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Mini review

Melamine, beyond the kidney: A ubiquitous endocrine disruptor and neurotoxicant?

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ABSTRACT

Melamine is commonly used in a variety of consumer products such as furniture, dining ware, and food utensils. The chemical infamously gained worldwide attention by its illegal addition to a variety of foodstuffs in order to falsify protein content, which led to serious, sometimes fatal, health impacts in children and pets. This resulted in a large amount of published primary studies and reviews of the impacts of melamine exposure on kidney function. However, a growing body of literature suggests that melamine may have impacts beyond renal dysfunction. We conducted a scoping review of this literature which yielded more than 40 studies with human, animal, and *in vitro* findings. Neurological impacts, reproductive function, and anthropometric outcomes were identified as possible candidates for systematic review based on evidence stream and replication of endpoints. The results of this analysis provide a basis for prioritizing future research on health impacts associated with melamine exposure.

1. Introduction

Melamine is a compound commonly used in a variety of common consumer products, such as laminates, resins, adhesives, glues, plywood, flooring, plastic molding compounds, paint pigments, furniture, food packaging, dinnerware, and food utensils (Lu et al., 2009; Hilts and Pelletier, 2008). It is also used as an ingredient in flame retardants, rust removers, metal cleaners, and chemical synthesis (Hilts and Pelletier, 2008; NLM, 2016). Melamine has become more frequently used in food contact items such as cups, plates, bowls, and utensils because they are dishwasher safe, inexpensive, and durable. These products are made from the polycondensation of melamine and formaldehyde, and both compounds have been shown to migrate out of melamine-ware (e.g., bowls, chopsticks, cups, plates, spoons, and tumblers) (Lu et al., 2009; Chik et al., 2011; Chien et al., 2011). When tested under conditions that simulated household uses, melamine and formaldehyde were shown to migrate out of melamine-ware after repeated microwaving and exposure to heat using an acidic food simulant. (Lund and Petersen, 2006; Ishiwata et al., 1986; Sugita et al., 1990) Repeated use likely makes these products an important source of exposure, due to increased probability of chemical leaching and ingestion during food preparation and consumption (Lund and Petersen, 2006; Mannoni et al., 2016).

Melamine is perhaps most well-known for the tragic consequences of its use in food products. Due to its high nitrogen content, analytical techniques identify melamine as an amino acid. Because of this, melamine has been added to wheat gluten, rice protein, and milk products in order to falsely increase the protein content of food commodities (Andersen et al., 2008; Chan et al., 2011). In 2008, melamine was discovered to be the cause of an outbreak of urinary tract stones and renal-failure resulting in illness and death in infants and children in China (Wei and Liu, 2012). Investigations revealed that the compound was added illegally to powdered milk and baby formulas with the intention of falsifying protein content. For example, the chemical was found in Chinese infant formula in concentrations as high as 2563 mg/kg, which far exceeded the 0.2 mg/kg World Health Organization (WHO) maximum tolerable daily intake. (Wang et al., 2014a) In 2007 several pet foods and treats were recalled because the chemical was

Abbreviations: 17β-HSD, 17β-hydroxysteroid dehydrogenase; ACh, acetylcholine; AChE, acetylcholinesterase; CA1, region 1 of hippocampus proper; CAT, catalase; ER, estrogen receptor; fEPSP, field excitatory postsynaptic potentials; GPx, glutathione peroxidase; HFS, high frequency stimulation; HSP70, heat shock protein; Ig, immunoglobin; IL, interleukin; LTD, long-term depression; LTP, long-term potentiation; MDA, malondialdehyde; MCH, mean corpuscular haemoglobin; MWM, Morris water maze test; NR2B, N-methyl D-aspartate receptor subtype 2B; PR, progesterone receptor; OHAT, Office of Health Assessment and Translation; PSD95, postsynaptic density protein 95; ROS, reactive oxygen species; SOD, superoxide dismutase; SYP, synaptophysin; WHO, World Health Organization

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found to be a contaminant in wheat gluten used in those products (Burns, 2007; Rumbeiha and Morrison, 2011; Brown et al., 2007). According to a US survey, this resulted in health effects and death in pets in 35 states, Puerto Rico, and in four Canadian provinces. (Rumbeiha et al., 2010)

