NTP Research Concept: Melamine/Cyanuric Acid

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Background

Melamine (1,3,5-triazine-2,4,6-triamine, CAS 108-78-1) is a high production-volume chemical that is used extensively by the industry in the preparation of polymers for the manufacture of a broad range of products, including countertops, fabrics, glues, houseware items, and flame retardants ***(1)***.

Cyanuric acid (1,3,5-triazine-2,4,6-triol, CAS 108-80-5), a deaminated derivative of melamine, is a high production-volume chemical that is used in the preparation of herbicides, dyes, resins, and antimicrobial agents, and as a stabilizer and disinfectant in swimming pool water ***(2)***.

In the spring of 2007, upon the sudden illness and death of a large number of cats and dogs, and the volunteer mass recall of pet food by a major manufacturer, the Food and Drug Administration initiated a wide scale investigation on the etiology of the pathology. The intentional adulteration of wheat and rice gluten used in the preparation of pet food with "scrap melamine" was soon identified as the probable cause for the observed toxicity. "Scrap melamine" is a residue from the melamine industry that in addition to melamine contains a variable proportion of a number of oxytriazines, including cyanuric acid. Given the high nitrogen content of melamine and related triazines, it is assumed that the adulteration was conducted with the intent of artificially increasing the gluten nitrogen content and as such its estimated protein content and commercial value.

Further confirmation that the etiology of the toxicity was due to exposure to melamine and cyanuric acid was obtained upon the necropsy of a number of affected animals, where the formation of melamine cyanurate (CAS 37640-57-6) crystals in the kidneys was identified as the probable cause for the observed nephrotoxicity and eventual renal failure.

A bulk of data available in the literature indicates that neither melamine nor cyanuric acid alone pose a significant toxicological hazard in a range of mammal species, with the LD50 values for the individual compounds ranging close to the LD50 for NaCl [$LD50_{rat,oral}$ = 3,161 mg/kg for melamine; $LD50_{mouse,oral}$= 3,296 mg/kg for melamine; $LD50_{rabbit,dermal}$ > 1,000 mg/kg for melamine; $LD50_{rat,oral}$ >10,000 mg/kg in for cyanuric acid, $LD50_{rabbit,\ dermal}$ > 7,940 mg/kg in for cyanuric acid ***(3, 4, 5)***].

There is a wealth of data available regarding the toxicity of the individual exposure to melamine or cyanuric acid in a number of species; however, the available toxicological data for the combined exposure to melamine and cyanuric acid are very limited in regard to the range of doses and number of test animals, and restricted to fish ***(6)***, hogs ***(6)***, cats ***(7)***, and rats ***(8)***. Acute renal toxicity, with formation of tubule-obstructing crystalline structures, was observed in all these models, suggesting a common mechanism of toxicity.

In a noteworthy study ***(8)*** a group of 10 rats was treated by gavage for 3 days with 400 mg/kg each of melamine and cyanuric acid and were sacrificed 24 hours after the last treatment. Acute renal failure was induced in all the animals and extensive crystal formation was observed in the kidneys. Interestingly, studies conducted in rodents with pre-formed melamine cyanurate suggest that the pre-formed salt presents very low toxicity ***(9)***.

These apparently unexpected results may be justifiable by the very low solubility of melamine cyanurate, leading to low absorption of melamine and cyanuric acid in the gastrointestinal (GI) tract of the animals. It has been hypothesized that the formation of melamine cyanurate crystals in the kidney is due to the extremely low water solubility of this compound (2.2 mg/L) in relation to the parent melamine (3,240 mg/L) and cyanuric acid (2,000 mg/L). It is assumed that melamine and cyanuric acid are absorbed in the GI tract, distributed systemically and, for reasons that have not yet been fully determined, precipitate in the kidney to form nephrotoxic melamine cyanurate crystals.

