Toxicological risk of consuming melamine contaminated food products in human body & its detection techniques

A project submitted

by

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This work is dedicated to my parents for their love and support.

Certification statement

This is to certify that this project titled “Toxicological risk of consuming melamine contaminated food products in human body & its detection techniques” is submitted for the partial fulfillment of the requirements for the degree of Bachelor of Pharmacy (Hons.) from the Department of Pharmacy, BRAC University constitutes my own work under the supervision of Dr. Raushanara Akter, Associate Professor, Department of Pharmacy, BRAC University. This project is the result of the author’s original research and has not previously been submitted for a degree or diploma in any university. To the best of my knowledge and belief, the project contains no material previously published or written by another person except where due reference is made in the thesis itself.

Signed,

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Counter signed by supervisor,

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Acknowledgement

At first, I would like to give thanks to the almighty Allah for giving me strengths and patience to complete my work properly.

I am truly grateful and obliged to my supervisor Dr. Raushanara Akter (Associate Professor of Pharmacy Department of BRAC University) who has continuously supported and guided me to do my work properly. She also inspired me and helped me to resolve any kind of inaccuracy that was present in my work. Besides, I must mention Professor Dr. Eva Rahman Kabir, Chairperson of the Department of Pharmacy, BRAC University, who has been a continuous inspiration to me and has guided me to develop and grow as a better student and a person over the past years.

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Abstract

Now-a-days, food adulteration is a burning issue in global food business and as well as for the people who purchase these food items. Adulteration not only disturbs the public health but also abolish the global business. Melamine is one of the poisonous substances that cause harmful effects in the organs of the human body. Melamine that is encompassed of a trimer of cyanamide, which is used as a constituent of plastic in the industry. It contains 66% of nitrogen by mass and due to this high nitrogen content, melamine is added to different nutritional substances raises their protein levels. This literature review revealed that, because of the presence of melamine in powder milk, it causes death of newborns. It was also found that huge number of people became hospitalized in China, Australia, Myanmar, Taiwan, and Bangladesh and in several countries for the issue of renal impairment, central nervous system dysfunction, dysfunction in digestive system and reproductive system due to increase consumption of melamine contaminated food products. According to the reports of WHO, EFSA and FDA, the normal tolerable range of melamine for the human body is 0.2 mg/kg body weight. When the normal range exceeds, it shows the toxicological effects in the body. Therefore, it is essential to determine the level of melamine contamination in food products such as powder milk, powder infant formula, liquid milk etc. There are different techniques available to detect melamine contamination and its level with certain techniques. Among them, HPLC UV, enzyme kit detector, synthetic dye, ion chromatography with UV detector are most importantly used to determine the concentration of melamine present in food products. Toxicokinetic study discovered that melamine might not stay in the body for a long time but it affects more organs of human body than we anticipated. This review work was focused to find out the information on toxicological risk of consuming melamine contaminated food products in human body & its detection techniques. Findings of this review showed that, a number of companies who were involved in the production of milk and baby formula, intentionally adulterated milk product to enhance the protein level. Due to this adulteration, different body organs are exposed to a number of toxicological problems as mentioned above. To conclude, it can be inferred that, this review information will help to create awareness among the general people regarding this issue and it will also help the scientists to get information about melamine toxicity and its detection techniques who want to explore further research in this area.

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List of acronyms

MEL = Melamine

CAS = Chemical Abstracts Service

CYA = Cyanuric Acid

STMR = Supervised Trial Median Residue

WHO = World Health Organization

EFSA = European Food Safety Authority

FDA = Food and Drug Administration

CAC = Codex Alimentarius Commission

SA = South Africa

HPLC-UV = High Performance Liquid Chromatography – UV

GC-MS = Gas Chromatography- Mass Spectrometry

LC = liquid Chromatography

ELISA = Enzyme-linked immune sorbent assay

BSA-MEL = Bovine serum albumin - melamine

RU = Responsive Unit

CBC = Complete blood count test

SPT = Serum proteins test

SBT = Serum bilirubin test

SLET = Serum lever enzyme test

SDS-PAGE = Sodium dodecylesulphate polyacrylamide gel electrophoresis

ROS = Reactive oxygen species

NOX = Nicotinamide adenine dinucleotide phosphateoxidase

VGSC = Voltage- gated sodium channels

ROS = Reactive oxygen species

TDI = Tolerable Daily intake

1. Introduction:

Melamine is a poisonous substance which causes formation of kidney stone, nephrolithiasis, kidney injury in infant as well as in adults by the ingestion of melamine contaminated food items, powder formula, powder milk etc. Sometimes it causes the formation of carcinogenic cell due to longer duration of exposure by melamine. According to WHO, the appraised value about 51,900 children was affected by the melamine-contaminated food in 2008-2009 (Chertow et al., 2009). Because of its high nitrogen content, it is used in the food products to increase the false protein level in food immorally. Not only that, but melamine also mixed in pet food product can cause the death of several pet because of kidney failure (Chen, 2009; Rumbeiha et al., 2010). Therefore, this poisonous substance creates much hazardous effect in human body in numerous behaviors.

Around 109-powder formula, were contaminated which confirmed by the China's General Administration of Quality Supervision, Inspection and Quarantine from 175-powder milk sample (Gabriels et al., 2015). According to World Health Organization (WHO) and Food and Drug Administration (FDA) proclamation, the standard acceptable labels of melamine per day in body weight is from 0.5mg per kg (mg/kgbw/day) to 0.2mg/kgbw/day and from 0.63mg/kgbw/day to 0.063mg/kgbw/day (Chien, Chen, Huang, Chou, Chang and Hsieh ,2011). However, the contaminated level of melamine is found to be 0.09 and 2563 mg/kg by melamine detection test. The highest levels of melamine has been found that 8.6 mg/kg (Chan, Griffiths & Chan, 2008) which causes toxicity in body.

Table 1.1 Maximum tolerable level of melamine intake daily (World Health Organization, European Food Safety Authority, Food and Drug Administration, Codex Alimentarius Commission, South Africa) (Gabriels et al., 2015)

Organization	Tolerable Daily intake (TDI)
WHO	❖ 0.2 mg/kg (2008) ❖ 10 mg for 50kg person ❖ 1 ppm (Infant formula) ❖ 2.5 ppm (Infant food)
EFSA	❖ 0.2 mg/kg (2010) ❖ 0.25 mg/kg (before 2010)
FDA	❖ 2.5 ppm (2008)
CAC	❖ 1 mg/kg (Infant formula) ❖ 2.5 mg/kg (Animal feed and food product)
SA	❖ 2.5 ppm

1.1 Melamine:

Melamine (2,4,6-triamino-1,3,5-triazine) is a kind of heterocyclic compound encompassing 6 molecules of nitrogen. Because of its arrangement, it encloses 66% nitrogen in its atom. It is a crude material in the manufactures of plastics, pesticides, cleaning operators, composts and numerous different items, e.g. melamine sap. Unadulterated melamine is an extremely thermostable crystalline substance, rather inadequately dissolvable in fluid media. However, the small dose of melamine is non-toxic substance (Suchý et al., 2009). In 2008, melamine, a synthetic substance was used to make amino resin and plastics (Harper, 2006). Besides, in view of its nitrogenous properties, it is utilized as plasticizer. However, the most burning issue is that melamine is contaminated in pet food as well as in baby food products. Defilement happens, partially, in light of the fact that generally utilized techniques for protein examination can't recognize nitrogen from protein sources and nitrogen from non-protein sources (WHO, 2009). This is demonstrating different toxicological consequence in infants, children and in addition to, in pets.

1.1.1 Structure of melamine:

Melamine has the chemical formula of $C_3H_6N_6$ with its mass number, 126.12 $g \cdot mol^{-1}$ and mainly has nitrogen base due to the present of 66% of nitrogen. Trimer cyanamide is synthetic chemical of melamine that structure resin with the formaldehyde (Lopez &Quereda, 2011). Melamine is also recognized as a nitrogen heterocyclic triazine complex. It is frequently referred to as triamines or protein essence and also identified as 2,4,6-triamino-1,3,5-triazine; 1,3,5-triazine-2,4,6-triamine; 2,4,6-triamino-s-triazine; melamine amide and cyanurictriamide (Zhang et al., 2011).

