Published in final edited form as:

Neurotoxicology. 2010 September ; 31(5): 409–423. doi:10.1016/j.neuro.2010.05.003.

DOMOIC ACID AS A DEVELOPMENTAL NEUROTOXIN

Lucio G. Costa^{1,2}, Gennaro Giordano¹, and Elaine M. Faustman^{1,3}

¹Dept. of Environmental and Occupational Health Sciences, University of Washington, Seattle, WA, USA

²Dept. of Human Anatomy, Pharmacology and Forensic Science, University of Parma Medical School, Parma, Italy

³Institute for Risk Analysis and Risk Communication, University of Washington, Seattle, WA, USA

Abstract

Domoic acid (DomA) is an excitatory aminoacid which can accumulate in shellfish and finfish under certain environmental conditions. DomA is a potent neurotoxin. In humans and in non-human primates, oral exposure to a few mg/kg DomA elicits gastrointestinal effects, while slightly higher doses cause neurological symptoms, seizures, memory impairment, and limbic system degeneration. In rodents, which appear to be less sensitive than humans or non-human primates, oral doses cause behavioral abnormalities (e.g. hindlimb scratching), followed by seizures and hippocampal degeneration. Similar effects are also seen in other species (from sea-lions to zebrafish), indicating that DomA exerts similar neurotoxic effects across species. The neurotoxicity of DomA is ascribed to its ability to interact and activate the AMPA/KA receptors, a subfamily of receptors for the neuroexcitatory neurotransmitter glutamate. Studies exploring the neurotoxic effects of DomA on the developing nervous system indicate that DomA elicits similar behavioral, biochemical and morphological effects as in adult animals. However, most importantly, developmental neurotoxicity is seen at doses of DomA that are one to two orders of magnitude lower than those exerting neurotoxicity in adults. This difference may be due to toxicokinetic and/or toxicodynamic differences. Estimated safe doses may be exceeded in adults by high consumption of shellfish contaminated with DomA at the current limit of 20 ug/g. Given the potential higher susceptibility of the young to DomA neurotoxicity, additional studies investigating exposure to, and effects of this neurotoxin during brain development are warranted.

Keywords

Domoic acid; Developmental neurotoxicity

1. Introduction

In the summer of 1961, hundreds of birds, mostly shearwaters, a normally non aggressive species that feeds on small fish, attacked the small seaside town of Capitola, in Northern California. The birds appeared to “cry like babies” as they dove into streetlamps, crashed through glass windows and attacked people on the ground (Trabing, 1961). This episode, together with a short story published in 1952 by Daphne du Maurier, is said to have inspired movie director Alfred Hitchcock, who with screenwriter Evan Hunter (a.k.a. crime fiction writer Ed McBain) created the now classical movie “The Birds”. Over the years, several

episodes of bizarre behavior, seizures and deaths in birds and sea lions were reported along the California coast and in other parts of the world (Lefebvre et al. 2002). In 1987, an outbreak of human poisoning occurred in Eastern Canada, which involved about 200 individuals with four fatalities (see Section 3a). In all cases, the causing factor is believed to be the neurotoxin domoic acid (DomA). Since then, a large number of studies have investigated the neurotoxic effects of DomA in non-human primates, rodents and other animal species; such studies have evidenced similar patterns of neurotoxic effects, involving various behavioral abnormalities, seizures, and neurodegeneration of the limbic system. In vitro studies have delineated the cellular and molecular mechanisms of neurotoxicity of DomA, which involve activation of a subtype of receptors for the excitatory amino acid glutamate, leading to oxidative stress-mediated neuronal cell death. A number of studies have also investigated the potential developmental neurotoxicity of DomA, following pre- or post-natal exposures. As these studies appear to indicate an enhanced susceptibility of the young to the toxic and neurotoxic effects of DomA, this review discusses the existing evidence for a possible definition of DomA as a developmental neurotoxin, and examines whether current regulatory guidelines would be protective of the young.

2. Characteristics and occurrence

DomA is a cyclic amino acid with a molecular weight of 311 Da; it has three carboxylic groups (Fig. 1) which are responsible for its high polarity and hydrophilicity. Its structure is very similar to that of another known neurotoxin, kainic acid (KA; Fig. 1). Several isomers of DomA exist, though they are present at very low concentrations in shellfish and display a much lower toxicity (Jeffery et al. 2004; Munday et al. 2008). Both DomA and KA are analogs of an important neurotransmitter, the excitatory amino acid glutamate, and indeed both mimic glutamate in its interaction with some of its receptor subtypes (see Section 4).

DomA was originally identified in 1957 from the seaweed *Chondria armata* off the coast of Southern Japan (Takemoto and Daigo, 1958), and seaweed extracts were used in Japan to treat intestinal parasite. The estimated dose of DomA for this use was ~20 mg, i.e. ~0.33 mg/kg for a 60 kg person. Based on the Canadian outbreak of 1987 (see section 3a) this dose is below that required to elicit any adverse effect (Todd, 1993). In the Canadian episode, marine diatoms of the genus *Pseudo nitzschia* (*P. pungens*) were found to be responsible for the production of DomA (Perl et al. 1990). Strains of *P. nitzschia* known to produce DomA also include *P. multiseriata*, *P. pseudodelicatissima*, and *P. australis* (Jeffery et al. 2004; Lefebvre and Robertson, 2010). Levels of DomA vary greatly with strain, geographical location, and environmental conditions (e.g. temperature) (Mos, 2001). Shellfish such as crabs, mussels, razor clams and scallops can accumulate DomA either by direct filtration of the plankton or by feeding directly on contaminated organisms (Jeffery et al. 2004). Cooking does not significantly reduce DomA concentration in shellfish (McCarron and Hess, 2006). DomA also accumulates in certain fish, such as anchovies, sardines, mackerel, and albacore, though levels are usually lower than in shellfish (Lefebvre et al. 2002). Table 1 contains a partial list of shellfish and fish found to be contaminated with DomA.

3. Neurotoxicity

DomA is a potent neurotoxin. Neurotoxicity has been observed in several animal species including humans, non-human primates, rodents, fish, marine mammals and birds. Its neurotoxic effects in adult animals have been the subjects of several studies and are summarized in various excellent reviews, and will only be briefly discussed here (Todd, 1993; Mos, 2001; Jeffery et al. 2004; Pulido, 2008; Lefebvre and Robertson, 2010; Grant et al. 2010).

3a. Humans

Between November 22 and 24, 1987, government agencies in the Canadian provinces of New Brunswick and Quebec received reports of three persons who had rapid onset confusion, disorientation, and memory loss, within 24 hours of having eaten mussels from Prince Edward Island (Perl et al. 1990). Within a four-week period, a total of 250 reports of illness were received in Eastern Canada related to consumption of mussels. Symptoms were primarily of gastrointestinal nature (nausea, vomiting, abdominal cramps, diarrhea), but included severe headache and loss of memory, particularly short term (Perl et al. 1990). More than twenty individuals (age >20-70) required hospitalization for 4 to 101 days; confusion, disorientation and inability to recall the recent past were prominent features of their clinical presentation (Perl et al. 1990). Seizures, myoclonus and abnormalities of arousal ranging from agitation to coma were also present. Four individuals died within four months of ingesting mussels. On neuropsychological testing several months later, twelve of the hospitalized patients had severe anterograde memory deficits and clinical and electromyographic evidence of motor or sensory-motor neuropathy (Teitelbaum et al. 1990). DomA was rapidly identified as being responsible for the toxic symptoms (Wright et al. 1989). As a main manifestation of poisoning was the destruction of short-term memory in about 25% of the exposed individuals, the syndrome of DomA intoxication was given the name of amnesic shellfish poisoning (ASP) (Todd, 1993; Jeffery et al. 2004). Positron-emission tomography showed decreased glucose metabolism in the medial temporal lobes. Neuropathological studies carried out in the four patients who died revealed neuronal necrosis or loss and astrocytosis, predominantly in the hippocampus and the amygdala (Teitelbaum et al. 1990). Cendes et al. (1995) reported clinical and neuropathology findings in an 84-year-old individual exposed to DomA, who died 3.5 years later of pneumonia. Initial symptoms included nausea, vomiting, confusion, coma, generalized seizures which resolved in a few weeks, and severe memory deficits. After one year the individual experienced complex partial seizures indicative of temporal lobe epilepsy. Autopsy revealed atrophy of the hippocampus, with complete neuronal loss in the CA1 and CA3 regions, almost total loss in the CA4 region, and moderate loss in the CA2 region; neuronal loss was also present in the amygdala.

Levels of DomA ingested by various individuals in the Canadian outbreak ranged from 15-20 mg (0.2-0.3 mg/kg bw) for an unaffected person, to 295 mg (4.2 mg/kg bw) for the most serious cases, while a dose of 60-110 mg (0.9-2.0 mg/kg bw) was sufficient to cause mild gastrointestinal symptoms (Todd, 1993). This indicates that DomA has a very steep dose-response curve, and that humans appear to be much more sensitive to DomA toxicity than rodents (see Sections 3b and 3e).

Since the 1987 intoxication in Eastern Canada, no confirmed cases of human poisoning with DomA have been reported, though in Washington State, USA, a series of illnesses were reported in 1991 upon consumption of contaminated razor clams and Dungeness crabs (Todd, 1993). In this case, estimated doses for mild gastrointestinal symptoms were even lower (0.16 mg/kg bw). However, the illness was not confirmed as ASP, and this poisoning incident was not well reported or substantiated (Jeffery et al. 2004).

3b. Non-human primates and rodents

Studies in non-human primates and in rodents (rats and mice) show a similar pattern of behavioral and histopathological effects upon exposure to DomA by the oral, parenteral and intravenous routes (Tryphonas et al. 1990a; 1990b; 1990c; Scallet et al. 1993; Sobotka et al. 1996; Colman et al. 2005; additional references in Pulido, 2008). In non-human primates, acute exposure results in chewing, teeth grinding, stereotypic scratching, vomiting and hypothermia (Tryphonas et al. 1990b; 1990c). In rodents, observed clinical symptoms include scratching, tremors and seizures (Iverson et al. 1989; Tasker et al. 1991). Cognitive changes (alterations

in spatial memory) were consistent with hippocampal damage (Petrie et al. 1992; Kuhlmann and Guilarte, 1997; Clayton et al. 1999). Acute brain damage is characterized by neurodegenerative changes consisting of neuronal shrinkage, vacuolization of the cytoplasm, and swelling of astrocytes (Pulido, 2008). The CA3 and CA4 regions of the hippocampus are primarily affected, followed by the CA1 region and the dentate gyrus, while the CA2 region is much less affected. Additional affected regions include the olfactory bulb, the amygdala, the lateral septum and the area postrema. As the area postrema is involved in the control of the vomit reflex, this latter finding may explain why vomiting was a prominent feature both in humans and in non-human primates administered DomA by the oral or parenteral route (Pulido, 2008). Damage to the area postrema is also observed in rodents, though these species cannot vomit.

3c. Other species

Seabirds and marine mammals are also affected by DomA (Bejarano et al. 2008). Work et al. (1993) reported neurological signs in brown pelicans and cormorants along the California coast, that had fed with DomA-contaminated fish. Several episodes of intoxication of California sea lions have also been reported. In 1998, several hundreds sea lions were exposed to DomA through contaminated anchovies and sardines; at autopsy, brains of sea lions that died acutely revealed lesions in the hippocampal region, particularly granule cells in the dentate gyrus, and pyramidal cells in CA4, CA3 and CA1, as well as marked gliosis (Scholin et al. 2000; Guillard et al. 2002; Silvagni et al. 2005). These lesions are similar to those found in humans, non-human primates and rodents, but appear to be more prominent (Pulido, 2008). In 2002, more than 2000 mammals (mostly female sea lions and male dolphins) presenting neurological signs, stranded in Southern California (Torres de la Riva et al. 2009). More recently, sea lions exposed to DomA that were rehabilitated, later developed epilepsy, suggesting that sublethal doses of DomA may cause delayed neurotoxicity (Goldstein et al. 2008). These observations in sea lions seem to agree with the report by Cendes et al. (1995) in a human. An interesting finding is that one fish species, the leopard shark, appears to be resistant to the neurotoxic effect of DomA, despite expressing the proper subunits of KA receptors (Schaffer et al. 2006). The authors suggest that these animals possess an endogenous ligand for the KA receptor, which may bind to the receptor and prevent its activation by DomA.

3d. Acute vs. repeated exposure

A few studies have investigated the effect of repeated exposure to DomA, at doses below those inducing overt clinical symptoms. In non-human primates, oral administration of DomA (0.5 mg/kg for 15 days followed by 0.75 mg/kg for 15 days) did not cause any behavioral and histopathological effect (Truelove et al. 1997). Similarly, rats dosed by gavage with 0.01 or 5 mg/kg DomA for 64 days, displayed no clinical abnormalities and unremarkable histopathological findings as assessed by light microscopy (Truelove et al. 1996). However, electron microscopy revealed morphologic changes (cytoplasmic vacuolization, mitochondrial damage) in the CA3 region of the hippocampus at the high dose (5 mg/kg) (Pulido, 2008). In a study in mice, a single i.p. dose of 0.5 mg/kg DomA did not cause any behavioral effects, nor did four separate exposures, 48 h apart (Peng et al. 1997). A single higher dose (2.0 mg/kg, i.p.) caused significant behavioral alterations, however, these did not increase with repeated dosing (four administrations every 48 h) (Peng et al. 1997). In a subsequent study, a single ip injection of DomA (1 or 2 mg/kg) to mice caused an impairment of functional memory, while repeated exposures to the same doses (four doses, every 48 h) appeared to cause a lesser effect (Clayton et al. 1999).