Melamine has also unintentionally become an agricultural food contaminant due to the use of the insecticide cyromazine. (Hilts and Pelletier, 2008) Cyromazine inhibits chitin synthesis and is commonly added to animal feeds to control flies in manure (Keiding, 1999; EPA, 2013). Additionally, cyromazine is used as an insecticide on beans, celery, onions, tomatoes, and leafy vegetables (EPA, 2013; Yokley et al., 2000; Li et al., 2011), and in plants, cyromazine is taken up by the roots and leaves (Karras et al., 2007). Not only can cyromazine undergo degradation to melamine in the environment, once ingested, it can also be converted to cyanuric acid and melamine *in vivo* (EPA, 2013; Li et al., 2011; Arnold, 1990). Recently, Rairat et al., demonstrated that cyromazine given to laying chickens at 10 mg/kg peaks in plasma at 3h and declines to undetectable levels at 24h post-administration. However, following 14d of administration, cyromazine remained in the liver for up to 3d and melamine accumulated in the liver and kidneys (but not muscle) at lower exposure levels (5 mg cyromazine/kg body weight) (Rairat et al., 2016). In studies evaluating melamine in the food chain, the compound has been shown to accumulate in the muscle of livestock animals (Sun et al., 2011; Battaglia et al., 2010; Shen et al., 2010; Lv et al., 2010; Yang et al., 2011; Bai et al., 2010; Qin et al., 2010; Wang et al., 2014b) and in crops such as corn, soybeans, and wheat (Qin et al., 2010), and has been detected in chicken eggs (Rairat et al., 2016; Yang et al., 2011; Bai et al., 2010). Studies have also shown that melamine accumulates in the milk of cows and goats following exposure to melamine containing feed (Sun et al., 2011; Battaglia et al., 2010; Shen et al., 2010; Baynes et al., 2010; Cruywagen et al., 2009). The compound has been detected in various food products, including several dairy products, processed frozen foods, eggs and dried egg products (Gossner et al., 2009), shrimp and fish (Andersen et al., 2008), and vegetable products (Tittlemier et al., 2010). This evidence indicates that melamine can accumulate in a variety of commonly consumed livestock and crops via melamine contaminated feed and cyromazine treated feed and crops.

Studies examining the pharmacokinetics of melamine in animal models have shown that the excretion of the compound depends on the species. In chicken eggs, melamine has been shown to reach undetectable levels (in whites) after 2d of withdrawal, following 14d of exposure to cyromazine and melamine at 5 mg/kg (Rairat et al., 2016). In contrast, melamine remained in pig muscle after 5d of withdrawal from 1000 mg melamine/kg given for 42d (Wang et al., 2014b). Pharmacokinetic analysis of the half-life of melamine in pigs ranged 3.94-4.07h following intravenous dosing (Baynes et al., 2008) and 9.90h after exposure via feed (Wang et al., 2014b). Further, Wang et al., modeled clearance from blood in pigs, and showed that complete clearance occurred after 92h following long-term high dose exposures (Wang et al., 2014b). Chu et al., found that doses of melamine (equivalent to doses detected in local diary food products) administered orally to pregnant rats had a half-life ranging 1.85-2.70h and 1.29-4.26h in pups, with longer half-life being observed in the youngest pups (Chu et al., 2013). Similarly, in non-pregnant female and male rats, half-lives were 1.92h and 1.62h, respectively (Jacob et al., 2012). In contrast, Xue et al., found the half-life of melamine in the plasma of rainbow trout to be 32.23h following exposure to a single dose at 5 mg/kg (Xue et al., 2011). In goats, a single dose of melamine at 40 mg/kg had a half-life of 11.12h and models indicated that concentrations would fall below detection limits after 120h (Baynes et al., 2010). These observations suggest that melamine exposure may, in fact, be occurring continuously due to estimations of rapid half-life and clearance time in animals like pigs, chickens, and rats combined as well as the ubiquity of the compound in food products.

It is not completely understood how these pathways contribute to human exposures. Melamine does not appear to undergo biotransformation, is excreted primarily via urine, (Kong et al., 2011; Mast et al., 1983) and has been detected in human urine. (Panuwet et al., 2012; Wu et al., 2015) In experimental models melamine has been shown to accumulate in the uterus, testes, liver, stomach, and spleen in rodents. (Sun et al., 2016) In an *ex vivo* model using human placenta, melamine was able to cross the placenta, was unaltered by placental metabolism, and transferred from maternal to fetal circulation (Partanen et al., 2012). Additionally, in rodent models, maternal exposure leads to accumulation of melamine in milk and the transfer of the compound to serum, amniotic fluid, and fetal tissues (e.g., brain, heart, lungs, kidney, and liver) (Chan et al., 2011; Chu et al., 2013, 2010; Jingbin et al., 2010). These findings suggest that melamine exposure can occur during critical periods of development (i.e., during fetal development) for both animals and humans.