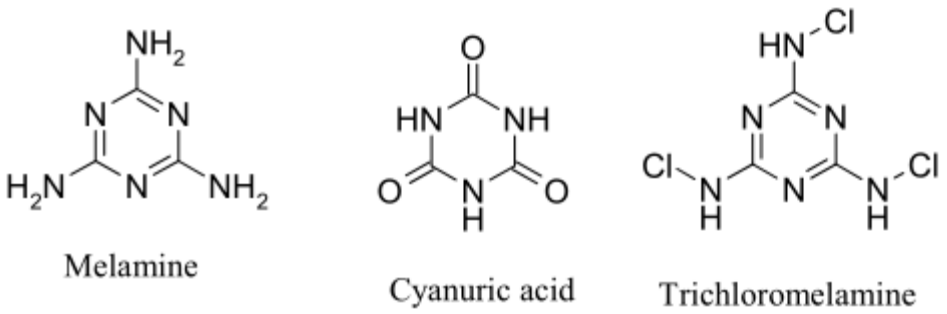
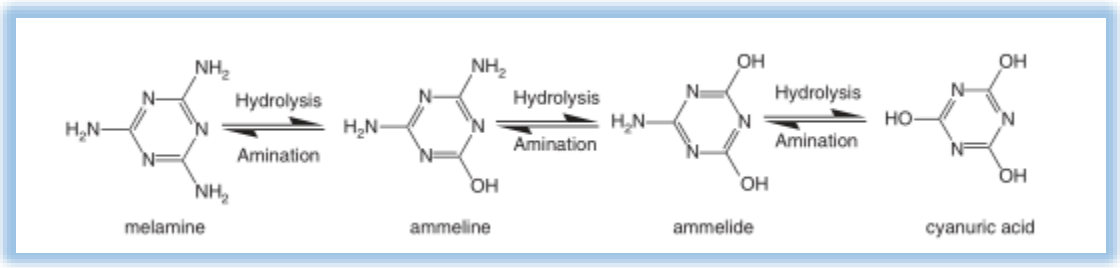


Figure 1.1 Structure of melamine and cyanuric acid (WHO, 2008).

1.1.2 Chemical conversion of melamine:



1.1 Figure Chemical conversion of melamine (Baynes & Riviere, 2010).

Melamine is converted into cyanuric acid by hydrolysis process and reverse in melamine form by amination process.

1.1.3 Physical and chemical properties of melamine:

Melamine was initially prepared in 1834 by Liebig by heating potassium thiocyanate with ammonium chloride. In additional, an added form is cyromazine which is metabolite situation in plant beneath environmental circumstance (Lei, Li, Yang, & Zhang, 2011). Overview of physical and chemical properties of melamine is given in the table 1.2.

Table 1.2 Physical and Chemical properties of melamine (Bhat, Ball, & McLellan, 2010; The Complete Book on Adhesives, Glues & Resins Technology).

Property	Data	References
Molecular formula	$C_3H_6N_6$	Hawley ,1981; Weast, 1984
CAS number	108-78-1	ChemlDPlus,2009
Molecular weight	126.13	Hawley ,1981; Weast, 1984
Physical state and color	White, monoclinic crystal or prism	Hawley ,1981
Melting point	354 °C	ChemlDPlus,2009;Hawley ,1981
Boiling point	280^0C	OECD 1998
Density	3.24mg/ml(experimental)	HSDB,2009
Storage temperature	-20^0C	Chemical book
Water solubility	3.240mg/L at 25^0C	Chemical book
pKa	5 at 25^0 C	ChemlDPlus,2009

The molecular weight of melamine ($C_3H_6N_6$) is 126.13. Its CAS number is 108-78-1. It is a white monoclinic crystal with a pKa of 5at25^0C. Its melting point is 354^0C and boiling point is 280^0C. The density of melamine is 3.24mg/ml (experimental) with a water solubility of 3.240mg/L at25^0C. Its storage temperature is -20^0C.

1.1.4 Sources of melamine production:

About 1.2 million tones melamine is fabricated in China and Western Europe from three different preparatory resources such as:

1. Urea,
2. Dicyandiamide or
3. Hydrogen cyanide.

However, mainly urea is used as raw materials in the commercial production of melamine. (WHO,2008). Melamine can also be degraded from plastic tableware, Cyromazine, Trichloro melamine. Here is a table which shows different kind of sources of melamine.

Table 1.3 Sources of melamine/cyanuric acid (WHO ,2008).

Sources	MEL/CYA	Reported level Mg/kg	Additional notes	References
Melamine migrated from the plastic tableware	MEL	❖ <1 ❖ <1 ❖ 0.18-42.9 ❖ 0.5-2.2	❖ Add water and ethanol at 60^0,70^0 and 95^0 ❖ Add 3% acetic acid both in 60^0 and 70^0 ❖ Add 4% acetic acid at 95^0temperature ❖ At 95^0 coffee, orange juice	Ishiwata, Inoue &Tanimura (1986); Bradley et al. (2005); Lund & Petersen (2006); Chinese Center for Disease Control and Prevention (unpublished data, 2008); Korea Food and Drug
Cyromazine	MEL	❖ 0.017-	❖ Tomato and	Japan Food

degradation		0.917 ❖ <1	lettuce celery residues from japan ❖ Beans vegetative residues part~10% of (STMR)supervised trial median residue in most crops	Safety Commission (unpublished data, 2007) Karras et al. (2007) FAO(2007 a,b)
Trichloromelamine	MEL	❖ 0.14		USFDA (unpublished data, 2008)

1.2 General use of melamine:

Melamine is mainly used in laminates, glues, adhesives, plastics, and some fertilizers (Lopez & Quereda, 2011). As we know that melamine is a highly nitrogenous substance, so it is used as fertilizer in several countries to fertilize the soil. It is also used as binding agent in industrial purpose, also as a flame retardant, and as a polymer in the construction of cooking utensils and plastics (Osborn, 2008; L. An, Li, & Zhang, 2014). As melamine is used in pesticide so, melamine is found in vegetables crops after spraying as pesticides (WHO, 2008).

1.3 Misuse of melamine:

Melamine, the poisonous substance is the evidence of forming kidney stone, nephrolithiasis kidney injury in infant as well as for the adult by the ingestion of melamine contaminated food items, powder formula, powder milk etc. Sometimes it causes the formation of carcinogenic cell due to longer duration of exposure by melamine**.** Because of, its high nitrogen content, it is used in the food product to increase the false protein level in food immorally (Bhat, Ball, & McLellan, 2010)**.** Because of this contamination, it causes difficult issue on wellbeing of children. It affects diverse body organ, and at some point, it causes malignancy in constant phases of influencing in

body. It is most troublesome for stomach to administrate melamine due to its complex structure. Hence, it causes poisonous quality in various organ of our body.

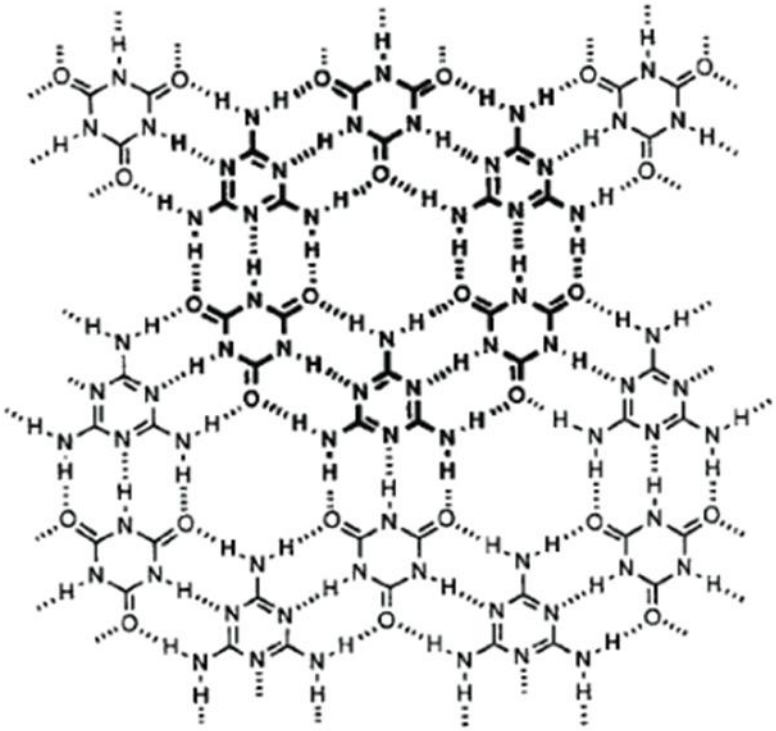


Figure 1.3 Complex structure of melamine that have intermolecular hydrogen bond with the aromatic ring stacking (WHO, 2008).

From this picture, it is easy to recognize that melamine is spreaded all through the world. In some nation melamine cases is accounted for, in some nations melamine takes as a notice issues and in some nation, melamine contaminated nourishment is banded for imports. This will mainly contribute strong visualization of melamine-threated country.

Table 1.4 Here some countries that report melamine contamination, some country takes it as warning issues and some country banned this contaminated food product (Bhalla, Grimm, Chertow, & Pao, 2009).

