Melamine in fish feed and implications for safety of aquaculture products

Iddya Karunasagar

Fish Utilisation and Marketing Service
Fisheries and Aquaculture Department, FAO, Rome, Italy
Iddya.Karunasagar@fao.org

A large outbreak of renal failure in cats and dogs in the United States of America (USA) in 2007 associated with pet food containing melamine and cyanuric acid and hospitalisation of over 50 000 children in China in 2008 linked to consumption of melamine-contaminated infant formula had focussed attention on the human heath hazards due to exposure to melamine through food. Melamine is a nitrogen-rich compound that could mimic proteins in some tests; melamine was therefore added to protein supplements used in feeds such as wheat gluten, corn gluten and rice gluten to artificially inflate protein levels. Melamine has also been detected in fish feed in several countries and the human health implications of melamine in fish feed is discussed here.

Chemical nature and sources of melamine

Melamine (2,4,6-triamino-1,3,5-triazine) is a chemical, which on reaction with formaldehyde, forms resins with many industrial uses such as the production of plastics, laminates, glues, adhesives, moulding compounds, coatings, paper, paperboard and flame retardants. It is the major component of pigment yellow 150 that is used as colourant in inks and plastics. Melamine is also used in some fertilisers. It is found as a metabolite of the pesticide cyromezine, that is used in plants[1] and in veterinary practice, as an ectoparasiticide on some animals such as sheep, goat, and rabbits. Trichloromelamine is approved for use as a sanitising agent on food processing equipments and utensils except for milk containers and equipment[2]. Trichloromelamine may decompose to melamine during this use.

Melamine is degraded by some soil associated bacteria in three successive deamination reactions to ammeline (4,6-diamino-2-hydroxy-1,3,5-triazine), ammelide (6-amino-2,4- dihydroxy-1,3,5-triazine) and cyanuric acid (s- triazine-2,4,6-triol) (Figure 1). Cyanuric acid may be found as an impurity in melamine. It is also found in swimming pool water as a dissociation product of dichloroisocyanurates used for water disinfection.

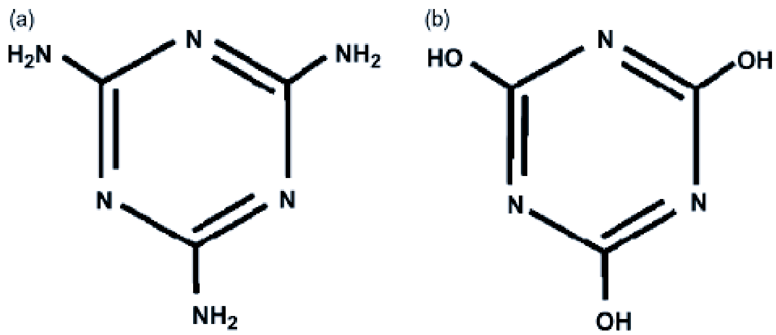

Figure 1. *Structure of (a) melamine and (b) cyanuric acid*

Due to its permitted usages, very low levels of melamine may be found in food. Some countries have legal limits regarding migration of melamine from food contact material to food. In the European Union (EU), the migration limit is 30mg/kg food (Commission Directive EC No. 2002/72). The level of melamine in food resulting from permitted usage on food contact surfaces has been estimated[2] to be less than 15 µg/kg (ppb). Under experimental conditions (hot, acidic conditions, 95°C for 30 min), levels of 0.54, 0.72, 1.42, 2.2 mg/kg were found in coffee, orange juice, fermented milk and lemon juice respectively as a result of migration from cup made of melamine-formaldehyde resin[3].

Melamine has been in industrial production for many years and its environmental distribution has been evaluated by several agencies. In factories involved in its production, 80-90 percent in waste water is eliminated by the waste water treatment plants (WWTPs)[4]. It has been reported that microorganisms in WWTPs can adapt to melamine, when continuously exposed. Water is the preferred environmental compartment for melamine and the environmental distribution has been estimated[4] as follows: air <0.0001 percent, water 99.99 percent, soil 0.006 percent, sediment 0.0001 percent. The Predicted Environmental Concentration (PEC) is 0.003 mg/l in site-specific water (based on estimates of a plant producing 300 t/yr) and 0.0042 mg/l in regional water based on European Union System for Evaluation of Substances (EUSES) model. Data of monitoring melamine in river water in Japan indicates levels ranging from below detection limit of 0.0001 to 0.0076 mg/kg in water, below detection limit of 0.01 to 0.40 mg/kg in sediment and below detection limit of 0.02-0.55 mg/kg in fish (OECD, 1998). The EUSES estimates for fish in local waters (near production facility) is 0.36-10.9 mg/kg. In common carp *Cyprinus carpio*, the bioconcentration factor (BCF) has been estimated[4] to be < 0.38. Melamine is metabolically inert and all studied animals excrete melamine or its analogues as such. Fish are reported to excrete melamine more slowly than rodents[2].

