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Toxicological analysis points to a lower tolerable daily intake of melamine in food

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ABSTRACT

Intensified food safety concern over melamine has prompted national authorities to assess its tolerable daily intake (TDI) for protection of general population including young children. TDI is calculated by dividing a no-observed-adverse-effect level (NOAEL) by a safety factor (SF). Based on appropriate choices of values, the US Food and Drug Administration determined two TDI values in the unit of mg per kg body weight per day as first 0.63 and then 0.063, while the World Health Organization, 0.5 and then 0.2, as a result of increasing the SF values in calculation. We used a similar procedure, with judicious selection of pertinent values, to obtain a TDI of 0.0081. Arguments in support of this lower TDI value were provided to alert the international community.

1. Background

Melamine is now a well known food adulterant and food contaminant worldwide (WHO, 2008a). Its occurrences in the ingredients of pet foods imported from China that resulted in killings of pets and its involvement in the "toxic milk" that caused kidney failure and deaths in infants in China have made melamine a focus of international food safety concern in recent years. In response to this critical situation, the US Food and Drug Administration (FDA)[1] promptly assessed a tolerable daily intake (TDI) value for melamine (FDA, 2007) using the data from a selected animal toxicity assay conducted by the National Toxicology Program (NTP) of the US Department of Health and Human Services (NTP, 1983). The TDI is defined as "the estimated maximum amount of an agent to which individuals in the population may be exposed daily over their lifetimes without appreciable health risk". Other national food safety authorities have acknowledged the TDI value given by FDA (2007), which was 0.63 mg per kg body weight per day (mg/kg bw/d), to derive their own appropriate TDI values. For example, the World Health Organization (WHO) set a TDI of melamine at 0.5 mg/kg-bw/d (WHO, 2008b) corresponding to the value of FDA. More recently, FDA revised and lowered its TDI to 0.063 mg/kg bw/d (FDA, 2008), but WHO, in a meeting of experts held in early December 2008 (WHO, 2008a), set a new TDI of 0.2 mg/kg-bw/d to be applied to "the whole population including infants".

2. TDI estimation by FDA

The procedure by which FDA determined its TDI value was briefly given as follows. A "no-observed-adverse-effect level" (NOAEL) of 63 mg/kg bw/d was selected from a single data point of a 13-week toxicity assay, in which six groups of 10 young male rats were fed diets containing different levels of melamine (NTP, 1983). Formation of bladder stones, or calculi, in animals was taken as toxic endpoint. The pertinent data are summarized as in Table 1.

Note that at the dose level of 63 mg/kg bw/d, the observed response was 10% greater than that of control, but nonetheless this single dose level was taken by FDA as NOAEL, which was then divided by a safety factor (SF) of 100 to obtain the TDI of 0.63 mg/kg bw/d. The SF of 100 accounted for extrapolation from rats to humans and variation within humans. More recently, a SF of 1000 was used by FDA to lower the TDI to 0.063 in view of additional uncertainties associated with a new exposure scenario of primary concern and the severity of effect in young children (FDA, 2008). These uncertainties include "the contaminated product being the totality of caloric exposure (such as milk) for some infants whose renal systems are not yet fully developed, the exposure being chronic over months, and the exposure being direct (through infant diets) not mitigated by previous passage through the digestive system of an animal (such as edible animal tissues)" (FDA, 2008). A conservative exposure scenario was used to calculate the "safe" level of melamine in solid food (SLM). In this scenario, it was assumed that an average person weighing 60 kg eats 3 kg of diet a day, of which 1.5 kg is solid food contaminated with melamine.

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[1] *Abbreviations used:* BMD, benchmark dose; BMDL, lower limit of BMD; $BMDL_5$, BMDL for 5% extra risk level; $BMDL_{10}$, BMDL for 10% extra risk level; BMR, benchmark response; EPA, US Environmental Protect Agency; FDA, US Food and Drug Administration; GFR, glomerular filtration rate; LOAEL, lowest-observed-adverse-effect level; NOAEL, no-observed-adverse-effect level; NTP, US National Toxicology Program; POD, point of departure; SF, safety factor; SLM, safe level of melamine in solid food; TDI, tolerable daily intake; WHO, World Health Organization.

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Table 1
Incidence of bladder stones formed in male rats in a 13-week feeding study (NTP, 1983) selected for this BMD analysis.