There have been many studies on the health effects of melamine. Outbreaks of nephrolithiasis and kidney damage in children (linked to the consumption of melamine adulterated milk and formula) led to studies assessing the impact of melamine on kidney function (Wei and Liu, 2012). Other studies indicate that melamine can cause health effects beyond kidney damage. However, non-renal effects of melamine exposure, including endocrine disruption, have not been reviewed. A recent review article did discuss the possible mechanisms of melamine neurotoxicity (Yang et al., 2012); however, the article was in Chinese only, and we did not evaluate it.

To understand the breadth of research of the non-renal effects of melamine, we conducted a scoping review. Scoping reviews are commonly used in the preclinical, clinical, and sociology fields (Arksey and O'Malley, 2005; Levac et al., 2010; Colquhoun et al., 2014) to characterize a particular area of research. More recently, they have been applied in the area of environmental health as an important tool in the problem formulation step of the systematic review process (NTP, 2015). Scoping reviews use systematic methodology to map a research area and identify the available evidence in order to pinpoint potential research questions for systematic review. Although they are extremely useful and powerful tools for generating evidence-based research questions, they do not themselves generate answers to research questions (Johnson et al., 2016, 2014; Koustas et al., 2014; Lam et al., 2014). In this scoping review, we reviewed the literature examining melamine exposure, including all effects (*in vitro* and *in vivo*), excluding renal effects. The aim was to identify specific endpoints that could be further explored via systematic review of the effects of melamine. The results of this analysis also identify research gaps, and prioritize further research.

2. Methods

2.1. Literature search and study identification

We conducted a comprehensive literature search to identify studies describing *in vivo* and *in vitro* effects of exposure to melamine. The search included all articles published for all years up to November 2016. Electronic searches were performed in Web of Science and PubMed using the following search criteria: “melamine OR (5-triazine and 6-triamine) OR s-triazine-6-triamine OR 6-triamino-s-triazine OR (triazine* and triamine) OR s-triazinetriamine OR s-triazine-triamine OR triamino-triazine OR triaminotriazine OR triaminotriazines OR triazine-triamine OR triazinetriamine OR cyanuramide OR cyanuric-triamide OR cyanurotriamide OR cyanurotriamine OR cyanuro-triamine OR isomelamine OR isomelamines OR 108-78-1” (the CAS registry number for melamine). For inclusion, the studies had to be primary literature, in the English language, and assess effects of melamine exposure *in vitro* and *in vivo* (excluding renal toxicity). All titles and abstracts were screened for inclusion independently by two reviewers using the software DistillerSR® (Evidence Partners, Ottawa, Ontario, Canada (Evidence Partners, 2015)). DistillerSR is software that allows

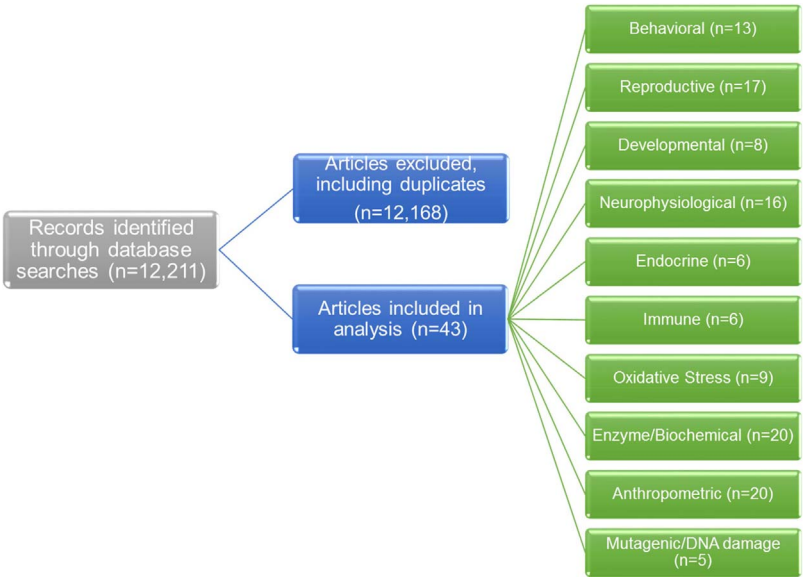

Fig. 1. Flow diagram with endpoint distribution.

for the identification of relevant studies via systematic and consistent evaluation of studies, and automates the process of conflict resolution. Conflicts or discrepancies were resolved by discussion between the two reviewers.

