Exposure to domoic acid through shellfish consumption in Belgium

M. Andjelkovic  a,⁎, S. Vandevijvere  a,⁎⁎, J. Van Klaveren  b, H. Van Oyen  a, J. Van Loco  a

a Scientific Institute of Public Health, Juliette Wytsmanstraat 14, B-1050 Brussels, Belgium
b Wageningen University, Wageningen, The Netherlands

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A B S T R A C T

A main known culprit causing amnesic shellfish poisoning in humans is domoic acid (DA). The toxin appearance in sea waters (by counting the toxin producing algae) and consequently in shellfish is closely monitored to prevent acute intoxications with gastrointestinal symptoms and neurological signs. However it is assumed that there might be some chronic problems with repetitive exposures to the toxin in animals. In humans this is greatly unknown and it is mostly assessed by relating reported toxin episodes and representative consumption data. Although in Belgium no alarming outbreaks have been reported in recent years, different concentrations of DA have been found in shellfish samples. In this study the human acute and chronic exposure to DA through shellfish consumption was evaluated by linking the data of DA concentrations in samples collected in the scope of the National Food control program in the period 2004–2009 and consumption data obtained from the National Belgian Food Consumption Survey including 3245 adults. The found level of toxin was highest in scallops while lowest in mussels. The mean usual long-term intake of molluscs such as scallops, mussels and oysters for the whole Belgian population was from 0.10 g/day for scallops to 1.21 g/day for mussels. With average portion size estimated to be 56–108 g/day depending on the shellfish source it was calculated that less than 1% of the population would be at risk of acute intoxication. Using a medium bound approach, 5–6% of the population shows chronic exposure exceeding the tolerable daily intake of 0.075 μg/kg bw per day with scallops being the most probable toxin vector when using lower (68.5%) and medium (45.6%) bound concentrations.

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1. Introduction

Domoic acid (DA) is a marine biotoxin causing amnesic shellfish poisoning (ASP) in humans. Symptoms of ASP include gastrointestinal effects (nausea, vomiting, diarrhoea or abdominal cramps) and/or neurological signs (confusion, loss of memory, or other serious signs such as seizure or coma) occurring within 24 to 48 h after ingestion, respectively. This toxin was first detected by monitoring services in Canada and first identified in 1987 (Bates et al., 2012), in the United States, and in a number of European countries (EFSA, 2009). The National Food Control Program in Belgium detects this toxin in the samples collected each year from either the harvest zone or retail or shell market.

Toxic blooms of DA-producing diatoms are a global and emerging issue that there might be some chronic problems with repetitive exposures to the toxin in animals. In humans this is greatly unknown and it is mostly assessed by relating reported toxin episodes and representative consumption data. Although in Belgium no alarming outbreaks have been reported in recent years, different concentrations of DA have been found in shellfish samples. In this study the human acute and chronic exposure to DA through shellfish consumption was evaluated by linking the data of DA concentrations in samples collected in the scope of the National Food control program in the period 2004–2009 and consumption data obtained from the National Belgian Food Consumption Survey including 3245 adults. The found level of toxin was highest in scallops while lowest in mussels. The mean usual long-term intake of molluscs such as scallops, mussels and oysters for the whole Belgian population was from 0.10 g/day for scallops to 1.21 g/day for mussels. With average portion size estimated to be 56–108 g/day depending on the shellfish source it was calculated that less than 1% of the population would be at risk of acute intoxication. Using a medium bound approach, 5–6% of the population shows chronic exposure exceeding the tolerable daily intake of 0.075 μg/kg bw per day with scallops being the most probable toxin vector when using lower (68.5%) and medium (45.6%) bound concentrations.

Abbreviations: NBPCS, National Belgian Food Consumption Survey; DA, domoic acid; ASP, amnesic shellfish poisoning; SPE, solid phase extraction; HPLC, high performance liquid chromatography; TDI, tolerable daily intake.

⁎ Corresponding author. Tel.: +32 2 642 5592; fax: +32 2 642 5736.
⁎⁎ Corresponding author. Tel.: +32 2 642 5029; fax: +32 2 642 5410. E-mail addresses: mirjana.andjelkovic@wiv-isp.be (M. Andjelkovic), stefanie.vandevijvere@wiv-isp.be (S. Vandevijvere).

