

CASE WESTERN RESERVE UNIVERSITY

# MELAMINE CONTAMINATION OF INFANT FORMULA

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## I. BACKGROUND

In 2007, the world witnessed again the extent to which environmental products can be misused and introduced into human body via contamination of food. Across the USA, cases of acute renal failure among cats and dogs were noticed by veterinarians as investigation revealed an epidemic of melamine contamination of pet food imported from China. The pet-food contamination occurred with a mixture of melamine byproduct that resulted in a very high toxicity in the body: cyanuric Acid. Combined, the two compounds formed crystals with a sphere-like shape that obstruct tubular passage of urine in the kidney, causing a pressure build-up that ends up killing the cells within the kidneys. The end result was mainly kidney failure in animals (Melamine Pet Food Recall of 2007, 2009). The scandal went under rigorous investigation until two Chinese companies and other members of USA companies were indicated months later for deliberately adding wheat gluten to increase the protein content of pet food. With such federal crime, one would think that lessons were learned by companies and personnel. By now, the risk associated with the contamination of pet food was the worry that human might be seriously affected through the consumption of poultry, meat or eggs, but the attention was quickly shifted as the world observed how events continued to unfold. Back in China, cases of kidney stone and kidney failure among babies caught the attention of pediatricians and nephrologists, and on September 11 2008, another interaction of environmental compound with the human body was announced (Chan, Griffiths, & Chan, 2008). The media reported a new outbreak of milk product contamination with melamine, causing a large number of children to be hospitalized in a small amount of time. Similar to the rationalization behind contaminating pet-food with melamine and cyanuric acid, melamine was also added to infant formula to increase the food's nitrogen content, thus falsely increasing milk protein value. Infant formula was the primary target, then milk product, and finally other food product such as beverage in general (Hau, Kwan, & Li, 2009).

Melamine (official UPAC name is tripolycyanamide) is an industrial chemical composed mainly of nitrogen (66%), carbon and hydrogen, and the compound is used in laminates, glues, adhesives, and plastics (Chan, Griffiths, & Chan, 2008). The compound was first introduced in the 1830s by a German scientist and became widely used in the 1930s. Melamine was combined with formaldehyde under high temperature, then cooled and dishwasher-safe dinnerware was made. Melamine has recently been used in its powder form to contaminate food to increase its protein content in order to increase the food's nutritional value (Pickert, 2008). Infant formula manufacturers were already facing problems of not meeting the required protein level for milk product, hence their justification behind the melamine contamination. Using the Kjeldahl method, Chinese manufacturers were able to identify the amount of nitrogen in protein, thus assessing the amount of protein in food. The method consists of reacting protein with sulfuric acid to produce carbon dioxide, ammonium sulfate and sulfur dioxide. The wanted product, ammonium sulfate is reacted with sodium hydroxide to form sodium sulfate and ammonium hydroxide, which in turn, is extracted to further react with sulfuric acid to yield water and more ammonium sulfate. The amount of ammonium sulfate is calculated by adding sodium carbonate with a methyl orange pH indicator. Following the Kjeldahl method, melamine can then be added into the food, depending on the amount desired:

an addition of 1g of melamine results in 0.4% increase in protein. The solubility of melamine in water renders it even more misleading when added in liquid milk, which can increase protein content by as much as 30% with 3.1g of melamine (Hau, Kwan, & Li, 2009).

In the preliminary investigation, WHO provided a list of products that had tested positive for melamine and the exportation of such products were powdered infant formula, liquid milk and yogurt, powdered milk product specifically in China. Products reported by other countries included all listed product from China in addition to frozen dairy products, snack foods, frozen processed foods, ammonium bicarbonate, nondairy creamer, protein powder, dried egg powder and liquid eggs, whole eggs, and animal feed (Gossner, et al., 2009).

## II. TOXICITY OF MELAMINE

To understand the toxicology of melamine, preliminary studies were done on animals using high dosage to investigate its metabolic pathway. Studies were conducted on pigs, rodents, and they indicated that each species had different rate of melamine elimination. Melamine toxicity has previously been studied decades ago, however with the pet-food incident, melamine analogous cyanuric acid research was underway (Afoakwa, 2008). Although the results differed between studies, information will serve toward solutions and will lead towards further research options. Absorption, distribution, metabolism and excretion of melamine were first investigated. In rats, maximum concentration in blood plasma occurred in 60 min, after a single dose administration, the half life was found to be about 2.7 h and urinary elimination was 93%. In pigs, a dose of 6.13 mg/Kg body weight was administered and the half-life was 4.04 h (Reimschuessel, Hattan, & Gu, 2009).