Cases reported	Warning issued	Imports banned/recalled
❖ China ❖ Hong Kong SAR ❖ Macau SAR ❖ Taiwan	❖ Bangladesh ❖ Myanmar ❖ Yemen ❖ Burundi ❖ Gabon	❖ Australia ❖ Argentina ❖ Bangladesh ❖ Bhutan ❖ Brunei ❖ Burundi ❖ Cameroon ❖ Canada ❖ Chile ❖ Colombia ❖ Dominican Republic ❖ European Union ❖ Gabon ❖ India ❖ Indonesia ❖ Japan

1.4 Melamine in food product:

Humans are exposed by melamine through food and environmental factors. Sources include breakdown of the pesticide cyromazine, which is permitted for use in several countries; to passage from accepted food packaging material to the contamination of certain foods (WHO, 2009). Because of this contamination, melamine causes life-threating toxicity, especially nephrotoxicity. Renal toxicity is acute for the infant instead of older child because their urinary tract have small lumens, tubules and blood vessel that is easy to irritate in earlier stage. The main foundations of melamine toxicity are food products, which are contaminated through the melamine immorally.

Table 1.5 Food items contaminate through the melamine (Gabriels, Lambert, Smith, Wiesner, & Hiss, 2015).

Food products	Melamine contamination levels (mg/kg)
Beverages (coffee/orange juice)	2
Powdered milk products	1-6.196
Powdered infant formula	0.1-2.563
Liquid milk	8.6
Contaminated foods	0.38 – 945
Processed food and ingredients	0.6 – 6.694
Whole eggs	2.9 – 4.7

1.5 Melamine in baby powder milk:

Milk is a central nutritional constituent in our daily food practice, mutually for the infant, children and adults. The straightforward element milk contains protein, carbohydrate, fat, vitamins and minerals (Jalili, 2017). Additionally, this is an essential component for the newborn baby as well as developing children. To provide all the necessary constituent of milk to the children, parents also have a preference to powder milk when the dairy farm is not easily accessible. Generally, powder milk is manufactured by the vaporization of dairy firm milk very cautiously. Unfortunately, now-a-days powder milk is contaminated by numerous kinds of substances such as water, detergents, formalin, alkaline and acidic compound and melamine. It has been found that milk is adulterate due to enormous demand and short supply, less existence of natural dairy milk, low purchase ability of customer, lack of technological facilities to detect this contaminated product (Jalili, 2017). In numerous investigations it has been evident that melamine is also present in powder milk of baby. About 29,000 infants were affected by

melamine contamination, 54,000 infants were hospitalized and about six were died due to this adulteration in 2008(WHO, 2009).

1.6 Melamine in pet food:

It had been found that in 2007 in North America an outbreak of death of cats occurred due to melamine contamination in the pet food product (Hau et al., 2009). Most of the animal died due to nephrological toxicity and others were diagnosed with advance kidney stone (Kim et al., 2011). Subsequently, a number of animal food items for consumption such as wheat, gluten, rice and protein were contaminated by the melamine poisonous substance (Brawn et al., 2007).

1.7 Rationale of this study

Melamine is generally used as coating material, adhesive, resin, fertilizer etc. It is typically the leading sources of preparing house hold belongings in our regular life. However, people used this melamine in food products as a consequence of its characteristics of falsie protein. In 2004, the first and in 2007, the second epidemic death of pet ensued in north American for renal nephrotoxicity and not only that, in 2008 China found a number of food product of infant which was contaminated by the melamine. (Brown et al.2007; Burns 2007; Puschner et al. 2007; Thompson et al.2008; Chan, Griffiths & Chan, 2008; EFSA, 2008a; WHO, 2008a, 2008b). After absorption of melamine through the digestive tract, it causes irritation in skin and eye mucous membrane, also disturbs the kidney function by formation of stones in kidney and bladder, which can sometimes become carcinogenic (Suchý et al., 2009).

Melamine is a high nitrogenous encompassing industrious chemical. However, recent studies have revealed that it is responsible for causing renal injury to animals and infants. Melamine also causes kidney stones, crystaluria following kidney failure. For this reason, most researches on melamine are based on the formation of renal stone. Melamine does not only affect the kidney, exposure to melamine might also affect eyes, skin respiratory system and might also affect the reproductive system. After conducting several experiments, it was exposed that uninterrupted exposure to melamine is accountable for producing testicular toxicity. Moreover, melamine taken in low dose could cause disruption of blood –testis barrier. Melamine has a very adverse effect on nervous system specifically on hippocampus. It is accountable for excessive generation of free radical and lipid peroxidation and affects oxidation –antioxidation hemostasis. Melamine alone or in combination with cyanuric acid also affects the humoral immunity of animals consequently obstructing their protection against infection (Yin et al., 2016).

Thus, this review work was devised to collect and compile information on melamine contamination in various food products and its toxicological effects on different organs of the human body. In addition to that, this study was also focused to gather information about the techniques used for the determination of melamine contamination.

1.8 Aim of the study:

The aim of the project is to study the toxicological risk of consuming melamine contaminated food products in human body & its detection techniques.

1.9 Objectives:

The main objective of this study is,

- To identify approximate chemistry of melamine and its sources of production.
- To estimate the presence of melamine in food products.
- To find the information regarding, toxicological effects of melamine in different organs of human body.
- To compile the information of different identification techniques of melamine in different food products.

Research methodology:

Thorough literature review was done to obtain all the information used in this review paper. The information was collected from various credible sources, including different peer-reviewed journals, online scholarly database, books, and newspapers. Following are the list of some of the many databases that were search extensively for the present study.

- Journal databases
- Library catalogue
- Subject specific professional websites
- Newspaper database

Keywords:

Keywords that were used to search for the information for this review paper are: Melamine, melamine sources, melamine toxicity, melamine contamination, powder milk, neurotoxicity, renal toxicity, urolithiasis, reproductive toxicity, clinical examination, detection techniques.

2. Melamine Contamination, Toxicity and Detection Techniques:

2.1 Unintentional and intentional contamination:

It has been found that, food products can be contaminated through the melamine intentionally and unintentionally to increase the false protein in nutritional substance.

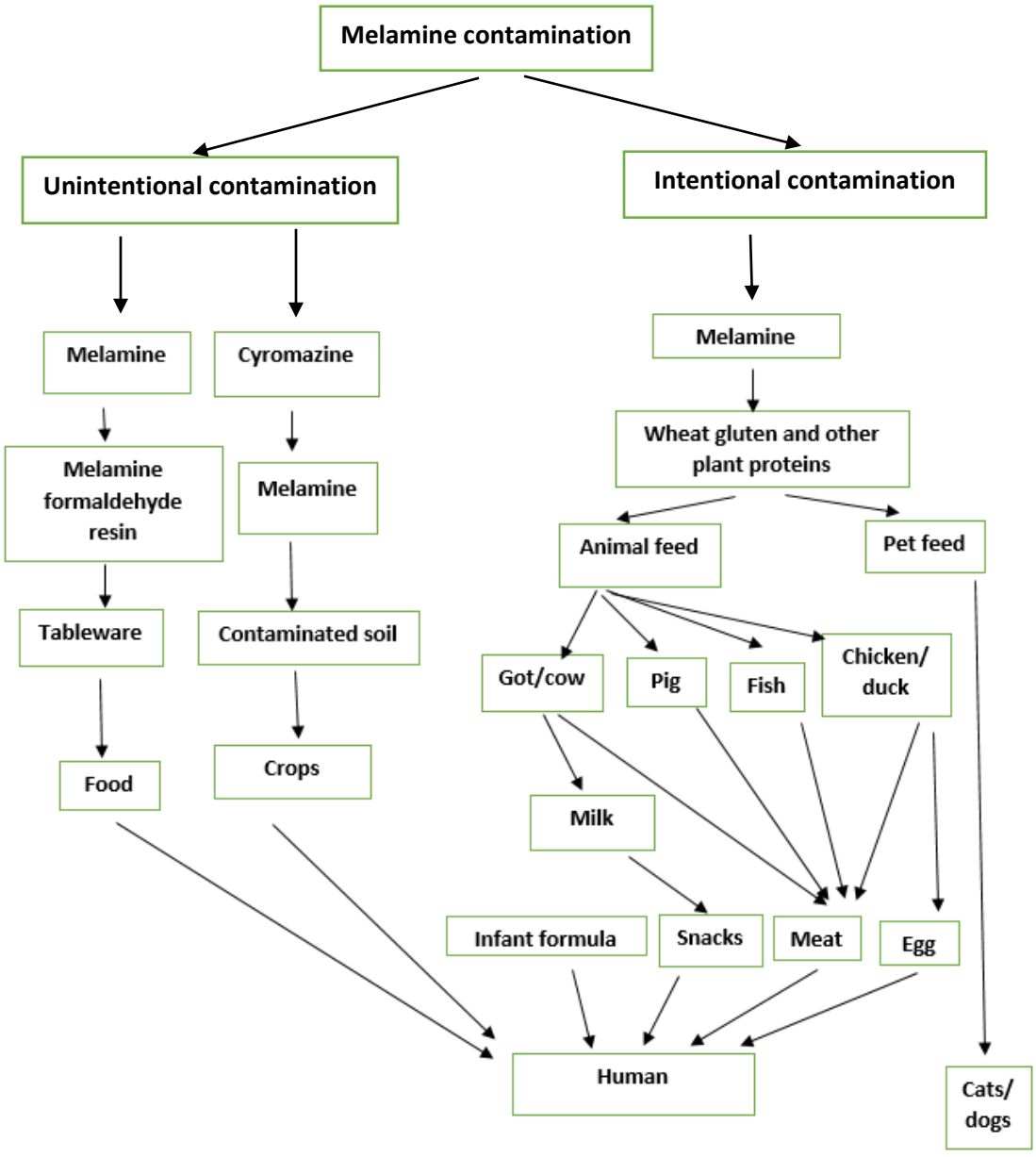


Figure 2.1 Food product contamination by melamine unintentionally as well as intentionally.