2.2. Data extraction

Data extracted included author information, year of publication, endpoint(s) evaluated, information about the model used (e.g., cell line and species), concentrations/doses tested, exposure duration, age at exposure, and route of exposure. The data were inputted by one reviewer and then quality checked by a second reviewer. Discrepancies were resolved by discussion.

3. Results

Our initial search logic yielded 2849 articles in PubMed and 9362 articles in Web of Science. These records were screened by title and abstract using the inclusion criteria. As shown in Fig. 1, a total of 43 studies were identified as relevant, and these studies underwent full text review.

As shown in Table 1, all included studies were published in 2010 or after. There were 11 studies in mice, 17 in rats, two in fish, two in chickens, and one in humans. Fifteen *in vitro* studies were conducted, primarily in animal cells. The one human study evaluated children exposed to melamine postnatally through early juvenile. Across all studies, many different outcomes were assessed, including neurological, reproductive, developmental, and anthropometric endpoints. Ages of exposure included prenatal, prepubescent/juvenile, pubescent, and adult.

Study characteristics for *in vivo* and *in vitro* studies are shown in Tables 2 and 3, respectively. The human study assessed the developmental (i.e., growth) effects of melamine in children exposed via melamine contaminated powdered milk formula. The animal studies evaluated endpoints such as learning and memory, hippocampal function, development, body weight, enzyme expression and activity (e.g., oxidative stress and hormone synthesis), antibody expression, lymphocyte function, sperm and oocyte parameters, and fertility. The 15 studies that assessed effects using *in vitro* models included neuronal, prostate, ovarian, and other cell lines.

Table 1
Summary of studies assessing the effects of melamine exposure.

	Number (%)		Number (%)
Total Number of Studies	43	Endpoints	
		Reproductive	17 (40)
Publication Date		Developmental	8 (19)
2016 – 2010	43 (100)	Neurophysiological	16 (37)
2009 – 2000	0 (0)	Endocrine	6 (12)
1999 – 1990	0 (0)	Immune	6 (12)
		Oxidative Stress	9 (21)
In vivo Model Used	32 (74)	Behavioral	13 (30)
Human	1 (2)	Enzyme/Biochemical	20 (47)
Animal	31 (72)	Anthropometric	20 (47)
Chicken	2 (5)	Mutagenic/DNA Damage	5 (12)
Fish	2 (5)		
Rat	17 (40)		
Mouse	11 (26)	Age of Exposure for *in vivo* models	
		Prenatal	5 (12)
In vitro Model (Cell Type) Used	15 (35)	Prepubescent/Juvenile	5 (12)
Human	2 (5)	Pubescent	12 (28)
Animal	11 (26)	Adult	19 (44)
Bacterial	3 (7)	Senescent	0 (0)

Percentages were determined by the total number of studies. Some studies included several models/methodologies.

4. Discussion

Studies examining the effects of melamine on non-renal endpoints have increased substantially in the last six years. This review identified three broad endpoint categories: reproductive, anthropometric, and neurophysiological (i.e., neurological and behavioral) as candidates for systematic review based on the current literature. The following is a discussion of the suitability of these endpoints for possible systematic reviews. We also discuss the types of future studies that could improve the body of evidence in order to more effectively use tools, such as the Office of Health Assessment and Translation (OHAT) systematic review framework, to determine the effects of melamine exposure on human health. The OHAT systematic review framework includes protocols to assess the validity of the studies, and integrates mechanistic (e.g., *in vitro*), animal, and human evidence in order to arrive at a human health hazard conclusion. There are many factors that are considered when selecting a topic for systematic review, however, ideally, the body of

Table 2
Studies assessing melamine *in vivo*.