Level in diet (ppm)	Dose received (mg/kg bw/d)	Incidence
0	0	1/10
750	63*	2/10
1500	126	5/10
3000	252	7/10
6000	502	9/10
12,000	1000	9/9

* Taken as NOAEL by FDA (2007).

The SLM corresponding to the TDI was calculated to be 2.52 ppm by the following equation,

$$\mathrm{SLM} = 0.063\ \mathrm{mg/kg\ bw/d} \times 60\ \mathrm{kg\ bw} \div 1.5\ \mathrm{kg\ diet/d} = 2.52\ \mathrm{mg/kg\ diet (2.52\ ppm)}. \quad (1)$$

Eq. (1) gives the conversion factor between TDI and SLM to be 40.0 kg bw-d/kg diet, i.e.,

$$\mathrm{SLM(mg/kg\ diet)} = 40.0\ (\mathrm{kg\ bw - d/kg\ diet}) \times \mathrm{TDI\ (mg/kg\ bw/d)}. \quad (2)$$

3. Benchmark dose analysis

A close examination of this assessment procedure revealed that the TDI of 0.063 mg/kg bw/d so derived by FDA to guard the food safety of an average person against melamine intoxication may not be statistically sound nor sufficiently conservative, because there is a finite probability that the dose at 63 mg/kg bw/d in Table 1 could be the "lowest-observed-adverse-effect level" (LOAEL) rather than NOAEL. Furthermore, the selection of a single dose level at 63 mg/kg/d for TDI calculation ignores the information implied in the whole set of data in Table 1 which show a strong dose–response relationship of bladder stones incidence over a broad range of dose levels.

This shortcoming can be improved by taking the "benchmark dose (BMD)" approach, which was established by the US Environmental Protect Agency (EPA) to incorporate statistical information involving the entire set of toxicity data to determine the "point of departure" (POD) (EPA, 2005, 2006). POD is defined as the lowest dose–response point that marks the beginning of a low-dose extrapolation without undue assumption about the shape of the dose–response curve up to that point. The POD is then used as NOAEL in the calculation of TDI as described above. In practice, POD is usually determined as the dose corresponding to the benchmark response (BMR) of 5% extra response or to the BMR of 10% extra response on the upper 95% confident interval of a dose–response curve fitting the entire set of toxicity data with linearity at low doses (EPA, 2005). The values of POD, also known as the lower limits of BMD (BMDL), as determined from data in Table 1 by the eight built-in mathematical models of the BMD software, BMDS2.0 (EPA, 2008), are tabulated in Table 2, excluding the data point of the highest dose level of 1000 mg/kg/d at which the response was 100%. We considered it appropriate to exclude this data point because the 100% response could occur at a dose lower than 1000 mg/kg bw/d but greater than 502 mg/kg bw/d. Inclusion of this data point having 100% response will result in under-estimation of risk. As discussed later, the EPA guidelines (EPA, 2005, 2006) suggest that only dose–response models that are linear at low doses be used for determining POD. Note that models 1–4, which were linear at low doses, give BMDL values for this set of data within a narrow range, and are considered appropriate for this analysis. It is interesting to note that use of the other models can result in either lower, or higher, POD estimates. Thus, BMDL for 5% extra risk level ($BMDL_5$) and $BMDL_{10}$ values are determined to be 8.04–8.13 (av. 8.09) mg/kg bw/d and 16.5–16.7 (av. 16.6) mg/kg bw/d, respectively. Since the smallest observed response for the NTP study is 10% over the control group (Table 1), and there was strong linear dose-dependence of the response, it is reasonable to assume that even at 5% extra risk level, a good dose–response relationship would still hold and hence $BMDL_5$ can be regarded as the POD of the dose–response curve, or NOAEL for TDI calculation. This assumption is substantiated by the value of $BMDL_5$ (8.09 mg/kg bw/d) being close to one half of $BMDL_{10}$ (16.6 mg/kg bw/d).

Using this statistically determined $BMDL_5$ as NOAEL and going through the same calculation procedure described in Section 2, a TDI for melamine and its corresponding SLM in diet for an average person of 60 kg body weight were calculated to be 0.00809 mg/kg bw/d and 0.324 ppm (or mg/kg diet), respectively:

$$\mathrm{TDI} = 8.09 \div 1000 = 0.00809\ \mathrm{mg/kg\ bw} \quad (3)$$

$$\mathrm{SLM} = 40.0 \times \mathrm{TDI} = 0.324\ \mathrm{mg/kg\ diet (0.324\ ppm)}. \quad (4)$$

4. Vulnerability of young children

Note that this new safe concentration of 0.324 ppm derived in this study to guard the food safety of an average person against melamine intoxication is only for 60 kg adults. It may not be adequate for guarding the safety of dairy products for children and infants for two reasons. Firstly, dairy products constitute a greater proportion in the diet of young children than in the diet of adults, and secondly, the sensitivity of children under 5 years old to melamine toxicity is likely greater than that of adults in view of lower

Table 3
Age-specific GFR as fraction of 25-year old adult value*.