(C. Y. Chu & Wang, 2013)

Generally, our daily life is surrounded by several kinds of chemical substances, which are warning for public health. Melamine is one of them. Melamine is mainly used in the manufacture of tableware, cans, plastic package, cleansing foam etc. However, even after knowing the harmful effect of melamine we use some plastic jars in micro oven that can only withstand in 140℃. When it is encountered with food product, it can easily enter into the human body. In this way if melamine is used in plasticizer as well as in fertilizer, it is easy to combine with the crops and that would be the reason of entering into human body. These type of contamination is known as unintentional contamination. However, when melamine is mixed with wheat glutens and other plant protein, it will be the reason of intentional contamination. It has been found that a distinguished company Sanlu sealed 2176 tons of powder milk that were contaminated by melamine in 2010. It became involved in human body inflow that shown in the figure(C. Y. Chu & Wang, 2013). So, melamine contamination through unintentional methods such as, in food and crops and intentional methods such as mixing of melamine in milk which is used, to produce infant's formula, snacks etc. effect our health badly.

2.2 Clinical Test for examination of melamine

It has been long time since it was seen that some outstanding brands, for example, dairy nourishment item, newborn child food and yogurt are also being immorally contaminated by melamine to increase the false nature of protein in food products (Hau, Kwan, & Li, 2009). The location of melamine in an assortment of human and animal nourishments proposes that, melamine adulteration of Chinese food items may at present exist. A current experiment with infant formula has shown that the concentration of melamine found in U.S. formulas is 10,000 times lower than that which is being manufactured in China. The concentration is also ten times lower than FDA acceptable amount for melamine in formula for infants. The main sources of infant food product are powder milk, which is highly contaminated by the melamine. In case of adult and older child, they mainly receive this poison by variety of food resources that they consume which helps to receive low amount of melamine.

The therapeutic evaluation of the influenced child may incorporate the accompanying examinations:

- Renal capacity test - serum electrolytes, urea nitrogen and creatinine
- Urinalysis (microscopic assessment of hematuria)
- Renal imaging - ultrasound (to preclude check)
- Counsel with a pediatric nephrologist for those youngsters in whom presence of melamine and prospective renal damage is affirmed.(Society, Nephrologists, Formula, & The, 2009)

2.3 Melamine health hazardous problem:

From the different kinds of study, it has been proved that, the toxic substance melamine can cause health hazardous problem in human body. It can cause anatomical, cellular and functional changes in different organ of human body such as in central nervous system, it can cause some cellular changes, known as hyperpolarization and deflect of learning and memory due to functional changes. In case of cardio vascular system it can cause crystal associated granuloma in epicardium and myocardium. Most of the effect of melamine is mainly visualized in kidney. Some cellular changes such as nephrolithiasis, hydronephrosis, urolithiasis occurs due to the melamine. Sometimes in chronic stages, it will be the reason of cancer cell development in kidney.

The poisonous melamine has a consequence on the different part of body as well as in different mechanism of actions of our body parts. However, in the majority of the case, the comprehensible mechanism of melamine in different body organ is approximately unidentified for everyone. As melamine has complex mechanism and its maximum portion is excreted through the urine, it has a reduced amount of impart on stomach. Melamine is absorbed in intestine instead of stomach.

When people are exposed to melamine, several kinds of changes occur in human body, which is found respectively by the complete blood count test (CBC), serum proteins test (SPT), serum bilirubin test (SBT), serum lever enzyme test (SLET), sodium dodecylesulphate polyacrylamide gel electrophoresis (SDS-PAGE) of serum protein test in animal (Rat) and presenting some

changes on liver, spleen, heart and tests (Baynes & Riviere, 2010). In CBC test, it has been found that melamine creates changes in liver enzyme.

Heart muscle damage was shown when treated with 20000-ppm melamine and less effect 5000-ppm range of melamine. After the overall investigation of melamine it was found to give its threaded consequences on heart (Baynes & Riviere, 2010).

2.4 Summary of melamine effect in different organs:

Table 2.1 Different kind of organs and its anatomical, functional and cellular changes due to melamine exposed (C. Y. Chu & Wang, 2013).

Organs	**Cellular changes**	**Anatomical changes**	**Functional changes**
Central nervous system	Hyperpolarization	No effect	Effect in learning and memory store
Cardio vascular system	Crystal association in granulomas in epicardium and myocardium region	No effect	No effect
Liver	# Granular degeneration # Liver cell vacuolation # Swelling of Hepatocytes	# Fatty degeneration # Liver fibrosis	Decreasing of liver function
Renal system	# Tubular dilation # Tubular hyperplasia # Inflammatory cellinfiltration	# Nephrolithiasis # Hydronephrosis # Urolithiasis	# Decreasing the kidney function # Kidney injury # Urinary bladder cancer

			# Calculas or stones formation
Reproductive system	# Decrease spermatogenesis # Decrease sperm number # Decrease sperm motility	# Disruption of seminiferous tubule structure # Coagulation of necrosis	No effect
Placenta	# Advancement in glomerular development	# Fetus skeletal anomalies # Delay in fetus growth	# Placental transfer

2.4.1 Melamine toxicity in kidney:

After different kind of investigation in rats, it has been establishing that the melamine affected kidney. It mainly had similar characteristics like light in color, edema, hemorrhagic plagues, apparent necrosis, autolysis, renal tubular dilation, epithelial, cell regeneration, and basophilic debris; interstitial fibrosis (C. Y. Chu & Wang, 2013).

Because of low solubility of melamine at 25^0℃, it is easily soluble in room temperature. For this reason, it is easily soluble in renal tubule and produces the crystal form in kidney. In different kinds of animal tests, it is demonstrated that melamine is rapidly excreted through the urine and can precipitate in distal renal tubules. Histopathological report also showed the melamine deposition in kidney of animal (Fundamentals of Toxicologic Pathology; Bhalla, Grimm, Chertow, & Pao, 2009).

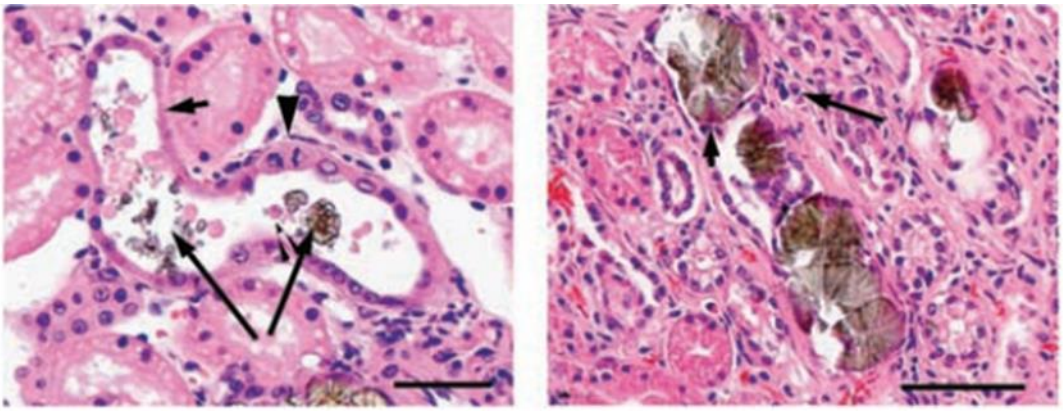


Figure 2.2 Green pigment region of this figure indicate the crystal from of melamine in distal tubules (Bhalla, Grimm, Chertow, &Pao, 2009).

Several experiment show that melamine produces urinary tract tumors if long term intake of melamine occurs. The urinary tract tumors can be observed in infant, adult as well as in cats and dogs due to contamination in food products. Generally, urinary tract stone is the most common issues in infant and adult when exposed by melamine. Several kinds of finding proved that there are different kind of kidney stone produced in kidney. One is definite stones, one is suspected stone and no stones. Most of the patients who are prone to kidney stones without other urinary tract abnormalities are less affected by urolithiasis than the patients who take melamine contaminated food for about 30 days or more. Urolithiasis is mainly known as stones or calculus formation in urinary tract. This is the common origin of blood in the urine and soreness in the abdomen, flank, or groin (Yang, Yang, & Zhang, 2010).