Sources of potential human exposure to melamine and cyanuric acid

- **Feed / food adulteration**

As a follow up to the FDA investigation, it was soon determined that melamine and analogs were present in several lots of feed for animals bred for human consumption (poultry, swine) and for aquaculture fish, thus, introducing the triazines in the human food chain. The available evidence indicates that this contamination was the result of the incorporation of contaminated "scraps" from the pet food industry in the animal feed. These "scraps" contained up to 1,950 ppm of melamine and up to 2,180 ppm of cyanuric acid ***(10)***.

This "spillage" of melamine and related compounds in the human food chain prompted the FDA/CFSAN to conduct an *Interim Melamine and Analogues Safety/Risk Assessment* ***(10)***. Based upon the assumption that melamine and its analogs presented an equal toxic potency, a number of intake scenarios, and the levels of triazines determined in edible tissues of animals given contaminated feed, the FDA determined that in this particular case the consumption of such tissues was "very unlikely to pose a human health risk".

A recently published study provides reasonable evidence that an earlier outbreak of renal failure in dogs in Asia in 2004 was also the result of the contamination of pet food with melamine and cyanuric acid ***(11)***. Furthermore, literature data indicate that in Italy, in the period between 1979 and 1987, the adulteration of meat and fish meal with melamine was a widespread practice, with up to 72% of samples testing positive for melamine, and with levels of contamination as high as 1.9% ***(12)***.

Considering the facts outlined above, it can be concluded that, on a worldwide scale, the adulteration of feed or feed ingredients with melamine and derivatives has been practiced for at least the past 29 years.

It should be emphasized that although the FDA/CFSAN *Interim Melamine and Analogues Safety/Risk Assessment* indicated that the scenario-driven consumption of meat products was "very unlikely to pose a human health risk" ***(10)***, the assessment did not take into account the currently known potent synergistic toxicological effect of melamine with cyanuric acid.

Of particular concern are the facts that it is not uncommon for the food industry to formulate both animal and human products within the same plant and that it was determined that the sacks containing the melamine-contaminated gluten were very poorly labeled. A direct melamine contamination of a human food product was and is an entirely feasible occurrence given this set of circumstances.

Another potential source of human exposure is vegetarian food preparation practices involving high levels of gluten. In such a scenario, the level of intake of melamine and its analogues could be similar to or even slightly higher than the levels that elicit toxicity in animal tests.

- **Other sources**

Other than the occupational exposure, a number of other potential human exposure sources should be considered with regard to melamine and its derivatives:

- Melamine resin, a hard thermosetting polymer made from melamine and formaldehyde, is widely used in the US in the form of kitchenware, including plates, bowls, mugs and utensils. Reports in the literature indicate that some kitchenware based on melamine resin leach considerable amounts of melamine monomer. A migration of up to 2.5 mg melamine/ 100 cm^2 was observed under conditions that simulate an exposure to hot acidic foods ***(13, 14)***.

- Cyanuric acid is an FDA-accepted component of feed-grade biuret, a ruminant feed additive. The additive can legally contain up to 30% of cyanuric acid (and triuret) ***(15)***.

- As previously indicated, cyanuric acid is used as a stabilizer and disinfectant in swimming pool water. Although concentrations between 30 and 50 ppm of cyanuric acid have been recommended, the maximum allowable limits, dictated by state or local codes are typically 100 ppm ***(16)***. A study on the water ingestion during swimming activities in a pool indicates that the average amount of water swallowed by non-adults (under 18 years old) was on average 37 mL and reached 154 mL ***(17)***. Considering this scenario and a cyanuric acid concentration of 100 ppm in a swimming pool, these volumes translate into an ingestion of up to 15.4 mg of cyanuric acid by non-adults. Furthermore, the possibility that non-professionally managed pools and hot tubs may accidentally be treated with much higher concentrations of cyanuric acid should not be disregarded.

Key Questions

The toxicological data currently available indicate that although melamine and cyanuric acid present very low individual toxicities in a range of species, the two triazines seem to present a

powerful synergistic nephrotoxic effect when co-administered to these same species. Pathology data from animals ingesting contaminated pet food and from animals exposed in the laboratory simultaneously to melamine and cyanuric acid indicate that the observed nephrotoxicity stems from the occlusion of the nephron's distal tubules with melamine cyanurate crystals, impairing the normal function of the kidney.