Overall, this limited information seems to indicate that subsymptomatic or symptomatic non lethal doses of DomA do not increase sensitivity or enhance neurotoxicity. This may be attributed to the fact that DomA is rapidly cleared from the serum (see Section 3f), and does

not accumulate. The findings also indicate that serum clearance of DomA is not altered by repeated exposures, suggesting that DomA itself does not affect renal clearance mechanisms (Truelove et al. 1996; Peng et al. 1997). In vitro studies in hippocampal slices from young (2-3 month-old) rats have shown that pre-conditioning with a low concentration of DomA (50 nM) would produce tolerance to the electrophysiological effects of higher concentrations (250-500 nM) (Kerr et al. 2002). This tolerance is not due to receptor desensitization, and was ascribed to a down regulation of high-affinity GTPase activity (Hesp et al. 2004). In preliminary experiments, we have found that chronic exposure of mouse cerebellar granule neurons (CGNs) to DomA (5 nM for 10 days) decreases the extent of apoptotic cell death caused by a higher concentration (100 nM) of DomA (Giordano and Costa, unpublished results). Such protective effect appears to be due to the ability of a low, sub-toxic concentration of DomA to upregulate glutathione (GSH) synthesis (Giordano and Costa, unpublished results; see also Section 4). In contrast to these findings, Qiu et al. (2006) reported that a 48 h incubation of rat mixed cortical cultures with 1 μ M DomA, increased their sensitivity to higher concentrations (3-10 μ M), suggesting that, in this case, pre-conditioning would actually increase susceptibility to a subsequent DomA exposure.

3e. Species differences in susceptibility

Susceptibility to acute DomA neurotoxicity shows significant species differences (Table 2). In humans, the oral NOAEL (No Observed Adverse Effect Level) calculated from the 1987 Canada outbreak was 0.2-0.3 mg/kg, while the LOAEL (Lowest Observed Adverse Effect Level) was ~0.9 mg/kg (Perl et al. 1990; Todd, 1993). In non-human primates the oral NOAEL was 0.75 mg/kg, and the LOAEL was 1.0 mg/kg (Truelove et al. 1997). In contrast, rodents are less sensitive to the acute oral toxicity of DomA, with NOAELs of 28 mg/kg (mice) and 60 mg/kg (rats) (Iverson et al. 1989). In these two rodent species, doses inducing neurotoxicity were 35 and 80 mg/kg, respectively. Thus, humans appear to be one to two orders of magnitude more sensitive than rodents to the acute oral toxicity of DomA. Sea-lions have been suggested to be as sensitive as humans and to thus represent a good sentinel species; however, no precise quantitative estimate has been made in this regard (Lefebvre and Robertson, 2010).

3f. Toxicokinetic considerations

Upon oral administration, which would be the only route of exposure in humans, DomA is poorly absorbed, as suggested by various lines of evidence. The NOAELs and LOAELs for DomA in mice were 1 and 2 mg/kg after i.p. administration, compared to 28 and 35 mg/kg after oral exposure (Iverson et al. 1989). Only 2% of the dose was excreted in the urine after oral administration of 5 mg/kg DomA to rats (Truelove et al. 1996), and similar results (4-7%) were reported in non-human primates (Truelove et al. 1997), suggesting that DomA is primarily excreted in the feces upon oral administration. Once absorbed, DomA appears to undergo very limited metabolism to compounds of greater hydrophilicity, with approximately 75% of the dose being excreted unchanged (Suzuki and Hierlihy, 1993). Serum clearance occurs by renal clearance, primarily by glomerular filtration (Suzuki and Hierlihy, 1993), though a role for active transporters in kidney has been suggested (Robertson et al. 1992). The elimination of DomA is rather rapid; half-lives of 20 and 110 min have been calculated in rats and non-human primates, respectively (Truelove and Iverson, 1994). This would explain why DomA was not detected in blood from individuals intoxicated in 1987 with DomA-contaminated mussels.

The transfer of DomA through the blood-brain barrier (BBB) has been examined by Preston and Hynie (1991). Though the central nervous system is the primary target of DomA toxicity, the BBB is poorly permeable to this toxin, as DomA permeates through the BBB only slightly more rapidly than sucrose, and does not utilize the anionic amino acid carrier used by glutamate (Smith, 2000). However, certain areas lacking a BBB (e.g. area postrema) would allow a higher permeation of DomA. DomA also crosses the placenta and has been detected in fetal brain and

in amniotic fluid (Maucher and Ramsdell, 2007; Brodie et al. 2006). It is also excreted through the milk (Maucher and Ramsdell, 2005). It should be noted that in all these latter studies DomA was administered by the i.v. or i.p. routes, and findings would have to be confirmed after oral administration.

4. Mechanism of neurotoxicity

It was apparent early on that the pattern of brain damage observed in humans, and subsequently in animals, following exposure to DomA, resembled that seen after administration of KA (Teitelbaum et al. 1990). Earlier studies showed that DomA has a higher affinity for KA receptors than KA itself (Zaczek and Coyle, 1982), and this was confirmed by electrophysiological studies in that rat dorsal hippocampus (Debonnel et al. 1989). A comparison of DomA and KA effects in vitro and in vivo confirmed that DomA acts via KA receptors, and is 3 to 20-fold more potent (depending on the measured end-point) than KA itself (Stewart et al. 1990).

Five KA receptor subunits have been cloned, GluR5, GluR6, GluR7, KA1 and KA2, which have been recently renamed by IUPHAR as GluK1-5, respectively, to mirror the corresponding gene names (Collingridge et al. 2009). They show approximately 40% sequence homology with four AMPA [(S)-2-amino-3-(3-hydroxy-5-methyl-4-isoxazolyl) propionic acid] receptors and a 25% homology with the five NMDA (N-methyl-D-aspartate) receptor subunits (Hampson and Manalo, 1998; Bleakman, 1999; Lerma, 2006). This large number of subunits comprises the family of ionotropic glutamate receptors, which in turn represents one family of receptors for this neurotransmitter, the other being that of metabotropic glutamate receptors. The human homologs of the KA receptors are 90-99% identical in their amino acid sequence to the rat proteins. KA receptors undergo several post-translational modifications, such as phosphorylation and palmitoylation, which are thought to be important modes of regulation of channel function (Hampson and Manalo, 1998; Pinheiro and Mulle, 2006; Coussen, 2009). Though KA and DomA have high affinity for KA receptors (Jane et al. 2009), they display some affinity for all classes of ionotropic glutamate receptors. Indeed, it has been shown that DomA neurotoxicity is mediated by its interaction with both KA and AMPA receptors (Hampson et al. 1992, Larm et al. 1997), while indirect activation of NMDA receptors can occur at higher DomA dose levels. Both KA and DomA bind with high affinity (K_i in the low nanomolar range) to recombinant homomeric subunits GluR6 or KA1; interestingly, GluR5 displays a substantially higher affinity for DomA than for KA in the spinal cord, in dorsal root ganglion neurons and in trigeminal neurons, in the hippocampus, the cerebellum, and the amygdala (Bleakman, 1999). The distribution of KA receptors coincides for the most part with the pattern of damage seen after administration of KA or DomA.

A number of studies have delineated the mechanisms of DomA neurotoxicity at the cellular level (Pulido, 2008). By activating AMPA/KA receptors, DomA causes an increase in the levels of calcium ions (Ca^{2+}), which results in the release of glutamate (Berman et al. 2002). This released glutamate in turn activates NMDA receptors and promotes further glutamate release. This combined action causes a rapid accumulation of Ca^{2+} , promotes GSH efflux, and causes a concomitant decrease in intracellular GSH. High Ca^{2+} levels and low intracellular GSH lead to the production of reactive oxygen species (ROS), which are probably of mitochondrial origin. Because there is insufficient GSH to scavenge ROS, there is an increase in lipid peroxidation, and this contributes to cell death, which is mostly necrotic in nature (Giordano et al. 2006). The central role of oxidative stress in mediating DomA neurotoxicity, which is similar to what had been previously observed with KA (Carriedo et al. 1998; Ceccon et al. 2000), is supported by various lines of evidence, which indicate that intracellular GSH can modulate DomA toxicity (Giordano et al. 2006). For example, depletion of GSH by chemical or genetic means increases susceptibility to DomA neurotoxicity; in contrast, increased GSH

levels and antioxidants decrease excitotoxicity of DomA (Giordano et al. 2006). In vivo studies have also shown that DomA increases brain levels of ROS (Bondy and Lee, 1993), and that antioxidants decrease its neurotoxicity (Ananth et al. 2003).

While both AMPA/KA and NMDA glutamate receptors are involved in necrotic neuronal death caused by higher concentrations of DomA (e.g. 10 μ M; Giordano et al. 2006), lower concentrations (50-100 nM) are primarily apoptotic (Giordano et al. 2007; 2009). Such low concentrations of DomA activate AMPA/KA receptors and cause a small and transient increase in Ca^{2+} levels, not sufficient to elicit the release of glutamate. This initial event is followed by an increase in mitochondrial Ca^{2+} levels, followed by loss of mitochondrial potential, increase in mitochondrial oxidative stress, and the opening of the permeability transition pore on the mitochondrial membrane. Cytochrome c is then released from mitochondria, followed by activation of caspase-3 and degradation of PARP (poly ADP ribose polymerase) (Giordano et al. 2007). Activation of p38 and of Jun N-terminal kinase (JNK) mitogen-activated protein kinases, has also been shown to be involved in DomA-induced apoptosis (Giordano et al. 2008a). DomA-induced apoptosis is more pronounced in neurons with low GSH content, such as those derived from Gclm ($-/-$) mice, which lack the modifier subunit of glutamate-cysteine ligase, the enzyme that carries out the first and rate-limiting reaction in the synthesis of GSH. For example, a concentration of DomA as low as 10 nM, had no effect in CGNs from wild-type mice, but caused a significant increase in apoptotic cell death in neurons from Gclm ($-/-$) mice (Giordano et al. 2009). Hippocampal neurons contain much less GSH than CGNs (5.5 vs. 12.4 nmol/mg of protein; Giordano et al. 2008b), and would thus be expected to be more sensitive to the toxicity of DomA.

5. Developmental neurotoxicity

Age is believed to be an important factor modulating the neurotoxicity of DomA. In the Prince Edward Island outbreak, Perl et al. (1990) observed that the four mortalities were elderly subjects, all over 70 years of age. Overall, the older adults were more likely to suffer memory loss. As DomA is rapidly cleared from serum, and agents that disrupt kidney function have been shown to increase DomA toxicity (Truelove and Iverson, 1994), it has been suggested that increased toxicity in older age groups may be due to decreased renal clearance of DomA. Young rats (5-6 month-old) were reported to be less sensitive than older animals (22-25 months) to KA-induced status epilepticus (Wozniak et al. 1991). Similar findings have been reported also for DomA (Kerr et al. 2002). Given the role of oxidative stress in mediating the neurotoxicity of DomA (see section 4), the lower levels of antioxidant defense mechanisms present in the aged rat brain (Liu, 2002; Sandhu and Kaur, 2002) may contribute to the higher sensitivity of aged rats to DomA neurotoxicity.

A number of studies, mostly in rodents, have addressed the issue of DomA toxicity and neurotoxicity upon perinatal exposure. Overall, DomA does not appear to act as a classic teratogen, however, both pre- and post-natal exposure to DomA have been found to cause hippocampal damage, seizure disorders and persistent behavioral abnormalities (Grant et al. 2010). Most importantly, such effects are seen at doses lower than those required to cause neurotoxicity in adult rodents. In some instances, when gender differences were found, this is indicated.

5a. Pre-natal exposure

Studies exploring the effects of pre-natal DomA exposure are summarized in Table 3. Three studies were carried out in rodents, two in zebrafish, and the last one is an observational study in sea lions. A study by Dakshinamurti et al. (1993) reported the effects of DomA (0.6 mg/kg, i.v.) in mice at different ages following in utero exposure at gestational day (GD) 13. While a higher dose of DomA (2.4 mg/kg, i.v.) caused seizures and death in the dams, the only effect

observed in dams following the lower dose was hypoactivity. Litter size, birth weight and hippocampal structure in pups at post-natal day (PND) 1 did not differ from controls. Increasing alterations in bipolar encephalogram (EEG) were found from PND 10 to 30. Mice also displayed a reduced seizure threshold to a second administration of DomA (0.6 mg/kg, i.v. on PND 10-30). Starting at PND 14, morphological changes were observed in the CA3 and dentate gyrus hippocampal regions, and alterations in the CA4 region were observed on PND 30. The CA1 and CA2 regions remained preserved (Dakshinamurti et al. 1993). Neurochemical analyses revealed an increase in kainate receptors, of glutamate levels, and of calcium influx, and a decrease of GABA (γ -aminobutyric acid) levels, in the hippocampus. The findings indicate that in utero exposure to a sub-convulsive dose of DomA causes hippocampal alterations that progress with age.

Levin et al. (2005) administered DomA (0.3, 0.6 and 1.2 mg/kg, s.c.) to pregnant rats on GD 13. No effects were seen in the dams, and litter size, birth weight and offspring growth did not differ from controls. Starting at 4 weeks of age, until week 13, mice were subjected to a series of behavioral tests. In the T-maze, there were no changes in percent alternation, but there was an increase in response latency. In the Figure-8 locomotor task, no overall differences in locomotion were observed, but the high dose group displayed increased rate of habituation. In the 8-arm maze, the normal gender differences (male performing more accurately than females) was diminished by pre-natal DomA exposure at the two highest doses. When challenged with an amnesic dose of scopolamine, mice pre-natally exposed to 1.2 mg/kg DomA performed significantly worse than controls (Levin et al. 2005). Overall, this study indicates that a single, low-dose exposure to DomA on GD 13 causes subtle but long-lasting behavioral impairments in the locomotor and cognitive domains (Grant et al. 2010).