Figure 2.3 Melamine stone from urinary tract of chines children (Wen, Liu, Wang, Li, & Wahlqvist, 2016).

Table 2.2 Signs and symptoms of kidney stone due to melamine consumption, which is investigate in-group of children group of children

Group	Oliguria	Unexplained crying	Edema	Kidney stones passing
Children with stones	3/47 (6.4%)	0/84	0/48	
Children are suspected with stones	6/109 (5.5%)	8/110 (7.3%)	1/110 (0.9%)	1/110 (0.9%)
Children without stones	10/419 (2.4%)	17/418 (4.1%)	4/416 (0.1%)	1/418 (0.2%)
All children	19/575 (3.3%)	25/576 (4.3%)	5/574 (0.9%)	2/576 (0.3%)
P value	0.20	0.08	1.00	0.47

* During urination unexplained crying most commonly happened

* P values were designed for the evaluation amongst the three subcategories of children with the use of Fisher's exact test.

From the table 4.2 we can see that most of the children do not have the oliguria who already had kidney stone. Most of the children showed the unexplained crying due to irritation during urination, edema and passing of kidney stones. Statistically it will show that there is no difference in the symptom of children who have stones, children who are suspected to stones and children without stones. Therefore, the symptoms due to melamine are not properly clear to compare with the affected and unaffected children (Guan, Ding, Zhao, Jiang et al.;2009).

2.4.2 Management of kidney stones:

The first management of kidney stones is to avoid intake of melamine-contaminated food such as melamine adulterated powder milk, supplement, candy, powder infant formula etc. Some therapeutic approach could be taken such as treatment by diuretics, antibiotics and rehydrate the stone when it is of small size. However, when the obstruction of kidney by the stone is larger, surgical way is always adopted. Most of the circumstances patient were treated by diuretics, antibiotics and rehydrate way due to less obstruction of kidney by the stones. Some easy way to hydrate the kidney are taking sufficient amount of water, injecting 5% sodium bicarbonate intravenously to make urine alkaline from, and taking an isodamine or atropine drugs, which work as antispasmodic drugs. Some other ways of treatment can include alteration of water, electrolyte and acid-base disproportions, carefully observing blood biochemistry, renal morphology (by ultrasound) and renal function (Wen, Liu, Wang, Li, & Wahlqvist, 2016).

2.4.3 Melamine causes inflammation in kidney:

Melamine indirectly increases the production of ROS (reactive oxygen species) due to activation of nicotinamide adenine dinucleotide phosphateoxidase (NOX) which is includes NOX1, NOX2 and NOX4. Melamine increases the pathways of NF-κB/COX-2 and NOX/ROS that are the reasons of inflammation in kidney by the production of ROS. Theses, ROS plays an important role for the increase of melamine toxicity. Several studies prove that, due to the activation of NF-κB signaling cascade through the melamine, it will increase the activity of NOX subunits. For

this reason, increase of NOX cause the production of ROS and then increase the inflammation due to melamine toxicity (Hau, Kwan, & Li, 2009).

2.4.4 Melamine toxicity in central nervous system:

Some previous toxicological study has shown that melamine not only affect the urinary tract but also affect central nervous system. In laboratories, different types of test were conducted on rats to show that melamine can affect some region in the brain such as cortex, striatum, hippocampus, cerebellum and brain stem (Wu et al., 2009). Melamine can give negative influence on brain, when considering the rat brain by the melamine toxin constituent. Ion channel in brain, mainly the target area of melamine gives its toxicological effect. Ion channels mainly work for creating signals, secretion, excitation, and targeting the toxins and drugs. Most of the damage in central nervous system mainly occurs due to the damage in ion channels by different kind of substances and melamine is one of them (Denac et al., 2000; Judge et al., 2007). The voltage- gated sodium channels (VGSC) is one part of ion channels. If any kind of damages occurs in VGSC it can cause the damages in central nervous system. The main work of VGSC is initiation and propagation of action potential, which is important for the central nervous system responses. Dysfunction of VGSC is mostly the reason for some harmful toxic constituents (Alekov et al., 2000; Amir et al., 2006; Berta et al., 2008; Takahashi et al., 2000; Tarnawa et al., 2007). Therefore, it has been under investigation that melamine can affect the central nervous system. (Yang & Zhang 2010).

2.4.5 Melamine toxicity in reproductive system:

Melamine shows the toxic effect in rat by disrupting seminiferous tubes in reproductive system. However, it is the cause of decreasing spermatogenetic cell series, which will give harmful effect during production of sperm or the number of sperm. Not only has that melamine showed it toxic effect in sperm but also effect in sperm mortality. It is also the reason of disrupting seminiferous epithelium and the asymmetrical character of interstitial cell nuclei (C. Y. Chu & Wang, 2013).

2.4.6 Melamine toxicity in placenta:

Melamine can promptly cross the placenta and easily be exposed to the embryo and fetus. Selectively, accumulation of melamine in various parts of the developing brain could prompt a danger of neuro developmental impairment. In specific, disturbance of hippocampal progression

could lead to changed learning and memory and additionally other antagonistic behavioral results. Melamine shows its toxic effect in neuronal activities when, melamine gives it toxicological effect in gestational stages of maternal period (Lei &Zhang 2014). Sometimes melamine gives the effect on fetus skeletal as well as in the growth of fetus (C. Y. Chu & Wang, 2013).

2.4.7 Melamine inducing the oxidative stress:

The harmful effect of melamine, which is used to adulterate the food, now is on important issues for the public health. In different kind of experiment, it has been proved that, melamine leaves it toxic effect on the cell as well as the growth of the cell. When eukaryotic cell are exposed by melamine, it suppresses growth of cell and also disturbs the cytomorphology and protein expression. However, it is the reason of cell apoptosis and disturbing in hemoestasis of calcium ion. Some experiment shows that melamine stimulates oxidative activities which is the reason of generating the free radical. Due to activation of free radical it will cause the modification of antioxidant activities. After the activation of antioxidant activities, it will aggravate the reactive oxygen species (ROS) scavenging enzyme in numerous organ systems. These have the killing response in immune cell due to microbial invasion. For this reason, it will cause different kinds of neurogenerative disorder and diseases in human body (An, Fu, & Zhang, 2015).

2.4.8 Melamine effect on Humoural immunity:

From some current researches carried on mice, scientists have revealed some of the harmful effects of melamine in conjunction with cyanuric acid on different body parts especially on the spleen lymphocytes of mice. The results proved that the effect of melamine is tremendously injurious for the immune system of animals. Humoral immunity is mainly responsible for protecting the body from various toxic effects. Humoural immunity is the feature of immunity that is arbitrated by macromolecules found in extracellular fluids such as secreted antibodies, complement proteins, and certain antimicrobial peptides. Humoral immunity is so-called because it contains substances found in the humors, or body fluids. For instance, melamine is responsible for reduction of blimp-1 in the animal's body. Decreased production of blimp -1 weakens the immune system. Recently scientists are conducting many researches concerning different types of toxicity of melamine with or without cyanuric acid (Yin et al., 2016).

2.5 Signs and symptoms of melamine:

To identify the presence of melamine, here are some signs and indications, which are apparently connected to melamine contamination powdered infant formula in Chinese infant (Gabriels et al., 2015)

- Sudden crying of baby during urination
- Vomiting
- Occurrence of fever due to urinary tract infection
- Macroscopic or microscopic hematuria (due to presences of red blood cell in urine it is colored red, pink or cola)
- Abstraction of urinary bladder by the stone producing kidney failure
- Discharge of stone through the urine
- High blood pressure
- Edema etc.

During 2007 to 2008, melamine caused toxicity among 58,000 infants and children worldwide. As melamine was present in baby supplements, it caused kidney stones. Melamine facilitates coagulation of calcium in tubules of nephron causing the formation of the stones. The substance under study is used as a false protein immorally to meet expected nutritional demands.

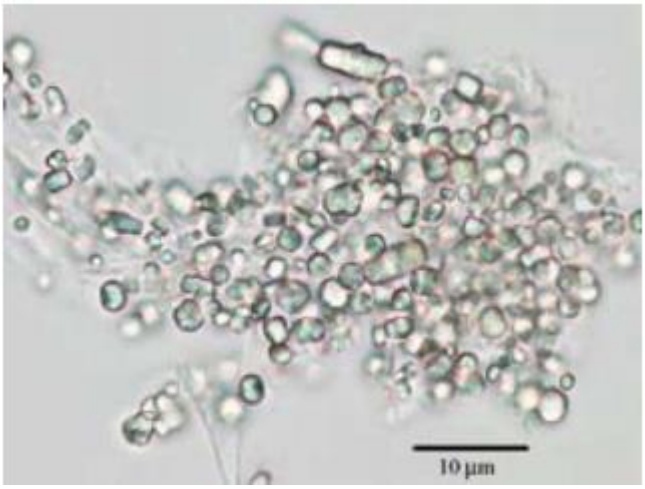


Figure 2.4 Magnified picture of melamine sedimentation in affected child (Langman et al., 2009).