Study	Model	Exposure Duration	Age at Exposure	Route of Exposure	Dose[a]	Endpoints Examined	Results
Abd-Elhakim et al., 2016	mouse	60 days	adult	gavage	50	immune	Exposure resulted in decreased immunoglobulin (IgG, IgM) concentrations. Exposure altered white and red blood cell counts, white blood cell function, and splenic histology.
An and Zhang, 2014b	rat	19 days	prenatal	gavage	400	reproductive; behavioral; anthropometric; neurophysiology	Exposure caused reduced litter size, reduced male:female sex ratio, and reduced hippocampal weight. Exposure also impaired spatial learning and memory in MWM tests: slower escape latency, faster swimming speed, longer path length, reduction in quadrant dwell time, and reduction in platform crossings. There was also a reduction of fEPSP amplitude after HFS of the hippocampus, indicating a reduction in synaptic plasticity.
An and Zhang, 2016	rat	gestation, or 20 days postnatal	prenatal, postnatal	gavage	400	behavioral; developmental; anthropometric; neurophysiology	Exposure caused spatial cognition deficits the MWM and dysfunction of synaptic transmission in the CA1 region of the hippocampus. Prenatal and postnatal exposures caused altered birth weight.
An et al., 2011	rat	4 weeks	pubescent	gavage	300	behavioral; anthropometric; neurophysiology	Exposure caused deficits in learning and memory (via MWM), reduction in body weight, and electrophysiological changes in neurons.
An et al., 2012	rat	4 weeks	pubescent	gavage	300	behavioral; enzyme/biochemical; anthropometric; oxidative stress; neurophysiology	Exposure caused reduced body weight gain, reduced spatial learning/ memory (via MWM), and neuropathological changes (reduced number of hippocampal neurons, morphological changes). Exposure also induced oxidative damage (increased ROS in the hippocampus, but not in other brain regions) and increased MDA.
An et al., 2013	rat	4 weeks	pubescent	gavage	300	behavioral; enzyme/biochemical; neurophysiology	Exposure caused disrupted reversal learning ability and disrupted electrophysiology in vitro. Exposure also decreased ACh and increased AChE in the hippocampus.
An and Zhang, 2014a	rat	28 days	pubescent	gavage	300	behavioral; anthropometric; neurophysiology	Exposure resulted in longer escape latencies, increased path lengths, and reduced platform crossings and quadrant dwelling times in MWM. There were also alterations in LTP and LTD, both functions of synaptic plasticity which may underlie the cognitive deficits in MWM performance.
An et al., 2015	rat	28 days	adult	oral	300	behavioral; enzyme/biochemical; oxidative stress; anthropometric; neurophysiology	Exposure resulted in reduced weight gain. In MWM tests, exposure decreased platform crosses and time spent in the quadrant with the platform. Histopathological analyses showed that the exposed group had more degenerated neurons, increased expression of oxidative stress, and markers of apoptosis in the hippocampus.
Chang et al., 2014	mouse	1 day and 5 days	adult	gavage	30, 140, 700	reproductive; endocrine; enzyme/ biochemical; cytotoxcity	Exposure induced sperm abnormalities and apoptosis in the seminiferous tubules, as well as a loss of vimentin filament staining in the sertoli cells, indicating collapse of the cytoplasmic skeleton.
Chu et al., 2015	rats	gestation	prenatal, adult	gavage	12.5, 25, 50	developmental; neurophysiology; reproductive	Exposure caused disruptions in crown rump length, number of somites, and brain development, as well as other developmental parameters.
Dai et al., 2015a	mouse	8 weeks	pubescent, adult	oral	10, 50	reproductive; enzyme/biochemical; oxidative stress	Exposure increased ROS as well as expression and activity levels of antioxidant enzymes (GPx and SOD) in oocytes. Histological evaluations showed increased atretic follicles and decreased numbers of normal oocytes.
Dai et al., 2015b	mouse	8 weeks	pubescent, adult	oral (drinking water)	10, 50	reproductive	The highest dose reduced *in vitro* fertilization rates. Exposure decreased the expression of Juno a receptor that permits the fusion of the egg and sperm. There was no effect on exocytosis of ovastacin or the zona pellucida.
Duan et al., 2015	mouse	8 weeks	pubescent, adult	oral (drinking water)	10, 50	reproductive; enzyme/biochemical; cytotoxicity	Exposure decreased ovary weights, polar body extrusion, and increased abnormal oocytes and apoptosis. Exposure also altered epigenetic modifications and decreased the number of offspring in females exposed during pregnancy.
Fu et al., 2016	rats	28 days	adult	gavage	300	behavioral; enzyme/biochemical; oxidative stress; neurophysiology	Exposure caused decreased cognitive performance in the MWM, and decreased LTP slopes (which can impact effective learning). Exposure also decreased the expression of PSD95, NR2B, and SYP. Exposure increased oxidative stress and markers of apoptosis in hippocampal neurons.
Kim et al., 2011	rat	15 days	prenatal, adult	gavage	200, 400, 800	developmental; anthropometric; endocrine; reproductive	Exposure in pregnant dams caused a decrease in body weight, decrease in food consumption, and changes in heart and adrenal gland weight. In the