In a recent study, mice were given a single injection of DomA (1.0 mg/kg, i.p.) on GD 11.5, 14.5 or 17.5 (Tanemura et al. 2009). No effects on the dams and on the pups at birth and during early development were noted. Between 4 and 11 weeks of age mice underwent a series of behavioral tests, including locomotor activity in an open field, the elevated plus maze test, the contextual/cued fear conditioned test, and the light/dark conditioned test. In the open field, no differences were observed in overall distance travelled, but mice exposed on GD 14.5 and 17.5 spent significantly more time in the center area. All mice showed alterations in the elevated plus maze test (an anxiety behavior test) with regard to time spent in the open area and distance travelled, while only mice exposed on GD 14.5 were affected in the light/dark conditioned test. In the contextual/cued fear conditioned test (a test of learning and memory), mice exposed on GD 14.5 and 17.5 performed significantly worse than controls. These results indicate that pre-natal exposure to DomA, particularly on GD 14.5 and 17.5 caused significant deficits in anxiety-related behaviors, and severe impairment in learning and memory (Tanemura et al. 2009). Immunohistochemical examination of the hippocampus at 11 weeks of age indicated an increased immunoreactivity against MAP-2 (microtubule associated protein 2, a marker of neuronal dendrites) in the lateral area of the CA3 region in all mice. Similar results were also found in the cerebral cortex, where a deficit in myelination was also detected. Differently from Dakshinamurti et al. (1993), no neuronal cell loss was found in the hippocampus.

In sum, these studies found behavioral, electrophysiological and morphological changes in mice exposed in utero to a single, low dose of DomA, suggesting that this toxin passes the placental barrier to affect the developing brain. This was confirmed by a study by Maucher and Ramsdell (2007) in rats. Animals were injected with DomA (0.6 or 1.6 mg/kg, i.v.) on GD 13 or GD20 and sacrificed one hour after exposure. DomA levels were measured by ELISA in plasma and brain of dams, amniotic fluid and fetal brain (Table 4). Results indicate that DomA crosses the placental barrier and enters the fetuses in which brain levels are about half of those of the dam.

Two studies examined the developmental toxicity of DomA in zebrafish upon in ovo microinjection (Tiedeken et al. 2005; Tiedeken and Ramsdell, 2007). Doses of DomA ranged between 0.12 and 16.8 mg/kg. At doses of 0.4 mg/kg and above DomA reduced hatching, possibly because of spinal cord deformities (Tiedeken et al. 2005). Neurotoxicity was a prominent effect, with uncontrolled pectoral fin motions and tonic-clonic convulsions. In a subsequent study, DomA was microinjected in ovo at doses of 0.12-1.26 mg/kg (Tiedeken and Ramsdell, 2007). Exposed zebrafish larvae displayed an increased sensitivity to the convulsant effects of pentylenetetrazole (PTZ), characterized by decreased latency to seizures and increase in their severity. Seizures were observed following in ovo exposure to sub-convulsive doses of DomA and challenges with normally sub-convulsive doses of PTZ (Tiedeken and Ramsdell, 2007). These findings seem to substantiate the earlier results of Dakshinamurti et al. (1993) in mice.

The increased number of cases of neurotoxicity in young sea-lions has also raised the possibility of effects due to prenatal exposures, because of maternal consumption of DomA-contaminated anchovies and sardines (Goldstein et al. 2008; Ramsdell and Zabka, 2008). Of interest is that levels of DomA in amniotic fluid of stranded sea lions (4-34 ng/ml; Brodie et al. 2006) are quite similar to those measured in rat amniotic fluid following i.v. administration of 0.6-1.6 mg/kg DomA (8-21 ng/ml; Table 4; Maucher and Ramsdell, 2007).

5b. Post-natal exposure

Various studies have investigated the effects of DomA exposure during the early post-natal period in rodents, usually the first two weeks of age (Table 5). It should be noted in this regard that this period of brain development in rodents is considered to be equivalent of human brain development during the third trimester of pregnancy (Dobbing and Sands, 1979). Thus, extrapolations to humans from the previous and the following studies, refer to in utero exposure at different times during pregnancy.

An earlier study (Bose et al. 1989) compared the toxicity of a shellfish extract, obtained from the 1987 Canadian outbreak and given by i.p. injection, in adult and PND 15 mice. Infant mice were found to be 3-4-fold more sensitive than adults to the extract's toxic effects (scratching, convulsions, death). The LOAELs of DomA for scratching and convulsions in this study were approximately 0.64 mg/kg in PND 15 pups, and 1.5-4.5 mg/kg in adult mice (Bose et al. 1990).

A later study by Xi et al. (1997) provided additional important observations. The acute toxicity of DomA was found to be higher in rat pups at PND 2 (i.p. LD₅₀ = 0.25 mg/kg), than at PND 10 (i.p. LD₅₀ = 0.7 mg/kg). A dose-response experiment in PND 5 pups with 0.02, 0.1, 0.2 mg/kg DomA i.p. showed that the lowest dose was the NOAEL for stereotypic behavior (scratching). The intermediate dose caused scratching, while the highest dose caused tonic-clonic convulsions. Behavioral testing was carried out after acute administration of DomA (0.05-0.8 mg/kg, i.p.) on PND 5 or PND 10. No major differences were detected between the two ages; a dose-dependent increase in behavioral symptoms (analyzed according to Tasker et al. 1991) was observed at all doses, with a LOAEL of 0.05 mg/kg (Xi et al. 1997). No morphological changes were found in the hippocampus of PND 2 rats 72 hours after i.p. treatment with 0.2 mg/kg DomA. Administration of 0.1 mg/kg DomA on PND 5 caused an increase in the expression of c-fos mRNA in brain, which was partially antagonized by an NMDA receptor antagonist, whereas in adult rats a dose of 0.5 mg/kg i.p. represented the LOAEL for the same effect (Peng and Ramsdell, 1996). Serum levels of DomA, measured 1 h after administration of different doses of DomA (0.05-0.8 mg/kg, i.p.) on PND 5 and PND 10, increased according to dose, and a good correlation was found between DomA levels and toxicity (Xi et al. 1997). A comparison of these results with those obtained in adult rats upon i.p. administration (Tryphonas et al. 1990a) indicates that rat pups are approximately 20-fold

more sensitive to DomA-induced seizure (LOAELs = 4.0 vs. 0.2 mg/kg), and approximately 20-40-fold more sensitive to DomA-induced stereotypic scratching (LOAELs = 2.0 vs. 0.05-0.1 mg/kg). A study by Sobotka et al. (1996), who administered DomA by i.p. injection to adult rats, reported a LOAEL for scratching and other behavioral effects of 0.93 mg/kg, and a LOAEL for seizures of 1.32 mg/kg. A comparison with the data of Xi et al. (1997) with those of Sobotka et al. (1996), indicates a differential sensitivity between neonate and adult rats of approximately 7-19-fold. Similarly, Peng and Ramsdell (1996) reported scratching in adult rats after 1 mg/kg DomA, and seizures after 2.0 mg/kg DomA (both i.p.), for an increased sensitivity of rat pups of approximately 10-20 fold. When comparing LD50 values, a difference of 2-6-fold can be calculated between adult (1.5 mg/kg; Sobotka et al. 1996) and neonate rats (0.25-0.7 mg/kg; Xi et al. 1997). An additional comparison between serum DomA levels and toxic effects in rat neonates and adults indicated that there is a similar sensitivity to internal DomA levels, suggesting that the higher susceptibility of neonates results from retention of higher levels of the toxin (Peng and Ramsdell, 1996; Xi et al. 1997). Such increased bioavailability of DomA may be due to an incomplete BBB or, more likely, to a decreased serum clearance due to lower renal clearance (Xi et al. 1997).

Wang et al. (2000) examined behavioral changes and spinal cord lesions in neonatal rats administered DomA (0.1, 0.17, 0.25, 0.33, 0.42, 0.50 mg/kg, s.c.) on PND 7. Behavioral changes included scratching and tail flicking, swimming-like movements, forced hindlimb extension followed by paralysis, respiratory failure and death. The approximate LD50 was 0.33 mg/kg, while the lowest dose tested (0.1 mg/kg) represented the LOAEL, both consistent with the findings of Xi et al. (1997). EEG measurements at the only dose tested (0.33 mg/kg) indicated significant alterations consistent with seizure activity. Animals treated with the same dose did not display any morphological alteration in various brain areas (e.g. hippocampus, cerebral cortex, hypothalamus), but presented significant changes in the spinal cord, characterized by hemorrhage, tissue swelling and neuronal degeneration, particularly in the ventral region, while the dorsal region was spared (Wang et al. 2000). Similar spinal cord lesions were also found in adult mice following administration of DomA (0.2-1.6 mg/kg, i.p.), and attributed to DomA-induced oxidative stress and mitochondrial dysfunction leading to apoptotic cell death (Xu et al. 2008). The LOAEL in this study was 0.2 mg/kg.

As a follow up to their previous pre-natal study, Levin et al. (2006) treated rats with 0.025, 0.05 and 0.1 mg/kg DomA, by s.c. injections, twice a day on PND 1 and PND 2. KA (1 mg/kg, s.c.) served as a positive control. Mortality was observed at all DomA doses, with all animals dying in the high dose group. The surviving offspring showed normal growth. Behavioral testing began in adolescence (weeks 4-8 of age) and continued through adulthood (weeks 8-13). No changes were found in the T-maze spontaneous alternation test, while the 0.05 mg/kg dose caused hypoactivity in the Figure-8 apparatus. No effects were seen in radial arm maze acquisition, nor in response to a scopolamine challenge. Overall, the effects were minor, however, hypoactivity, which is seen immediately upon exposure, was still present several weeks later. This finding suggests that early post-natal exposure DomA may cause subtle but very persistent behavioral effects. A comparison with the results of the pre-natal exposure study (Levin et al. 2005) indicate that effects were more modest upon early post-natal exposure at PND 1 and 2, corresponding to about the 24th week of gestation in humans. The LOAEL from this study is between 0.05 and 0.1 mg/kg, similar to that reported by Xi et al. (1997).

The comparative behavioral toxicity of DomA and KA was evaluated in neonatal rats following i.p. administration on PND 0, 5, 14 and 22, with doses ranging from 0.05 to 10 mg/kg and 0.05-30 mg/kg, respectively (Doucette et al. 2000). A behavioral toxicity rating scale, which included a number of effects associated with exposure to DomA (e.g. scratching, masticatory behavior, hindlimb hyperextension etc.) was used as the end-point for age, dose and compound comparisons. For DomA, the toxicity score increased with age; the ED50 (Effective Dose 50)

increased from 0.12 mg/kg on PND 0, to 0.15 mg/kg on PND 5, to 0.30 on PND 14, to 1.06 on PND 22 (a 9-fold increase). These results are consistent with those of Xi et al. (1997). In adult mice, the i.p. ED50 for behavioral toxicity was reported as 3.9 mg/kg (Tasker et al. 1991). A comparison with KA (but with both compounds given s.c.) was carried out on PND 8 and PND 14; at both ages KA was 5-6-fold less toxic than DomA (Doucette et al. 2000). The authors also noted some small differences in behavioral response between neonatal and adult rodents after administration of DomA. However, a prominent effect was hindlimb scratching, which is a characteristic feature of DomA toxicity in neonates and adults. Some differences were also noted between DomA and KA; for example, duration and intensity of scratching were less upon KA administration, and KA-induced convulsions were characterized by higher rigidity than those seen after DomA (Doucette et al. 2000).

A series of studies examined the behavioral effects of exposure to daily DomA s.c. injections from PND 8 to PND 14. An initial study by Doucette et al. (2003) again compared the effects of DomA (0.005 and 0.02 mg/kg, s.c.) and KA (0.025 and 0.1 mg/kg, s.c.) on neurobehavioral development. Neither compound had any effect on weight gain, acoustic startle, ultrasonic vocalization or maternal retrieval. In contrast, the higher dose of DomA (0.02 mg/kg, which thus represents the LOAEL in this study) decreased the age of eye opening and affected olfactory conditioning. KA had also an effect on locomotor activity at both dose levels (Doucette et al. 2003). A subsequent study, using the same exposure paradigm, investigated long-term effects on a number of behavioral, biochemical and morphological end-points (Doucette et al. 2004). On PND 120 animals of all groups (DomA and KA) displayed symptoms that were named “novelty-induced seizure-like syndrome”, when placed on the escape platform in a Morris water maze. Similar findings were obtained when animals were placed in a novel water maze at PND 240, and in an open field arena on PND 270, suggesting that the observed symptoms (hunched body posture, facial clonus, repetitive head extension and bobbing, eye blinking, mastication with tongue protrusion, ear twitching) were associated with exposure to a novel environment. These effects were observed even in rats dosed with the lowest dose (0.005 mg/kg), which thus represents the LOAEL in this study. In contrast, no differences from controls were found in standard measures of Morris water maze performance (Doucette et al. 2004). DomA also increased expression of BDNF (brain-derived neurotrophic factor) in the hippocampal CA1 region, but did not affect expression of neuropeptide Y. Hippocampal abnormalities, assessed at approx. PND 510, included enhanced mossy fiber sprouting (suggestive of new synapse formation), and increased cell loss in the CA1, CA3b and CA3c regions. Such alterations are similar to those seen in animal models of temporal lobe epilepsy. Morphological analysis of the hippocampus at PND 90, following the same PND 8-14 exposure of rats to DomA (0.02 mg/kg, s.c.), showed cytoarchitectural changes, characterized by an increase in mossy fiber sprouting in the CA3 region, but no increase in neuronal cell loss (Bernard et al. 2007). This finding differs from that of Doucette et al. (2004) who found hippocampal cell loss on PND 510. This would suggest that upon this developmental paradigm of exposure, damage to the hippocampus is progressive, resulting in increasing damage as the animal ages. A proposed explanation is that between 3 and 7 months of age animals may experience more bouts of undetected spontaneous seizure activity that would induce further damage (Bernard et al. 2007). An increase in trkB receptors (the high affinity receptors for BDNF) was also found in the hippocampus. These results clearly show that a brief post-natal exposure to DomA causes long-lasting, irreversible changes in the rat hippocampus, that appear to be progressive in nature.