2.6 Diagnosis of melamine affected people:

There are several diagnostic tools used to recover the affected infant who were exposed to melamine. Here are some basic diagnostic procedure towards the affected infant:

- Measurement of blood pressure frequently as well as examine nutritional and hydration level
- Inspection of urine, microscopically.
- The level of creatinine, blood urea nitrogen, and serum electrolytes and glomerular filtration rate are examining properly
- To identify stone in kidney, accomplish ultrasound examination.
- To identify kidney dysfunction radionuclide renal scans must be proceeding (Langman et al., 2009).

2.7 Melamine detection process:

In 1960s and 1970s, it was found that nitrogen-comprising melamine was used as an anticancer agent. However, due to its toxicity and not having as much of benefits, it was banded. Not only that, but many plastic materials which were prepared by melamine were banded due to its decomposition by heat to give harmful effects (Gabriels, Lambert, Smith, Wiesner & Donavon Hiss ;2015). In Ruminants during 1950 and 1960 melamine was used as a number of protein sources in food product. Not only that, but it was also used as a nitrogen sources for the food crops. However, melamine was banded in 2007 to 2008 due to outburst of melamine contamination in food products of infant (Narayan & Veeranna, 2014).

In 2008, it has been published that Australia's Diploma and Red Cow, Denmark's Dano Full Cream, China's Yashili-1, Yashili-2 and Sweet Baby-2, and New Zealand's Nido Fortified were the band which were contaminated by melamine. Several investigations publish that 2.5 parts per millions of melamine is tolerable for the human body but all of this product exceed the normal label (Kamol, 2008).

Because of melamine contamination, many infants died and many were hospitalized in 2008. At that time, to detect melamine in food product and also for the banding of several powder milk brands, many detection process were used as follow.

2.7.1 Summary of melamine detection process:

Melamine can easily combine with the solvent and sample assortment. The UV spectrometer is an instrument which distinguish the melamine from the mixture at 240nm. An additional detection test of melamine is HPLC, LC/MS/MS, GC/MS, and ELISA, comparatively problematical and time consuming techniques (Cai et al., 2008; Filigenzi et al., 2008; Kim et al., 2008; Muniz-Valencia et al., 2008; Yokley et al., 2000).

Table 2.3 Different kind of melamine detection process

Detection process	**Sensitivity**	**Selectivity**	**Expenses**	**Purposes**	**References**
PH indicator (Synthetic Dye)	Medium	Medium	Low	To detect melamine from contaminated food	Narayan and Veeranna; 2014
HPLC-UV	Low	Low	Medium	To detect melamine	(WHO Reports;2009) (Salman, Hameed, Al-Amoudi et. al.;2012)
GC-MS	Medium	Medium	High	Screening and conforming melamine in food products	WHO Reports; 2009
Visual detection	Medium	Medium	Low	Detection of melamine	Narayan and Veeranna; 2014
Enzyme kit	Low	Medium	Low	Rapid and specific detection from contaminated sample	Krebs ; 2014
Ion chromatography	High	Medium	High	Determination of melamine from powder	Lang li, jiLuo, Rhorer et. Al.;2009

				milk	
ELISA	Low	Low	Low	Screening	WHO Reports; 2009

2.7.2 Melamine detection by using synthetic dyes:

In visual detection process, some pH indicators are used to detect the melamine from the adulterate food product. Some synthetic dyes such as bromophenol, methyl red and alizarin reds are used to detect the presence of melamine. This dyes are mainly mixed with melamine contaminated food product at different concentrations. Generally, if the concentration of melamine is seen in an increased amount, the color of the mixture of melamine and dye change along with the pH of the system. In short, change of melamine- dye color is directly proportional to the solution contain melamine. Main advantage of this process is that it is a cost effective process for the determination of melamine from the adulterate food product.

Table 2.4 Dyes and its concentration level to detect the melamine. (Narayan & Veeranna; 2014)

Synthetic Dye	**Color change**	**Concentration of melamine that can be detectable**
Bromophenol	Present	3-30(mg dm−3)
Methyl red	Present	10-50(mg dm−3)
Alizarin red-s	Present	41-206.8(mg dm−3)

Here, bromophenol dye is used in solution to detect 3-30 mg dm^{-3} concentration of melamine. Methyl red and alizarin red-s are used to detect 10-50 (mg dm^{-3}) and 41-206(mg dm^{-3}) of melamine from the solution.

When melamine is mixed with the bromophenol solution, different color would be visualized due to increasing in ratio of melamine.

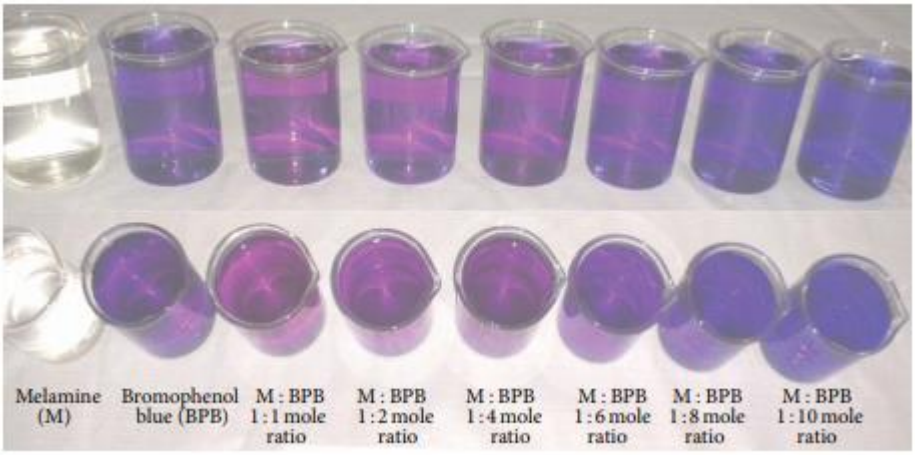


Figure 2.5 Melamine and Bromophenol solution at different ratio. (Narayan &Veeranna; 2014)

Methyl red expressed different types of color when mixed with different ratio of melamine solution.

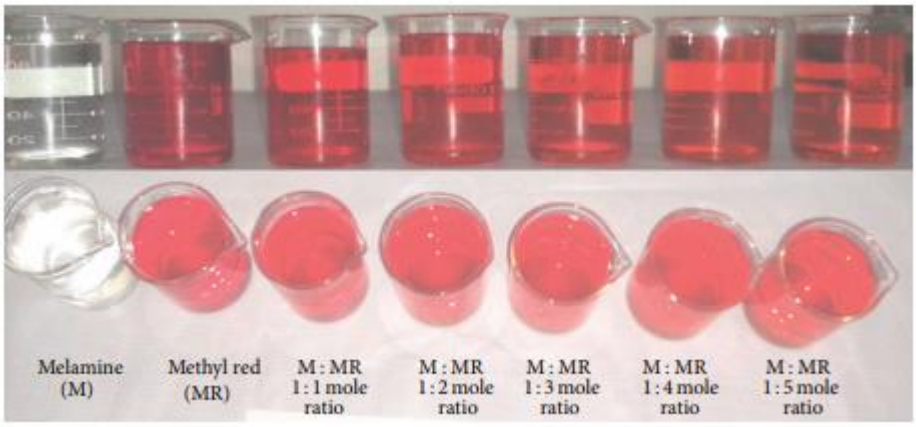


Figure 2.6 Melamine and methyl red solution at different ratio (Narayan & Veeranna; 2014).

The color of melamine and alizarin red-s mixture are darker when the concentration of melamine increases.

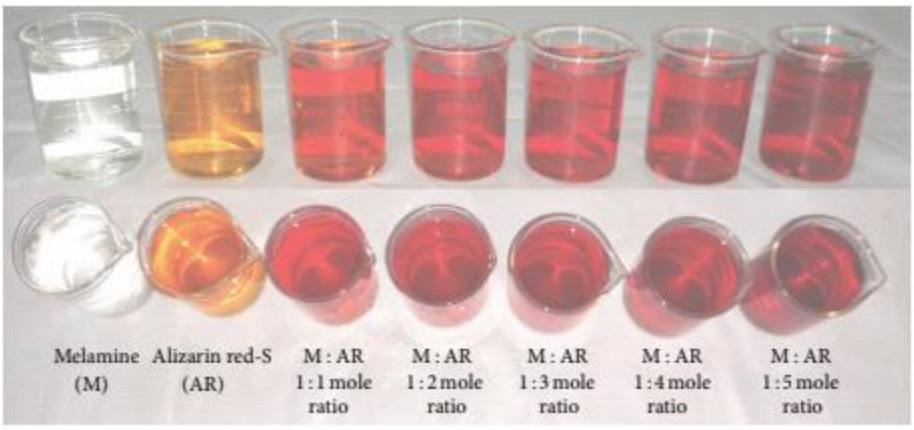


Figure 2.7 Melamine and alizarin red-s solution at different ratio (Narayan &Veeranna; 2014).

