Functional Neurotoxic Effects	Time Neurotoxic Effects Examined^	Neuropathological Effects	Time Pathology Examined^	Citation
Mice											
Oral and ip	8.8-104 mg/kg oral; 12-233 mg/kg ip	1x	Urine: not detected; Feces: 102% of dose	Males and Females	Hindlimb scratching, seizures, and death at >23mg/kg ip; death at >47 mg/kg oral	<30 min			Hippocampus and hypothalamus: ND at 5 mg/kg ip and 35 mg/kg oral	~1 h	Iverson et al., 1989
ip	55-630 mg of shellfish protein extract/kg	1x		Males	Motor activity, stereotypic behaviors, convulsions, respiratory distress, deaths	<60 min					Bose et al., 1989
ip	5-20 mg/kg	1x		Females	Forelimb tremors at 5 mg/kg, hypoactivity at 20 mg/kg	<60 min					Munday et al., 2008
ip	4 mg/kg	1x		Females	Catatonic state, hindlimb scratching, convulsions, death	1 min			Hippocampus: ND at 4 h, largest damaged area at 168 h	4,24,48, 72, and 168 h	Strain & Tasker, 1991
ip	4 mg/kg	1x		Males	Hypo-/hyperactivity, hindlimb scratching, seizures, death	<1 h			Hippocampus, olfactory, and septal areas: ND	48 h	J. C. Ryan et al., 2011
ip	4 mg/kg	1x		Females					Hippocampus, amygdala, olfactory areas, septal area: ND and axonal degeneration	72 h	Colman et al., 2005
ip	2, 3, and 7 mg/kg	1x							Hypothalamus and area postrema: No changes at 2-3 mg/kg; ND at 7 mg/kg	30 min, 60 min, 24 h, 48 h, and 3 d	Bruni et al., 1991
ip	2 mg/kg	1x		Males and Females	Behavioral toxicity, not otherwise described		↓ learning and memory performance	1-14 d			Petrie et al., 1992
ip	1-15 mg/kg	1x		Females	Hypoactivity, stereotypic hindlimb scratching, tremors, and death at >4 mg/kg	1 min					Tasker et al., 1991
ip	~1.3-50 mg/kg	1x		Females	Hypoactivity, hindlimb scratching, death	<1 h					Grimmelt et al., 1990
ip	1-4 mg/kg	1x		Females					CA1/2/3: ND; ↑ transient c-Fos at 4 mg/kg	15-240 min	Peng et al., 1994
ip	1 and 4 mg/kg	1x		Males and Females	Hypoactivity, hindlimb scratching, head weaving, tremors, convulsion	<30 min			Whole brain: 800 differentially expressed genes analysis per time point	30, 60, and 240 min	J. C. Ryan et al., 2005
ip	0.25-4.0 mg/kg	1x	Serum: 50-1000 ng/ml for low and high dose, 60 min post-exposure	Females	Hypoactivity, hindlimb scratching at >1 mg/kg, tremors, convulsions, death, convulsions at >2 mg/kg	<1 h			Whole brain: ↑ c-Fos mRNA at >1 mg/kg	60 min	Peng & Ramsdell, 1996
ip	1 and 2 mg/kg	1x and 4x over 7 d		Males	Hypoactivity, hindlimb scratching at >1 mg/kg, tremors, convulsions, death, convulsions at >2 mg/kg with repeat and single doses	<1 h	↓ working memory performance for both doses of single exposures and 1 mg/kg multiple exposure	7 d	Whole brain: ND at both 2 mg/kg, single and multiple exposures	25 d	Clayton et al., 1999
ip	0.5 and 2 mg/kg	1x and 4x with 48 h between doses	Serum: ~550-1230 ng/ml for low and high dose, 60 min post exposure for repeat and single doses	Females	Hindlimb scratching, tremors, convulsions, death at <2 mg/kg; no symptoms at 0.5 mg/kg	<1 h					Peng et al., 1997
ip	2 mg/kg	Daily, for 21 d		Males	Seizures, but not death		↓ learning and memory	5 wk	Hippocampus: ND; ↑ GFAP+ astrocytes; ↑ IL-1b, TNF-α, Cox-2, and NOS	6 wk	Lu et al., 2013
ip	2 mg/kg	Daily, for 28 d		Males	Seizures, but not death		↓ learning and memory	5 wk	Hippocampus: ND; ↑ NADPH/ROS; ↑ SAPK/JNK/ FoxO1/Fas	6 wk	Wu et al., 2012
ip	2 mg/kg	Daily, for 28 d		Males	Seizures, but not death		↓ learning and memory	5 wk	Hippocampus: ND; ↑ ROS and mitochondrial dysfunction	6 wk	Wu et al., 2013
ip	2 mg/kg	Daily, for 28 d		Males	Not reported		↓ object recognition, learning and memory		Hippocampus: ↑ mitochondrial dysfunction; ↑ ROS; ↓ NRF-1	5 wk	D. Wang et al., 2018