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From non-bivalve species, crabs may accumulate DA. However their ability to accumulate and eliminate the toxin is different from bivalves. Whereas in the latter DA is excreted, in crabs it is rapidly and extensively absorbed from the digestive system (Schultz et al., 2008). On the other hand there is no evidence that DA is toxic to fish although they can accumulate (e.g. salmon) it up to levels of 1800 μg/g bodyweight. The fish is depurated within few days but may be a vector of toxin to higher fish eating animals during this period.

Enforcement in the EU of the regulatory limit for DA of 20 μg/kg (Regulation EC, 2074/2005) implies that samples with DA levels below this value would be released on the market and get into the food chain (Bates et al., 2012; EC, 2074/2005). Consumption of these products may not result in an acute intoxication with DA but it is not yet clear what would be the effect of their long-term consumption. Furthermore, some population groups like children and infants are at higher risk due to their lower body weight. In addition in rats transition of the toxin via milk to neonates was proven to be also a route (Maucher and Ramsdell, 2005).

Risks to human health via low level exposure are not yet fully understood, as there are only studies on animals or model systems. The toxicological studies with animals show clinical signs and brain lesions mostly consistent with those in naturally exposed humans and sea lions. Those clinical signs included seizures, periods of marked lethargy and inappetence, vomiting, muscular twitching, central blindness, blepharospasm (often unilateral) and abnormal behaviour (Goldstein et al., 2008). Based on the data for sea lions the authors suggested that the conversion from acute to chronic exposure is possible. In a model system with rats, seizures with increasing magnitude over a period of 6 months were induced in animals by application of low repetitively DA doses (Muha and Ramsdell, 2011).

Low levels of DA (0.20–0.75 ppm) show no toxic symptoms in humans and non-human primates, but clinical effects are apparent in them and in humans, at a concentration of 1.0 ppm. The tolerable daily intake (TDI) of DA for humans is calculated as 0.075 ppm, whereas for razor clams and crabs, the TDIs are 19.4 and 31.5 ppm respectively (Costa et al., 2010; Kumar et al., 2009).

The marine mammals (sea lions, sea otters and dolphins) were the marine species most often affected by DA in recent years. Moreover, the rate of intoxication events due to DA appears to be increasing (Lefebvre and Robertson, 2010). The toxin is transferred by dietary consumption among the species. Humans consume many of these marine species. Although no alarming outbreaks have been reported in recent years, DA has been found in different concentrations in shellfish samples in Belgium. In this study the human acute and chronic exposure to DA through shellfish consumption was evaluated by linking the data of DA concentrations collected in the scope of the Federal Agency for the Safety of the Food Chain control program in the period 2004–2009 and consumption data obtained from the National Belgian Food Consumption Survey (NBFCS) in adults (2004).

2. Materials and methods

2.1. Shellfish samples

During the period 2004–2009 the Federal Agency for the Safety of the Food Chain collected 224 mussel samples, 217 oyster samples, 319 scallop samples and 45 shellfish samples of unspecified origin, indicated hereafter as molluscs. The samples originated from the National control program and were analysed by the Scientific Institute of Public Health for the presence of domoic acid. The samples were delivered fresh and analysed within a maximum 4 days upon arrival. The cleaning and initial preparation were done according to the procedure described by the European Reference Laboratory for marine toxins (EURL-MB, 2008).

2.2. Reagents and solutions

Solutions used for the HPLC analysis were methanol and acetonitrile of HPLC grade supplied by Merck and Millipore-Q cleaned water. Certified DA standard was purchased from the National Research Council of Canada (NRC) for periodic calibration curve.

The DA standard (SIGMA-Aldrich, Bornem, BE) was used in the routine analyses for preparing the standard solution used for spiking the blank sample (one part) and preparing the control sample.

2.3. Toxin extraction

Extractions were carried out according to the method of Quilliam (1995) with some modifications. After homogenizing 4 g of a sample the extraction was performed with aqueous 50% methanol (4:1) mixing in a vortex for 3 min. After 10 min of centrifugation at 5000 rpm, the supernatant was purified through SPE-SAX (SupelClean, 3 ml, Supelco, Bornem — Belgium) columns filtered into a screw-cap autosampler vial with a filter (0.45 mm, Milipore milllex — HV). In order to control the recovery, the blank sample and the control samples were analysed in each injection series. The latter was spiked with a standard solution to a final concentration of 25 μg/g.