An additional series of studies, carried out in the past five years, reported various long-term behavioral effects of neonatal exposure to DomA (0.02 mg/kg, s.c., from PND 8 to PND 14). Tasker et al. (2005) found that conditioned odor preference on PND 13 was affected by DomA in an NMDA receptor-sensitive manner. In a study by Doucette et al. (2007) female, but not male rats had significant impairments in learning new platform locations in the Morris water

maze. Furthermore, in the elevated plus maze, female rats (but not males) spent more time in the open arm of the maze. Small, but persistent alterations in locomotor activity were found in rats on PND 18, 36 and 150 (Burt et al. 2008a), with some gender differences. This study also reported of changes in novelty-seeking behavior, in male rats only, at PND 150. In a related study, adolescent rats (PND 36), neonatally exposed to DomA, showed no response to nicotine in a place conditioning test, suggesting an alteration of the mesocorticolimbic pathway involved in the response to the rewarding properties of nicotine (Burt et al. 2008b). Adams et al. (2008) reported that neonatal DomA exposure lowered pre-pulse inhibition in adult males, and caused an increased startle response in adult females, while Perry et al. (2009) confirmed the altered behavior (“novelty-induced seizure-like syndrome”) when rats were exposed to a novel environment (Doucette et al. 2004), but no changes in the response to mild stress. Adams et al. (2009) reported that neonatal DomA administration caused persistent alterations in learning and memory, as assessed in the radial arm maze (PND 34-36) and the Morris water maze (PND 132-134), with female animals being more affected. These findings differ from those of Doucette et al. (2004), who found no changes in Morris maze performance. Interestingly, animals exposed neonatally to DomA demonstrated superior spatial and working memory in these tests compared to controls (Adams et al. 2009). Finally, a study by Gill et al. (2010) reported a loss of GABAergic neurons and of parvalbumin-containing immunoreactivity in the hippocampus, with no changes in somatostatin expression. These effects were more pronounced in male, than in female, rats.

While all these studies involved direct administration of DomA to pups, lactational exposure to DomA was specifically addressed by a study of Maucher and Ramsdell (2005). Rat dams were given 1.0 mg/kg DomA (i.p) on lactational day 12, and levels of DomA in milk were measured 1-24 h after administration. At the 1 h time-point, concentration of DomA in milk was about 5% of that present in the dam’s blood, however, traces of DomA were still detectable in milk 24 h after exposure when no DomA was present in blood, indicating a longer retention time of DomA in milk. When pups were given milk collected from DomA-treated dams 4 h after exposure, no DomA could be detected in their blood, whereas milk spiked with 0.3 or 1.0 mg/kg DomA produced blood levels of approximately 15-25 ng/ml in pups. Though with limitations, this study shows that DomA is excreted in milk and exposure of pups may occur at early time-points after exposure of the dams.

Altogether, the results of experiments carried out with early post-natal exposure to DomA indicate that subtle, but persistent behavioral alterations can be detected, together with some biochemical and morphological changes that increase with age. The data also provide evidence of a greater susceptibility of pups to DomA neurotoxicity, compared to adult animals.

5c. Additional considerations on age-dependent susceptibility

A comparison of the neurotoxic effects of DomA during brain development and in adulthood indicates that this neurotoxin causes, for the most part, the same behavioral, biochemical and morphological alterations. However, the data also suggest that such effects may be elicited during development at lower doses of DomA (Table 6). A direct age comparison should take into account species differences, and most importantly, route of administration. Indeed, there are significant quantitative differences between oral and parental routes of administration because of the poor absorption of DomA through the gastrointestinal tract. As there are no developmental neurotoxicity studies in which DomA was given by gavage, any quantitative consideration only takes into account the parental routes. In the few pre-natal studies in rodents, doses of DomA of 0.3 to 1.2 mg/kg were utilized, and the overall LOAEL appears to be 0.6 mg/kg in both mice and rats (Dakshinamurthy et al. 1993; Levin et al. 2005). In the post-natal studies, a greater range of DomA doses as used, and the LOAELs were 0.05-0.1 mg/kg after a single administration (Xi et al. 1997; Wang et al. 2000; Doucette et al. 2000; Levin et al.

2006), and 0.005-0.02 mg/kg after repeated administrations from PND 8 to PND 14 (Doucette et al. 2003;2004). In adult rats, reported LOAELs for different DomA effects upon i.p. administration include values of 0.5 mg/kg (Peng and Ramsdell. 1996), 0.9-1.3 mg/kg (Sobotka et al. 1996), 1.0 mg/kg (Peng et al. 1997), 1.5 mg/kg (Kuhlman and Guilarte, 1997), and 2.0 mg/kg (Tryphonas et al. 1990;Scallet et al. 2005). In mice, a LOAEL of 0.8 mg/kg i.p. was reported (Tasker et al. 1991). The overall ratio of these values between developing and adult animals ranges from 5 to 400.

Comparison of values other than the LOAELs indicates similar age-related differences. For example, the ED50 for behavioral effects of DomA (i.p.) was 0.12 mg/kg in PND 0 rats, and 3.9 mg/kg in adult mice (a 32-fold difference) (Doucette et al. 2000; Tasker et al. 1991). When comparing LD50 values, these range from 0.25-0.7 mg/kg in pups to 3.6-4.0 mg/kg in adult rodents, a 5 to 16-fold difference (Xi et al. 1997; Tasker et al. 1991; Grimmelt et al. 1990). Thus, overall, it would appear that young rodents are one to two orders of magnitude more sensitive to DomA-induced toxicity and neurotoxicity than adult animals.

5d. Possible mechanisms of developmental neurotoxicity

As previously discussed (Section 4) the primary targets of DomA are the receptors for KA. In vitro evidence suggests that while higher concentrations of DomA exert neurotoxicity by a dual action mediated by AMPA/KA and by NMDA receptors (the latter activated by released glutamate), low concentrations of DomA act almost exclusively through the AMPA/KA receptors (Giordano et al. 2006; 2007). During development, gene expression for the individual KA receptor subunits can be detected as early as embryonic day 12 in rat, two days earlier than when their proteins can be detected by autoradiography (Bahn et al. 1994; Simeone et al. 2004). All of the subunit genes undergo peak expression during the late embryonic or early postnatal period, however, transcripts for different subunits have different developmental time-courses; for example, KA1 transcripts appear at embryonic day 17-19 and increase gradually with hippocampal maturation, while expression of GluR5 peaks between PND 0 and PND 5 and then declines (Simeone et al. 2004). In the rat hippocampus, all KA receptor subunits were expressed to higher levels early postnatally, with a decline by PND 35 (Ritter et al. 2002). However, there was a significant difference in the level of expression for different subunits and in different hippocampal subfields. For instance, the KA1 subunit was maximally expressed in the CA2, CA3 and CA4 regions on PND 4-8, but less so in the dentate gyrus or the CA1 region (Ritter et al. 2002). An elevated binding of [³H] KA in the first two postnatal weeks, compared with adult levels, was also reported (Miller et al. 1990; Garica-Ladona and Gombos, 1993). In human fetal brain, only the KA2 and GluR7 subunits were expressed at significant levels as early as gestational week 8, with several peaks of higher expression at gestational weeks 11, 13 and 19 (Ritter et al. 2001). Binding of [³H]-KA was also detected at gestational week 8, and peaked at gestational week 11, and then again at gestational week 15 (Ritter et al. 2001).

Expression of AMPA receptor subunits in rat brain can be detected as early as embryonic day 16, gradually increases until a peak around PND 10 to PND 14, and then declines to adult levels (Simeone et al. 2004). However, not all subunits develop at the same rate; for example, the GluR1 subunit does not change post-natally in the rat hippocampus, while the other three subunits peak between PND7 and PND 18 (Ritter et al. 2002). In the rat mesencephalon, the GluR1-3 subunits were detected from embryonic day 13, and were found to plateau on embryonic day 19 (Lilliu et al. 2001). In human fetal cerebral cortex, transient periods of high expression of AMPA receptor subunits were found at gestational weeks 11, 13 and 19, similar to what observed for the KA receptor subunits; however, levels of AMPA receptor proteins (as inferred by [³H]- AMPA binding) were lower than those of KA (Ritter et al. 2001).

The development of NMDA receptor subunits has also been investigated (Simeone et al. 2004; Ritter et al. 2001; 2002; Law et al. 2003). Low levels of expression are found in the rat fetal brain, but NMDA receptor subunits increase dramatically after birth, with some differences among subunits. In the rat hippocampus, the NR1 subunit peaks around PND 7 and PND 14, while the NR2B subunit declines progressively from birth to adulthood (Ritter et al. 2002). The NR1, NR2B, and NR2D subunits were those mostly expressed in human fetal cortex (Ritter et al. 2001), while in human hippocampus NR1 and NR2B expression increased and decreased, respectively, from 5-20 to 22-51 weeks of age (Law et al. 2003).

In sum, all the putative targets for DomA are present in the developing brain, both in animals and in humans. Though these studies indicate the presence of the specific subunits and not necessarily the presence of functional receptors, the findings that developmental exposure to DomA causes behavioral, biochemical and morphological effects very similar to those found in adult animals, would argue that AMPA/KA and perhaps NMDA receptors, represent the primary target for DomA in the developing brain. A possible explanation for the enhanced susceptibility of the neonates to DomA neurotoxicity is that there are structural and/or functional differences in AMPA/KA receptors at different ages. This has been suggested by some investigators (Doucette et al. 2000; Stewart et al. 2009), who noted that in some brain circuits excitatory synapses tend to develop pre-natally, while inhibitory processes are not mature until PND 14/15 (Schwartzkroin, 1981). Studies with KA have shown that the developing brain appears to be less sensitive to its neurotoxicity (neurodegeneration) compared to adults (Coyle, 1983; McDonald and Johnston, 1990). However, this is based on exposure on PND7 and histological examination on PND 12 (Coyle, 1983). As shown earlier, neuronal loss may be substantially delayed upon early post-natal exposure (Doucette et al. 2004; Bernard et al. 2007). Furthermore, neonatal animals are very sensitive to KA-induced seizures (McDonald and Johnston, 1990), and this would contribute to neurotoxicity. Indeed, it has been pointed out that it is difficult to clearly separate direct effects of KA from those deriving from seizure activity.

Based on limited in vivo information in adult animals, and on more detailed studies in vitro (see Section 4), one would expect that activation of AMPA/KA receptors in the developing brain results in increased oxidative stress. The developing brain is particularly sensitive to oxidative stress, possibly due to its richness in free iron and its limited antioxidant capacity (Sola et al. 2007). Oxidative stress plays a relevant role in contributing to the pathological apoptosis in the developing brain (Blomgren et al. 2007). Thus, DomA-induced oxidative stress may be a relevant contributor to its developmental neurotoxicity.

It has been suggested that DomA may cause developmental neurotoxicity by activating microglia, which would in turn release neurotoxic free radicals, cytokines, eicosanoids and matrix metalloproteases (Mayer, 2000); however, this hypothesis has not been supported by experimental findings of the same proponent investigators (Mayer et al. 2007).

Others have suggested that DomA developmental neurotoxicity may be secondary to an action on thyroid hormones (Arufe et al. 1995; Alfonso et al. 2000), as hypothyroidism is a known cause of developmental neurotoxicity (Schalock et al. 1977; Zoeller and Crofton, 2005). In adult rats, DomA (0.5 and 1.0 mg/kg, i.p.) decreased serum levels of T₄ at 2 and 3 h after treatment, and increased levels of TSH (thyroid stimulating hormone), without altering T₃ levels (Arufe et al. 1995). In a subsequent study, DomA (1 mg/kg, i.p.), as well as KA (30 mg/kg, i.p.), were found to increase levels of T₄, T₃ and TSH at 5 min to 1 h after administration to adult rats (Alfonso et al. 2000). Thus, the available data do not support the hypothesis that hypothyroidism may contribute to the developmental neurotoxicity of DomA.

Additional explanations as to why the developing nervous system may be more susceptible to DomA toxicity have focused on toxicokinetic factors. These include an increase entry of DomA in the brain because of the incomplete development of the blood-brain barrier (Mayer, 2000), the presence of a reduced renal clearance in neonates (Xi et al. 1997; Maucher and Ramsdell, 2005), or possible prolonged pre-natal exposure through the amniotic fluid (Ramsdell and Zabka, 2008). All such hypotheses need to be further investigated.

6. Considerations for human health risk assessment

A few investigators and advisory/regulatory agencies have proposed acceptable acute or repeated levels of exposure to DomA that would be protective of human health, based on the results of animal studies or data obtained from the 1987 Canadian outbreak. A summary of this information is shown in Table 7.

Slikker et al. (1998) derived an oral ARfD (Acute Reference Dose) of 0.034 mg/kg bw, based on a LOAEL of 0.5 mg/kg for DomA give i.v. to non-human primates (Scallet et al. 1993). Uncertainty factors (UFs) included an UF of 10 for LOAEL to NOAEL extrapolation, an UF of 3 for interspecies differences, and an UF of 10 for intraspecies differences, in addition to an adjustment for deriving oral exposure (using a value of 5% absorption rate). Using the same data set and the same corrective UFs, Slikker et al. (1998) also calculated an ARfD of 0.018 mg/kg bw, based on a benchmark dose of 0.26 mg/kg i.v., calculated from the study of Scallet et al. (1993) in non-human primates.