Table 2D: Adult Clinical and Preclinical Neurotoxic Effects of Domoic Acid, continued

Route of Exposure	Dose	Duration of Exposure	Biomarker of Exposure	Subject Characteristics	Overt Neurotoxic Effects	Time Onset^	Functional Neurotoxic Effects	Time Neurotoxic Effects Examined^	Neuropathological Effects	Time Pathology Examined^	Citation
Mice, continued											
ip	2 mg/kg	Daily, for 30 d		Males	Seizures		↓ learning and memory		Hippocampus: ND and ↓ PCG-1a, NRF-1, TFAM; ↓ mitochondrial and antioxidant activity	30 d	Lu et al., 2012
ip	0.2-1.6 mg/kg	Daily, up to 30 d		Females	None		↓ hind limb motor coordination	10 d	Spinal cord: lesions with TUNEL staining, ↓ proteasome activity after 10 days, ↑ ROS production	0, 7, 15, 20, 25 and 30 d	Xu et al., 2008
ip	0.75-0.82 mg/kg	1x/wk, up to 25 wk		Females	None		↓ memory on the radial water tread maze and increased locomotion in open field at 25 wk, recoverable after 9 wk without exposure	1, 6, 25 wk and 9 wk	Hippocampus: No changes in cell count for both young and aged mice	22 wk	Lefebvre et al., 2017
ip	0.75-0.82 mg/kg	1x/wk, for 22 wk		Females	None				Hippocampus: No changes in neurons or astrocytes with NeuN and GFAP; no changes in VGluT2 or VGAT; CA1: ↑ VGluT1	22 wk	Moyer et al., 2018
intracisternal	25 ng	1x		Males	Scratching, running, seizures	<10 min					Wood et al., 1982
Fish											
oral and ic	0.8 mg, oral; 38-194 mg/kg, ip		2.7 mg/kg in liver; 0.4 mg/kg in body	Anchovies	Spiral swimming, head shaking, death	40 min					Lefebvre et al, 2001
ip	5 mg/kg	1x	DA detection by immunohistochemistry	Killifish	Hyperactivity, spiral swimming	<3 h			Optic areas: ↑ c-Fos	3 h	Salierno et al., 2006
ic	0.47 or 1.2 mg/kg	1x		Zebrafish	Erratic darting, circle and spiral swimming, death	<20 min			Whole brain: 223 and 106 differentially expressed in 0.47 and 1.2 mg/kg, respectively	6 h	Lefebvre et al., 2009
ic	0.31 mg/kg for 6 wks followed by 0.18 mg/kg	1x/wk for 6 wk, plus 1x/every other wk for another 6-30 wk		Zebrafish	None				Whole brain: no changes in pathology at 36 wk; 52-239 differentially expressed genes per timepoint; ↓ mitochondrial respiration capacity	2-36 wk	Hiolski et al., 2014

^reported in time post-initial dose

Abbreviations: BBB – blood-brain barrier; DG – dentate gyrus; ECoG – electrocorticography; EEG – electroencephalogram; GABA - γ-aminobutyric acid; GFAP – glial fibrillary acidic protein; ic – intracoelomic; ip – interparental; iv – intravenous; ND – neuronal degeneration; NOS – nitric oxide synthase; ROS – reactive oxygen species; sc - subcutaneous