2.4. Quantification of domoic acid

Liquid chromatography was performed on a Varian HPLC-UV system (Model 310) equipped with a binary pump (Varian, model 230) and an autosampler (Varian, model 410). Data collection was performed by the Varian Star Chromatographic Workstation. The column used was a C18 Bondapak HPLC column (Waters 300×3.9 mm, 10 μm) with isocratic gradient of the mobile phase containing 87% aqueous solution of 8.5% phosphoric acid adjusted to pH 2.5 and 13% of 100% acetonitril. This solution was prepared freshly before each analysis. The injection volume was 20 μl and analysis time was set at 20 min. Detection wavelength was set at 242 nm.

Calibration was performed by a full set of calibration standards of DA (1–25 mg/ml). Calibration curves obtained with a double point calibration were always linear, with a correlation coefficient of 0.99. Under these conditions the detection limit was 0.8 μg/g tissue.

2.5. Food consumption data

Consumption data from the National Food Consumption survey 2004 were used to perform the exposure assessments. Aims, design and methods of this survey are described elsewhere (De Vriese et al., 2005; Vandevijvere et al., 2009). The target population comprised all Belgian inhabitants of 15 years or older.

The sample included 3245 participants randomly selected from the National Register, using a multi-stage stratified procedure. Information on dietary intake was collected by two non-consecutive 24-hour recalls in combination with a food frequency questionnaire. The 24-hour recall was carried out using EPIC-SOFT software (Slimani et al., 2002). This program allows obtaining very detailed information about the foods consumed and the recipes used in a standardized way.

3083 participants (1357 women and 1546 men) completed two 24-hour recalls. Participants were categorized into four age groups: 15–18 years (n = 760), 19–59 years (n = 830), 60–74 years (n = 789) and 75 years or older (n = 704).

2.6. Statistical analysis

2.6.1. Acute exposure to domoic acid

The acute dietary exposure to domoic acid was calculated via a probabilistic approach using the “Monte Carlo risk assessment program (MCRA),” Release 6.2, available for registered users at the RIVM website (de Boer and Van der Voet, 2007). For the estimation of daily acute
exposure, daily consumption patterns selected from the consumption database, were multiplied with randomly selected domoic acid levels from the concentration database for each food group (mussels, scallops, oysters and shellfish of undetermined origin) and expressed per kg bodyweight. Because a person eating shellfish will not eat the same portion size containing the same level of toxin each time, the probabilistic calculation includes all combinations of the different occurrence and consumption data. To determine the exposures at each percentile, 100,000 iterations were performed for the general population.

2.6.2. Chronic exposure to domoic acid

Similarly like above the long-term dietary exposure to domoic acid was calculated via a probabilistic approach using MCRA. All daily consumption patterns were multiplied with the mean domoic acid concentration value at the food group level (mussels, scallops, oysters and shellfish of undetermined origin), and summed over foods consumed per day and individual. The estimated exposures were adjusted for the individual’s body weight. A distribution of daily exposures, calculated as described above using mean concentrations of domoic acid per food or food group, includes both the variation between individuals and between the days of one individual. However, to assess the long-term intake within a population only the former type of variation is of interest, since in the long run the variation between different days of one individual will level out.

Therefore, to calculate a long-term dietary exposure distribution, the within-person (between days) variation should first be removed from the distribution of daily exposures using statistical models. The relatively new beta-binomial-normal (BBN) model was used (Slob, 2006).

To remove the within-person variation from the daily exposures, the BBN model transforms the daily exposure distribution into a normal distribution using a logarithmic function. After removal of the within-person variation, the normal distribution is back-transformed and is then considered a long-term dietary exposure distribution. Transformation of the daily exposure distribution into a normal distribution is an important prerequisite to be able to use statistical models that assess the long-term exposure. To determine the exposures at each percentile, 1,000,000 iterations were performed for the general population.

For both chronic and acute exposure lower, medium and upper bound scenarios were calculated. In the lower bound scenario all non detects were set at half of the limit of quantification, LOQ=(mg/kg) was highest in oysters. Both mean concentration and mean positive concentration were lowest in mussels. Among all samples investigated, the percentage of positive samples was highest for scallops (22%).

