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Bioaccumulation of Melamine in Catfish Muscle Following Continuous, Low-Dose, Oral Administration

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ABSTRACT: In this study, catfish muscle was analyzed for melamine (MEL) and cyanuric acid (CYA) residues following experimental feeding with low doses of MEL and MEL and CYA (MEL+CYA) and with the insoluble melamine−cyanurate complex (MEL=CYA). Catfish were daily fed 0.1 mg/kg BW of MEL for 15, 28, or 42 days, 0.1 mg/kg BW of MEL+CYA for 28 days, 2.5 mg/kg BW of MEL+CYA for 14 days, or 400 mg/kg BW of MEL=CYA for 3 days. Residues in the tissue were determined by LC-MS/MS. MEL was extracted with acidic acetonitrile, followed by defatting with dichloromethane, and isolated with cation exchange solid phase extraction (SPE). For CYA analysis, fish were extracted with dilute acetic acid, defatted with hexane, and cleaned up with a graphitic carbon SPE. Catfish fed 0.1 mg/kg BW of MEL reached a maximum muscle residue concentration of 0.33 ± 0.04 mg/kg (ppm) after 28 days of continuous feeding. The same concentration was found for MEL+CYA feeding at the 0.1 mg/kg BW level for 28 days. Feeding at 2.5 mg/kg BW of MEL+CYA yielded muscle concentrations above the 2.5 mg/kg level of concern for most of the study fish. Finally, catfish fed high levels of the MEL=CYA complex (400 mg/kg BW) accumulated relatively little MEL in the muscle (0.14 ± 0.07 mg/kg) and, unlike treatment with MEL+CYA, did not form renal melamine−cyanurate crystals. Appreciable concentrations of CYA were not detected in any of the muscles tested. These studies provide data to model the bioaccumulation of triazine residues into edible fish tissue as a result of the continuous consumption of adulterated feed.

KEYWORDS: melamine, cyanuric acid, fish, dosing study

INTRODUCTION

Tandem episodes of melamine adulteration of animal feeds and food were exposed in 2007 and 2008 after thousands of cases of illness and renal failure resulted from the ingestion of contaminated pet foods and milk products, including infant formula.[1−3] The addition of melamine to food or feed ingredients such as grain, glutens, and milk amplified the apparent protein content of these products and led to the recall of thousands of tons of ingredients, animal feeds, and finished food products worldwide. During this time, melamine contaminations as high as 3200 mg/kg in pet food and 4700 mg/kg in infant formula were reported.[4] Although melamine by itself is not highly toxic, ingestion of melamine in combination with cyanuric acid has been found to cause insoluble melamine−cyanurate crystals to form in and block kidney tubules, leading to renal failure.[5−8] Most risk assessors and the Codex Alimentarius Commission have now concluded that melamine levels below 2.5 mg/kg in foods and feeds, other than in infant formula, are unlikely to pose health risks to consumers.[9] A tolerable daily intake (TDI) of 0.2 mg of melamine/kg of body weight (BW) has been established by both the World Health Organization and the European Food Safety Authority.[10,11] Recent studies have calculated TDIs that are 2−7 times lower on the basis of possible concurrent exposure to both melamine and cyanuric acid.[8]

Melamine is an industrial chemical with widespread use in the production of plastics and resins, adhesives, concretes, and fire retardants. It is also used in plastic dishware and food packaging materials and is a metabolite of cyromazine, a pesticide with approved agricultural and veterinary uses. Therefore, melamine and related triazine compounds are known to be present in the food supply from a variety of sources resulting from contact with food preparation and packaging materials, environmental contamination from industrial manufacturing, and direct application to crops and livestock.[11] Exposure from these nonadulterated sources is unavoidable, yet exposure levels are expected to be below the TDI. Only limited data are available for the bioaccumulation and potential transfer of triazines through the food chain as a result of contaminated animal feed, despite numerous reports[12−18] of animal feed contamination from around the world. In a 2007 survey of seafood products on the market in the United States, melamine residues were found at low levels (0.01−0.24 mg/kg) in 31% of tested products (n = 105).[19] Residues were found in shrimp, tilapia, catfish, salmon, dace, and eel products originating from at least five different countries. Several recent studies have correlated the level of melamine and/or cyanuric acid fed to animals with the resulting concentration found in edible muscle of fish and shrimp,[19−22] ruminants,[23,24] swine,[25,10,26] poultry and/or eggs,[27−30,24,31,32] and milk.[33,34] Despite health advisories and international regulation, there continues to be evidence of melamine adulteration, stockpiling, and relabeling of contaminated food ingredients for