Studies of acute toxicity were first performed in rodents, and the median lethal dose (LD<sub>50</sub>) was 3161 mg/kg body weight for rodent males and 3828 mg/Kg body weight for females. Chronic toxicity was investigated during a two-week and thirteen-week study. In the two-week study, rodents were given a diet containing 5,000 to 30,000 mg/Kg (250-1,500 mg/Kg body weight) and none were affected. When the dose was increased to 15,000 mg/Kg and higher, reduced body weight and body weight were observed. An increased in dosage between 20,000 and 30,000 mg/Kg showed the development of hard crystalline solid in the urinary bladder of both male and female rodents. In the thirteen-week study, the administered dosage ranged between 750-18,000 mg/Kg (given 38-900 mg/Kg body weight per day) and toxicity in the body was shown by reduced body weight gain and decreased body weight. Stone formation was found in urinary bladder along with hyperplastic epithelial changes, prominent capillaries and occasional oedema and scattered mast cells in the submucosa (Reimschuessel, Hattan, & Gu, 2009).

A sheep study in 1953 examined the acute renal toxicity of melamine by giving a single oral dose of 100-g and all the animals died on the 11<sup>th</sup> day. When the dose was reduced to 25 to 50g for a period of seven to nine days, the animals still died. The acute toxicity was associated with elevated blood urea nitrogen and creatinine, oliguria then death. Chronic toxicity study was also done in 1953 on dogs, feeding them 30,000ppm melamine for one year.

Changes observed were increased urinary output, melamine crystalluria, and proteinuria with microscopic hematuria. Furthermore, the analysis on carcinogenicity of melamine did not yield conclusive results from animal studies, thus WHO classified melamine as group three carcinogenic, referring to inadequate information from studies (Hau, Kwan, & Li, 2009).

### **III. RISK ASSESSMENT**

Following the outbreak, U.S. Food and Drug Administration advised not to buy milk product from China but assured that any other products following the US regulations would be safe. Investigation included laboratory measures to detect any melamine in milk product using the same method adopted to detect melamine and cyanuric acid in pet food by veterinary laboratories. Liquid chromatography triple quadrupole tandem mass spectrometry is the method used beginning with the extraction of the powdered samples with 2.5% formic acid in a 50mL polypropylene centrifuge tube. Extracts undergo a series of filtration followed by dilution with acetonitrile and analyzed by LC-MS/MS. Melamine is separated from cyanuric acid using a zwitterionic HILIC column, then melamine is detected in positive ion mode and cyanuric acid is detected in a negative ion mode (Turnipseed, Casey, Nochetto, & Heller, 2008).

Like mentioned in the toxicity section, melamine has very low acute toxicity from an observation of mice and rats study – the lethal dose was found to be at 3,000mg/Kg body weight. The main risk assessment of melamine contamination at both acute and chronic level has been bladder calculi formation on a dose dependent level. Reports from hospitals indicated that most affected children were contaminated between a period of three months and cases included mainly infant less than 3 years old with “irritability, dysuria, difficulty in urination, renal colic, hematuria, or stone passage”. Severe cases included hypertension, edema, or oliguria (Hau, Kwan, & Li, 2009). In rat studies, the tolerable daily intake of melamine was found to be 0.2 mg/kg body weight and an estimated comparison pointed out that the daily intake of melamine in formula ranged between 8.6 to 23.4 mg/Kg body weight per day, which is about 40-120 times the tolerable daily intake (Gossner, et al., 2009).

### **IV. IMPACT ON VULNERABLE POPULATIONS**

The most vulnerable population has been, evidently, infants younger than 3 years old. As of December 1, 2009, it is estimated that 51,900 children have been impacted, six cases of deaths have been recorded and 29,400 cases of milk product consumption were announced by the Chinese Ministry of Health. All 51,900 cases reported were hospitalized and among that number, 51,039 were discharged after hospitalization, 154 were in serious condition following hospitalization, 861 cases were still in the hospital, six died, and 74 children were hospitalized with renal failure or renal stones. With such recent events, health officials and professionals realized that many cases of kidney stones have been underreported and the number of cases might be increasing with better testing and thorough studies (Gossner, et al., 2009).

One reason for the impact on infants is because formula is the single source of food where they get all the nutrients they need. Adults were not affected at all because their diet is varied and the amount of melamine ingested might have not produced an effect like in infants who

until a certain age, only depend on formula, hence the formation of stone due to a chronic exposure (Brown & Brown, 2010). In reference to studies, children ingest more melamine per body weight unit than adults.