2.7.3 High Performance Liquid Chromatography – UV Detector:

HPLC UV locators are utilized with superior fluid chromatography to recognize and distinguish analytes in the sample. An UV visible HPLC indicator utilizes light to break down samples. By estimating the sample's assimilation of light at various wavelengths, the analytes can be recognized. HPLC UV indicators can be utilized by any lab utilizing HPLC, including genomic, biology and biochemistry research centers, to examine nucleic acids, proteins, and to do lethal and helpful medication testing. Two kinds of HPLC UV indicators are single and variable wavelength locators. Single wavelength indicators measure the samples retention of a solitary wavelength, while variable wavelength identifiers measure ingestion of numerous wavelengths.

The high performances liquid chromatography test was used to rapid detection of melamine from the powder formula about 50 µg/Kg in China (China National Standardizing Committee, 2008a, 2008b). Through this process, it was easy to detect the high levels of melamine from the infant formula instead of low levels of melamine from the infant formula. There several HPLC methods were used to detect the melamine from the infant formula that was quite beneficial for the detection of melamine. To analyze biological matrices, a pharmaceutical preparation HPLC was one of the standard methods.

In this process, to recognize and discrete the melamine from the powder formula symmetry, C18 column is used and mobile phase which contains buffer of citric Acid and sodium 1-octane

sulfonate acetonitrile. A UV detector is used to detect the melamine from the chromatograph at 240nm wavelengths.

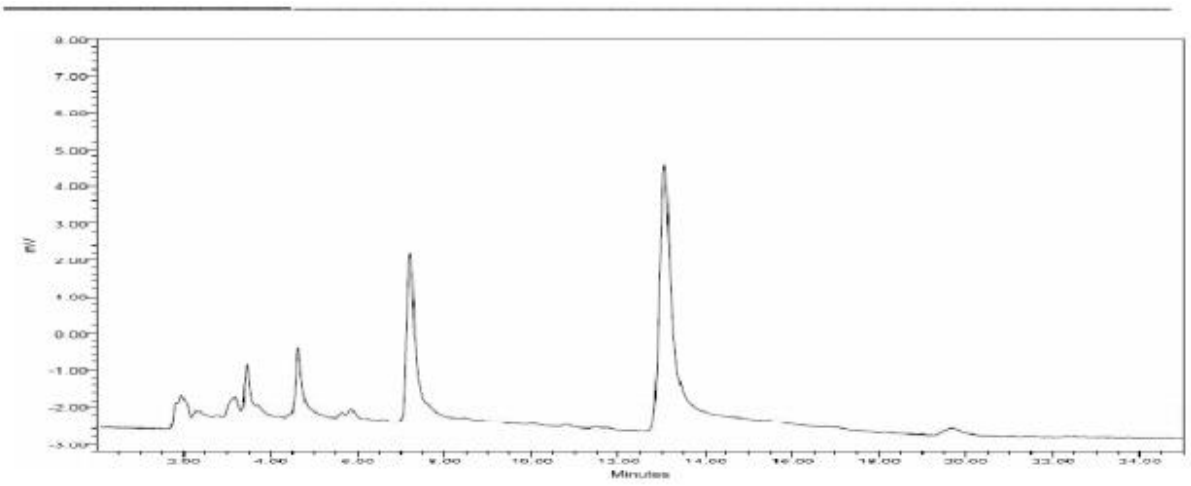


Figure 2.8 Chromatograph of infant formula without melamine contamination in UV detector (Salman et al., 2012).

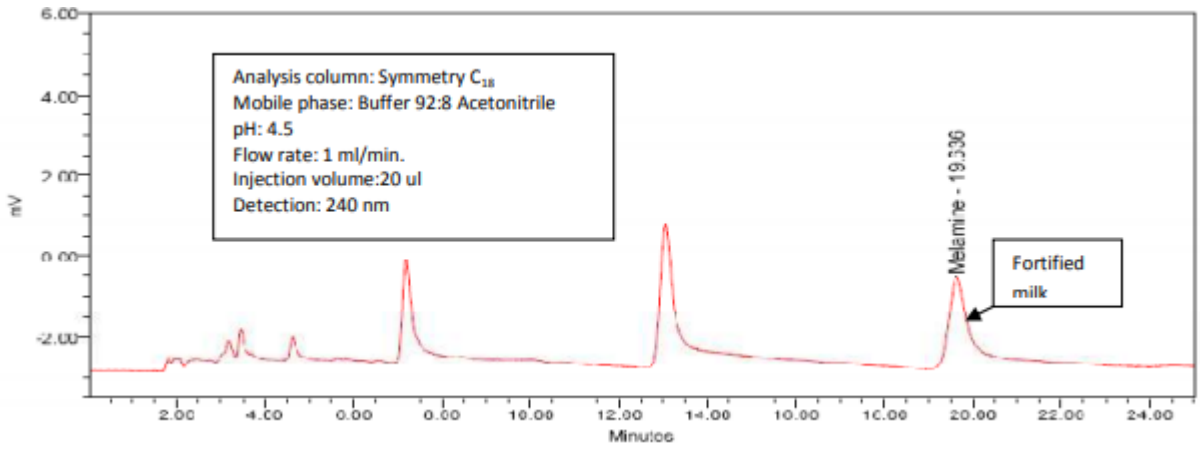


Figure 2.9 Chromatograph of infant formula with melamine contamination in UV detector (Salman et al., 2012).

In first figure, there are no peaks in the retention time of the melamine observed because no melamine was added in the chromatograph. However, in second one, melamine is distinguished in 19.3 minutes in retention time (Salman et al., 2012).

2.7.4 Enzyme kit detection process:

Melamine, which is present in the contaminated food product, can be detected by the use of microbial enzyme known as melamine deaminase. This enzyme causes the hydrolysis of melamine and then transforms it into ammeline and ammonia form. From the figure below, it is easy to visualize that when melamine associates with the enzyme, it will demonstrate larger peak.

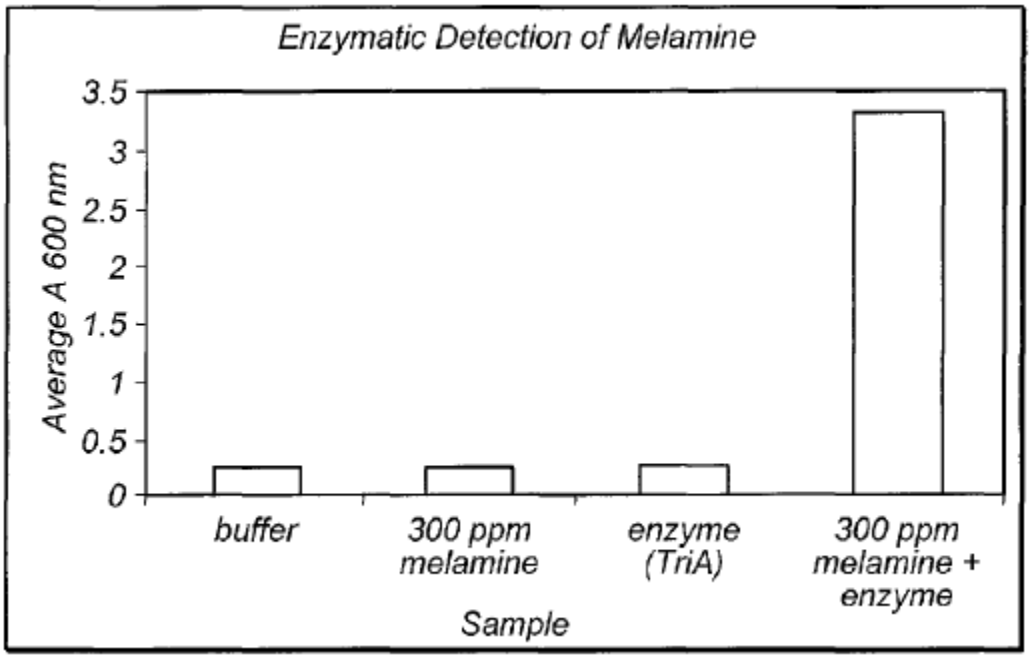


Figure 2.10 Enzymatic detection of melamine (Krebs; 2014).