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resale.[35−38] As recently as July 2010, contaminated milk powder that had been recalled in 2008 was resold for use in the production of ice cream.[39] Chinese authorities continued to make arrests and confiscate contaminated milk powder through the end of 2010.[40] Thousands of tons of melamine-contaminated ingredients and finished foods and feeds have been recalled but have not yet been destroyed.[41] Considering the vast quantities of food and feed ingredients and finished products adulterated with melamine worldwide, the bioaccumulation of melamine from contaminated feeds into animal products should be expected.[3]

The muscle concentration or bioaccumulation of melamine into edible catfish muscle from low-dose feeding experiments is the focus of the present work. Four sets of experiments were designed to supplement a larger body of published work by the U.S. Food and Drug Administration on triazine dosing, depletion, and renal crystal formation in fish.[6,19,21,20,42,22] In the first experiment, fish were fed a low daily dose (0.1 mg/kg BW) of melamine for 15−42 days to determine the maximum muscle accumulation. This feeding level is of particular interest as it models the scenario of fish consuming a constant diet of feed contaminated with 2.5 mg/kg of melamine, which is the new CODEX limit for melamine in feeds.[9] The second and third experiments tested the bioaccumulation of triazines in catfish muscle based on daily dosing with either 0.1 and 2.5 mg/kg BW of both melamine and cyanuric acid. These levels represent doses 5 times below and 5 times above the no observable adverse effects level (NOAEL) (0.5 mg/kg BW), a level at which renal crystals were not observed in catfish following daily dosing.[42] Finally, this paper also presents the first report of muscle accumulation and kidney histopathology following oral administration of the insoluble melamine−cyanurate complex.

MATERIALS AND METHODS

Additional details about animal experiments[42] and analytical methods for melamine[19] and cyanuric acid [20] residues in muscle tissue can be found in previously published work.

Chemicals and Reagents. For both standard preparation and animal dosing, melamine (MEL; 99% purity, CAS Registry No. 108-78-1) and melamine−cyanurate (MEL=CYA; product R426555, CAS Registry No. 37640-57-6) were obtained from Sigma-Aldrich (St. Louis, MO). Cyanuric acid (CYA; 98% purity, CAS Registry No. 108-80-5) was obtained from TCI America (Portland, OR) for standard preparation and from Sigma-Aldrich for animal dosing. Deionized water was purified to 18.2 MΩ·cm (Millipore, Bedford, MA). All other chemicals used were of reagent grade or better.

Animal Experiments. This study was approved by the USFDA/CVM/OR Institutional Animal Care and Use Committee. Channel catfish, *Ictalurus punctatus*, were obtained from a commercial source and acclimated for at least 1 month before use. Catfish were fed 1−2% BW per day using a commercial extruded diet containing 35% protein, 8% fat, and 5% fiber (Burris Aquaculture, Franklinton, LA). Catfish were acclimated in individual 60 L flow-through tanks (1 fish/tank) for at least 1 day prior to administration of chemicals. Catfish received doses of melamine alone (MEL), the combination of melamine and cyanuric acid (MEL+CYA), or the melamine−cyanurate complex (MEL=CYA). Chemical doses were transferred into individual gelatin capsules (size 2 or 4, Torpac, Fairfield, NJ; or size 4, Capsuline, Pompano Beach, FL). Quantities of MEL or CYA used in each dose were based on the starting weight of the individual fish. Combination doses (MEL+CYA) were prepared separately and combined into one capsule just prior to dosing. Low-concentration doses of 0.1 mg/kg BW were prepared by dispensing an aliquot of MEL or CYA stock solution (in methanol) into half of the two-piece gelatin capsule and then evaporating the methanol to dryness under nitrogen. Control catfish received an empty gelatin capsule on each day of the study. Capsules were administered to each fish via an intragastric feeding tube or, in the case of the 42 day feeding study, by embedding the capsule within a gelatin feed nugget and observing that the fish swallowed it whole. All commercial feed diets and gelatin capsules were tested using a feed analysis method[43] and found to be absent of melamine and cyanuric acid above the 0.5 mg/kg detection level for that method.