Toxicity of melamine

Toxicity test has been performed in some aquatic animals. The no-observable-effect- concentration (NOEC) in chronic toxicity estimations for the aquatic invertebrate *Daphnia magna* was 500 mg/l based on macroscopic observation and <125 mg/l based on microscopic observation[4]. The $EC_{50,\,48h}$ for this organism in acute/prolonged toxicity estimation was >2000 mg/l. For fish, the LC_{50} was as follows: *Leuciscus idus melanota*, $LC_{50,48h}$ >500 mg/l; *Oryzias latipes*, $LC_{50,48h}$ 1000 mg/l; *Poecilia reticulate* $LC_{50,96h}$ >3000 mg/l; *Poecilia reticulata* $LC_{50,48h}$ >2000 mg/l (OECD, 1998). Data for chronic toxicity are available in two fish *Jordanella floridae* (NOEC 500 mg/l) and *Salmo gairdneri* (NOEC >1000 mg/l)[4].

A 13-week rat study established a no-observed-adverse-effect level (NOAEL) of 63 mg/kg and based on this data and adopting a 100-fold safety factor, FDA[5] designated 0.63mg/kg as the tolerable daily intake (TDI). Considering the increased toxicity of melamine and cyanuric acid, FDA[2] applied an additional 10-fold safety factor and revised the TDI to 0.063mg/kg. This would mean, for a 60 kg person, a 3.78 mg melamine and its analogues/day. Using a worst case exposure scenario, in which 50 percent of diet (estimated 3 kg, typically composed of 1.5 kg liquid and 1.5 kg solid food) is contaminated with melamine, it was estimated that if the level in the contaminated food is 2.5 mg/kg (ppm), the daily intake would be 0.063 mg/kg bw/d. Based on these calculations, FDA[2] concluded that levels of melamine and its analogues below 2.5 ppm in foods other than infant formula is not of public health concern.

The European Food Safety Authority (EFSA)[6] recommended a TDI of 0.5 mg/kg bw/day for the total of melamine and its analogues ammeline, ammelide, cyanuric acid (EFSA, 2008).

A recent Expert Meeting of the World Health Organization (WHO) used dose-response modelling and benchmark dose approach for deriving TDI[7]. The benchmark dose for 10 percent response rate ($BMDL_{10}$) was calculated to be 35 mg/kg body weight per day. Applying a safety factor of 200 to this value, the Expert Meeting determined TDI to be 35/200= 0.175, rounded to 0.2mg/kg body weight per day.

Melamine in aquacultured fish

Since the price of fish meal depends on the protein content, there is a possibility of adulterating this ingredient of fish feed with melamine to artificially inflate protein levels. Recently, there have been reports of detection of melamine (up to 150 ppm) in fish meal and fish feed in different countries. Method for determination of melamine in catfish tissue up to 10 ng/g (ppb) using Triple quadrupole LC-MS-MS has been reported[5]. This method was further improved[8] to achieve a limit of detection (LOD) of 3.2 µg/kg (ppb) and melamine could be detected in edible portion of fish fed with melamine alone or with cyanuric acid at 400 mg/kg. In catfish (*Ictalurus punctatus*), the levels ranged from 81-210 mg/kg; tilapia, (*Oreochromis* sp.), 0.02-177 mg/kg; trout (*Oncorhynchus mykiss*) 34-80 mg/kg; salmon (*Salmo salar*) 58-94 mg/kg. Melamine was not detected in muscle tissue of non-dosed catfish or tilapia, but levels ranging from 0.04-0.12 mg/kg were found in non-dosed trout and salmon. This was attributed to the presence of melamine at 0.5 mg/kg and 6.7 mg/kg commercial trout and salmon feed, respectively. Two salmon that were dosed 380 mg/kg for longer withdrawal study died at 7 and 11 days. Fish dosed with combination of melamine and cyanuric acid had lower cyanuric acid residues in tissue compared to fish dosed with cyanuric acid alone. Melamine-cyanurate crystals were observed in kidney and intestinal tissue but not in edible tissue[8]. Maximum cyanuric acid residue level detected was 5.8 mg/kg in trout, 11.2 mg/kg in catfish, 27.7 mg/kg in tilapia. In the case of salmon, after 1 day withdrawal, 1200 mg/kg was found in tissue, but levels were 1.7 and 0.43 mg/kg after 6 and 10 days withdrawal[9]. Two salmon that received lower dose of 200 mg/kg melamine and cyanuric acid had residue levels of 0.57 and 0.78 mg/kg after 14 days withdrawal and kidneys of these fish had crystals[9].