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Table 2 *(continued)*

Study	Model	Exposure Duration	Age at Exposure	Route of Exposure	Dose[a]	Endpoints Examined	Results
							offspring, exposure reduced fetal weight and increased the incidence of skeletal malformations.
Phromkunthong et al., 2015a	fish	12 weeks	juvenile	oral (diet)	10 g/kg feed	behavioral; development; anthropometric; enzyme/biochemical; oxidative stress	Exposure decreased growth rates and other metabolic parameters (such as protein utilization). Exposure also increased HSP70 and decreased GPx expression in the liver but had no impact on CAT expression.
Phromkunthong et al., 2015b	fish	12 weeks	juvenile	oral (diet)	10 g/kg feed	developmental; enzyme/biochemical; immune; anthropometric; oxidative stress	Exposure decreased average body weight and growth weight. Expression of HSP70 was increased and GPx decreased in the liver while CAT remained unaffected. Exposure also impacted several blood parameters (white blood cell counts, hematocrit, lysozyme activity).
Son et al., 2014	rat	50 days	pubescent	oral	63	endocrine; enzyme/biochemical; anthropometric; reproductive	Exposure caused an increase in body weight and in thyroid weight. Exposure did not affect testis, epididymal, adrenal, nor liver weights, or alter blood chemistry.
Stine et al., 2014	rat	10 days	prenatal, adult	gavage	1000	developmental; enzyme/biochemical; immune; anthropometric; reproductive	Exposure resulted in increased fetal death, decreased litter size, decreased fetal body weight, increased number of runts, and decreased crown-to-rump length. Exposure also caused altered heart and body weight in adults.
Suchy et al., 2014	chicken	5 weeks	adult	oral (diet)	100 mg/kg feed	enzyme/biochemical; immune; reproductive	Exposure caused an increase in erythrocytes and a decrease in MCH. Further, there was decreased shell strength in weeks 3 and 4 compared to week 0 and 1, indicating eggshells became weaker due to MEL exposure (although this was not compared to controls). Egg yolk weights increased during the experiment. Yolk color was also altered during the experiment.
Sun et al., 2016a	rat	28 days	pubescent,adult	gavage	10, 20, 40	endocrine; anthropometric; cytotoxicity; enzyme/biochemical; oxidative stress; reproductive	Exposure did not alter body, ovarian, or uterine weights, and did not impact estrous cyclicity or alter levels of E2 or P. The highest dose increased atretic follicles and apoptotic cells in the ovary. Exposure also altered the expression of antioxidant enzymes and altered the expression of steroid synthesizing enzymes (17β-HSD I and II).
Sun et al., 2016b	mouse	28 days	adult	gavage	2, 10, 50	endocrine; anthropometric; enzyme/biochemical; reproductive	Exposure did not decrease testes weight however sperm count and leydig cell numbers were decreased sperm abnormalities were increased. Exposure also decreased testosterone levels and altered the expression of steroid synthesizing enzymes StAR, P450scc and 17β-HSD.
Tu et al., 2015	rat	3 days	adult	gavage	500, 1000, 2000	mutagenic/DNA damage; anthropometric	Exposure resulted in reduced weight gain. Exposure did not alter micronuclei in reticulocytes or red blood cells but there were decreases in reticulocytes after exposure to higher doses.
Wang et al., 2014a	human	≥ 1 month	≤ 4 years	oral	n/a	developmental; anthropometric	Children exposed via contaminated milk powder had delayed growth (length, weight, and head circumference).
Xu et al., 2013	rat	4 weeks	pubescent	oral	300	behavioral; neurophysiology	Exposure caused a deficit in learning and spatial memory (via the MWM test), and changes in neural activity.
Yang et al., 2011a	rat	28 days	pubescent	oral	300	behavioral; developmental; neurophysiology	Exposure decreased body weight and caused reduced spatial learning and memory (via MWM test). Exposure also caused changes in electrophysiology of the brain, *in vivo* and *in vitro*.
Yang et al., 2012a	rat	1 h	pubescent, adult	oral	5, 25	behavioral; neurophysiology	Exposure caused neurotoxicity (impaired hippocampal synaptic plasticity), and reduced time to immobility in the forced swim test, indicating depressed behavior.
Yin et al., 2013	mouse	14 days	adult	gavage	2, 10, 50	reproductive; anthropometric; cytotoxicity	Exposure caused (non-quantified) changes in histopathology and ultrastructure of the testes and increased rate of sperm abnormalities. Exposure also increased apoptosis of spermatogenic cells.
Yin et al., 2014b	mouse	30 days, 2 x per day	adult	gavage	2, 10, 50	reproductive; endocrine; enzyme/biochemical; anthropometric;	Exposure in males resulted in increased apoptotic mechanisms in the testes. In females, exposure caused decreased expression of PR and ER-alpha in the ovaries.
Yin et al., 2014a	mouse	30 days	adult	gavage	2, 10, 50	immune; enzyme/biochemical; anthropometric	Exposure increased the expression of apoptotic genes in the spleen and altered the expression rate of CD8+ cells and the CD4+/CD8+ ratio. Exposure did not alter body weight, splenic parameters, or lymphocyte stimulation.
Yin et al., 2016	mouse	once every 2 days for 30 days	adult	gavage	2, 10, 50	immune; anthropometric	Exposure altered IgG expression but did not impact Blimp-1, sIgA or C3. Exposure altered levels of IL-4, IL-6 and IL-10 in Th2 cells. There was no