Experimental Design. Specific design details for each experiment are described below. All four experiments shared necropsy and analytical methodologies.

Low-Dose Melamine Threshold Experiments. Catfish were dosed daily with 0.1 mg/kg BW of MEL for 15, 28, or 42 days. Fish were sacrificed 24 h after the final dose. Six catfish were used for each of the 15 and 28 day experiments; two control catfish were used for each of these experiments. Eight catfish were used for the 42 day experiment in case there were any mortalities after the prolonged dosing regimen. The 42 day experiment had 4 control fish; 2 early controls were sacrificed after 3 days, and 2 controls were sacrificed after 42 days of empty-capsule feeding. Actual average melamine doses for 15, 28, and 42 day fish based on necropsy weight were 0.106, 0.113, and 0.098 mg/kg BW, respectively.

Low-Dose (^{1}/5 NOAEL) Melamine and Cyanuric Acid Accumulation Experiments. Six catfish were dosed daily with 0.1 mg/kg BW of MEL and 0.1 mg/kg BW of CYA for 28 days and sacrificed 24 h following the final dose. These fish were dosed concurrently with the 28 day (0.1 mg/kg BW) melamine-only catfish described above and shared the same set of two control catfish. The actual average doses based on necropsy weight were 0.114 mg/kg BW for MEL and 0.114 mg/kg BW for CYA.

Melamine and Cyanuric Acid (5× NOAEL) Accumulation Experiments. Six catfish received nominal doses of 2.5 mg/kg BW of MEL and 2.5 mg/kg BW of CYA for 14 days (actual = 2.512 mg/kg BW). The actual average dose based on necropsy weight was 2.785 mg/kg BW. One control fish was fed empty gelatin capsules. Fish were sacrificed 3 days after the final dose.

Melamine−Cyanurate Dosing. Three catfish were dosed with the MEL=CYA complex at a nominal dose of 400 mg/kg BW for each of 3 days. One control fish was fed empty gelatin capsules. The actual average dose based on necropsy weight was 457 mg/kg BW. Fish were sacrificed 3 days after the final dose.

Necropsy and Kidney Analysis. After the various withdrawal times, fish were stunned with a sharp blow to the head and euthanized by severing the cervical spine followed by double pithing. The skin was removed, and both filets were wrapped in foil and stored at −80 °C until analysis. The caudal kidney was also removed for subsequent histopathology and chemical analysis.[42,44,45] The average body weight at necropsy for all low-dose catfish was 806 g (±271 g, n = 32) and average length, 40 cm (±4 cm); smaller catfish were used for the MEL=CYA dosing experiment and had an average weight of 150 g (±39 g, n = 3) and average length of 25 ± 2 cm.

Analytical Methods. Catfish muscle was analyzed for melamine and cyanuric acid using previously validated and published LC-MS/MS methods[19,20] with method detection levels (MDL) for both compounds in catfish of 0.003 mg/kg (ppm). Analytical procedures are only briefly outlined below.

Muscle Processing. Thawed fish filets were cut into small pieces and blended with dry ice in a blender/homogenizer with pulsed action until contents were uniform and had the consistency of a fine powder. After degassing overnight, the homogenate was stored at −80 °C until analysis. Positive control (fortified at 0.025 and 0.050 mg/kg) and negative control samples were extracted and analyzed with each set of study samples to ensure method performance criteria were met for MEL and CYA residue determination.

