Research Fund Secretariat, Food and Health Bureau

Summary report on commissioned research studies related to the melamine incident

Key Messages

1. The Hong Kong SAR Government acted promptly to investigate the potential risks to the Hong Kong population resulting from melamine-tainted milk products (MTMP) originating in Mainland China.
2. The Food and Health Bureau commissioned a series of basic and applied research studies following stringent peer review.
3. Ten studies valued at over HK$6.5 million were supported.
4. Taken together, the results of this commissioned research suggest that there were no long-term adverse health consequences for Hong Kong inhabitants who had consumed MTMP.
5. The Hong Kong SAR Government has enhanced measures to ensure the continued safety of imported foodstuffs.

Background

In September 2008, melamine was found in infant formula, and infants in Mainland China were reported to have suffered from kidney stones and kidney failure after consuming such infant formula. By the end of November 2008, the Ministry of Health of the People's Republic of China, reported that 294 000 infants had been affected by melamine-contaminated infant formula, of whom more than 50 000 were hospitalised and six had died.[1]

There was public concern in Hong Kong that melamine-contaminated milk products (MTMP) might also be available locally, posing a potential risk to health, particularly in children. Responding swiftly, the Food and Health Bureau and the Hospital Authority established free screening services at 18 Designated Clinics and 8 Special Assessment Centres on 23 September 2008. The screening services were operated effectively and were able to meet the public's demands. The Designated Clinics had provided screening for 56 847 children before closing on 1 April 2009, due to declining demand, whereas the Special Assessment Centres handled 27 616 cases before closing on 9 April 2009.

A total of 15 children with renal stones suspected to be related to MTMP were reported, two by private doctors and the rest by the Hospital Authority. The notification of the last case was on 13 March 2009; no report was received thereafter.

On 26 September 2008, the Food and Health Bureau established the Expert Group on Melamine Incident, chaired by the Secretary for Food and Health, to address public health and food safety issues arising from the incident.

To address the public's concerns over the incident, the Centre for Health Protection operated a dedicated telephone enquiry hotline from 21 September 2008 to 1 April 2009 to provide an enquiry service for those who suspected themselves or their children of having consumed MTMP. Apart from answering public enquiries on the melamine incident, health advice was also disseminated to the callers when responding to their enquiries. Between 23 and 27 September 2008, the number of enquiries handled by the hotline peaked at over 1000 per day. Thereafter, the number of calls decreased continuously until termination of the dedicated hotline on 1 April 2009.

At the early stage of the incident, very little was known about the health effects of MTMP on humans; most information was based on animal studies and well-publicised overseas cases of melamine-contaminated pet food in 2007. With the development of the incident and accumulation of experience and scientific understanding, a number of studies on the health effects of melamine were published, some from the Mainland and others from local institutions. Despite limitations in methodology and data collection, they provided substantial information about the relationship between exposure to melamine and nephrolithiasis in infants and children.

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Commissioned Research

Given the novel nature of melamine-related disorders, the Group recommended commissioning research studies to assess the potential medium- and long-term health effects associated with melamine exposure, including laboratory studies and basic science research. Invitations to submit proposals for projects were sent to The Chinese University of Hong Kong, Hospital Authority, and The University of Hong Kong on 18 November 2008. Priority areas for research included (1) follow-up studies of affected persons, particularly those at higher risk of adverse health outcomes, (2) laboratory testing of melamine and analogues, and (3) research involving animal models or other basic science.

All eligible applications were evaluated under a two-tiered peer review system comprising expert reviewers and an Assessment Panel. At the Assessment Panel meeting held on 20 January 2009, ten projects were recommended for funding, which addressed each of the three priority areas identified by the Group. The funds granted for the ten projects amounted to over $6.5 million. A summary of the projects is shown at the Table.

Contracts were issued in early March 2009. All projects commenced in March or early April 2009, and interim reports on the progress of each project helped to monitor and ensure the quality of each project.