3. Results

3.1. Domoic acid concentration in shellfish samples

The domoic acid concentrations found in the different samples of molluscs, mussels, oysters, and scallops can be found in Table 1. Mean concentration was highest in scallops whereas mean positive concentration (representing the concentration above the legislative norm of 20 mg/kg) was highest in oysters. Both mean concentration and mean positive concentration were lowest in mussels. Among all samples investigated, the percentage of positive samples was highest for scallops (22%).

3.2. Consumption of shellfish

The mean usual long-term intake of scallops, molluscs, mussels and oysters for the whole Belgian population was 1.21 g/day for mussels, 0.14 g/day for oysters and 0.10 g/day for scallops, and 0.04 g/day for molluscs. The mean portion sizes for consumption days only were 108 g/day for mussels, 103 g/day for oysters and 56 g/day for scallops (Table 2).

3.3. Acute exposure to domoic acid

The median acute intake of domoic acid (consumption days only, medium bound approach) was 0.53 μg/kg bw/day, while at the 99th percentile the intake was 17.16 μg/kg bw/day. The acute reference dose of 30 μg/kg bw/day was exceeded between the 99th percentile and 99.9th percentile. This means that less than 1% of the population would be at risk (Table 3). Scallops were the biggest contributor to acute intake (43.4%), followed by mussels (36%), molluscs (14.9%) and oysters (5.7%). At the upper 5% of the distribution, the contributions were 62.9% for scallops, 21.9% for molluscs, 11.7% for mussels and 3.5% for oysters (Tables 4a, 4b).

3.4. Chronic exposure to domoic acid

The usual long-term intakes of domoic acid were 0.02, 0.05, and 0.16 μg/kg bw per day at the 90th, 95th and 99th percentile, respectively, using the lower bound concentrations. The usual intakes of domoic acid were 0.05, 0.08, and 0.17 μg/kg bw per day at the 90th, 95th and 99th percentile, respectively, using the medium bound concentrations. When using upper bound concentrations the usual intakes were 0.04, 0.05, and 0.16 μg/kg bw per day at the 90th, 95th and 99th percentile, respectively, using the upper bound concentrations.

Table 1

<table>
<thead>
<tr>
<th>Food</th>
<th>Mean concentration (mg/kg)</th>
<th>Mean positive concentration (mg/kg)</th>
<th>Range (mg/kg)</th>
<th>Positive samples</th>
<th>Total samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molluscs n.s.</td>
<td>6.5</td>
<td>48.75</td>
<td>0.80–187.70</td>
<td>6</td>
<td>45</td>
</tr>
<tr>
<td>Mussels</td>
<td>0.06</td>
<td>0.82</td>
<td>0.53–1.67</td>
<td>16</td>
<td>224</td>
</tr>
<tr>
<td>Oysters</td>
<td>0.35</td>
<td>77</td>
<td>77</td>
<td>1</td>
<td>217</td>
</tr>
<tr>
<td>Scallops</td>
<td>7.14</td>
<td>32.54</td>
<td>0.80–203.40</td>
<td>70</td>
<td>319</td>
</tr>
</tbody>
</table>

n.s., non specified.

Table 2

Mean consumption of shellfish in Belgium (National Food Consumption survey, 2004).

<table>
<thead>
<tr>
<th>Food</th>
<th>Mean consumption (g)</th>
<th>Mean, consumption days only (g)</th>
<th>Number of consumption days</th>
<th>Total number of days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All days</td>
<td>p95</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Molluscs</td>
<td>0.04</td>
<td>0</td>
<td>250</td>
<td>6488</td>
</tr>
<tr>
<td>Mussels</td>
<td>1.21</td>
<td>0</td>
<td>107.93</td>
<td>6488</td>
</tr>
<tr>
<td>Oysters</td>
<td>0.14</td>
<td>0</td>
<td>103.24</td>
<td>6488</td>
</tr>
<tr>
<td>Scallops</td>
<td>0.10</td>
<td>0</td>
<td>56.45</td>
<td>6488</td>
</tr>
</tbody>
</table>

n.s., non specified.

Table 3

Percentiles of intake of domoic acid (acute exposure assessment), medium bound approach, consumption days only, including uncertainty of percentile distribution.