Marien (1996) suggested a TDI (tolerable daily intake) for DomA of 0.075 mg/kg bw. This value was derived from an acute oral NOAEL in non-human primates (0.75 mg/kg; Truelove et al. 1997) divided by an UF of 10 for intraspecies differences. An interspecies UF was not deemed important, as non-human primates and humans display similar dose-response relationships for DomA for different end-points (Marien, 1996). Note, however, that the calculated NOAEL for humans is slightly lower (0.2-0.3 mg/kg; Todd et al. 2003).

In 2004, the Joint FAO/WHO/IOC ad hoc Expert Consultation on Biotoxins in Molluscan Bivalves determined an acute oral reference dose (ARfD) for DomA of 0.1 mg/kg bw. This value was derived from the LOAEL in humans of 1 mg/kg, estimated from the 1987 Canadian outbreak, divided for an UF of 10 to account for intraspecies differences (FAO, 2004; Toyofuku, 2006). Since DomA does not appear to be a cumulative neurotoxin, it was suggested that it would be “unlikely that people who habitually consume small amounts of DomA would experience any chronic effects; thus, the ARfD may be considered a provisional chronic TDI (FAO, 2004; Toyofuku, 2006).

More recently, the European Food Safety Agency (EFSA) indicated an ARfD of 0.030 mg/kg bw (EFSA, 2009). This value was derived from a LOAEL of 0.9 mg/kg, estimated from the Canadian outbreak, with a UF of 3 for extrapolation to a NOAEL (given the steep dose-response of DomA), and an UF of 10 for intraspecies variability. EFSA also calculated an ARfD from the Tryphonas et al. (1990b) study in non-human primates. In this case the point of departure (PoD) was a LOAEL of 5 mg/kg, with an UF of 3 to extrapolate from LOAEL to NOAEL, a 10 UF for intraspecies variability, and a 4 UF for interspecies variability in toxicokinetics, for an ARfD of 0.040 mg/kg bw.

Overall, these ARfD and TDI values, which range from 0.018 to 0.1 mg/kg bw vary by about 5.5-fold (Table 7). As shown in Tables 8 and 9, these levels would be exceeded in certain instances in adults upon consumption of shellfish contaminated with the current limit of DomA (20 ug/g). Additionally, values of ARfD and TDI shown in Table 7 were derived from data obtained in adult animals or humans. Hence, it is important to determine whether they would be protective of subsensitive populations, i.e. whether the 10 UF for intraspecies difference

would be sufficient in this regard. It was noted that individuals with impaired renal functions may be more susceptible to DomA (EFSA, 2009); however, quantification of this enhanced susceptibility is difficult to ascertain. Furthermore, aged individuals appear to be more sensitive to DomA neurotoxicity, because of lower renal clearance, diminished antioxidant capacity, or decreased compensatory ability (Perl et al. 1990; Kerr et al. 2002; Hesp et al. 2004; Liu, 2002; Sandhu and Kaur, 2002; Ginsberg et al. 2005). More germane to the topic of this review is the potential age-related susceptibility with regard to enhanced susceptibility of the young. Information available on the neurotoxic effects of DomA during development, discussed in Section 5, seem to indicate that most behavioral, biochemical, and morphological effects of DomA are qualitatively similar in neonatal and adult animals. However, in all cases, such effects are seen during development at doses of DomA that are one to two orders of magnitude lower than in adult animals. If a similar enhanced susceptibility of the young would exist in humans, one should consider whether an additional UF to account for developmental neurotoxicity might be warranted.

7. Human exposure and advisories

Exposure of humans to DomA can occur primarily through the consumption of contaminated shellfish (mussels, clams), while exposure through contaminated fish (e.g sardines) is less likely. Following the 1987 Canadian outbreak, limits of 20 ug DomA/g shellfish meat have been imposed in Canada, and this limit has been adopted in the USA, European Union, New Zealand and Australia. This value derives from an estimate of a concentration of 200 ug DomA/g mussel which had caused illness in 1987, by applying a 10-fold safety factor (Wekell et al. 2004).

Limited information exists on the consumption of shellfish and on potential exposure to DomA, particularly in potentially sensitive population. Tsuchiya et al. (2008) determined shellfish and finfish consumption in women of childbearing age from two Japanese and Korean communities living in Washington State. Finfish consumption was similar between the two groups, though there were differences in the type of fish consumed. In contrast, Koreans consumed nearly 70% more shellfish on a daily basis (23 g/person) than the Japanese (14 g/person) (Table 8; Tsuchiya et al. 2008). No significant differences were found with regard to age for Japanese and Korean populations, or pregnancy status (for the Japanese population only) in fish consumption. Fish consumption values reported by Tsuchiya et al. (2008) are comparable to those seen in tribal fish consumption studies (CRITFC, 1994; Toy et al. 1996; Sechena et al. 1999; Suquamish, 2000). Assuming a DomA level of 20 ug/g in shellfish, potential exposure to DomA was calculated, and is shown in Table 8. While mean exposure was well below any ARfD, the 95th percentile consumption of shellfish provided exposure close to the proposed ARfDs (Table 7).

The European Food Safety Agency provided information on shellfish consumption in various European populations (Table 9; EFSA, 2009). Overall, shellfish consumption appears to be higher than that found in the previous study, particularly in Germany and the Netherlands. Based on a DomA level of 20 ug/g, the estimated exposure to this neurotoxin appears to exceed in several cases the ARfD or TDI values. These considerations would represent a worst-case scenario, as they imply consumption of shellfish contaminated with the maximal allowable level of DomA (20 ug/g). In reality, monitoring programs in different parts of the world indicate that levels of DomA of <5 ug/g are usually found (Vale and Sampayo, 2001; James et al. 2005; Trainer et al. 2007), though peaks as high as 2800 ug/g (more than 3-times those found in the Canadian outbreak) have been reported (James et al. 2005). EFSA estimated that for the ARfD not be exceeded, a 400 g portion of shellfish (representing the 95th percentile value of EU consumption) should contain a maximum level of DomA of 4.5 ug/g. In the EU, 3.5% of shellfish normally exceed this value, though the 95th percentile of DomA concentration in

shellfish in the EU is 2.5 ug/g. Thus a 400 g portion of shellfish would normally provide 0.017 mg/kg bw of DomA, below the ARfD (EFSA, 2009). However, as said, the ARfD and TDI values shown in Table 7 are derived from studies and observation in adult animals and humans, without any consideration for a potential higher susceptibility of the young. If an additional UF were applied to the ARfD, even consumption of shellfish with levels of DomA contamination lower than the current 20 ug/g limit, would lead to exceed these safety values.

8. Overall perspective and research needs

DomA is a potent neurotoxin in humans and in animals. In humans and in non-human primates oral exposure to a few mg/kg DomA elicits gastrointestinal effects, while slightly higher doses cause neurological symptoms, seizures, memory impairment, and limbic system degeneration. In rodents, which appear to be less sensitive than humans or non-human primates, oral doses cause behavioral abnormalities (e.g. hindlimb scratching), followed by seizures and hippocampal degeneration. Similar effects are also seen in other species (from sea-lions to zebrafish), indicating that DomA exerts similar neurotoxic effects across species. Such effects are ascribed to the ability of DomA to interact and activate the AMPA/KA receptors, a subfamily of receptors for the neuroexcitatory neurotransmitter glutamate. Studies exploring the neurotoxic effects of DomA on the developing nervous system have evidenced that DomA elicits similar behavioral, biochemical and morphological effects as seen in adult animals. However, these effects are produced at doses that are one to two orders of magnitude lower. The reason for these quantitative differences is unclear, but may be ascribed to toxicokinetic and/or toxicodynamic differences.

Since the 1987 outbreak of human DomA poisoning, guidelines have been implemented to limit DomA contamination in shellfish to 20 ug/g. Though this limit has served public health well, as no additional proven cases of human poisoning have been reported, limited studies have shown that such limit may actually be too high, as shellfish consumption may in some cases lead to exceed calculated ARfD and TDI values. More importantly, such values have not taken into account the potential higher susceptibility of fetuses, infants and children. If DomA were an industrial chemical or any other chemical requiring registration prior to commercialization, one may argue that an additional UF for developmental neurotoxicity may be warranted, to ensure full protection of children. Nevertheless, given the paucity of knowledge currently available, acquisition of additional information and further considerations would be important.

First, since the oral route is the only expected route of exposure in humans, studies are needed that investigate the oral dose-response of DomA given pre-natally and/or post-natally, assessing a number of behavioral, biochemical, electrophysiological, and morphological end-points. Second, studies are needed to confirm the placental transfer of DomA to the fetus and its excretion to the milk upon oral exposure. In this regard, additional studies addressing toxicokinetic parameters in pregnant animals and in pups would be of relevance. Third, longitudinal experimental studies need to be carried out to determine whether DomA may cause delayed, progressive or silent neurotoxicity, as recently suggested (Grant et al. 2010; Stewart, 2010). Of particular relevance is the recent suggestion that pre-natal exposure to subsymptomatic doses of DomA may represent a risk factor for temporal lobe epilepsy (TLE; Stewart, 2010). As the onset of TLE is thought to be triggered by an initial insult to the brain occurring early in development, and the highest incidence is seen in children and the elderly (Bowie, 2008), the potential role played by DomA should be addressed. Fourth, the effects of acute vs. chronic exposure should be better investigated, to fully understand whether a chronic low level exposure would be devoid of effects, and possibly provide protection toward higher acute exposure, or to the contrary, sensitize to DomA neurotoxicity. Such studies may also include the use of more novel approaches in the “omics” field, as shown for example by some

preliminary results in zebrafish (Lefebvre et al. 2009). Fifth, given the increasing relevance of gene-environment interactions in understanding human susceptibility to environmentally-induced diseases (Costa and Eaton, 2006), the issue of whether genetic background may render some individuals more susceptible to DomA neurotoxicity should be addressed. Evidence already suggests that genetic factors play a role in KA and DomA-induced seizure-mediated cell death (Schauwecker, 2003; Peng et al. 1997). Furthermore, in vitro data indicate that genetic variations leading to lower antioxidant defense mechanisms may also increase susceptibility to DomA neurotoxicity (Giordano et al. 2006; 2007). Sixth, some attention should be given to the possibility that other excitatory amino acids present in shellfish (e.g. glutamate or aspartate) may exert additive or synergistic neurotoxic effects with DomA, as suggested by earlier investigations (Novelli et al. 1992). Seventh, additional studies are needed in human populations to ascertain the level of shellfish consumption, particularly in women of child-bearing age, pregnant or lactating, as done by Tsuchiya et al. (2008). Eighth, though not a focus of this review, possible effects of developmental exposure to DomA on the cardiovascular, immune, neuro-endocrine, and gastrointestinal systems should also be investigated (Pulido, 2008). Finally, while such endeavors would lead to a better understanding of the potential developmental effects of DomA, and to a better characterization of human risks, efforts should continue in concert with scientists in oceanography and marine science, to increase knowledge on the modes and mechanisms of DomA production and bioaccumulation in marine organisms, in order to limit or better predict potential human exposure.

Acknowledgments

Studies by the authors were supported in part by grants from the National Science Foundation and the National Institute of Environmental Health Sciences (ES012762/NSF-OCE-0434087, NSF-OCE-0910624, and ES07033).

References

- Adams AL, Doucette TA, Ryan CL. Altered pre-pulse inhibition in adult rats treated neonatally with domoic acid. *Amino Acids* 2008;35:157–60. [PubMed: 17973071]
- Adams AL, Doucette TA, James R, Ryan CL. Persistent changes in learning and memory in rats following neonatal treatment with domoic acid. *Physiol. Behav* 2009;96:505–12. [PubMed: 19146865]
- Alfonso M, Duran R, Arufe MC. Effect of excitatory amino acids on serum TSH and thyroid hormone levels in freely moving rats. *Horm. Res* 2000;54:78–83. [PubMed: 11251371]
- Ananth C, Gopalakrishnakone P, Kaur C. Protective role of melatonin in domoic acid-induced neuronal damage in the hippocampus of adult rats. *Hippocampus* 2003;13:375–87. [PubMed: 12722978]
- Arufe MC, Arias B, Duran R, Alfonso M. Effects of domoic acid on serum levels of TSH and thyroid hormones. *Endocr. Res* 1995;21:671–80. [PubMed: 7588435]
- Bahn S, Volk B, Wisden W. Kainate receptor gene expression in the developing brain. *J. Neurosci* 1994;14:5525–47. [PubMed: 8083752]
- Bejarano AC, VanDola FM, Gulland FM, Rowles TK, Schwacke LH. Production and toxicity of the marine biotoxin domoic acid and its effects on wildlife: a review. *Hum. Ecol. Risk Assess* 2008;14:544–67.
- Berman FW, LaPage KT, Murray TF. Domoic acid neurotoxicity in cultured cerebellar granule neurons is controlled preferentially by the NMDA receptor Ca^{2+} influx pathway. *Brain Res* 2002;924:20–9. [PubMed: 11743991]
- Bernard PB, McDonald DS, Gill DA, Ryan CL, Tasker RA. Hippocampal mossy fiber sprouting and elevated trkB receptor expression following systemic administration of low dose domoic acid during neonatal development. *Hippocampus* 2007;17:1121–33. [PubMed: 17636548]
- Bleakman D. Kainate receptor pharmacology and physiology. *Cell. Mol. Life Sci* 1999;56:558–66. [PubMed: 11212306]
- Blomgren K, Leist M, Groc L. Pathological apoptosis in the developing brain. *Apoptosis* 2007;12:993–1010. [PubMed: 17453164]