Follow-up studies of affected persons

In the project MI-FU-08, Lau and Tu conducted a case-control study of Sichuan children (n=44; mean age, 25.7

Table. Summary of commissioned research studies related to the melamine incident

Project no.	Title	Funds expended (total=6 552 984)	Subjects
MI-BS-06	Melamine toxicity in rat foetuses and infants	982 760	Rats (n=10 per group) were treated with a single dose of melamine (21.4 mg/kg body weight)
MI-BS-16	Impact of melamine-tainted milk on foetal kidneys and disease development later in life	1 154 090	Mice (n=6-10 per group) were treated with both melamine and cyanuric acid (40 mg/kg/day) for 3 days
MI-BS-12	Renal and vascular function in pregnant and neonatal rats exposed to melamine and related compounds	808 417	Rats (n=6 per group) were treated with melamine (60, 300, and 600 mg/kg/day) for 3 months
MI-BS-07	Effects of melamine on urine crystallisation kinetics and cell responses	737 578	Human (tubular, gastric) and canine (tubular) cell lines
MI-BS-18	Mechanism of melamine-induced human urinary bladder carcinoma	278 760	Human bladder epithelial cells
MI-LAB-01	Urinary free to complex melamine ratio: a confirmatory test of melamine-induced urinary calculus	0	Urine samples from affected children in Mainland China with ultrasonographic evidence of urinary stones (n=30)
MI-LAB-02	Diagnostic tools for detection of intoxication by melamine and its analogue	846 059	Biological samples from melamine-exposed pregnant women (n=74) and controls (n=78). Urine samples from melamine-exposed, high-risk children (n=302)
MI-FU-08	Case-control study of Sichuan and Hong Kong children with melamine-associated renal stones: renal ultrasonography and urinary IL-8 and MCP-1 levels	462 100	Sichuan (n=44) and Hong Kong (n=22) children with melamine-associated renal stones
MI-FU-04	Prevalence of melamine exposure in Hong Kong children	300 373	Hong Kong school children with elevated urine melamine levels (n=46)
MI-FU-01	Two-year follow-up for children with melamine exposure in Hong Kong: a multicentre study	982 847	Hong Kong children with ultrasound (n=62) or urine (n=321) abnormalities

months) and Hong Kong children (n=22; mean age, 75 months) with melamine-associated renal stones. The number of renal stones in Sichuan children[2] was significantly higher than that in Hong Kong children. Urinary interleukin (IL)-8 (a marker for localised inflammation) concentration was higher in children with renal stones in Sichuan but similar in Hong Kong children with or without renal stones. About 28% of Sichuan and 48% of Hong Kong children still had renal stones at the 1-year follow-up. The authors recommended that Mainland Chinese children with persistent melamine-associated renal stones should have long-term follow-up to monitor possible renal interstitial inflammation and fibrosis. For Hong Kong children, it was unlikely that their renal stones were related to melamine and no significant clinical harm would likely to result from these stones.

In the project MI-FU-04, Kong et al conducted a prospective follow-up study (median follow-up duration, 23.5 months) in Hong Kong school children aged 6 to 20 years (n=46) with elevated urine melamine levels (urine melamine/creatinine ratio, >7.1 μg/mmol). About 9% of Hong Kong school children (n=502) had elevated urinary melamine levels. There was no significant association between milk consumption and urinary melamine level. The Hong Kong school children with high urine melamine levels appeared to have a benign clinical course in the short-term. The investigators suggest that long-term follow-up be conducted on those with persistently high urine melamine levels to identify any significant adverse clinical outcomes.[3]

In the project MI-FU-01, Lam et al conducted a multicentre 2-year follow-up study to investigate the