Muscle Extraction and Cleanup. (a) Melamine. Catfish homogenate (5 g) was extracted with an acidified solution of acetonitrile/water (50:50 v/v). Following mixing and centrifugation, a portion of supernatant was extracted with dichloromethane, and then the dichloromethane layer was back-extracted with water. Aqueous extracts were combined and cleaned up with a mixed-mode cation exchange SPE cartridge (Oasis MCX, Waters Corp., Milford, MA). The cartridge was washed with hydrochloric acid (0.1 N) and methanol and then dried under vacuum. Melamine was eluted from the cartridge (5% ammonium hydroxide in methanol), evaporated, and reconstituted in mobile phase (95:5 (v/v) acetonitrile/ammonium formate (20 mM)).

(b) Cyanuric Acid. A 5 g portion of catfish homogenate was extracted with an acetic acid solution (0.04% vol), mixed, and heated in a water bath (84 °C). A mixed and centrifuged portion of the cooled mixture was extracted with hexane. The defatted aqueous portion was cleaned up using a graphitic carbon black SPE cartridge (Envi-Carb Supelco, Bellefonte, PA). The cartridge was washed with water, dried under vacuum, and then eluted with methanol. The eluate was evaporated to dryness and then reconstituted in a formic acid solution (0.1% vol).

LC-MS/MS Parameters. A Thermo (San Jose, CA) TSQ Quantum triple-quadrupole mass spectrometer coupled to a Thermo Surveyor LC-MS pump and autosampler was used for both MEL and CYA determination.

(a) Melamine. The LC-MS/MS with electrospray ionization was operated in positive ion mode with selected reaction monitoring (SRM) using previously described parameters.[19] Two SRM transitions of m/z 127 $[MH]^+ \rightarrow$ 85 (collision energy = 7 V) and m/z 127 $\rightarrow$ 68 (collision energy = 23 V) were monitored. The LC column was an Atlantis HILIC silica column (3 μm, 3.0 × 50 mm, Waters Corp.), and the mobile phase program consisted of a binary gradient of acetonitrile and 20 mM aqueous ammonium formate, with an initial composition by volume of 95% acetonitrile decreasing linearly to 50% acetonitrile over 5 min, then returning to and re-equilibrating at 95% acetonitrile. The flow rate was 350 μL/min, and the injection volume was 10 μL.

(b) Cyanuric Acid. The electrospray interface was operated in negative ion mode with SRM using previously described parameters.[20] Collision energies were optimized on SRM transitions m/z 128 $[M - H]^- \rightarrow$ 42 (collision energy = 20 V) and 128 $\rightarrow$ 85 (collision energy = 11 V). A Hypercarb LC column (5 μm, 100 × 2.1 mm, Thermo) heated to 30 °C was used with a solvent gradient of acetonitrile and 0.1% (vol) formic acid in water. The initial acetonitrile concentration was 10%, increasing to 100% over the run, followed by a return to and re-equilibration at the initial conditions. The flow rate was 200 μL/min, and the injection volume was 10 μL.

Detection and Confirmation of Melamine and Cyanuric Acid in Muscle by LC-MS/MS. To positively confirm residues, the retention time of MEL or CYA found in a sample had to match within 5% of that for standards analyzed on the same day, and the relative abundance of the two SRM transitions had to match within 10% of that for the standards.[46]

(a) Melamine. Concentration was calculated from the peak area of the m/z 127 $\rightarrow$ 85 SRM transition using a 7-point calibration curve generated for that transition from melamine solvent standards with concentrations ranging from 0.005 to 1.0 μg/mL (equivalent to mg/kg). Samples with >1.0 mg/kg of melamine were appropriately diluted and reanalyzed.

(b) Cyanuric Acid. Concentration was calculated from the peak area of the m/z 128 $\rightarrow$ 42 transition using a calibration curve generated from five or six cyanuric acid matrix-matched standards with concentrations ranging from 0.010 to 1.0 μg/mL.

Statistical data comparisons were made on the basis of p value calculations (two-tailed t test with equal variance and $\alpha = 0.05$) using Microsoft Excel Data Analysis tools.