Table 3*: Prenatal Exposure and Neurodevelopmental Effects of Domoic Acid*

Route of Exposure	Dose	Duration of Exposure	Maternal Toxicities	Offspring Sex	Offspring Biomarker of Exposure	Period of Study	Overt Signs of Neurotoxicity	Affected Developmental Domains	Unaffected Developmental Domains	Neuropathological Effects	Citation
Monkeys											
maternal, oral	0.075 or 0.15 mg/kg/day	1x/d in gestation (~5-6 mth)	Tremors	Males and Females	Plasma: 0.44 ng/ml and 1.26 ng/ml	Infancy	None		Physical		Burbacher et al., 2019
maternal, oral	0.075 or 0.15 mg/kg/day	1x/d in gestation (~5-6 mth)	Tremors	Males and Females	Plasma: 0.44 ng/ml and 1.26 ng/ml	Infancy	None	Cognition	Early Reflexes		Grant et al., 2019
Rats											
maternal, sc	0.3, 0.6 or 1.2 mg/kg	1x on GD13	None	Males and Females		Weaning-adulthood	None	Activity, Cognition	Physical		E. D. Levin et al., 2005
Mice											
maternal, oral	1 or 3 mg/kg/d	1x on GD10-17	None	Males and Females		Neonatal period-adulthood	None	Physical, Emotionality, Activity	Physical, Early Reflexes, Sensory/Motor Processing, Cognition		Shiotani et al., 2017
maternal, ip	1 mg/kg	1x on GD11.5, 14.5 and 17.5		Males		Adulthood		Emotionality, Cognition, Activity	Activity	Whole brain: no pathology changes; Cortex: ↓ MAG; ↑ MAP; CA3: ↑ MAP	Tanemura et al., 2009
maternal, sc	1.5 mg/kg	1x on GD16		Males and Females		Behavior: adolescence; MRI: adulthood		Social Behavior	Emotionality	MRI tracts of anterior cingulate cortex and infralimbic and orbital regions: ↑ connectivity; MRI tracts of dorsal retrosplenial and CA3: ↓ connectivity	Mills et al., 2016
maternal, sc	1.5 mg/kg	1x on GD16		Males and Females		Adolescence		Sensory/Motor Processing, Social Behavior, Emotionality	None	CA1/3, basolateral amygdala, infralimbic and prelimbic cortices: no changes in parvalbumin+ cells; Lateral amygdala and DG: ↑ in parvalbumin+ cells	Zuloaga et al., 2016
maternal, iv	0.6 or 1.2 mg/kg	1x on GD13	Hindlimb scratching, seizures, death	Males (0.06 mg/kg) and Females (1.2 mg/kg)		Adolescence and adulthood			Spontaneous Seizures	Hippocampus: no change in hilar neurons or mossy fiber sprouting	Demars et al., 2018
maternal, iv	0.6 mg/kg	1x on GD13	Slight hypoactivity, temporary immobilization	Males and Females		Neonatal period, weaning, and adolescence	None	Electrophysiology (EEG, ↓ seizure threshold)	Physical	Age-related, selective ND: PND1-No obvious cellular damage; PND14- In CA3 and DG: ↑ cell damage; PND30- Hippocampus and cerebral cortex:↓ GABA and ↑ glutamate	Dakshinamurti et al., 1993
Zebrafish											
in ovo	0.12 to 17 mg/kg (DA/egg weight)	Microinjection of fertilized eggs at the 128-to 512 cell stages	N/A			Hatching-5 d postfertilization	Seizures, rapid and continuous fin movement	Early Reflexes			Tiedeken et al., 2005
in ovo	0.12 to 1.26 ng/mg (DA/egg weight)	Microinjection of fertilized eggs at 1K-cell and high-oblong cell stages	N/A			7 d	Seizures, rapid and continuous fin movement	Physical, Early Reflexes, Activity, Electrophysiology (EEG, ↓ seizure threshold)		↑ spinal deformities	Tiedeken & Ramsdell, 2007
in ovo	1, 10, 100, and 1000 ng/L	Cocultured for 72 h	N/A			72 h postfertilization	Death			↑ spinal deformities, yolk sac edema	Hong et al., 2015
iv	0.09-0.18 ng/fish	1x on a single day within 1-4 d postfertilization				5-7 d postfertilization	Fin flapping and convulsions at some doses/times	Physical, Early Reflexes		Whole brain: widespread necrosis at highest dose; reduced myelin and altered structure at some timepoints; 10 differentially expressed genes at all timepoints	Panlilio, Aluru, & Hahn, 2020