<table>
<thead>
<tr>
<th>Percentile</th>
<th>Domoic acid intake (μg/kg bw/day)</th>
<th>Uncertainty of percentiles of domoic acid intake (μg/kg bw/day) (confidence limits)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>0.53</td>
<td>0.41, 0.48, 0.57, 0.62</td>
</tr>
<tr>
<td>90</td>
<td>1.61</td>
<td>1.36, 1.48, 1.74, 2.03</td>
</tr>
<tr>
<td>95</td>
<td>2.54</td>
<td>1.86, 2.21, 3.05, 3.47</td>
</tr>
<tr>
<td>99</td>
<td>17.16</td>
<td>6.18, 13.82, 24.79, 41.91</td>
</tr>
<tr>
<td>99.9</td>
<td>177.33</td>
<td>77.03, 133.13, 223.81, 545.64</td>
</tr>
<tr>
<td>99.99</td>
<td>545.64</td>
<td>199.77, 357.12, 545.64, 545.64</td>
</tr>
<tr>
<td>Mean</td>
<td>1.61</td>
<td>1.09, 1.37, 1.88, 2.34</td>
</tr>
<tr>
<td>Maximum</td>
<td>545.64</td>
<td>235.25, 395.5, 545.64, 545.64</td>
</tr>
</tbody>
</table>

bw, body weight.

Number of simulations (consumers): 100,000 out of 3244.

Positive intakes: 100,000 out of 100,000 consumptions.
of domoic acid were 0.07, 0.10, and 0.18 μg/kg bw per day at the 90th, 95th and 99th percentile. When taking only consumption days into account, usual intakes of domoic acid were 5.24, 7.24, and 12.76 μg/kg bw per day at the 90th, 95th and 99th percentile using the upper bound concentrations (data not shown). The tolerable daily intake (TDI) of 0.075 μg/kg bw per day was exceeded between the 94th and the 95th percentile for the total population using medium bound concentrations.

Regarding the species contribution to the total usual domoic acid intake, scallops contributed most to the total usual domoic acid intake when using lower (68.5%) and medium (45.6%) bound concentrations but mussels contributed most to domoic acid intake when upper bound concentrations were used (47.6%). However, oysters contributed the least, independent of the approach used (4.9%, 6.5%, and 7.3%, respectively). When using the lower bound concentrations the second contributor species were samples marked as molluscs (19.4%) followed by mussels (7.2%) whereas mussels were the second contributor species to the intake when using medium bound concentrations (34.7%), followed by molluscs (13.1%). When using upper bound concentrations mussels contributed most to domoic acid intake (47.6%), followed by scallops (34.9%), molluscs (10.2%) and oysters (7.3%) (Fig. 1).

### Table 4b
Summary characteristics of the upper acute intake distribution of domoic acid using the medium bound approach.

<table>
<thead>
<tr>
<th>Food</th>
<th>Relative contribution to domoic acid intake (%)</th>
<th>Mean (μg/kg bw/day)</th>
<th>P97.5 (μg/kg bw/day)</th>
<th>Uncertainty of percentiles of domoic acid intake (μg/kg bw/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(confidence limits)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.50% 25% 75% 97.50%</td>
</tr>
<tr>
<td>Molluscs n.s.</td>
<td>14.9</td>
<td>0.24</td>
<td>0</td>
<td>0 0 20.5 37.3</td>
</tr>
<tr>
<td>Mussels</td>
<td>36</td>
<td>0.58</td>
<td>2.78</td>
<td>20.9 30.6 42.8 53.6</td>
</tr>
<tr>
<td>Oysters</td>
<td>5.7</td>
<td>0.09</td>
<td>1.02</td>
<td>1.3 3.6 8.7 14.7</td>
</tr>
<tr>
<td>Scallops</td>
<td>43.4</td>
<td>0.7</td>
<td>1.19</td>
<td>22.1 36.6 51.7 65.4</td>
</tr>
</tbody>
</table>

### Table 4c
Summary characteristics of the upper acute intake distribution of domoic acid using the medium bound approach. Characteristics per food of the upper 5.0% of the distribution corresponding to a total intake higher than 2.5439 μg/kg bw/day.