RESULTS AND DISCUSSION

The experiments described in this paper were designed to provide supplementary information about muscle residue accumulation in catfish following triazine feeding. Earlier work has focused on high-level dosing (approximately 400 mg/kg BW) of MEL, CYA, and MEL+CYA and on residue depletion following mid-level dosing (20 mg/kg). The high-level studies were intended to model feeds contaminated at similar levels as some adulterated pet foods.[4] In cases when adulterated wheat gluten was used in the manufacturing of fish feeds, data were needed to determine both the toxicological effect high doses of triazines would have on fish[6] and how much MEL and CYA would accumulate in fish muscle following experimental feeding.[19,20] Depletion studies have focused on how long after a single 20 mg/kg BW dose of MEL, CYA, or MEL+CYA would the compounds or melamine−cyanurate crystals persist in the muscles or kidneys, respectively.[21]

In addition to high (400 mg/kg BW) and medium (20 mg/kg BW) dosing studies, which were designed to give fish only one or three daily doses of triazines, it is also important to understand the effect of constant low-dose feeding. For example, in an aquaculture operation, fish might consume a contaminated batch of feed for a prolonged period during production. A daily dose of 0.5 mg/kg BW was recently determined to be the NOAEL, or the level at which renal crystals of melamine−cyanurate were not found following 14 days of dosing with both MEL and CYA in catfish.[42] Bioaccumulation into edible muscle from low-dose feeding experiments near the NOAEL is the focus of the present work.

Low-Dose Melamine Threshold Experiments. The first experiment was designed to study the resulting edible muscle concentration of melamine following daily dosing with MEL alone at a level of 0.1 mg/kg BW for 15, 28, or 42 days. All fish accumulated melamine in the muscle, with average muscle concentration for the three treatment durations ranging from 0.22 to 0.33 mg/kg (ppm). Data from individual catfish are shown in Table 1. Melamine was found in three of the eight control fish from this study; however, concentrations were all at extremely low levels (<0.006 mg/kg). Maximum accumulation levels in treated fish were not yet reached after 15 days of dosing (0.22 mg/kg; p value < 0.001 for 15 vs 28 day dosing). Maximum MEL accumulation was reached after 28 days of dosing (0.33 mg/kg), with no significant additional accumulation occurring after 42 days of dosing (0.29 mg/kg; p value = 0.07 for 28 vs 42 day dosing).

On the basis of a 4% feeding rate, the daily 0.1 mg/kg BW dose is equivalent to the catfish consuming a contaminated feed with a concentration of approximately 2.5 mg/kg of melamine, the level of "no concern" concluded by many risk assessments. Although this dose is below the MEL+CYA NOAEL,[42] it should be pointed out that edible tissues from fish raised on "safe levels" of MEL-contaminated feeds do provide a non-negligible source of melamine exposure to humans.

Low-Dose ($^1/_5$ NOAEL) Melamine and Cyanuric Acid Accumulation Experiments. In addition to MEL alone, catfish were also treated with both triazines at the same 0.1 mg/kg BW low dose. As shown in Table 1, six fish receiving 0.1 mg/kg BW of MEL and 0.1 mg/kg BW of CYA for 28 days had melamine muscle residues of 0.32 ± 0.07 mg/kg (ppm). These values are equivalent to melamine accumulation in fish that received only MEL for 28 days (0.33 mg/kg; p value = 0.80 for 28 day MEL vs MEL+CYA dosing) and suggest that the presence of CYA in feed at low levels does not decrease the bioaccumulation of MEL into edible fish muscle.