Shrimp fed for 14 days with feed containing 50 or 100 mg/kg melamine had 217 and 51 µg/kg in muscle, while shrimp fed with non-dosed feed had 41 µg/kg. Shrimp feed had background level of 170 µg/kg. Shrimp given feed with 100 mg/kg were found to feed poorly[8]. Cyanuric acid could not be detected in edible tissues of shrimp fed with melamine and cyanuric acid, though melamine residues could be detected[9].

In a survey[8] of market-ready shrimp, catfish, tilapia, salmon, eel and other types of fish in the USA, 31.4 percent had melamine at concentrations above LOD. 10/105 samples (9.5 percent) had melamine at levels ranging from 51-237 µg/kg.

Methods for analysis of melamine in food and feed were reviewed by the WHO Expert Meeting. Enzyme-linked immunosorbant analysis (ELISA) kits are available and these tests can be performed without sophisticated laboratory. These are useful for screening with detection limits ranging from 0.1 to 25 mg/kg[7] depending upon the matrix being analysed and sample extraction method employed, but positive results should be confirmed using methods like High Performance Liquid Chromatography-ultravoilet/diode array detection (HPLC-UV/DAD) which has a detection limit of 0.05-65 mg/kg.

The levels found in market survey and in non-dosed fish/shrimp in experimental studies were lower than 2.5 ppm concern level suggested by FDA. At these levels, there is no health hazard for fish/shrimp consumers. However, studies with experimental feeding show that if the feed is contaminated with high levels (reports indicate up to 150 ppm), some fish species may have levels that are of concern. Therefore, it would be important to ensure that fish feed is not adulterated with melamine and its analogues.

[1]WHO/FAO. 2006. Pesticide Residues in foods. Report of Joint FAO/WHO Meeting on Pesticide Residues. 400pp.

[2]FDA. 2008. Interim Safety and Risk Assessment of Melamine and Analogues in food for humans. accessed October 3, 2008. (http://www.cfsan.fda.gov/~dms/meamra3.html).

[3]Ishiwata, H., Inoue,T., Yamazaki,T., & Yoshihira,K. 1987. Liquid chromatographic determination of melamine in beverages. J. Assoc. .Off. Anal. Chem. 70: 457-460.

[4]OECD. 1998. Screening Information Data Set for Melamine. CAS No. #108-78-1. Available at http://www.chem.unep.ch/irptc/sids/OECDSIDS/108781.pdf, 17 Sept, 2008.

[5]FDA. 2007. Interim Melamine and Analogues Safety/Risk Assessment, May 25, 2007. (http://www.cfsan.fda.gov/~dms/meamra.html).

[6]European Food Safety Authority (EFSA). 2008. Statement of EFSA on risks for public health due to the presence of melamine in infant milk and other milk products in China. The EFSA Journal 807: 1-10.

[7]WHO. 2009. Toxicological and Health aspects of melamine and cyanuric acid. Report of a WHO Expert Meeting in collaboration with FAO supported by Health Canada. http://www.who.int/foodsafety/fs_management/infosan_events/en/index.html.

[8]Andersen, W.C., Turnispeed, S.B., Karbiwnyk, C.M., Clark, S.B. Madson, M.R., Gieseker, C.M., Miller, R.A., Rummel, N.G., & Reimschuessel, R. 2008. Determination and confirmation of melamine residues in catfish, trout, tilapia, salmon and shrimp by liquid chromatography with tandem mass spectrometry. J. Agric. Food Chem. 56: 4340-4347.

[9]Karbiwnyk, C.M., Andersen, W.C., Turnispeed, S.B., Storey, J.M., Madson, M.R., Miller, K.E., Gieseker, C.M., Miller, R.A., Rummel, N.G., & Reimschuessel, R. 2008. Determination of cyanuric acid residues in catfish, trout, tilapia, salmon and shrimp by liquid chromatography-tandem mass spectrometry. Anal. Chim. Acta doi:10.1016/j.aca.2008.08.037.