### 4. Discussion

During the period 2004–2009 the Belgian Food Control Program screened shellfish samples for domoic acid. The percentage of the positive samples was highest for scallops and molluscs. A similar study in Croatia revealed that of those species in the period from 2006 to 08 domoic acid was the most prevalent in blue mussel (Mytilus galloprovincialis), followed by the European flat oyster (Ostrea edulis), the Mediterranean scallop (Pecten jacobaeus) and proteus scallop (Flexopecten proteus) (Ujević et al., 2010).

After the first reported and severe poisoning with amnesic shellfish toxins in 1987 in Canada there were other episodes with less severe outcomes. Reports presented levels of domoic acid in razor clams and scallops even up to 230 and 2900 ppm, respectively (Ramsdell, 2007 and references therein). According to these occasional cases EFSA stated that less than 1% of the population would be at risk for exceeding the acute reference dose. Our study confirms this for Belgian population. In order to protect high consumers against acute effects of marine biotoxins, the CONTAM Panel of EFSA identified 400 g of shellfish meat as an appropriate estimate of a large portion size consumed in Europe to be used in the risk assessments. According to the CONTAM panel for a 60 kg adult, the chance of exceeding a dietary exposure of 1.8 mg sum DA, corresponding to the ARfD of 30 μg DA/kg bw, is about 1%, when consuming shellfish currently on the European market. The chance of exceeding the deterministic dietary exposure estimate of 8 mg sum DA, corresponding to consumption of a portion of 400 g containing DA at the level of the current EU limit value of 20 mg sum DA/kg, is less than 0.1%. EFSA refers to DA as a sum of domoic acid and epi-domoic acid which may also be measured in samples.

With regard to the long-term intake of domoic acid in Belgium the tolerable daily intake may be exceeded between the 94th and the 95th percentile for total population using medium bound concentrations. This estimate corresponds to the high consumers of shellfish products. However the clinical consequences of the long-term exposure to domoic acid in humans are not known. There are animal models evaluating certain symptoms like DA-induced epilepsy (Ramsdell and Muha, 2011) but these are also pointing out to age-related vulnerabilities. Some groups, like pregnant and lactating women, newborns, individuals with impaired renal clearance may be more susceptible to the toxins requiring special attention when developmental studies and environmental regulatory policies are considered (Grant, 2010 24862/id).

Another important public health implication is the DA heat stability. Although cooking does not destroy the toxin, normal home cooking processes, such as boiling and steaming, could reduce the amount of DA in shellfish meat due to partial leaching of the toxin into the cooking fluids (Vidal et al., 2009; McCarron and Hess, 2006). The effect of cooking on the domoic acid concentration and the toxin concentration in different species may be various. Toxin concentration may decrease in the cockle but may increase in soft tissue of Manila clam after cooking (Vidal et al., 2009). These changes in toxin quantity may have an
important impact when assessing the acute and chronic DA intakes provided that sample pre-treatment may be different e.g. cooking of muscles in order to open them easier before analysing. This is not always accounted for in the risk assessment studies and safeguarding the consumer health.

Data collected for regulatory control of food are often products of targeted sampling in the expected problematic and designating harvesting areas, while ideal sampling for exposure assessments should be random (Verger and Fabiansson, 2008). This is not always possible and may be costly. Only an evidence based approach to risk assessment like only conducting research after outbreaks may be disadvantageous in comparison to a knowledge based approach where we react according to processing already existent data and predict emerging problems. On the other hand, food frequency surveys give insight into the frequency with which the listed food items are consumed during a specific time period (e.g. daily, weekly) but usually they do not give the number of eating occasions (Vandevijvere et al., 2009; Illner et al., 2010). A 24-h recall or one or more days of dietary record are needed to provide more detailed information such as the source of food, time of day and the place where foods are consumed. Dietary information can be collected at three different levels of aggregation: national food supply data, data at the household level and data at the individual level (Verger and Fabiansson, 2008).

5. Conclusions

The present study showed that domoic acid occurs in shellfish in the Belgian food chain. From routine testing it was found that less than 1% of the Belgian population would be at risk of acute DA intoxication. These would mostly be the high consumers of scallops. Although the effects of long term exposure to domoic acid are not yet clear, there is also a possibility of delayed or latent DA neurotoxicity. The present study showed that in Belgium around 5% of the population may be exposed to unsafe DA doses over longer periods. Given this and the severity of DA toxicity more tight control programs and dose–response studies are required.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.envint.2012.08.007.

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