This is a provisional English translation of an excerpt from the original full report.

Risk Assessment Report

Okadaic Acid group toxins in bivalve molluscs

(Natural toxins and mycotoxins)

Food Safety Commission of Japan (FSCJ)
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ABSTRACT

The Food Safety Commission of Japan (FSCJ) conducted a risk assessment of okadaic acid (OA)-group toxins in contaminated bivalve molluscs based on documents of FAO/IOC/WHO, EFSA and others. The consumption of contaminated bivalve molluscs can pose Diarrhoeic Shellfish Poisoning (DSP) in humans.
The data used in the assessment were from epidemiological studies of DSP in humans, as well as acute toxicity, subacute toxicity and genotoxicity studies in experimental animals and so on.

The OA-group toxins has been detected in bivalve molluscs ingesting toxin-producing planktons. The DSP associated with consumption of the contaminated bivalve molluscs has been reported since the 1970's. The OA-group toxins includes OA (CAS No.78111-17-8) and its derivatives dinophysis toxins (DTXs), which are DTX1 (CAS No.81720-10-7), DTX2 (CAS No.139933-46-3) and DTX3. All of the DTXs were considered in this assessment.

Cases of DSP have been reported in many countries including Japan, the United States and European countries. However, there are only a few epidemiological investigations which succeeded in estimating the amount of bivalve molluscs and the toxins consumed by affected people, and in identifying types of the toxins. One of these investigations is on DSP outbreaks occurred in France in 2009. The amount of mussel consumed and personal information (e.g., weight) of people involved in the outbreaks were recorded. Based on the data, the lowest-adverse-effect-level (LOAEL) was estimated 0.8μg OA equivalents (eq)/kg.

Gastrointestinal disorders such as diarrhea and adverse effects in the liver were observed in acute toxicity studies of OA-group toxins in rodents. The toxicity depends on administration route, being lower by oral administration than intraperitoneal administration. No long-term toxicity/carcinogenicity studies have been reported, but OA and DTX1 were identified as tumor promoters in a two-stage carcinogenicity study in rodents. Although some genotoxicity tests (e.g. chromosomal aberration test) showed positive results, bacterial reverse mutation assay (Ames), hypoxanthine-guanine phosphoribosyltransferase (HPRT)-mutation assay and an *in vitro* unscheduled DNA synthesis assay showed negative results. Based on these

findings, FSCJ concluded that OA is not a genotoxic carcinogen.

FSCJ did not establish a TDI, but considered it appropriate to establish an acute reference dose (ARfD) based on the available human data. The ARfD was established considering the following findings:
There are no available data on chronic toxicity of the OA-group toxins; OA-group toxins are known to cause acute toxic effects in humans; there are seasonal variations in the occurrence and population density of toxic plankton, which may be ingested by bivalve molluscs, and in the toxin accumulation in bivalve molluscs; and it is unlikely that people consume bivalve molluscs on a daily basis which may accumulate toxins.

FSCJ adopted the LOAEL of 0.8 μg OA eq/kg b.w. taking into account the DSP outbreaks in France in 2009. An uncertainty factor of 3 was applied to the LOAEL, since the LOAEL was based on data from various DSP cases in both sexes of people at a wide range of age in different countries. It was also taken into account that symptoms in affected people are gastrointestinal disorders represented by diarrhea which resolve within several days.

FSCJ thus specified an ARfD of 0.3 μg OA eq/kg b.w. for the OA-group toxins.

Executive Summary

The Food Safety Commission of Japan (FSCJ) conducted a risk assessment on okadaic acid (OA)-group toxins upon request from the Ministry of Health, Labour and Welfare of Japan (MHLW) toward establishment of regulatory levels of Diarrhoeic Shellfish Poisoning DSP toxins in bivalve molluscs. The assessment was based on reference materials and documents submitted by MHLW, articles published in domestic and international journals, and documents of FAO/IOC/WHO and EFSA.

The DSP is caused by consumption of bivalve molluscs such as scallops and mussles that accumulated OA-group toxins through ingestion of OA-group toxins producing planktons. Symptoms of DSP including diarrhea appear 0.5–4 hours after consumption of OA-group toxins-contaminated foods, and these are temporary and usually resolve within 72 hours.

In Japan, risk management of DSP-toxins is implemented based on the regulatory level of DSP-toxins, voluntary surveillance by monitoring of bivalve molluscs in harvesting area, and the notified method of mouse bioassay (MBA). MBA is performed by intraperitoneal injection of a lipid soluble fraction of shellfish meat to mice by using lethality of the mice an index of toxicity. This method can detect OA-group toxins, pectenotoxin (PTX) -group toxins and yessoyoxin (YTX) -group toxins as a total activity of mouse lethality, but cannot separately detect each of these toxins. Although PTX-group toxins and YTX -group toxins exert lethal toxicity in MBA, they do not induce diarrhea in mice when given orally. No human health effects have been reported related to these toxins. Therefore, this assessment deals with the OA-group toxins, which have been observed to cause DSP in humans.