Table 1. Melamine Residues in Catfish Filets (mg/kg, ppm) Following Multiple-Day Dosing

0.1 mg/kg BW dose				2.5 mg/kg BW dose
15 day MEL only	28 day MEL only	42 day MEL only	28 day MEL+CYA	14 day MEL+CYA
0.24	0.37	0.30	0.42	3.88[a]
0.20	0.36	0.25	0.37	3.05
0.24	0.26	0.27	0.32	2.44
0.22	0.33	0.28	0.27	2.90
0.20	0.31	0.30	0.25	3.27[a]
0.21	0.32	0.31	0.27	3.58[b]
		0.31		
		0.30		
	Mean			
0.22	0.33	0.29	0.32	3.19
	SD			
0.02	0.04	0.02	0.07	0.51

[a] Trace CYA found in this fish with concentration between 0.003 and 0.009 mg/kg. [b] CYA concentration found was 0.010 mg/kg.

Melamine and Cyanuric Acid (5× NOAEL) Accumulation Experiments. The third experiment was designed to test muscle concentrations of fish that received a high enough low dose of MEL+CYA to produce minor renal effects (5× NOAEL). Dosing catfish at 2.5 mg/kg BW MEL+CYA for 14 days represented a level at which the majority of subject catfish (11 of 12) were found to have class 1 or 2 ("one" or "few", respectively) scattered melamine−cyanurate crystals throughout the kidneys.[42] Muscle analysis was conducted on six of those class 2 catfish (Table 1). The average MEL concentration was 3.19 ± 0.51 mg/kg (ppm). Five of the six catfish had MEL muscle residues above the 2.5 mg/kg level of no concern. CYA analysis was also performed on these samples. CYA was positively identified in three of the six catfish above the MDL, but only one fish had a quantifiable CYA residue level of 0.01 mg/kg (Table 1). Considerably lower CYA accumulation in fish muscle as compared to MEL is consistent with previous studies.[21] Although the melamine accumulation found in these experiments is considerable, the lack of significant CYA muscle accumulation is also important in providing further evidence that fish exposed to relatively low, yet violative, levels of both MEL and CYA in contaminated feed are not likely to provide a simultaneous dietary source of the dangerous combination of both MEL and CYA residues.

Melamine muscle residue determination has been the subject of recent low-dose mammalian and avian feeding studies,[23,24,26−28,31,32] summarized in Table 2. In these studies, animals were provided access to melamine-containing feed, rather than receiving an oral supplement. For comparison to our studies, the 0.1 and 2.5 mg/kg BW oral doses are roughly equivalent to fish daily consuming feed containing MEL (or MEL+CYA) at a level of 2.5 and 62.5 mg/kg, respectively, based on fish consuming 4% of their body weight per day. Although actual experimental dose and duration varied between the studies as detailed, it can be concluded that fish accumulate melamine in muscle tissue to a greater extent than do ruminants and poultry. Allowing for differences in feeding dose and duration, fish muscle concentrations were roughly an order of magnitude higher than muscle concentrations in ruminants, and ≥2 times higher than poultry muscle concentrations. Accumulation in pig muscle was 2−14 times lower than catfish accumulation depending on the feeding level. Qin and co-workers reported similarly high levels of melamine accumulation in trout as compared with other animals.[24,47] This is consistent with fish also requiring more time to eliminate melamine muscle residues compared with other species.[6,21,23,24]

Melamine−Cyanurate Dosing. Muscle concentration and kidney wet mount examination following oral administration of the melamine−cyanurate complex (MEL=CYA) was determined for the first time. Aside from ongoing dosing studies at the National Center for Toxicological Research,[48] there are only a few published animal studies reporting toxicity from direct exposure to complexed melamine−cyanurate.[49,50] In those studies, relatively low toxicity for the complex was observed following intragastric exposure, with an LD_{50} of 4110 mg/kg BW in rats. Muscle concentrations and kidney examinations were not included in those papers. In our study, an average concentration of 0.14 ± 0.07 mg/kg of melamine was found in the muscle from fish given a nominal dose of 400 mg/kg BW of MEL=CYA. In these fish, cyanuric acid was not detected in the muscle, nor were melamine−cyanurate crystals observed in the kidneys with either wet mount microscopic or histopathological examination.