Abbreviations: GABA - γ-aminobutyric acid; GD – gestational day; ip – interparental; iv – intravenous; MRI – magnetic resonance imaging; PND – postnatal day; sc – subcutaneous

*Table 4: Postnatal Exposure and Neurodevelopmental Effects of Domoic Acid**

Route of Exposure	Dose	Duration of Exposure	Period of Study	Sex	Overt Signs of Neurotoxicity	Affected Developmental Domains	Unaffected Developmental Domains	Neuropathological Effects	Citation
Rats									
ip	0.05-0.4 mg/kg	1x on PND2, 5, or 10	Neonatal	Males and Females	Dose-dependent hyperactivity, scratching, seizures			Hippocampus: no morphological changes; Whole brain: ↑ c-fos mRNA	Xi et al., 1997
sc	0.1-0.50 mg/kg	1x on PND7	Neonatal	Males and Females	Paralysis, tremors, seizures, death	Electrophysiology (EEG)		Whole brain: no pathology changes; Spinal cord: ↑ lesions	J. Wang et al., 2000
sc	25-100 µg/kg	2x/d on PND1 and 2	Neonatal period-adulthood	Males and Females	Death (highest dose)	Activity	Physical, Cognition		E. D. Levin et al., 2006
sc	20 or 60 µg/kg	1x/d on PND8-14	Neonatal period-adulthood	Males	None	Emotionality	Physical, Activity, Social behavior, Emotionality	Limbic areas: ↑ binding of yohimbine to α2-adrenoceptors in at 20 µg/kg and ↓ binding at 60 µg/kg	Thomsen et al., 2016
sc	30 µg/kg	1x/d on PND10-14	Adolescence and adulthood	Males	None	Activity	Physical		Jandová et al., 2014
sc	20 µg/kg	1x/d on PND8-14	Adulthood	Males		Electrophysiology (EEG)			D. A. Gill et al., 2009
sc	20 µg/kg	1x/d on PND8-14	Adulthood	Males		Electrophysiology (EEG), ↓ threshold to chemically induced seizures		DG and CA3: ↑ MFS	D. A. Gill, Bastlund, et al., 2010
sc	20 µg/kg	1x/d on PND8-14	Neonatal period-adulthood	Males and Females	None	Cognition	Physical, Early reflexes		Adams et al., 2009
sc	20 µg/kg	1x/d on PND8-14	Neonatal period-adulthood	Males and Females	None		Physical, Early reflexes	Hippocampus: ↑ in MFS; ↑ density of trkB receptors; no cell loss	Bernard et al., 2007
sc	20 µg/kg	1x/d on PND8-14	Neonatal period-adulthood	Males and Females	None	Physical, Activity, Drug-seeking behavior	Physical, Early reflexes		Burt et al., 2008a
sc	20 µg/kg	1x/d on PND8-14	Adolescence	Males and Females	None	Drug-seeking behavior	Activity		Burt et al., 2008b
sc	20 µg/kg	1x/d on PND8-14	Neonatal period-adulthood	Males and Females	None	Cognition, Emotionality, Activity, Behavioral seizures	Physical, Early reflexes, Cognition	Hippocampus and amygdala: Sex-specific alterations α2 adrenoceptors; no changes in corticotropin-releasing factor receptors I/II or D2 receptors	D. A. Gill et al., 2012
sc	20 µg/kg	1x/d on PND8-14	Adulthood	Males and Females	None			Ventral hippocampus: ↓ GAD; Dorsal/mid hippocampus: sex-specific ↓ parvalbumin+ cells; no changes in somatostatin	D. A. Gill, Ramsay, & Tasker, 2010
sc	20 µg/kg	1x/d on PND8-14	Neonatal period-adulthood	Males and Females	None	Cognition, Sensory/Motor Processing	Physical, Early reflexes		Marriott et al., 2012
sc	20 µg/kg	1x/d on PND8-14	Adulthood	Males and Females		Cognition			Marriott et al., 2014
sc	20 µg/kg	1x/d on PND8-14	Adulthood	Males and Females	None	Cognition	Physical	Hippocampus and prefrontal cortex: no changes in D1, D2, TH, GAD65 or GAD67 proteins	Marriott et al., 2016
sc	20 µg/kg	1x/d on PND8-14	Neonatal period-adulthood	Males and Females	None	Behavioral seizures	Physical, Early reflexes	Hippocampus and hypothalamus: no change in glucocorticoid and mineralocorticoid receptors	Perry et al., 2009
sc	20 µg/kg	1x/d on PND8-14	Adulthood	Males and Females	None	Social behavior	Physical		C. L. Ryan et al., 2011
sc	20 µg/kg	1x/d on PND8-14	Neonatal	Males and Females	None	Cognition			Tasker et al., 2005
sc	5 or 20 µg/kg	1x/d on PND8-14	Neonatal period-preadolescence	Males and Females	None	Physical, Cognition	Physical, Early reflexes, Emotionality, Activity		Doucette et al., 2003
sc	5 or 20 µg/kg	1x/d on PND8-14	Adulthood	Males and Females	None	Behavioral seizures	Physical	Hippocampus: ↑ MFS, ↓ cell counts, ↑ brain derived neurotrophic factor (BDNF)	Doucette et al., 2004
sc	5 or 20 µg/kg	1x/d on PND8-14	Adulthood	Males and Females	None	Cognition, Emotionality			Doucette et al., 2007