Following the observation of many children with urinary tract stones from hospital records and doctor reports, the Chinese government authorized cross-sectional study to further assess the association between melamine exposure through milk product and the formation of kidney stones. One study recruited participants to collect information on milk consumption and symptoms associated with urinary tract stones. All children who were 36 months or younger were considered at risk if they had been consuming formula for the past thirty days. Melamine contamination was categorized as: high melamine content (formula with a concentration greater than 500 ppm), moderate melamine content (formula with a concentration less than 150 ppm), and no-melamine formula. Laboratory testing included urinalysis, renal-function and liver-function tests, urinary tests, for biochemical markers and the calcium: creatinine ratio, and ultrasonography. A total of 589 children were screened and 421 children were found to have ingested contaminated formula. Stone findings were grouped as: definite stones, suspected stones (based on increased, sporadic, punctiform echogenicity in the kidneys or pyelocalyceal system), or no stones. Of all the children found without stones, 68 had high melamine content, 223 had moderate melamine content and 136 had no melamine content. Among children with suspected stones, 30 had high melamine content, 58 had moderate, and 24 had no melamine content in their formula. The results showed a prevalence of 8.5% of kidney stones among the 589 children, and estimate risk calculations showed that children who consumed contaminated milk were 7.0 times more likely to develop stones than children who were not exposed (Guan, et al., 2009). Although the study provides some data to explore and an idea of the magnitude of the incidence, the impact of the outbreak on children is uncertain (Langman, 2009).

## V. SOLUTION

Following the release of infant formula contamination news, the WHO provided international information through the WHO/Food and Agricultural Organization International Food Safety Authorities Network where all the list of contaminated products were published and all the information on melamine dosage tolerance in food for adults and infant (Gossner, et al., 2009). With such as relatively recent incident, the quickest action involved stopping importation of any milk product from China.

The first solution was to assess countries affected by melamine nephrotoxicity, and a total of sixty-four countries were affected of the melamine contamination among which China, Hong Kong SAR, Macau SAR, and Taiwan reported cases. The Chinese government issued warning to a list of five countries that might also be facing the outbreak: Bangladesh, Myanmar, Yemen, Burundi, and Gabon. The remaining forty-two countries decided to completely ban, recall or tighten regulations on milk products from China. Among these countries were the USA, all European Union members (27), Australia, Argentina, Bangladesh, Bhutan, Brunei, Burundi, Cameroon, Canada, Chile, Colombia, Dominican Republic, Gabon, India, Indonesia, Ivory Coast,

Japan, Laos, Macaus SAR, Malaysia, Maldives, Mali, Myanmar, Nepal, New Zealand, Papua New Guinea, Paraguay, Peru, Philippines, Qatar, Russia, Singapore, South Korea, Sri Lanka, Suriname, Taiwan, Tanzania, Thailand, Togo, United Arab Emirates, Vietnam (Bhalla, Grimm, Glenn, & Pao, 2009).

In the USA, the USFDA regulated that milk product must contain less than 1 ppm of melamine and the government established an agency that will continuously investigate products from China being exported to the USA. The list of products and brands included: "Fresh and Crispy Jacobina Biscuits, Koala's March Crème filled Cookies, YILI Brand Sour Milk Drink, YILI Brand Pure Milk Drink, Blue Cat Flavoured Drinks, White Rabbit, Mr. Brown Mandheling Blend Instant Coffee (3-in-1), Mrs. Brown Arabica Instant Coffee (3-in-1), Mr. Brown Blue Mountain Blend Instant Coffee (3-in-1), Mr. Brown Caramel Macchiato Instant Coffee (3-in-1), Mr. Brown French Vanilla Instant Coffee (3-in-1), Mr. Brown Mandheling Blend Instant Coffee (2-in-1), Mr. Brown Milk Tea (3-in-1), and all infant formula manufactured in China" (Bhalla, Grimm, Glenn, & Pao, 2009).

The second solution involved judicial actions, and investigation led to the sentencing to life of the chairwomen of the San Lu Company, and two other members were executed. The jurisdiction of the case was proving once again that no misdemeanor of such kind should go unpunished because lives have been put in danger for personal gain and market purposes (Brown & Brown, 2010).