If the melamine−cyanurate complex separated in vivo, the 400 mg/kg BW dose of MEL=CYA would correspond to roughly 200 mg/kg BW of melamine and 200 mg/kg BW of cyanuric acid. For comparison to previous experiments, six catfish given a single oral dose of both melamine and cyanuric acid at $^1/_{10}$ the level of the MEL=CYA experiment (20 mg/kg BW of MEL plus 20 mg/kg BW of CYA), followed by a 3 day withdrawal, had a mean muscle melamine concentration of 2.05 ± 1.18 mg/kg.[21] Five of those six catfish also developed renal crystals. Thus, fish receiving one dose of the separate chemicals (MEL+CYA) developed renal crystals and had 15 times higher melamine tissue residues than fish given three doses of the complex (MEL=CYA) and at 10× the dose. In addition, the 0.14 mg/kg melamine accumulation found in MEL=CYA dosed animals was less than half of the melamine found in the muscles of fish described earlier in this

Table 2. Comparison of Melamine Muscle Concentrations Following Experimental Animal Feeding

animal	MEL orally administered dose (mg/kg BW)	MEL concn in feed or feed equivalent (mg/kg)	dose duration[a] (days)	MEL concn found in muscle (mg/kg)	ref
catfish	0.1	2.5 (equivalent)	42	0.29	this study
trout		2	60	0.20	47
lamb		2	60	0.02	23
lamb		10	60	0.05	23
sheep		2	60	0.02	24
calf		2	60	0.02	24
pig		2	60	0.02	24
chicken		2	40	0.09	24
duck		2	40	0.04	24
duck (laying)		2	40	0.01	24
catfish	2.5[b]	62.5 (equivalent)	14[c]	3.19	this study
trout		20	14	0.83	47
trout		100	60	3.60	24
lamb		30	14	0.08	23
lamb		100	14	0.32	23
sheep		100	60	0.43	24
calf		100	60	0.06	24
cow		100	40	0.42	24
pig		30	42	0.25	26
pig		100	42	0.98	26
pig		100	60	1.20	24
chicken		100	40	1.70	24
chicken		100	42[d]	0.62	28
chicken		200	28	1.80	27
chicken		300	42	5.10	28
chicken (laying)		50	14	0.42	32
chicken (laying)		100	14	0.78	32
chicken (laying)		100	40	0.88	24
chicken (laying)	8.6	125	34	0.40	31
duck		100	40	1.86	24
duck (laying)		100	40	0.10	24

[a] One day or less withdrawal time unless noted. [b] Dosed as 2.5 mg/kg BW of MEL and 2.5 mg/kg BW of C YA. [c] Three day withdrawal time. [d] Seven day withdrawal time.

paper that received a 0.1 mg/kg BW dose (effectively 2000 times smaller dose) of either MEL alone (0.33 mg/kg) or MEL+CYA (0.32 mg/kg). From these experiments, it is clear that the insoluble MEL=CYA complex has much lower bioavailability in fish than MEL and CYA coadministered as separate chemicals or MEL given individually. Because future cases of animal feed contamination or adulteration may include triazines as individual chemical compounds (MEL, CYA) or as the melamine−cyanurate complex, it is important to better understand the bioaccumulation characteristics of the complex.

In summary, these studies provide additional details to help refine melamine exposure models to assess risk to humans. Catfish fed a constant diet of MEL and/or MEL+CYA at the level of no concern (0.1 mg/kg BW; 2.5 mg/kg feed equivalent) accumulate approximately 0.3 mg/kg (ppm) of MEL in the edible tissue. This results in a source of melamine exposure for humans that is comparable to the exposure that results from food contact materials.[11] We also found that a 14 day exposure to 2.5 mg/kg BW MEL+CYA (62.5 mg/kg feed equivalent) is the concentration required to produce levels above the 2.5 mg/kg limit for MEL in edible tissue for the majority of dosed fish. CYA residues at the 2.5 mg/kg BW exposure level were found to be negligible. Finally, we found that in fish, oral administration of even high levels of the MEL=CYA complex does not lead to significant accumulation of MEL or CYA in muscles and does not cause the formation of melamine−cyanurate crystals in the kidneys. In all of the studies reported herein, cyanuric acid had exceedingly low or no accumulation.

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