Summary of results	Publications (as at 30 Nov 2013)
• About 80% of melamine was found in mother's serum after administration of a single dose of melamine to pregnant rats by gavage • Melamine reached the foetuses and the kidneys of neonates through placental transfer, and was later excreted into the amniotic fluid in utero • In lactating rats, about 40% of maternal intake of melamine was transferred to breast milk • Distribution of melamine in all postnatal organs was higher than that in prenatal organs • Postnatal kidneys in early infants had the highest maximum concentration and the lowest clearance of melamine than the other postnatal organs	Chu et al.[5] 2010 Chan et al.[6] 2011 Chu et al.[7] 2013
• 34-45% of the added melamine was transferred to foetal circulation in the ex vivo human placenta perfusion model, but cyanuric acid did not influence such transfer • When the supply of drinking water was normal, no renal stones were formed in the treated mice • When drinking water was restricted, stone formation was observed	Partanen et al.[8] 2012 Peng et al.[9] 2012
• Melamine significantly reduced renal blood flow and impaired renovascular function in renal vasculature and kidney • Melamine-induced renal fibrosis and inflammatory changes • Melamine and cyanuric acid caused nephropathies in neonatal rats and impacted on postnatal rats resulting from exposure during pregnancy	Nil
• Melamine crystallised out from human urine under acidic conditions • Melamine caused the precipitation of other lithogenic salts • Citrate and bicarbonate therapy could reduce melamine crystallisation • Melamine caused physical damage to renal cells and humoral type of immune response	Poon et al.[10] 2012
• Melamine-associated crystals induced apoptosis in bladder epithelial cells, which might contribute to renal failure • Melamine stimulated the growth of bladder epithelial cells by activating mitogen-activated protein kinases • In combination with other carcinogens, melamine could facilitate the cellular transformation of bladder epithelial cells	Nil
• A pilot study showed that the liquid chromatography-tandem mass spectrometry analytical method led to negative values for complex melamine in 30-40% of cases • Pilot data showed that the hypothesised relationship was not useful	Nil
• There was no significant increase of melamine content in different biological samples collected from pregnant women and their neonates with history of low melamine exposure. • Developed protocols and quantified melamine and cyanuric acid in various biological samples from melamine-exposed pregnant women • Introduced neutrophil gelatinase-associated lipocalin as a surrogate marker for the detection of kidney injury in the urine samples from melamine-exposed, high-risk children • Demonstrated co-contamination to be the likely cause of melamine-cyanurate nephrolithiasis	Panesar et al.[4] 2010
• The number of renal stones in Sichuan children was significantly higher than that in Hong Kong children • Urinary IL-8 was higher in children with renal stones in Sichuan but similar in Hong Kong children with or without renal stones • About 28% and 48% of Sichuan and Hong Kong children respectively still had renal stones at the 1-year follow-up	Wang et al.[2] 2011
• About 9% of Hong Kong school children (n=502) had elevated urinary melamine levels • There was no significant association between milk consumption and urinary melamine level • Hong Kong school children with high urine melamine levels appeared to have benign clinical course in the short term	Kong et al.[3] 2011
• No clinically significant differences were noted between children exceeding the World Health Organization melamine tolerable daily intake and those who did not • Renal function parameters were normal in all subjects • No associations were noted between the estimated melamine exposure and the medium- to long-term adverse renal outcomes	Nil

medium- and long-term renal outcomes in children (≤12 years old) with a history of melamine exposure in Hong Kong. In those with ultrasound (n=62) or urine (n=321) abnormalities, no clinically significant differences were noted between children exceeding the World Health Organization melamine tolerable daily intake (TDI) of 0.2 mg/kg body weight and those who did not. Renal function parameters were normal in all subjects. No associations were found between estimated melamine exposure and the medium- to long-term adverse renal outcomes.

Laboratory testing of melamine and analogues

In the project MI-LAB-01, Mak et al conducted a pilot study to determine if melamine complex could be detected in urine samples from affected children in Mainland China with ultrasonographic evidence of urinary stones. Preliminary results showed that the proposed liquid chromatography-tandem mass spectrometry (LC-MS/MS) analytical method was unsatisfactory and no further evaluation was conducted.

In the project MI-LAB-02, Wong et al developed protocols and quantified the trace amounts of melamine and cyanuric acid present in various biological samples (serum, urine, amniotic fluid, breast milk, and placenta) collected from melamine-exposed pregnant women using a LC-MS/MS method. They also introduced neutrophil gelatinase-associated lipocalin (NGAL, lipocalin-2), a biomarker of kidney function, as a surrogate for kidney injury in urine samples from melamine-exposed high-risk children. They also demonstrated that melamine could not be converted to cyanuric acid by mammalian cells, suggesting that renal pathology involving deposition of melamine-cyanurate crystals resulted from the two compounds having been consumed simultaneously.[4]

Animal models and basic science research

In the project MI-BS-06, Wang et al investigated melamine toxicity in foetal and infant rats during and after pregnancy. Their results showed that about 80% of melamine was found in the mother's serum after administration of a single dose of melamine (21.4 mg/kg body weight) by gavage to pregnant rats. Melamine reached the foetuses and the kidneys of the neonates through placental transfer, which was later excreted into the amniotic fluid in utero. In lactating rats, melamine accumulated in mammary glands and about 40% of the maternal intake was transferred to breast milk.[5,6] Distribution of melamine in postnatal organs was higher than that in prenatal organs. Postnatal kidneys in early infants had the highest maximum concentration and the lowest clearance of melamine, compared to other postnatal organs.[7]

