Received 1 March 2016
Received in revised form 5 May 2016
Accepted 14 June 2016
Available online 17 June 2016
Peer Reviewed
2157118X,6,1,P1

Transfer of Melamine in Food in the Egg of Laying Hen

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Abstract A carry-over study examined the transfer of melamine from the feed in the hens egg. Carried out in a compound, a feeding study on laying hens which with three graduated levels of melamine (2.5; 100 and 400 mg of melamine per kg of feed) had been supplemented, on laying hens kept in group housing was used as feed. the levels of melamine in the eggs were analyzed led each differentiated according to egg white and egg yolk, leading to the derivation of specific transfer council. the investigation period consisted of a 28 day main period (feeding of melamine containing feed) and one up to twenty day follow-up period (backend ütterung control lining without the addition of melamine. the carry over study results were between to a health assessment and discussed in terms of the health and consumer protection.

Keywords: Hen egg, Melamine, Food, Forensic science.

1 Introduction

In 2008, sufferers in china more than 50000 children after consumption of melamine containing infant foods. the pretence a increased article content in the final product, what the milk powder for the manufacture of foods was used, specifically added to melamine. the melaminaufnahme on the infant had at least six infants death due to acute renal failure, tens of thousands of infants wore heavy renal damage (Wei and Liu 2011).

Melamine (1,3,5 - 5-triazine - 2,4,6 - triamin) is a synthetic chemical, 5-triazine based, mainly for industrial use state, for example in the manufacture of plastics, as a shelter or as flame retardants. during the manufacturing process by one to three desaminierungsreaktionen the strukturanaloga ammelin, ammelid and cyanursäure are formed.

Studies have shown that melamine and its strukturanaloga tend to complex formation. dose dependent, it can, while intake of melamine and its strukturanaloga with food both in humans and in animals to nierendysfunktionen and training of kidney stones to death by acute renal failure. a sole ingestion of melamine (without strukturanaloga) is 90% of the ingested melamins within 24 h with the urine (EFSA, 2010).

Since 2007, a number of health assessment of melamine and its strukturanaloga by international bodies (efsa, published in 2007, 2008, 2010: us fda in 2007, 2008; who 2008). since the year 2010, there is a between the world health organization (who) and the european food safety authority behürde harmonised reference value for the lifelong tolerable daily intake (tolerable daily intake, tdi) of 0.2 milligrams (mg) per kilogram (kg) of body weight (bw) and day (d) for the intake of melamine in humans efsa 2010; who 2008). the efsa in its opinion, however, is the fact that the tdi of 0.2 mg / kg bw and d exclusively for the sole inclusion of melamine is applied and for the simultaneous opening of cyanursäure, ammelid, ammelin, or a combination of these substances (efsa, 2010).

Furthermore, the european commission, as a result of the melaminskandals in china in 2008, special measures for the import of milk and milk products containing the origin or the origin of china is adopted (commission decision 2008 / 757 / ec) of 26. september 2008; commission decision 2008 / 798 / ec) 14 october 2008). with the entry into force of regulation (ec) no 1135 / 2009 is a maximum level for melamine of 2.5 mg / kg for the products originating in or coming from china, the milk, dairy products, soy, or sojaerzeugnisse

included a ban on the import of these products and, if the particular erna¨hrungsbedürfnisse of infants and young children in accordance with directive 2009 / 38 / ec are intended. a maximum of 2.5 mg / kg was first in 2011 for feedingstuffs (with a moisture content of 12%) (regulation (eu) no 574 / 2011), and has existed since 2012 on food (regulation (eu) no 594 / 2012). except, in powder infant and follow on formulae ", for which a melamine content of 1 mg / kg was defined.

Since 2008, a large number of studies both on toxicology, as well as the analysis of melamine and its strukturanaloga published in various matrices. this work mainly originated from china. on the website of the food and environmental hygiene department of the centre for food safety in hong kong was a list published in 2008, the first by a detection of melamine in eggs of laying hens have been reported (centre for food safety, 2008). further studies showed that a transfer (carry over) of melamine and its strukturanaloga) from the feed in eggs of laying hens (bai et al. in 2010, chen et al. 2010; gallo et al. 2012; yang et al. 2011), ducks (gao et al. 2010) and quail (zhang et al. 2012) takes place. in dong, et al. (2010) the first, the egg yolk and egg white samples separately analysed and different data depending on the sample matrix. based on the determined transfer rate includes whole eggs with their shell removed was empirically for the transition of melamine in the food in the egg laying hen was 1.2% (dong et al. in 2010).

The results of the present study add to the above studies, a classification in terms of consumer health protection. to this end, the analytical data of health assessments. the present study contributes to the identification and assessment of potential health risks to humans and animals by ingestion of melamine on the consumption of food and animal feed.

2 Materials and Methods

2.1 Major experimental analysis

Each feeding study consisted of three phases: pre -, main - and nachperiode (tab. 1). in a 14 day period the hens received the compound without the addition of melamine (kontrollfutter). the previous period was followed by a 28 day trial period (each main period) in the chickens of group a fodder with 400 mg / kg melamine, the animals of group b with 2.5 mg / kg melamine feed the chickens of group c food with 100 mg of melamine per kg. in a nachperiode received all groups that kontrollfutter (without the addition of melamine. the nachperiode included in group a a period of 14 days in groups b and c a, each of 21 days.