- Bondy SC, Lee DK. Oxidative stress induced by glutamate receptor agonists. *Brain Res* 1993;610:229–33. [PubMed: 8319085]
- Bose R, Glavin GB, Pinsky C. Neurotoxicity and lethality of toxic extracts from Atlantic coast shellfish. *Prog. Neuro-Psychopharmacol. Behav. Psychiat* 1989;13:559–62.
- Bose R, Pinsky C, Glavin GB. Sensitive murine model and putative antidotes for behavioral toxicosis from contaminated mussel extracts. *Can. Dis. Wkly Rep* 1990;16(Suppl. 1):91–8. [PubMed: 2101746]
- Bowie D. Ionotropic glutamate receptors and CNS disorders. *CNS Neurol. Disord. Drug Targets* 2008;7:129–43. [PubMed: 18537642]
- Brodie EC, Gulland FMD, Greig DJ, Hunter M, Jaakola J, St.Leger J, Leighfield T, Van Dolah FM. Domoic acid causes reproductive failure in California sea lions (*Zalophus californianus*). *Mar. Mamm. Sci* 2006;22:700–7.
- MA, Burt; CL, Ryan; TA, Doucette. Altered response to novelty and drug reinforcement in adult rats treated neonatally with domoic acid. *Physiol. Behav* 2008a;93:327–36.
- Burt MA, Ryan CL, Doucette TA. Low dose domoic acid in neonatal rats abolishes nicotine induced conditioned place preference during late adolescence. *Amino Acids* 2008b;35:247–9. [PubMed: 17701097]
- Carriedo SG, Yin HZ, Sensi SL, Weiss JH. Rapid Ca^{2+} entry through Ca^{2+} -permeable AMPA/kainate channels triggers marked intracellular Ca^{2+} rises and consequent oxygen radical production. *J. Neurosci* 1998;18:7727–38. [PubMed: 9742143]
- Ceccon M, Giusti P, Facci L, Borin G, Imbesi M, Floreani M, Skaper SD. Intracellular glutathione levels determine cerebellar granule neuron sensitivity to excitotoxic injury by kainic acid. *Brain Res* 2000;17:83–9. [PubMed: 10799672]
- Cendes F, Andermann F, Carpenter S, Zatorre RJ, Cashman NR. Temporal lobe epilepsy caused by domoic acid intoxication: evidence for glutamate receptor-mediated excitotoxicity in humans. *Ann. Neurol* 1995;37:123–6. [PubMed: 7818246]
- Clayton EC, Peng YG, Means LW, Ramsdell JW. Working memory deficits induced by single but not repeated exposures to domoic acid. *Toxicon* 1999;37:1025–39. [PubMed: 10484738]
- Collingridge GL, Olsen RW, Peters J, Spedding M. A nomenclature for ligand-gated ion channels. *Neuropharmacology* 2009;56:2–5. [PubMed: 18655795]
- Colman JR, Nowocin KJ, Switzer RC, Trusk TC. Mapping and reconstruction of domoic acid-induced neurodegeneration in the mouse brain. *Neurotoxicol. Teratol* 2005;27:753–67. [PubMed: 16109471]
- Costa, LG.; Eaton, DL. *Gene-Environment Interactions: Fundamentals of Ecogenetics*. John Wiley & Sons; Hoboken, NJ: 2006. p. 557
- Coussen F. Molecular determinants of kainate receptor trafficking. *Neuroscience* 2009;159:25–35. [PubMed: 18358623]
- Coyle JT. Neurotoxic action of kainic acid. *J. Neurochem* 1983;4:1–11. [PubMed: 6306166]
- CRITFC (Columbia River Treaty Tribes Fish Consumption Study). *Fish Consumption Survey of the Umatilla, Nez Perce, Yakima, and Warm Springs Tribes of the Columbia River Basin*. Columbia River Intertribal Fish Commission; Portland, Oregon: Oct. 1994 CRITFC Technical Report No. 94-3
- Dakshinamurti K, Sharma SK, Sundaram M, Watanabe T. Hippocampal changes in developing postnatal mice following intrauterine exposure to domoic acid. *J. Neurosci* 1993;13:4486–95. [PubMed: 8105041]
- Debonnel G, Beauchesne L, De Montigny C. Domoic acid, the alleged “mussel toxin”, might produce its neurotoxic effect through kainate receptor activation: an electrophysiological study in the rat dorsal hippocampus. *Can. J. Physiol. Pharmacol* 1989;67:29–33. [PubMed: 2540893]
- Dobbing J, Sands J. Comparative aspects of the brain growth spurt. *Early Hum. Dev* 1979;3:79–83. [PubMed: 118862]
- Doucette TA, Strain SM, Allen GV, Ryan CL, Tasker RA. Comparative behavioral toxicity of domoic acid and kainic acid in neonatal rats. *Neurotoxicol. Teratol* 2000;22:863–9. [PubMed: 11120392]
- Doucette TA, Bernard PB, Yuill PC, Tasker RA, Ryan CL. Low doses of non-NMDA glutamate receptor agonists alter neurobehavioral development in the rat. *Neurotoxicol. Teratol* 2003;25:473–9. [PubMed: 12798964]

- Doucette TA, Bernard PB, Husum H, Perry MA, Ryan CL, Tasker RA. Low doses of domoic acid during postnatal development produce permanent changes in rat behaviour and hippocampal morphology. *Neurotox. Res* 2004;6:555–63. [PubMed: 15639787]
- Doucette TA, Ryan CL, Tasker RA. Gender-based changes in cognition and emotionality in a new rat model of epilepsy. *Amino Acids* 2007;32:317–22. [PubMed: 17068662]
- EFSA (European Food Safety Authority). Marine biotoxins in shellfish- domoic acid. Scientific opinion of the Panel on Contaminants in the Food Chain. *EFSA J* 2009;1181:1–61.
- FAO (Food and Agriculture Organization). Report of the Joint FAO/IOC/WHO ad hoc Expert Consultation on Biotoxins in Bivalve Molluscs. Oslo, Norway: Sept 26–30. 2004 p. 31
- Garica-Ladona FJ, Gombos G. Regional distribution of transient [³H]kainic acid-binding sites in the central nervous system of the developing mouse: an autoradiographic study. *Neurosci. Lett* 1993;151:209–13. [PubMed: 8389436]
- Gill DA, Ramsay SL, Tasker RA. Selective reductions in subpopulations of GABAergic neurons in a developmental rat model of epilepsy. *Brain Res.* 2010 in press.
- Ginsberg G, Hattis D, Russ A, Sonawane B. Pharmacokinetic and pharmacodynamic factors that can affect sensitivity to neurotoxic sequelae in elderly individuals. *Env. Health Perspect* 2005;113:1243–9. [PubMed: 16140636]
- Giordano G, White CC, McConnachie LA, Fernandez C, Kavanagh TJ, Costa LG. Neurotoxicity of domoic acid in cerebellar granule neurons in a genetic model of glutathione deficiency. *Mol. Pharmacol* 2006;70:2116–26. [PubMed: 17000861]
- Giordano G, White CC, Mohar I, Kavanagh TJ, Costa LG. Glutathione levels modulate domoic acid-induced apoptosis in mouse cerebellar granule cells. *Toxicol. Sci* 2007;100:433–44. [PubMed: 17804861]
- Giordano G, Klintworth HM, Kavanagh TJ, Costa LG. Apoptosis induced by domoic acid in mouse cerebellar granule neurons involves activation of p38 and JNK MAP kinases. *Neurochem. Int* 2008a; 52:1100–5. [PubMed: 18164102]
- Giordano G, Kavanagh TJ, Costa LG. Neurotoxicity of a polybrominated diphenyl ether mixture (DE-71) in mouse neurons and astrocytes is modulated by intracellular glutathione levels. *Toxicol. Appl. Pharmacol* 2008b;232:161–8. [PubMed: 18656495]
- Giordano G, Li L, White CC, Farin FM, Wilkerson HW, Kavanagh TJ, Costa LG. Muscarinic receptors prevent oxidative stress-mediated apoptosis induced by domoic acid in mouse cerebellar granule cells. *J. Neurochem* 2009;109:525–38. [PubMed: 19200344]
- Goldstein T, Mazer JAK, Zabka TS, Langlois G, Colegrove KM, Silver M, Bargu S, Van Dolah F, Leighfield T, Conrad PA, Barakos J, Williams DC, Dennison S, Haulena M, Gulland FMD. Novel symptomatology and changing epidemiology of domoic acid toxicosis in California sea lions (*Zalophus californianus*): an increasing risk to marine mammal health. *Proc. R. Soc. B* 2008;275:267–76.
- Grant KS, Burbacher TM, Faustman EM, Grattan L. Domoic acid: neurobehavioral consequences of exposure to a prevalent marine biotoxin. *Neurotoxicol. Teratol* 2010;32:132–41. [PubMed: 19799996]
- Grimmelt B, Nijjar MS, Brown N, MacNair N, Wagner S, Johnson GR, Ahmend JF. Relationship between domoic acid levels in the blue mussel (*Mytilus edulis*) and toxicity in mice. *Toxicon* 1990;28:501–8. [PubMed: 2389251]
- Guilland FMD, Haulena M, Fauquier D, Langlois G, Lander ME, Zabka T, Duerr R. Domoic acid toxicity in California sea lions (*Zalophus californianus*): clinical signs, treatment and survival. *Veter. Rec* 2002;150:475–80.
- Hampson DR, Huang XP, Wells JW, Walter JA, Wright JLC. Interaction of domoic acid and several derivatives with kainic acid and AMPA binding sites in rat brain. *Eur. J. Pharmacol* 1992;218:1–8. [PubMed: 1383007]
- Hampson DR, Manalo JL. The activation of glutamate receptors by kainic acid and domoic acid. *Nat. Toxins* 1998;6:153–8. [PubMed: 10223631]
- Hesp BR, Wrightson T, Mullaney I, Kerr DS. Kainate receptor agonists and antagonists mediate tolerance to kainic acid and reduce high-affinity GTPase activity in young, but not aged, rat hippocampus. *J. Neurochem* 2004;90:70–9. [PubMed: 15198668]

- Iverson F, Truelove J, Nera F, Tryphonas L, Campbell J, Lok E. Domoic acid poisoning and mussel-associated intoxication: preliminary investigations into the response of mice and rats to toxic mussel extract. *Food Chem. Toxicol* 1989;27:377–84. [PubMed: 2792967]
- James KJ, Gillman M, Fernandez Amandi M, Lopez-Riviere A, Fernandez Puente P, Lehane M, Mitrovic S, Furey A. Amnesic shellfish poisoning toxins in bivalve molluscs in Ireland. *Toxicon* 2005;46:852–8. [PubMed: 16289180]
- Jane DE, Lodge D, Collingridge GL. Kainate receptors: pharmacology, function and therapeutic potential. *Neuropharmacology* 2009;56:90–113. [PubMed: 18793656]
- Jeffery B, Barlow T, Moizer K, Paul S, Boyle C. Amnesic shellfish poison. *Food Chem. Toxicol* 2004;42:545–57. [PubMed: 15019178]
- Kerr DS, Razak A, Crawford N. Age-related changes in tolerance to the marine algal excitotoxin domoic acid. *Neuropharmacology* 2002;43:357–66. [PubMed: 12243765]
- Kuhlmann AC, Guilarte TR. The peripheral benzodiazepine receptor is a sensitive indicator of domoic acid neurotoxicity. *Brain Res* 1997;751:281–8. [PubMed: 9099816]
- Larm JA, Beart PM, Cheung NS. Neurotoxin domoic acid produces cytotoxicity via kainate- and AMPA-sensitive receptors in cultured cortical neurons. *Neuroche. Int* 1997;31:677–82.
- Law AJ, Weickert CS, Webster MJ, Herman MM, Kleinman JE, Harrison PJ. Expression of NMDA receptor NR1, NR2A and NR2B subunit mRNAs during development of the human hippocampal formation. *Eur. J. Neurosci* 2003;18:1197–205. [PubMed: 12956718]
- Lefebvre KA, Bargu S, Kieckhefer T, Silver MW. From sand dabs to blue whales: the pervasiveness of domoic acid. *Toxicon* 2002;40:971–7. [PubMed: 12076651]
- Lefebvre KA, Tilton SC, Bammler TK, Beyer RP, Srinouanprachan S, Stapleton PL, Farin FM, Gallagher EP. Gene expression profiles in zebrafish brain after acute exposure to domoic acid at symptomatic and asymptomatic doses. *Toxicol. Sci* 2009;107:65–77. [PubMed: 18936300]
- Lefebvre KA, Robertson A. Domoic acid and human exposure risks: a review. *Toxicon*. 2010 in press.
- Jerma J. Kainate receptor physiology. *Curr. Op. Pharmacol* 2006;6:89–97.
- Levin ED, Pizarro K, Pang WG, Harrison J, Ramsdell JS. Persisting behavioral consequences of prenatal domoic acid exposure in rats. *Neurotoxicol. Teratol* 2005;27:719–25. [PubMed: 16054336]
- Levin ED, Pang WG, Harrison J, Williams P, Petro A, Ramsdell JS. Persistent neurobehavioral effects of early postnatal domoic acid exposure in rats. *Neurotoxicol. Teratol* 2006;28:673–80. [PubMed: 17046199]
- Lilliu V, Pernas-Alonso R, Trelles RD, di Porzio U, Zuddas A, Perrone-Capano C. Ontogeny of AMPA receptor gene expression in the developing rat midbrain and striatum. *Mol. Brain Res* 2001;96:133–41. [PubMed: 11731018]
- Liu RM. Down-regulation of gamma-glutamylcysteine synthetase regulatory subunit gene expression in rat brain tissue during aging. *J. Neurosci. Res* 2002;68:344–51. [PubMed: 12111865]
- Marien K. Establishing tolerable Dungeness crab (*Cancer magister*) and razor clam (*Siliqua patula*) domoic acid contaminant levels. *Environ. Health Perspect* 1996;104:1230–6. [PubMed: 8959413]
- Maucher JM, Ramsdell JS. Domoic acid transfer to milk: evaluation of a potential route of neonatal exposure. *Environ. Health Perspect* 2005;113:461–4. [PubMed: 15811837]
- Maucher JM, Ramsdell JS. Maternal-fetal transfer of domoic acid in rats at two gestational time points. *Environ. Health Perspect* 2007;115:1743–6. [PubMed: 18087593]
- Mayer AMS. The marine toxin domoic acid may affect the developing brain by activation of neonatal brain microglia and subsequent neurotoxic mediator generation. *Med. Hypoth* 2000;54:837–41.
- Mayer AMS, Guzman M, Peksa R, Hall M, Fay MJ, Jacobson PB, Romanic AM, Gunasekera SP. Differential effects of domoic acid and *E.coli* lipopolysaccharide on tumor necrosis factor- α , transforming growth factor- β 1 and matrix metalloproteinase-9 release by rat neonatal microglia: evaluation of the direct activation hypothesis. *Mar. Drugs* 2007;5:113–35. [PubMed: 18458762]
- McCarron P, Hess P. Tissue distribution and effects of heat treatments on the content of domoic acid in blue mussels, *Mytilus edulis*. *Toxicon* 2006;47:473–9. [PubMed: 16488458]
- McDonald JW, Johnston MV. Physiological and pathophysiological roles of excitatory amino acids during central nervous system development. *Brain Res. Rev* 1990;15:41–70. [PubMed: 2163714]