In the project MI-BS-16, El-Nezami et al determined the consequences of exposure of mouse foetuses to melamine and cyanuric acid. Their results showed that 34 to 45% of the added melamine was transferred to the foetal circulation in the ex vivo human placenta perfusion model, but cyanuric acid did not influence the transfer.[8] In mice treated with both melamine and cyanuric acid (40 mg/kg/day) for 3 days, no renal stones were formed when the supply of drinking water was normal. However, when drinking water was restricted, stone formation ensued, accompanied by high levels of serum urea, creatinine, urine haemoglobin, and glucose.[9]

In the project MI-BS-12, Wong et al investigated the impact of 3-month oral ingestion of melamine (60, 300, and 600 mg/kg/day) on renal and vascular function in pregnant and neonatal rats. Preliminary data demonstrated that melamine significantly reduced renal blood flow and impaired renovascular function associated with overexpression of three pro-inflammatory markers (TGF-β1, BMP1, and COX-2) within the renal vasculature. Melamine also induced renal fibrosis and inflammatory changes. Their study revealed that melamine and cyanuric acid caused nephropathies in neonatal rats and affected the health of postnatal rats exposed to melamine and cyanuric acid during pregnancy.

In the project MI-BS-07, Ng et al measured melamine and its effects on urine crystallisation kinetics and cell responses. In vitro studies revealed that melamine crystallised out from human urine under acidic conditions (pH 5.0). The presence of melamine caused the precipitation of other lithogenic salts (uric acid, calcium, oxalate, and phosphate).[10] Citrate and bicarbonate therapy significantly reduced melamine crystallisation. Such crystals caused physical damage to renal cells.

In the project MI-BS-18, Yue et al investigated the cellular effects and mechanisms of melamine and its crystals on cultured human bladder epithelial cells. They found that melamine-associated crystals induced apoptosis in bladder epithelial cells, which might contribute to the renal failure in affected subjects. They also found that melamine stimulated the growth of bladder epithelial cells by activating mitogen-activated protein kinases. In combination with other carcinogens, melamine facilitated the cellular transformation of bladder epithelial cells.

Conclusions

The commissioned projects provide a range of findings to better understand the risk to human health posed by melamine and its analogues. The effects of melamine and its analogues are wide-ranging and complex. A variety of experimental methods is needed to determine the potential adverse health effects of melamine and its analogues. Overall, the medium- and long-term health effects of MTMP in the Hong Kong population are considered minor. At least nine high-impact peer-reviewed journal publications have been produced from the research supported by the commissioned programme.

Lessons learned and perspectives

Scientific limitation of animal models

Because of insufficient human data, it is necessary to rely on toxicological studies on laboratory animals to characterise the human health risks related to melamine in food. Humans and most primates do not possess the enzyme urate oxidase, which is responsible for the conversion of uric acid to allantoin in most other mammals.[11] As a result, the melamine dosage needed to produce melamine-uric acid stones in normal rats with normal urate oxidase function may be higher than that in primates. It is therefore important to consider the normal endogenous uric acid concentration when using animal models for risk assessment. It may be informative to conduct studies to better understand melamine toxicokinetics in animal models that reflect uric acid levels in humans, especially neonates. Comparative toxicokinetics in humans and other species would also be valuable.

Need for improved analytical methods

In order to avoid adulteration with fraudulent non-protein nitrogen sources, such as melamine, development of effective and rapid methods for protein analysis that do not include non-protein nitrogen is important. Sensitive detection of real-world food and water contaminants using a portable and automated optofluidic surface enhanced Raman spectroscopy (SERS) microsystem may be useful. It exhibits up to two orders of magnitude improvement compared with conventional microfluidic SERS.[12] Using the optofluidic SERS device, melamine was detected at low concentrations, with an estimated limit of detection of 63 parts per billion. This could lead to highly sensitive and automated sensing systems for on-site detection of food and water contaminants. In addition, a simple and rapid surface-assisted laser desorption/ionisation MS approach also holds great potential for screening melamine in food (with a 5 nM detection limit).[13] Alternatively, a simple and efficient ambient ionisation method based on paper spray combined with tandem MS allows rapid detection and quantitation of various contaminants (eg clenbuterol, melamine, plasticiser, and Sudan red) in various foodstuffs (eg meat, milk, sports drinks, and chili powder).[14]