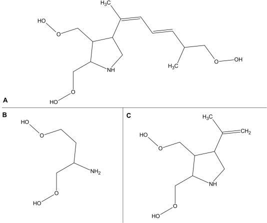
*No biomarkers of exposure were reported.

Abbreviations: D – dopamine; GABA - γ-aminobutyric acid; GAD – glutamic acid decarboxylase; MFS – mossy fiber sprouting; PND – postnatal day; TH – tyrosine hydroxylase

Table 5: Domoic Acid Human Health Risk Assessments and Suggested Regulatory Limit

Suggested DA limit in shellfish (ppm)	Estimated Seafood Consumption in 1 Meal (g)	Suggested Consumption Limit (mg DA/kg bw)	Citation
19.4	270	0.075	Mariën, 1996
16-24	250-380	0.1	WHO - Toyofuku, 2006
12	200	0.03	LOAEL - Slikker et al., 1998
10	200	0.03	1 in 10,000 risk - Slikker et al., 1998
6.4	200	0.018	Benchmark Dose - Slikker et al., 1998
4.5	400	0.03	EFSA - Alexander et al., 2009
2	135	0.003	Benchmark Dose – Stuchal et al., 2020

Abbreviations: bw – body weight; EFSA – European Food Safety Authority; LOAEL – lowest-observed-adverse-effect-level; WHO – World Health Organization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O
NH
H3C
O
OH
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HO
A
B
HO
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NH2
O
HO
C
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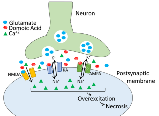
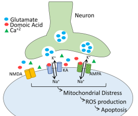
HO
O
NH
O
HO


Neuron
Glutamate
Domoic Acid
Ca+2
K+
K+
NMDA
KA
AMPA
Na+
Na+
Postsynaptic
membrane
Overexcitation
Necrosis


Neuron
Glutamate
Domoic Acid
Ca+2
K+
K+
NMDA
KA
AMPA
Na+
Na+
Mitochondrial Distress
ROS production
Apoptosis