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Table 2 *(continued)*

Study	Model	Exposure Duration	Age at Exposure	Route of Exposure	Dose[a]	Endpoints Examined	Results
							impact on splenic gata-3 expression.
Zapletal et al., 2016	chicken	40 days	juvenile	oral (diet)	50, 100 mg/kg feed	developmental; anthropometric	Exposure did not affect body weight or survival in chickens.
Zhang et al., 2011	mouse	5 days	adult	oral	400, 800, 1600	behavioral; reproductive; mutagenic/DNA damage	Exposure decreased testes weight, sperm number, and sperm motility, and increased rate of sperm abnormalities.

MWM: morris water maze; fEPSP: field excitatory postsynaptic potentials; HFS: high frequency stimulation; ROS: reactive oxygen species; MDA: malondialdehyde; ACh: acetylcholine; AChE: acetylcholine esterase; LTP: long-term potentiation; LTD: long-term depression; PR: progesterone receptor; ER: estrogen receptor; MCH: mean corpuscular haemoglobin; GPx: glutathione peroxidase; SOD: superoxide dismutase; CA1: region 1 of hippocampus proper; Ig: immunoglobin; IL: interleukin; 17β-HSD: 17β-hydroxysteroid dehydrogenase; CAT: catalase; HSP70: heat shock protein 70; PSD95: postsynaptic density protein 95; NR2B: N-methyl D-aspartate receptor subtype 2B; SYP: synaptophysin.

[a] Dose is in mg/kg/day unless indicated.

evidence would include all three evidence streams and contain similar endpoints evaluated across all studies. It should be noted, however, that inclusion of all three evidence streams is not necessary to determine a hazard classification (NTP, 2015; Rooney et al., 2014).

4.1. Neurophysiological outcomes

There were 16 studies of neurological function and 13 studies that observed behavioral effects following melamine exposure. In several cases, the studies that evaluated behavioral endpoints also included neurological mechanistic experiments that may support the biological plausibility of the behavioral observations. Of the 13 behavioral studies, ten assessed learning and memory across life-stages. The majority (i.e., 15) of the neurological observations evaluated hippocampal function *in vivo* and *in vitro*, including long-term potentiation, long-term depression, neurotransmitter release, and oxidative stress. These studies could biologically support the behavioral observations in the context of systematic review. Due to the robust number of studies in this area, a *meta*-analysis could be used to determine effect estimates for potential learning and memory deficits. In addition, *meta*-analytical techniques could be used to determine sources of heterogeneity (i.e., timing of exposure, sex differences, and experimental design considerations such as blinding), which may influence the significance and estimate of the effect size. Although the neurophysiological body of evidence contains only two of the three evidence streams (i.e., animal and mechanistic), it is a strong candidate for systematic review because similar endpoints are evaluated across the studies and the mechanistic data is highly relevant to the animal data.

4.2. Reproductive outcomes

We identified 17 studies evaluating the potential reproductive effects of melamine. Both female and male reproductive function were examined in the literature, including sperm and oocyte parameters such as sperm count/motility and follicular atresia. Interestingly, the reproductive literature, particularly with respect to oocyte competence, is mixed; some studies show effects while others do not. Observations suggest that melamine exposure affects female fertility, however, the mechanism(s) has not been clearly identified. This is not surprising, given the very recent interest in research in this area. Our search did find several papers that examined the hormonal action of melamine; namely, its effect on hormone levels, steroid receptor expression, and steroid synthesizing enzyme expression (see Table 2). These studies biologically support the reproductive outcomes assessed. A systematic review of the reproductive endpoints would only contain two of the three evidence streams (mechanistic and animal). Additionally, this body of evidence contains many different endpoints, and some of these were not replicated.