Choice of exposure measure

Several different biomarkers can be used to predict renal damage following exposure to melamine. It has been reported that renal papillary antigen-1 (RPA-1) may serve as a non-invasive urinary biomarker for the detection and monitoring of obstructive nephropathy associated with melamine and cyanuric acid exposure.[15] Alternatively, a mixture of melamine- and cyanuric acid–induced metabolites (including hydroxyproline) may be useful non-invasive urinary biomarkers for the detection of acute kidney injury, which is potentially associated with kidney fibrosis.[16,17] Further studies are required to understand the mechanism of toxicity and subtle renal alterations induced by subchronic exposure to low-dose melamine or short-term exposure to intermittent high doses. Biomarkers should be sought to evaluate the long-term effects of early-life melamine exposure in humans. Population-level urine tests may be valuable as biomarkers to indicate potential problems prior to stone formation (eg the detection of crystals of melamine-cyanuric acid or melamine-uric acid in urine).

Due to the short half-life of melamine (approximately 3 to 4 hours in mammals), very sensitive methods are required to measure the low level of melamine crystals present in urine. LC-MS/MS and gas chromatography-MS/MS can be used as confirmatory and/or screening methods due to their high selectivity and high sensitivity. However, these high-technology, high-cost methods are not cost-effective screening approaches. Development of more specific low-cost biomarkers and diagnostic techniques is needed.

Apart from nephrotoxicity, neurotoxicity may be induced by acute low-dose melamine, which affects hippocampal synaptic plasticity and behaviour in rats.[18] This selective neurotoxicity of melamine in the hippocampus may be associated with oxidative damage.[19] Further studies are warranted to assess the neurotoxicity of melamine.

Exposure to low-dose melamine in Hong Kong

The World Health Organization has established a TDI of 0.2 mg/kg body weight for melamine. This TDI is applicable to infants. The dietary exposure based on the consumption of MTMP in China was estimated to range from 8.6 to 23.4 mg/kg body weight per day, based on data provided by the Chinese Center for Disease Control and Prevention.[20] This is about 40 to 120 times the TDI, which may explain the dramatic health outcomes in Chinese infants. In contrast, all infant formulas in the Hong Kong market (84 samples) were tested by the Hong Kong Centre for Food Safety and found to comply with the legal standards. Thus, estimated potential exposure of Hong Kong infants to melamine from powdered infant formula containing adulterated milk was well below the TDI.[21]

The estimated melamine intake of the 15 Hong Kong children with renal stones was between 0.09 mg/kg/day and 0.91 mg/kg/day. The widespread and severe outbreak of melamine-related kidney stones observed in Mainland China did not occur in Hong Kong. Results from the Hong Kong screening programme suggest that large-scale and urgent screening programmes may not be informative or cost-effective for other populations who have been exposed to low-dose melamine.[22,23]

Need for continued assessment

In view of the lack of evidence to guide the government initially and the large number of severely affected children in Mainland China, a large-scale territory-wide urgent screening programme was justified at the time. As these

screening programmes showed no obvious medium- or long-term adverse effects from exposure of the Hong Kong population to MTMP, there is no compelling reason for continued follow-up of affected cases.

Research output

Apart from academic outputs, such as journal publications, the impact of the commissioned studies was further evaluated via completion of research payback questionnaires conducted 2 years after the completion of each research project. These allowed better understanding of the translation of research findings into health care service delivery and practice and contribution to health policy formulation in Hong Kong. The principal applicants of all eight projects that were completed as approved submitted a research evaluation questionnaire 2 years after project completion. Six projects (75%) resulted in publication of at least 10 peer-reviewed papers (Table). Five projects (62.5%) resulted in either gain of additional qualifications or career advancement for project team members that could be attributed in part to participation in the research. One project led to an award of additional research funding, and the results of another project led to further research by other groups. Engagement with fellow researchers and health professionals through conferences, seminars, workshops, and the general public through other media were also widely reported.

International collaboration and interdisciplinary approaches

The deliberate adulteration of food products is probably as old as the food processing and production systems themselves. With globalisation and rapid distribution systems, these incidents can have an international impact with far-reaching and sometimes fatal consequences. A well-coordinated and harmonised system to establish standards and regulations is important. The International Food Safety Authorities Network (INFOSAN) is a joint initiative between the World Health Organisation and the Food and Agriculture Organization of the United Nations. This global network includes 177 member states (including Mainland China). Each has a designated INFOSAN emergency contact point for communication between national food safety authorities and the INFOSAN secretariat in urgent events.

Reports of the Expert Group on Melamine Incident

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