Thousands of cases of DSP including children have been reported in Japan, Europe, North America and others. However, epidemiological data including the amount of bivalve molluscs and toxins consumed by affected individuals as well as information on types of toxins have been reported for a limited number of DSP cases

In 2009, 11 outbreaks of DSP occurred in association with consumption of mussels in France involving totally 45 cases from 11 to 65 years of age. DTX3 was suspected to be the cause. Epidemiological data such as the amount of mussel consumed by affected people and their body weights were reported. The smallest serving size of the mussels was 150 g, which contained 36 g of edible parts. The toxin intake from this size was estimated to be 45 μg OA eq. The body weight of the case of the lowest intake was 58 kg. The LOAEL was thus estimated to be 0.8 μg OA eq/kg b.w.

In 1976 and 1977, outbreaks of DSP occurred in Japan involving 164 affected people from 10 to 68 years of age who had consumed mussels and scallops. DTX1 was suspected to be the cause. Epidemiological data of eight people of both sexes involved in the outbreaks indicated that the lowest intake in the affected people was as little as 12 mouse unit (MU) of the toxin per person noted in two affected persons. Upon the assumption that one MU equivalents to 4.0 μg, the lowest intake for symptoms was estimated to be 48 μg OA eq per person in the outbreaks in Japan.

Gastrointestinal disorders such as diarrhea and adverse effects in the liver were observed in acute toxicity study of OA-group toxins in rodents. The toxicity depended on administration routes, being lower by oral administration than intraperitoneal administration. No long-term toxicity/carcinogenicity studies have been reported, but OA and DTX1 were identified as tumor promoters in a two-stage carcinogenesis study in rodents. Although some genotoxicity tests (e.g. chromosomal aberration test) showed positive results, bacterial reverse mutation assay (Ames), hypoxanthine-guanine phosphoribosyltransferase (HPRT)-mutation assay and an *in vitro* unscheduled DNA synthesis assay showed negative results. Therefore, FSCJ concluded that OA is not a genotoxic carcinogen.

FSCJ did not establish a TDI, but considered it appropriate to establish an acute reference dose (ARfD) based on the available human data. The ARfD was established considering the following findings: There are no available data on chronic toxicity of the OA-group toxins; OA-group toxins are known to cause acute toxic effects in humans; there are seasonal variations in the occurrence and population density of toxic plankton, which may be ingested by bivalve molluscs, and in the level of toxins in bivalve molluscs; and it is unlikely that people consume bivalve molluscs on a daily basis which may accumulate toxins.

In documented DSP cases, uncertainties are associated with the estimation of dietary exposure to OA in humans, but FSCJ adopted the LOAEL of 0.8 μg OA eq/kg b.w. taking into account the DSP outbreaks which occurred in France in 2009. This LOAEL was near to the LOAEL derived from epidemiological data in Japan. FSCJ decided to apply an uncertainty factor of 3 to the value, 0.8 μg OA eq/kg b.w., to specify ARfD, since the LOAEL was based on data from various DSP cases in both sexes of people at a wide range

of age in different countries. It was also taken into account that symptoms in affected people are gastrointestinal disorders represented by diarrhea which resolve within several days.

FSCJ thus specified an ARfD of 0.3 μg OA eq/kg b.w. for OA-group toxins.

Exposure estimates for OA-group toxins through consumption of bivalve molluscus in people in Japan could not be performed due to limited data, but the concentration of OA- group toxins in bivalve molluscus that will not result in intake exceeding the ARfD were calculated as described in the appendix below.

<Appendix >

MHLW estimated the average daily consumption of each speices of bivalve molluscus on the basis of investigations in Japan from FY 2005 to 2007. Though data were limited, the maximum serving sizes of average consumption and 95.5 percentile consumption of mussels were 72.2 g and 148.0 g, respectively. The maximum serving size of scallops was 279.0 g. The maximum serving size of bivalve molluscus was noted in 360.0 g of cultivated oysters.

Based on these data, the concentration of OA-group toxins in bivalve molluscs (edible shellfish portion) that will not exceed the ARfD (0.3 μg OA eq/kg b.w.) were estimated fbased on 55.1 kg b.w. for adults, the average body weight in Japan, to be 229 μg/kg, 111μg/kg, 56 μg/kg and 25 μg/kg under the assumption of serving sizes of bivalve molluscus of 72 g, 148 g, 300 g, and 360 g, respectively.

Since the maximum serving size of bivalve molluscus was 360.0 g and that of 99 percentile consumption was 300 g, a large size may be occasionally consumed in a day. If edible parts of bivalve molluscus contain OA-group toxins at a concentration of 0.16 mg/kg and such parts is consumed with a serving size over 103 g, intake of the toxin will exceed the ARfD. However, shellfish toxins are known to accumulate in the midgut gland of edible parts. Therfore, adverse health effects by consumption of intoxicated shellfish would be reduced by removing the midgut gland. The regulation level of the toxins (0.05 MU per g edible parts) is the amount of toxins causing deaths of 2 or 3 among 3 mice within 24 hours with the notified MBA. This level was estimated to be over 0.16 mg OA eq per kg edible part in the assessment by FAO/IOC/WHO (2004).