Lessons can be taken from previous cases of environmental chemical poisoning of the pet food with melamine and cyanuric acid. Similarly to infant formula contamination, the outbreak started in China and products found their way to North America. The turn of events, however, is that clinical symptoms in dogs and cats were found about three years later, in 2007, after the events occur in 2003-2004 in China. Laboratory findings from the Minnesota Urolith Center confirmed that the canine stones examined from animals indicated an exposure that has probably been occurring since 2002. From this case, American authorities realized that people have not narrowly escaped the event of infant formula contamination. The fact of the matter is to keep a watchful eye among children feeding on formula, thus the third solution is to monitor the population, especially the most vulnerable population, which happens to be infant less than 3 years for possible cases of nephrolithiasis. Even though no cases have been reported in the USA, pediatricians, nephrologists, urologists and radiologists must stay alert to the signs of calculi formation due to a chronic exposure. The toxicity of melamine is still not clearly known and the information on melamine nephrotoxicity is only based on animal studies (Brown & Brown, 2010).

Data must be shared internationally in such events to ensure better analysis and coherent development of methodology and testing. The fourth solution was introduced in WHO expert meeting in 2009: authorities requested exchange of information and sharing of analysis with the Chinese authorities. Although this was not mentioned, a system of food and safety needs to be closely regulated through the creation of risk-based import-inspection systems that will set safety measures for import and export products (Gossner, et al., 2009)

WHO has also published solutions that should be taken by affected country. It was discovered that infants were taken as much as 0.16 – 0.7 mg/Kg body weight as a daily intake, but this number is only estimate. WHO recommended that research continues to be conducted (Expert Meeting to Review Toxicological Aspects of Melamine and Cyanuric Acid, 2008).

## VI. FURTHER RESEARCH

The problem with melamine contamination is the recent occurrence of events; therefore, authorities have their hands full because of the profile of melamine itself as a compound and its effect in the body. The two most critical challenges that chemists are faced with is the fact that melamine has a very low toxicity and second melamine is rapidly excreted from the body, leaving little room for lengthy research.

The first area of research should focus on developing testing method in the laboratory. Testing to detect the level of melamine was based on veterinary methods that were developed during pet food melamine contamination. With that, analytical variability needs to be taken into account, and the results can only be estimated for humans rather than animals (Expert Meeting to Review Toxicological Aspects of Melamine and Cyanuric Acid, 2008). In the future, all milk products can be further tested and melamine can be precisely detected.

The second area of research should be centered on understanding melamine and its analogous forms. During the pet food melamine contamination, cyanuric acid was the main reason for a higher mortality in dogs and cats. Since cyanuric acid is an analogous of melamine, like ammeline and ammelide, its effect can also mimic toxicity of melamine when used alone in the food; however when combined with melamine, they increased their toxicity in the excretory system (Expert Meeting to Review Toxicological Aspects of Melamine and Cyanuric Acid, 2008). Like melamine, none of the three analogous have been very well studied, thus in a case of another outbreak, authorities in countries affected would have not much background information on any of the analogous. If infant formula was contaminated with melamine and an addition of any of its analogous, the death toll would have been greater in a very short amount of time.

The third area of research needs to involve some sort of clinical research using better epidemiology methods. Since cross-sectional studies are only a snapshot of the population at a particular time and exposure and disease are assessed at the same time, the complexity of nephrotoxicity that arise from melamine exposure will not be well understood. Also, referring back to the article of the cross-sectional study conducted by Guan et Al., the results were not conclusive and can only be used to estimate the risk without giving an absolute or relative risk (Langman, 2009). At this point, a prospective cohort study can be designed collecting participants who have infants feeding on formula from milk product manufacturers. The study could give insight on multiple risk factors that have contributed to kidney stone formation. Furthermore, a cohort study can serve as follow-up monitor for infants who might develop condition other than stones, such as cancer (Expert Meeting to Review Toxicological Aspects of Melamine and Cyanuric Acid, 2008).

The fourth area of research that needs to be developed is identifying biomarkers and diagnostic techniques to predict renal damage after melamine exposure. This can be done by studying urine tests of a whole population which will serve as biomarkers for problems before the formation of kidney stone, or crystals in urine. Techniques developed need to be very sensitive to measure small levels of crystals in the urine because melamine is rapidly excreted from the body and has a short half-life. The fifth and final area of research should be focused on melamine toxicokinetics to identify the uric acid level in humans and neonates (Expert Meeting to Review Toxicological Aspects of Melamine and Cyanuric Acid, 2008).

Laboratory testing for melamine is available, however, a better method to detect even small amount of melamine in blood serum and urine needs to be developed. From the study findings, the advantage is that if small amount of melamine is found once better testing is available, people should not worry about developing any illness later in life (Melamine in Food Products Manufactured in China, 2008). This is a little positive note that CDC has provided at the end of its report on melamine.

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