4.3. Anthropometric outcomes

Our review identified 20 anthropometric studies, including body weight, body length, and fetal growth. These studies were carried out at several life-stages. Due to many studies in this area, the best endpoint for *meta*-analysis is body weight, and analysis of heterogeneity of these data could potentially identify sensitive life-stages of exposure. This endpoint also contains both animal and human evidence streams, however, there are no relevant mechanistic data.

4.4. Potential areas of future research

Of the three broad categories, neurophysiological outcomes appear to have the most replicated studies, and thus is the most favorable for systematic review. However, human epidemiological studies on melamine exposure and neurodevelopment (especially learning and memory), as well as more mechanistic studies would be extremely

Table 3
Studies assessing melamine *in vitro*.

Study	Model	Endpoint(s)	Concentrations Tested
An et al., 2014	PC12	cytotoxicity; apoptosis; oxidative stress; caspase activity	990 μg/mL
Chu et al., 2015	rat embryo	embryotoxicity	50 μg/mL
Li et al., 2015	*Tetrahymena thermophila*	proliferation; sexual reproduction; genotoxicity; apoptosis; MTT1 expression	1, 2, 3, 4 g/L
Sun et al., 2010	Herring sperm DNA	genotoxicity; reproduction	10^{-4} M
Tu et al., 2015	*Salmonella typhimurium*, CHO	genotoxicity; mutagenicity	62.5–1000 μg/plate; 75–300 μg/mL
Wang et al., 2011	primary hippocampal neurons	cytotoxicity; Ca2+ concentration; caspase activity	156, 312, 625 μg/mL
Wang et al., 2015	PC12	cytotoxicity; oxidative stress; autophagy	99, 330, 990, 1980, 3300 μg/mL
Xie et al., 2015	DNA	genotoxicity	0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5 × 10^{-3} mol/L
Yang et al., 2010a	CA1 hippocampal pyramid neurons	voltage gated sodium current	5 × 10^{-6}–5 × 10^{-4} g/mL
Yang et al., 2010b	PC12 and CA1 hippocampal pyramid neurons	cytotoxicity; transient outward potassium current; delayed rectifier potassium current	5 × 10^{-6}–5 × 10^{-4} g/mL
Yang et al., 2011a	Hippocampal slices	spontaneous excitatory postsynaptic currents	5 × 10^{-5} g/mL
Yang et al., 2012a	Hippocampal slices	field excitatory postsynaptic potentials; synaptic plasticity	50, 250, 500 μg/mL
Yu et al., 2014	PC3	cytotoxicity; Ca2+ concentrations	300 to 2500 μM
Zhang et al., 2011	L02 and CHO	genotoxicity; mutagenicity	8–5000 μg/well; 0.16–4 mM
Zhang et al., 2016	Hippocampal slices	eEPSCs of NMDA receptors; autophagy	5 × 10–6, 5 × 10-5, 5 × 10–4 g/mL

Ca2+: calcium; ROS: reactive oxygen species; MTT1: metallothionein; eEPSCs: evoked excitatory postsynaptic currents; NMDA: N-methyl-D-aspartic acid.

useful for clarifying the effects on human health. Similarly, more mechanistic and human evidence would be helpful for both anthropometric and reproductive outcomes. For the reproductive outcomes, additional research replicating the endpoints would allow for *meta*-analysis, as well as more a complete systematic review of the topic.

Our review revealed several endpoints that were not well-studied with respect to melamine exposure. These included immune, mutagenic/DNA damage, and hematological endpoints. There are also many endpoints that have not been examined at all, such as cardiovascular health, respiratory heath, and metabolic diseases. Although these endpoints and others would be interesting to examine with future experimental studies—in particular, immune effects have been shown to be important when examining endocrine disruption—it is important to keep in mind that research and resources should be carefully allotted for the maximum protection of human and environmental health.

5. Conclusion

This scoping review revealed several studies indicating that melamine has endocrine disruptive and neurotoxic properties. Scoping reviews are an effective research synthesis tool that can determine the best specific topics for future systematic reviews. The summary level data from scoping reviews can also help to prioritize future research efforts. This scoping review highlighted three areas that have sufficient bodies of evidence to complete systematic reviews to determine the potential hazard of melamine exposure in humans: neurophysiological, anthropometric, and reproductive effects. Of these three, the neurophysiological literature is the most robust for systematic review. This area also appears to have sufficient mechanistic research that could be used to support the biological plausibility of the effects on learning and memory, should the relationship be sufficiently compelling. Thus, we recommend a systematic review of learning and memory be conducted to determine if melamine is a hazard to human health.

Disclosure of interest

The authors report no conflict of interest.

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