- Miller LP, Johnson AE, Gelhard RE, Insel TR. The ontogeny of excitatory amino acid receptors in the rat forebrain. II. Kainic acid receptors. *Neuroscience* 1990;35:45–51. [PubMed: 2163032]
- Mos L. Domoic acid: a fascinating marine toxin. *Environ. Toxicol. Pharmacol* 2001;9:79–85. [PubMed: 11167152]
- Munday R, Holland PT, McNabb P, Selwood AI, Rhodes LL. Comparative toxicity to mice of domoic acid and isodomoic acids A, B and C. *Toxicon* 2008;52:954–6. [PubMed: 18977379]
- Novelli A, Kispert J, Fernandez-Sanchez MT, Torreblanca A, Zitko V. Domoic acid-containing toxic mussels produce neurotoxicity in neuronal cultures through a synergism between excitatory amino acids. *Brain Res* 1992;577:41–8. [PubMed: 1355695]
- Peng YG, Ramsdell JS. Brain Fos induction is a sensitive biomarker for the lowest observed neuroexcitatory effect of domoic acid. *Fund. Appl. Toxicol* 1996;31:162–8.
- Peng YG, Calyton EC, Means LW, Ramsdell JS. Repeated independent exposures to domoic acid do not enhance symptomatic toxicity of outbred or seizure-sensitive inbred mice. *Fund. Appl. Toxicol* 1997;40:63–7.
- Perl TM, Bedard L, Kosatsky T, Hockin JC, Todd EC, Remis RS. An outbreak of toxic encephalopathy caused by eating mussels contaminated with domoic acid. *New Engl. J. Med* 1990;322:1775–80. [PubMed: 1971709]
- Perry MA, Ryan CL, Tasker RA. Effects of low dose neonatal domoic acid administration on behavioural and physiological response to mild stress in adult rats. *Physiol. Behav* 2009;98:53–9. [PubMed: 19375434]
- Petrie BF, Pinsky C, Standish NM, Bose R, Glavin GB. Parenteral domoic acid impairs spatial learning in mice. *Pharmacol. Biochem. Behav* 1992;41:211–4. [PubMed: 1539071]
- Pinheiro P, Mulle C. Kainate receptors. *Cell Tissue Res* 2006;326:457–82. [PubMed: 16847640]
- Preston E, Hynie I. Transfer constants for blood-brain barrier permeation of the neuroexcitatory shellfish toxin, domoic acid. *Can. J. Neurol. Sci* 1991;18:39–44. [PubMed: 2036614]
- Pulido OM. Domoic acid toxicologic pathology: a review. *Mar. Drugs* 2008;6:180–219. [PubMed: 18728725]
- Qiu S, Pak CW, Curras-Collazo MC. Sequential involvement of distinct glutamate receptors in domoic acid-induced neurotoxicity in mixed cortical cultures: effect of multiple dose/duration paradigms, chronological age, and repeated exposure. *Toxicol. Sci* 2006;89:243–56. [PubMed: 16221958]
- Ramsdell JS, Zabka TS. *In utero* domoic acid toxicity: a fetal basis to adult disease in the California sea lion (*Zalophus californianus*). *Mar. Drugs* 2008;6:262–90. [PubMed: 18728728]
- Ritter LM, Unis AS, Meador-Woodruff JH. Ontogeny of ionotropic glutamate receptor expression in human fetal brain. *Dev. Brain Res* 2001;127:123–33. [PubMed: 11334999]
- Ritter LM, Vazquez DM, Meador-Woodruff JH. Ontogeny of ionotropic glutamate receptor subunit expression in the rat hippocampus. *Dev. Brain Res* 2002;139:227–36. [PubMed: 12480137]
- Robertson H, Renton K, Kohn J, White T. Patterns of Fos expression suggest similar mechanisms of action for the excitotoxins domoic and kainic acid. *Ann. N.Y. Acad. Sci* 1992;648:330–4. [PubMed: 1637064]
- Sandhu SK, Kaur G. Alterations in oxidative stress scavenger system in aging brain and lymphocytes. *Biogerontology* 2002;3:161–73. [PubMed: 12075135]
- Scallet AC, Binienda Z, Caputo FA, Hall S, Paule MG, Rountree RL, Schmued L, Sobotka T, Slikker W Jr. Domoic acid-treated cynomolgus monkeys (*M. fascicularis*): effects of dose on hippocampal neuronal and terminal degeneration. *Brain Res* 1993;627:307–13. [PubMed: 8298975]
- Scallet AC, Schmued LC, Johannessen JN. Neurohistochemical biomarkers of the marine neurotoxicant, domoic acid. *Neurotoxicol. Teratol* 2005;27:745–52. [PubMed: 16203121]
- Schaffer P, Reeves C, Casper DR, Davis CR. Absence of neurotoxic effects in leopard sharks, *Triakis semifasciata*, following domoic acid exposure. *Toxicon* 2006;47:747–52. [PubMed: 16566956]
- Schalock RL, Brown WJ, Smith RL. Neonatal hypothyroidism: behavioral, thyroid hormonal and neuroanatomical effects. *Physiol. Behavior* 1977;19:489–91.
- Schauwecker PE. Genetic basis of kainate-induced excitotoxicity in mice: phenotypic modulation of seizure-induced cell death. *Epilepsy Res* 2003;55:201–10. [PubMed: 12972174]

- Scholin CA, Gulland F, Doucette GJ, Benson S, Busman M, Chavez FP, Cordaro J, DeLong R, De Vogelaere A, Harvey J, Haulena M, Lefebvre K, Lipscomb T, Loscutoff S, Lowenstine LJ, Marin R, Miller PE, McLellan WA, Moeller PD, Powell CL, Rowles TPPP, Silvagni M, Silver T, Spraker V, Trainer V, Van Dolah FM. Mortality of sea lions along the Central California coast linked to a toxic diatom bloom. *Nature* 2000;403:80–4. [PubMed: 10638756]
- Schwartzkroin PA. Development of the rabbit hippocampus: physiology. *Brain Res* 1981;254:469–86. [PubMed: 6272945]
- Sechena, R.; Nakano, C.; Liao, S.; Polissar, N.; Lorzana, R.; Truong, S.; Fenske, R. Asian and Pacific Islander Seafood Consumption Study. King County; Washington: May 27. 1999 EPA 910/R-99-03
- Silvagni P, Lowenstine LJ, Spraker T, Lipscomb TP, Gulland FM. Pathology of domoic acid toxicity in California sea lions (*Zalophus californianus*). *Vet. Pathol* 2005;42:184–91. [PubMed: 15753472]
- Simeone TA, Sanchez RM, Rho JM. Molecular biology and ontogeny of glutamate receptors in the mammalian nervous system. *J. Child Neurol* 2004;19:343–60. [PubMed: 15224708]
- Slikker W Jr, Scallet AC, Gaylor DW. Biologically-based dose-response model for neurotoxicity risk assessment. *Toxicol. Lett* 1998;102-103:429–33. [PubMed: 10022291]
- Smith QR. Transport of glutamate and other amino acids at the blood-brain barrier. *J. Nutr* 2000;130:1016S–22S. [PubMed: 10736373]
- Sobotka TJ, Brown R, Quander DY, Jackson R, Smith M, Long SA, Barton CN, Rountree RL, Hall S, Eilers P, Johannesen JN, Scallet AC. Domoic acid: neurobehavioral and neurohistological effects of low dose exposure in adult rats. *Neurotoxicol. Teratol* 1996;18:659–70. [PubMed: 8947943]
- Sola A, Rogido MR, Deulofeut R. Oxygen as a neonatal health hazard: call for detente in clinical practice. *Acta Paediatrica* 2007;96:801–12. [PubMed: 17537007]
- Stewart GR, Zorumski CF, Price MT, Olney JW. Domoic acid: a dementia-inducing excitotoxic food poison with kainic acid receptor specificity. *Exp. Neurol* 1990;110:127–38. [PubMed: 2170163]
- Stewart I. Environmental risk factors for temporal lobe epilepsy-Is prenatal exposure to the marine algal neurotoxin domoic acid a potentially preventable cause? *Med. Hypoth* 2010;74:466–81.
- Suquamish. Fish Consumption Survey of the Suquamish Indian Tribe of Port Madison Indian Reservation, Puget Sound Region. 2000. The Suquamish Tribe, 15838 Sandy Hook Road, Post Office Box 498, Suquamish, WA 98392
- Suzuki CA, Hierlihy SL. Renal clearance of domoic acid in the rat. *Food Chem. Toxicol* 1993;31:701–6. [PubMed: 8225127]
- Takemoto T, Daigo K. Constituents of *Chondria armata*. *Chem. Pharmacol. Bull* 1958;31:701–6.
- Tanemura K, Igarashi K, Matsugami TR, Aisaki KI, Kitajima S, Kanno J. Intrauterine environment-genome interaction and children's development (2): brain structure impairment and behavioral disturbance induced in male mice offspring by a single intraperitoneal administration of domoic acid (DA) to their dams. *J. Toxicol. Sci* 2009;34(Special Issues 2):SP279–86. [PubMed: 19571481]
- Tasker RAR, Connell BJ, Strain SM. Pharmacology of systemic administered domoic acid in mice. *Can. J. Pharmacol. Physiol* 1991;69:378–82.
- Tasker RAR, Perry MA, Doucette TA, Ryan CL. NMDA receptor involvement in the effects of low dose domoic acid in neonatal rats. *Amino Acids* 2005;28:193–6. [PubMed: 15714252]
- Teitelbaum JS, Zatorre RJ, Carpenter S, Gendron D, Evans AC, Gjedde A, Cashman NR. Neurologic sequelae of domoic acid intoxication due to the ingestion of contaminated mussels. *New Engl. J. Med* 1990;322:1781–7. [PubMed: 1971710]
- Tiedeken JA, Ramsdell JS, Ramsdell AF. Developmental toxicity of domoic acid in zebrafish (*Danio rerio*). *Neurotoxicol. Teratol* 2005;27:711–7. [PubMed: 16061356]
- Tiedeken JA, Ramsdell JS. Embryonic exposure to domoic acid increases susceptibility of zebrafish larvae to the chemical convulsant pentylenetetrazole. *Environ. Health Perspect* 2007;115:1547–52. [PubMed: 18007982]
- Todd ECD. Domoic acid and amnesic shellfish poisoning-A review. *J. Food Protect* 1993;56:69–83.
- Torres de la Riva G, Johnson CK, Gulland FMD, Langlois GW, Heyning JE, Rowles TK, Mazet JAK. Association of unusual marine mammal mortality event with *Pseudo-Nitzschia* spp. Blooms along the Southern California coastline. *J. Wildl. Dis* 2009;45:109–21. [PubMed: 19204340]