In this procedure, enzymatic deaminase is used, which is converted in ammonia after chemical reaction. After that, the ammonia detection process detects this ammonia. Generally, more the melamine present in sample, the more of creating color in the mixture of melamine and enzyme kit.

Microbes have the capability to convert the harmful organic chemical substance, which opposes threat for the environment. Some bacteria present in the environment sometimes utilize the melamine and use it as a source of carbon preparation reason of creating positive environment for their growth. One bacterium named *Pseudomonas sp.* transforms melamine into carbon sources for their growth.

MDA enzyme

Melamine + H_2O ⟶ Ammeline + NH_3 (Krebs; 2014)

Due to the presence of melamine deaminase enzyme, melamine hydrolysis happens and then transform into ammeline and ammonia from. The main sources of this melamine is milk, powder milk, cream, ice cream, infant formula and also some pharmaceutical products etc. After that ammonia can be, detect by several kind of method such as using salicylic acid produce indophenol (green blue color at 620nm). (Krebs; 2014)

NH_3 + Salicylic Acid ⟶ Indophenol (green- blue color at 620nm) (Krebs; 2014)

2.7.5 Ion Chromatography with UV detector:

Melamine can also be detected by ion-pairing HPLC method using Acclaim 120 C18 column and other one is HPLC based Acclaim Mixed-Mode WCX column. Both of the procedures are mainly used to separate the cationic and hydrophobic type melamine from the powder milk. (Rhorer et. al., 2009)

2.7.6 ELISA process for melamine detection:

All the process which are described before, are expensive as well as less effective to detect the melamine from the adulterate food product. However, the ELISA process is cost effective and takes less time in comparison to the other one. Sometimes this process shows some inaccurate result due to melamine and enzyme reaction time as well as antibodies may have cross intervention from melamine equivalents. There are three types of immunoassays employed, specifically direct assay, displacement assay and competitive assay. To increase the sensitivity 14-60 times, during this process bovine serum albumin is used which conjugated with the melamine (BSA-MEL).

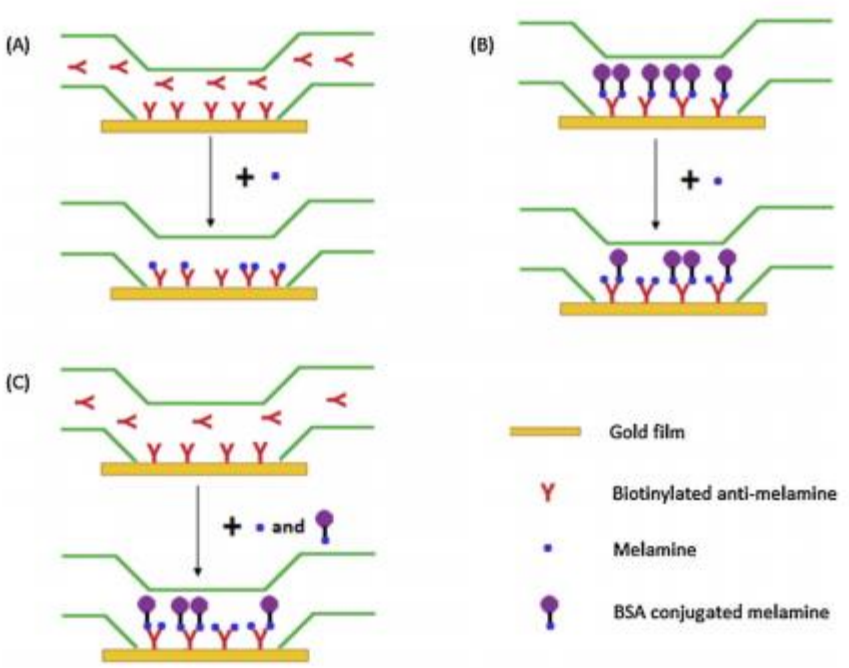


Figure 2.11 (A) Direct assay, (B) displacement assay, (C) competitive assay (Wu, Li, Chua, & Li, 2013)

In case of the simplest method of direct immunoassay, melamine directly binds with the antibodies, which are shown in the figure number (A). In another, competitive assay method, (B) BSA-MEL and melamine are mixed together and binding in the site of sensor. The amount of melamine is reversely proportional to the responsive units (RU), if we keep the amount of BSA-MEL constant. More sensitive method known as displacement assay method, the antibody BSA-MEL mixture mind with melamine when found the melamine in contaminated products. (H. Wu, Li, Chua, & Li, 2013)

In our daily life, we are exposed by melamine from different kind of sources such as food, environmental sources, household things etc. Sometimes, contamination of food is done intentionally and sometimes unintentionally. Some immoral business person contaminates this food by melamine to increase the false protein level, especially, baby food products. After digesting this melamine contaminated food many infants are affected by nephrolithiasis, hydronephrosis, urolithiasis due to exposed by this for a long time. Not only that, it also gives serious effect in central nervous system, digestive system as well as in reproductive system.

Therefore, it is high time to concern about the melamine contamination in food product to get rid of this health hazardous problem.

- Melamine mostly found in Australia's Diploma and Red Cow, Denmark's Dano Full Cream, China's Yashili-1, Yashili-2 and Sweet Baby-2, and New Zealand's Nido Fortified, which are actual well-known brand. (kamol, 2008)
- From the report of the Chinese Ministry of Health, 294 000 newborn children had been influenced by melamine before the finish of November 2008. In excess of 50 000 newborn children have been hospitalized, and six passing's have been affirmed (WHO report 2009).
- Melamine produce nephrotoxicity, kidney and bladder stone of children who consume the melamine contaminated food and sometimes it converted into carcinogen from.
- All in all, in the event that if someone come in contact of melamine containing substance in large time, it is very possible to make renal malfunction, eye and skin irritation, and in addition liver, heart, spleen, planetary poisonous quality.

3. Discussion:

In China 2008, different kinds of food products like powder milk and powder formula were contaminated by melamine poisonous substance. Due to this adulteration, many infants and adults were associated with renal damage, consequently having urinary stone. After this melamine scandal about eight years ago, food adulteration is still a burning issue in different countries. Therefore, this study is made to detect food industry's unethical preparation as well as to find out in what way this toxic substance affects different organs of human body. From the distinctive sort of study, it has been demonstrated that, the harmful substance melamine can causes dangerous effects in human body. It can cause anatomical, cellular and utilitarian changes in various organs of human body. The vast majority of the impact of melamine is predominantly seen in kidney. Some cellular changes happen in kidney because of melamine are, nephrolithiasis, hydronephrosis, urolithiasis. In some cases, it will be the reason of producing malignant cell development in kidney. At that time, death of many infants happened due to exposure by melamine contaminated baby food products. Some helpful approach could be taken, for example: treatment by diuretics, anti-biotic and rehydrate the stone when it is in small size. When the kidney stone is bigger than the normal size, operating method is constantly received. Consequently, to prevent this contamination and identify the poisonous amount of melamine, there are various kinds of detection process applied by the investigators such as HPLC UV, enzyme kit detector, synthetic dye, ion chromatography with UV detector. Most of the techniques gave positive report during the test, because of melamine present in food that exceed from its considerable range. So lastly, it can be said that, to avoid food contamination in food industry, awareness must be created among the general people and some preventive steps must be taken to abolish the poisonous contamination in food products.

4. Conclusion:

Unwanted expansion of substances present in food products ought to be checked from the earliest starting point of food chain thus, to reduce the contamination in food products and downsizing the health adverse effect by taking appropriate preventive steps in health sectors. It has been found that melamine was added to powder milk and food products to raise the protein level erroneously. The harmful effects of melamine mainly detected by different kinds of animal tests as well as available evidence in human body when exposed to melamine such as renal impairment, central nervous system problems and dysfunction in digestive and reproductive system. The foremost effect of melamine toxicity in human body was primarily found in urinary system. Due to this adulteration, many newborns had died and many were hospitalized with serious health issues. To detect the level of melamine contamination in food products there are several kinds of detection techniques such as HPLC, UV, enzyme kit detector, synthetic dye, ion chromatography with UV detector are being more importantly used. According to WHO and other organization the normal TDI of melamine is 0.2 mg/kg, which must be maintained in food product to make it tolerable during consumption of melamine containing food. Setting up this TDI levels in food industry is used to identifying and tracing all the chemical substance from the food products when necessary. All these types of preventive steps can reduce global disaster in health sectors as well as increase the trust issues in general people. So, it is high time to create awareness among the general people about the contamination of food products through the melamine.

❖ Future prospect:

- ❖ To find out the mechanism of cancer, caused by the consumption of melamine contaminated food product, since the clear mechanism of carcinogenic cell formation is still unknown.
- ❖ To assess melamine-drug interactions in different cases, because in some cases melamine showed interactions with the drugs taken for treating different diseases.
- ❖ To investigate whether melamine has any toxicological effect on cardiovascular system or not though the adverse effect on different organ of human body that has already been proved.

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