Age	Male	Female
1 month	0.126	0.134
3 month	0.145	0.152
6 month	0.171	0.178
1 year	0.209	0.218
5 year	0.336	0.364
10 year	0.556	0.619
15 year	0.841	0.907
25 year	1	1

* Taken from Haber et al. (2005). Mean GFR for 25-year old is 123.39 (Clewell et al., 2002).

Table 2
BMDL values calculated by the software BMDS2.0 from the data shown in Table 1.

Model name	Gamma	Multi-stage	Weibull	Quantal-linear	Logistic	Log-logistic	Probit	Log-probit
Model number	1	2	3	4	5	6	7	8
BMDL10*	16.8	16.6	16.7	16.5	39.6	11.5	40.9	29.1
BMDL5*	8.16	8.08	8.13	8.04	20.7	5.46	21.1	20.2

* BMDL10 and BMDL5 (in mg/kg bw/d) represent respectively the POD's for 10% extra risk level and 5% extra risk level on the upper 95% confidence interval of the dose-response curve fitting the entire data points in Table 1, excluding the data point giving 100% (9/9) response.

Table 4
Differences in the values used and obtained in calculations among the three studies presented in this paper.

Data & parameters	WHO (2008a)	FDA (2008)	This study
Data used	Two 13-week rat studies	One selected 13-week rat study	Same as FDA
BMDL10 (mg/kg bw/d)	35	Not given	16.6
BMDL5 (mg/kg bw/d)	Not given	Not given	8.09
NOAEL used for TDI estimation (mg/kg bw/d)	35	63	8.09
Safety factor used for TDI estimation	200	1000	1000
TDI estimated to one significant number (mg/kg bw/d)	0.2	0.06	0.008

glomerular filtration rate (GFR) for melamine in children than in grown adults (25 years old) as shown in Table 3 (Haber et al., 2005). For a chemical such as melamine, which is relatively highly absorbed, widely and evenly distributed throughout the body, very poorly metabolized, and completely excreted in urine, the renal clearance that depends largely on GFR would drive the steady state concentration in the blood. Thus the steady state concentration of melamine in the blood of children under 5 years old can be three times or more of that in adults based on data in Table 3. More detailed pharmacokinetic studies are needed to elucidate the fate of melamine in kidney.

Taken together, all the toxicological factors known and the data available to date point to a TDI for children lower than 0.00809 mg/kg bw/d and a tolerable concentration of melamine in dairy products lower than 0.324 ppm. To protect the general population including young children with a singular food safety standard, it is advisable that the standard be set at or below 0.3 ppm in human foods and dietary ingredients.

5. TDI estimation by WHO

At the end of the Ottawa convention of their experts in early December 2008 (WHO, 2008a), WHO issued a report indicating that a similar BMD analysis was performed on the data from two 13-week studies including the same set of data in Table 1 (NTP, 1983). A $BMDL_{10}$ of 35 mg/kg bw/d was obtained which was regarded as an appropriate NOAEL for estimating TDI. A safety factor of 200 was used to assess a TDI of 0.175 mg/kg bw/d,

$$\text{TDI} = 35\ \text{mg/kg bw/d} \div 200 = 0.175\ \text{mg/kg bw/d} \quad (5)$$

WHO rounded the value 0.175 to one significant number and designated 0.2 mg/kg bw/d as the TDI of melamine alone in food. The TDI designated by WHO is 3 times and 25 times, respectively, of the values determined by FDA (0.063 mg/kg bw/d) and by this study (0.00809 mg/kg bw/d). The significant discrepancy among the values determined by the three studies is a result of differences in the choice of NOAEL and SF values used in the calculation procedure, as summarized in Table 4. Part of the difference between WHO and this study also comes from the inclusion of the data point having 100% response in the WHO's BMD analysis, but this same data point was excluded from our analysis in order to avoid possible under-estimation of risk, or over-estimation of TDI, as elaborated in Section 3.