- Toy, KA.; Polissar, NL.; Liao, S.; Mittelstaedt, GD. A Fish Consumption Survey of the Tulalip and Squaxin Island Tribes of the Puget Sound Region. 1996. Tulalip Tribes, Department of Environment, 7615 Totem Beach Road, Marysville, WA 98271
- Toyofuku H. Joint FAO/WHO/IOC activities to provide scientific advice on marine biotoxins (research report). Mar. Pollut. Bull 2006;52:1735–45. [PubMed: 16979668]
- Trabing, W. Seabird invasion hits coastal homes; thousand of birds floundering in streets. Santa Cruz Sentinel: Aug 18. 1961 p. 1
- Trainer VL, Cochlan WP, Erickson A, Bill BD, Cox FH, Borchert JA, Lefebvre KA. Recent domoic acid closures of shellfish harvest areas in Washington State inland waterways. Harmful Algae 2007;6:449–59.
- Truelove J, Iverson F. Serum domoic acid clearance and clinical observations in the cynomolgus monkey and Sprague-Dawley rat following a single i.v. Bull. Env. Contam. Toxicol 1994;52:479–86. [PubMed: 8167439]
- Truelove J, Mueller R, Pulido O, Iverson F. Subchronic toxicity study of domoic acid in the rat. Food Chem. Toxicol 1996;34:525–9. [PubMed: 8690311]
- Truelove J, Mueller R, Pulido O, Martin L, Fernie S, Iverson F. 30-Day oral toxicity study of domoic acid in cynomolgus monkeys: lack of overt toxicity at doses approaching the acute toxic dose. Nat. Toxins 1997;5:111–4. [PubMed: 9285915]
- Tryphonas L, Truelove J, Nera E, Iverson F. Acute neurotoxicity of domoic acid in the rat. Toxicol. Pathol 1990a;18:1–9. [PubMed: 2362984]
- Tryphonas L, Truelove J, Todd E, Nera E, Iverson F. Experimental oral toxicity of domoic acid in cynomolgus monkeys (*Macaca fascicularis*) and rats. Preliminary investigations. Food Chem. Toxicol 1990b;28:707–15. [PubMed: 2276699]
- Tryphonas L, Truelove J, Iverson F. Acute parenteral neurotoxicity of domoic acid in cynomolgus monkeys (*M. fascicularis*). Toxicol. Pathol 1990c;18:297–303. [PubMed: 2268382]
- Tsuchiya A, Hardy J, Burbacher TM, Faustman EM, Marien K. Fish intake guidelines: incorporating n-3 fatty acid intake and contaminant exposure in the Korean and Japanese communities. Am. J. Clin. Nutr 2008;87:1867–75. [PubMed: 18541579]
- Vale P, Sampayo MA. Domoic acid in Portuguese shellfish and fish. Toxicon 2001;39:893–904. [PubMed: 11137551]
- Wang GJ, Schmued LC, Andrews AM, Scallet AC, Slikker W, Binienda Z. Systemic administration of domoic acid-induced spinal cord lesions in neonatal rats. J. Spinal Cord Med 2000;23:31–9. [PubMed: 10752872]
- Wekell JC, Hurst J, Levebvre KA. The origin of the regulatory limits for PSP and ASP toxins in shellfish. J. Shellfish Res 2004;23:927–30.
- Work TM, Barr B, Beale AM, Fritz L, Quilliam MA, Wright JLC. Epidemiology of domoic acid poisoning in brown pelicans (*Pelecanus occidentalis*) and Brandt's cormorants (*Phalacrocorax penicillatus*) in California. J. Zool. Wildl. Med 1993;24:54–62.
- Wozniak DF, Stewart GR, Miller JP, Olney JW. Age-related sensitivity to kainate neurotoxicity. Exp. Neurol 1991;114:250–3. [PubMed: 1748199]
- Wright JLC, Boyd RK, de Freitas ASW, Falk M, Foxall RA, Jamieson WD, Laycock MV, McCullough AW, McInnes AG, Odense P, Pathak VP, Quilliam MA, Ragan MA, Sim PG, Thibault P, Walter JA, Gilgan M, Richard DJA, Dewar D. Identification of domoic acid, a neuroexcitatory amino acid, in toxic mussels from eastern Prince Edward Island. Can. J. Chem 1989;67:481–90.
- Xi D, Peng YG, Ramsdell JS. Domoic acid is a potent neurotoxin to neonatal rats. Nat. Toxins 1997;5:74–8. [PubMed: 9131593]
- Xu R, Tao Y, Wu C, Yi J, Yang Y, Yang R, Hong D. Domoic acid induced spinal cord lesions in adult mice: evidence for the possible molecular pathways of excitatory amino acids in spinal cord lesions. NeuroToxicology 2008;29:700–7. [PubMed: 18534681]
- Zaczek R, Coyle JT. Excitatory amino acid analogues: neurotoxicity and seizures. Neuropharmacology 1982;21:15–26. [PubMed: 7063105]
- Zoeller RT, Crofton KM. Mode of action: developmental thyroid hormone insufficiency–neurological abnormalities resulting from exposure to propylthiouracil. Crit. Rev. Toxicol 2005;35:771–81. [PubMed: 16417044]

Fig. 1.
Chemical structures of glutamate, kainic acid and domoic acid.

Table 1

Examples of shellfish and finfish that contain DomA

Shellfish *	Finfish *
Crab (<i>Cancer magister</i>)	Anchovy (<i>Engraulis mordax</i>)
Mussel (<i>Mytilus edulis</i>)	Albacore (<i>Thunnus alalunga</i>)
Razor clam (<i>Siliqua patula</i>)	Jack mackerel (<i>Thracurus symmetricus</i>)
Scallops (<i>Pecten maximus</i>)	Pacific halibut (<i>Hippoglossus stenolepis</i>)
Furrow shell (<i>Scrobicularia plana</i>)	Pacific sardine (<i>Sardinops saga</i>)

From: Lefebvre et al. (2002), Jaffery et al. (2004), Lefebvre and Robertson (2010)

* DomA is present most often in shellfish meat, but in finfish viscera.

Table 2

Acute oral toxicity of DomA in different species

Species	NOAEL (mg/kg)	LOAEL (mg/kg)	NTD (mg/kg)	Reference
Humans	0.2 – 0.3	0.9 – 2.0	1.9 – 4.2	Perl et al. 1990; Teitelbaum et al. 1990; Todd, 1993
Non-human primates	0.75	1.0		Truelove et al. 1997
Mice	28		35	Iverson et al. 1989
Rats	60		80	Iverson et al. 1989

NTD = Dose causing neurotoxicity

Table 3

Developmental neurotoxicity of DomA: pre-natal exposure

Species	Dose (mg/kg)	Route	Age	Effect	Reference
CD-1 mice	0.6	i.v.	GD13	EEG alterations; decreased seizure threshold; morphological alterations in hippocampus; biochemical changes	Dakshinamurti et al. 1993
SD rats	0.3, 0.6, 1.2	s.c.	GD13	Alterations in T-maze, Figure-8 maze. Attenuation of gender differences; increased susceptibility to scopolamine	Levin et al. 2005
C57BL/6 mice	1.0	i.p.	GD11.5, GD14.5, GD17.5	Deficits in cognitive and anxiety-related behaviors; morphological changes in cortex, hippocampus	Tanemura et al. 2009
Zebrafish	0.12-16.8	microinjection	In ovo	Reduced hatching, uncontrolled pectoral fin motions, tonic-clonic convulsions	Tiedeken et al. 2005
Zebrafish	0.12-1.26	Microinjection	In ovo	Increased sensitivity to PTZ	Tiedeken et al. 2007
Sea lions	NA	oral	NA		Goldstein et al. 2008; Ramsdell and Zabka, 2008

Table 4

Maternal and fetal DomA levels following exposure during pregnancy

	GD13	GD13	GD20	GD20
	0.6 mg/kg	1.6 mg/kg	0.6 mg/kg	1.6 mg/kg
Maternal plasma	166 ng/ml	716 ng/ml	654.7 ng/ml	1600 ng/ml
Maternal brain	NA	NA	5 (FC), 5.5 (Hy), 4.3 (H) ng/g tissue	18 (FC), 29 (Hy), 24 (H) ng/g tissue
Fetal brain	7.5 ng/g tissue	16.9 ng/g tissue	5.3 ng/g tissue	11.4 ng/g tissue
Amniotic fluid	8.7 ng/ml	21.0 ng/ml	11.1 ng/ml	31.2 ng/ml

Pregnant rats were administered DomA by i.v. injection on GD 13 or GD 20 and sacrificed after one hour. FC = frontal cortex; Hy = hypothalamus; H = hippocampus; NA = not available. Data derived from Maucher and Ramsdell (2007).

Table 5

Developmental neurotoxicity of DomA: post-natal exposure

Species	Dose (mg/kg)	Route	Age	Effect	Reference
CD -1 mice	0.6	i.v.	PND10, PND 20, PND 30	EEG abnormalities	Dakshinamurti et al. 1993
LE rats	0.05-1.5	i.p.	PND2, PND5, PND10	Scratching, convulsions, death; c-fos induction	Xi et al. 1997
SD rats	0.1, 0.17, 0.25, 0.33, 0.42, 0.50	s.c.	PND7	Behavioral changes; EEG alterations; spinal cord degeneration	Wang et al. 2000
SD rats	0.05-1.0	i.p.	PND0, PND5, PND14, PND22	Various behavioral effects (scratching, convulsions)	Doucette et al. 2000
SD rats	0.005, 0.02	s.c.	PND8-14	Delayed eye opening; altered olfactory conditioning and locomotor activity	Doucette et al. 2003
SD rats	0.005, 0.02	s.c.	PND8-14	Novelty-induced seizure-like syndrome; increased BDNF levels; increased hippocampal mossy fiber sprouting; hippocampal cell loss	Doucette et al. 2004
SD rats	0.02	s.c.	PND8-14	Altered conditioned odor preference	Tasker et al. 2005
SD rats	0.025, 0.05, 0.1, 1.0	s.c.	PND1-2	Hypoactivity	Levin et al. 2006
SD rats	0.02	s.c.	PND8-14	Increased hippocampal mossy fibers sprouting; increased trkB receptors	Bernard et al. 2007

Species	Dose (mg/kg)	Route	Age	Effect	Reference
SD rats	0.005, 0.02	s.c	PND8-14	Impaired spatial learning; alterations in elevated plus maze	Doucette et al. 2007
SD rats	0.02	s.c.	PND8-14	Altered locomotor activity; changes in novelty-seeking behavior	Burt et al. 2008a
SD rats	0.02	s.c.	PND8-14	Decrease pre-pulse inhibition; increased startle response	Adams et al. 2008
SD rats	0.02	s.c.	PND8-14	Novelty-induced seizure-like syndrome	Adams et al. 2009
SD rats	0.02	s.c.	PND8-14	Alterations in radial arm maze and Morris water maze performances	Perry et al. 2009
SD rats	0.02	s.c	PND8-14	Reduced GABAergic neurons in hippocampus	Gill et al. 2010

Table 6

Age-related susceptibility to DomA toxicity (parenteral routes)

Age	LOAEL (mg/kg)	Reference
Adult	0.5 – 2.0	Peng and Ramsdell, 1996; Sobotka et al. 1996; Peng et al. 1997; Kuhlmann and Gilarte, 1997; Tryphonas et al. 1990a, ^b ; Scallet et al. 2005
Prenatal	0.6	Dakshinamurti et al. 1993; Levin et al. 2005
Postnatal (single exposure)	0.05 - 0.1	Xi et al. 1997; Wang et al. 2000; Doucette et al. 2000; Levin et al. 2006
Postnatal (repeated exposures)	0.005 – 0.02	Doucette et al 2004; Doucette et al.2003

Table 7

Summary of proposed oral ARfDs or TDIs for DomA

ARfD or TDI	PoD	UFs	Reference
0.034 mg/kg	LOAEL in non-human primates (0.5 mg/kg, i.v.; Scallet et al. 1993)	10 (LOAEL to NOAEL) 3 (animals to humans) 10 (intraspecies) 5% absorption of oral dose	Slikker et al. 1998
0.018 mg/kg	BMD in non-human primates (0.26 mg/kg, i.v.; Scallet et al. 1993)	10 (LOAEL to NOAEL) 3 (animals to humans) 10 (intraspecies) 5% absorption of oral dose	Slikker et al. 1998
0.075 mg/kg	NOAEL in non-human primates (0.75 mg/kg, oral; Iverson and Truelove, 1994)	10 (intraspecies)	Marien, 2006
0.10 mg/kg	LOAEL in humans (1.0 mg/kg, oral; Perl et al. 1990; Todd, 1993)	10 (intraspecies)	FAO, 2004; Toyofuku, 2006
0.03 mg/kg	LOAEL in humans (0.9 mg/kg oral; Perl et al. 1990; Todd, 1993)	3 (LOAEL to NOAEL) 10 (intraspecies)	EFSA, 2009
0.04 mg/kg	LOAEL in non-human primates (5 mg/kg, oral; Tryphonas et al. 1990b)	3 (LOAEL to NOAEL) 4 (animal to human) 10 (intraspecies)	EFSA, 2009

ARfD = Acute Reference Dose; TDI = Tolerable Daily Intake; PoD = Point of Departure; UF = Uncertainty Factor; NOAEL = No Observed Adverse Effect Level; LOAEL = Lowest Observed Adverse Effect Level.

Shellfish consumption and potential exposure to DomA in Washington State's Korean and Japanese women of child -bearing age (18-45 yr of age)

Table 8

Population (n)	Mean consumption (g/person)	At 20 ug/g (ug/person)	DomA (mg/kg bw)	95 th percentile consumption (g/person)	At 20 ug/g (ug/person)	DomA (mg/kg bw)
Japanese (106)	14	280	0.0047	59	1180	0.0197
Korean (108)	23	460	0.0077	84	1680	0.0280

Shellfish consumption data are from Tsuchiya et al. (2008)

BW = body weight (60 kg)

Table 9

Shellfish consumption and potential exposure to DomA in the European Union

Country (n)	Mean consumption (g/person)	At 20 ug/g (ug/person)	DomA (mg/kg bw)	95 th percentile consumption (g/person)	At 20 ug/g (ug/person)	DomA (mg/kg bw)
France (218-962)	10-32	200-640	0.0033-0.0107	94	1880	0.031
Germany (150)	107	2140	0.0357	400	8000	0.133
Italy (212)	47	940	0.0157	NA	----	----
Netherlands (47)	136	2720	0.045	465	9300	0.155
United Kingdom (212)	114	2280	0.038	NA	----	----

Shellfish consumption data are from EFSA (2009)

BW = body weight (60 kg)

NA: data not available