Taking into consideration the information outlined above, it is clear that a number of sources of exposure to melamine and cyanuric acid can be found in the normal daily life of an average individual. Furthermore, the possibility that the human food chain may be indirectly or directly contaminated with these triazines cannot be ignored. This possibility is of particular concern given the fact that in the US an estimated 13% of adults aged 20 or older present physiological evidence of chronic kidney disease ***(18)*** and as such may be particularly susceptible to such an exposure.

On May 24, 2007 the FDA had its *Interim Melamine and Analogues Safety/Risk Assessment* peer reviewed by a panel of 6 external experts. The review panel issued a number of recommendations, including the need to determine the combined toxicological implications of a concurrent exposure of different species to melamine and derivatives, the need to assess the possible occurrence of biomarkers predictive of renal failure upon exposure to melamine and derivatives, and studies to understand better the pharmacokinetics of melamine ***(19)***.

Strategy

The strategy outlined below addresses key questions raised by the *Interim Melamine and Analogues Safety/Risk Assessment* external review panel ***(19)*** and by an FDA interagency panel, as reiterated in the December 3, 2007 Science Board to the FDA (Science Board) meeting ***(20)***

It is proposed to conduct the study in a phased manner, as outlined below.

1. Conduct a pharmacokinetic study in rats with melamine and cyanuric acid when administered individually, in a time-staggered manner as a separate base and acid, simultaneously as a separate base and acid and simultaneously as a pre-formed compound (melamine cyanurate).

   It should be noted that although previous pharmacokinetic rodent studies have been conducted individually with melamine or cyanuric acid, the data stemming from those studies are limited and do not address fully issues such as bioavailability of the individual triazines as the free acid and base ***(21, 22)***. A full toxicokinetic evaluation of the individual triazines and their combinations is essential for the conduct of an integral risk assessment.

2. Based on the results of the pharmacokinetic studies, design and conduct 28-day and 90-day studies in rats to determine the NOAEL of a combined treatment with melamine and cyanuric acid. Additional studies including an evaluation of systemic toxicity and/or specialized organ systems toxicity after long term (chronic) exposure will be considered following an evaluation of the subchronic study data.

3. Investigate the occurrence of metabolomic and proteomic early biomarkers of melamine + cyanuric acid-induced nephrotoxicity, obtainable by non-invasive methods.

4. Investigate the pharmacokinetics and determine the NOAEL of a combined exposure to melamine and cyanuric acid in a miniature pig model deemed to be representative of the human kidney anatomy and physiology.

   The data currently available suggest that the formation of melamine cyanurate in the kidney of animals exposed simultaneously to melamine and cyanuric acid is due to the very poor solubility of this lattice crystal in relation to the respective acid and base. Although the details of the crystallization mechanism remain unknown at the present time, it is reasonable to assume that the process will be affected by factors such as pH, osmolarity, concentration of particular anions or cations, rate of filtration and physical characteristics of the nephron structure. As such, the choice of the animal model that is used to generate human risk assessment data could become crucial.

   The swine kidney is known to be anatomically close to the human kidney and the physiology of digestion in pigs is similar to humans ***(23)***. As such, the miniature pigs should constitute a good model to simulate both the absorption of melamine and cyanuric acid and the urinary disposition of these compounds. Therefore, we propose to conduct a limited study in miniature pigs and confirm the NOAEL value obtained in the rat study and the biomarkers predictive of renal toxicity.

Significance and Expected Outcome

It is critical for the FDA agencies to develop a good basic science understanding of the nephrotoxic potential of the concurrent human exposure to melamine and cyanuric acid.

It is expected that the qualitative and quantitative data stemming from the proposed studies should greatly increase the knowledge base on the combined toxicity of these triazines, and provide the data required to conduct an updated and authoritative estimate of human risk.

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