6. Issues on uncertainties

Our choice of using $BMDL_5$ instead of BMDL10 for TDI estimation is in keeping with the guidelines of EPA as elaborated in Section 3. We agree with FDA that the safety factor of 1000 is appropriate in view of the additional uncertainties enumerated by FDA in their revised assessment (FDA, 2008), as well as the uncertainty of using data of a sub-chronic study of 13 weeks for estimating TDI that has a lifetime implication. It is a common practice in health risk assessment that when NOAEL needs to be derived from LOAEL, NOAEL is assumed to be one-tenth of LOAEL (Dourson et al., 1996). Further justification of the greater SF value is in view of limitedness of data available for analysis and the small numbers of animals used in the toxicity assay chosen. The factor of 2, rather than 10, used in the WHO report (WHO, 2008a) to account for the "additional uncertainties" in TDI estimation would need more explicit justifications. Although in the WHO report, it was stated that the TDI of 0.2 mg/kg bw/d they derived "is conservative, as no bladder stones were observed in weanling rats exposed to melamine in the diet at a dose of about 168 mg/kg bw/d for 4 weeks", it is important to realize that a response not seen in a sub-chronic study may appear when sufficient time is allowed for observation.

Studies have shown that about 5% of women and 12% of men in the US will develop a kidney stone during their lifetimes (Coe et al., 2005), and the prevalence has been rising in both genders. This pre-existence condition in humans may have a profound implication on the TDI of melamine if the mechanism of kidney stones induced by melamine is similar to that occurring in the background incidence. It could compromise the protective capacity of a TDI derived from experimental animals that are free of this same condition. This condition represents another uncertainty to be taking into account when the TDI values presented in this report are used for setting up a food quality standard of melamine for human protection.

Based on all the toxicological considerations presented in this report, we cannot support the WHO's conclusion that 0.2 mg/kg bw/d is a "conservative" TDI applicable to "the whole population including infants".

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References

Clewell, H., Teeguarden, J., McDonald, T., Sarangapani, R., Lawrence, G., Gentry, R., Covington, T., Shipp, A., 2002. Review and evaluation of the potential impact of age- and gender-specific pharmacokinetic differences on tissue dosimetry. Crit. Rev. Toxicol. 32, 329–389.

Coe, F.L., Evan, A., Worcester, E., 2005. Kidney stone disease. J. Clin. Invest. 115, 2598–2608.

Dourson, M.L., Felter, S.P., Robinson, D., 1996. Evolution of science-based uncertainty factors in noncancer risk assessment. Regul. Toxicol. Pharmacol. 24, 108–120.

EPA, 2005. Guidelines for Carcinogen Risk Assessment, EPA/630/P-03/001F. Available from: http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=116283).

EPA, 2006. A Framework for Assessing Health Risks of Environmental Exposures to Children, EPA/600/R-05/093F. Available from: http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=158363.

EPA, 2008. Benchmark Dose Software (BMDS). Available from: http://www.epa.gov/ncea/bmds/.

FDA, 2007. Interim Melamine and Analogues Safety/Risk Assessment, May 25. Available from: http:// www.cfsan.fda.gov/~dms/melamra.html.

FDA, 2008. Interim Safety and Risk Assessment of Melamine and its Analogues in Food for Humans, November 28. Available from: http://www.cfsan.fda.gov/~dms/melamra3.html#ftn1).

Haber, L., Hack, C.E., Zhao, J., 2005. Meeting materials for a peer consultation on a draft framework to evaluate whether the default uncertainty factor for human kinetic variability is adequate for protecting children, Draft Framework, TERA. Available from: http://www.tera.org/peer/AdultChildTK/HKAdultChildFramework.pdf.

NTP, 1983. Carcinogenesis bioassay of melamine in F344/N rats and B6C3F1 mice (feed study), NTP Tech. Rep. Ser. No. 245.

WHO, 2008a. Melamine-contamination Event, China, 2008, Report from Expert Meeting to Review Toxicological Aspects of Melamine and Cyanuric Acid, Held in Collaboration with FAO and Supported by Health Canada, Ottawa, Canada, 1–4 December 2008. Available from: http://www.who.int/foodsafety/fs_management/infosan_events/en/index.html.

WHO, 2008b. Melamine and Cyanuric acid: Toxicity, Preliminary Risk Assessment and Guidance on Levels in Food, 25 September 2008—Updated 30 October 2008. Available from: http://www.who.int/foodsafety/fs_management/Melamine.pdf.