Each hen were presented daily 150 g compound in 12 animals (group a), a total of 1800 g of food equivalent to or in six animals (gruppenb and (c) progruppe 900 grams of food per day. the quantification of the average food intake was the amount of feed consumed by rückwaage once daily (every morning) for the whole group and under the assumption that no significant differences in the individual food intake in the animals were. the average daily feed intake amounted to 120 grams of feed per animal. the futtervorlage was an aliquot of sample together every day and in this the melamine content.

The eggs of all animals were collected daily during the entire experimental period and the marks. As a classification of the eggs to the individual hens was not possible, was the daily analysis for each group. the eggs were stored at 4 °C to homogenization.

2.2 Analytical detection of melamine in the egg whites and yolks of compound matrices.

For the detection of melamine in the egg whites and yolks were compound matrices, different extractions, aufreinigungsschritte and methods used. this was determined by the analysis matrix of the melamine concentration in versuchsfutter or the expected melamine concentration in egg white and yolk, and the derived necessary limit of determination in various matrices.

The detection of melamine in animal feed is done by GC-MS, and the quantification using internal standardization with $^{13}C_3$-melamine was made. this was first measured to confirm the trimethylsilylderivat and four other ions.

The analysis of egg white and yolk samples was done by LC-MS/MS with positive elektrosprayionisation melamine. it was shown wobeidrei transitions of melamine were measured. the quantification was also carried out using internal standardization (for details see supplementary).

2.3 Statistical Analysis

The regression analysis and the calculation of the mean, the median, and the standard deviation was made using microsoft office excel 2003.

3 Results and Discussion

This carry-over study investigated the transfer of melamine from the feed in the hens egg. To a feeding experiment with laying hens was carried in a laying hen feed with three graduated levels of melamine was supplemented. The studies stretched from a high dose (400 mg of melamine per kg of feed) about a medium dosage (100 mg of melamine per kg of food) in the low-dose range (2.5 mg of melamine per kg of food).

The different dosages were chosen to answer different issues in relation to a possible carry over event. The use of melamine in the high-dose range (test series A) was planned as a pilot study for further experiments to answer general questions in relation to a possible transfer. In addition to this was the focus of the test series B and C on the transferability of the results of the pilot study on the transfer of melamine in the low dose range, as well as on the applicability and implementation of feed - and food law regulations.

A melamine supplementation was chosen for test series B No. 574/2011 corresponded to the maximum

Table 1. Experimental design.

Groups	Previous period / Control Period (14 days)	Main period (28 days)	After period (14 or 21 days)
Group A (pilot study) —> 12 animals	Food free from melamine	Melamine-supplemented diet (400 mg melamine / kg feed)	Food free of melamine (14 days)
Group B —> 6 animals	Food free from melamine	Melamine-supplemented diet (2.5 mg melamine / kg feed)	Food free of melamine (21 days)
Group C —> 6 animals	Food free from melamine	Melamine-supplemented diet (100 mg melamine / kg feed)	Food free of melamine (21 days)

Table 2. Mean value (MV) and standard deviation of the melamine amounts [lg absolutely] Melamine amount recorded in the egg white and yolk of the eggs of Group C in relation to the with the feed (compound feed).

Day of the experiment	Middle melamine content in feed [mg/kg = µg/g]	Medium melamine intake per hen per day * [µg absolutely]	Melamine amount (MW and standard deviation) in the egg white [µg absolutely]	Melamine amount (MW and standard deviation) in the yolk [µg absolutely]
Previous period (feed without melamine additive)				
0–14	< LOD	< LOD	< LOD	< LOD
Main period (100 mg of melamine per kg of feed)				
1	63	7560	45.0 ± 2.0	2.7 ± 1.3
2	73	8760	53.7 ± 6.1	3.6 ± 0.9
3	70	8400	56.2 ± 6.8	4.6 ± 1.2
4	70	8400	52.6 ± 10.6	6.6 ± 2.3
5	71	8520	58.0 ± 3.1	6.7 ± 0.5
6	65	7800	53.6 ± 5.2	8.0 ± 1.9
7	75	9000	57.9 ± 13.4	8.8 ± 3.4
8	79	9480	62.0 ± 6.6	9.2 ± 1.7
9	65	7800	56.7 ± 5.4	9.7 ± 1.8
10	75	9000	55.3 ± 6.8	9.3 ± 1.8
11	68	8160	58.0 ± 8.6	9.0 ± 1.2
12	66	7920	59.5 ± 4.5	9.5 ± 1.1
13	70	8400	58.5 ± 7.0	9.5 ± 1.8
14	67	8040	53.8 ± 6.6	10.0 ± 1.5
15	68	8160	59.2 ± 5.7	9.5 ± 0.7
16	69	8280	55.6 ± 8.3	11.4 ± 3.6
17	74	8880	52.4 ± 3.3	9.0 ± 1.0
18	61	7320	50.8 ± 5.5	9.8 ± 1.7
19	56	6720	58.2 ± 6.4	9.7 ± 1.2
20	65	7800	74.5 ± 7.6	11.0 ± 1.3
21	69	8280	57.3 ± 1.9	10.7 ± 1.2
22	93	11160	71.7 ± 7.5	8.1 ± 1.1
23	96	11520	83.1 ± 13.7	9.8 ± 3.8
24	104	12480	73.9 ± 7.1	8.3 ± 2.7
25	99	11880	75.4 ± 9.4	8.5 ± 0.8
26	101	12120	81.3 ± 10.3	12.0 ± 3.4
27	89	10680	81.0 ± 4.5	10.7 ± 2.0
28	90	10800	82.4 ± 5.3	10.9 ± 1.3
After period (feed without melamine additive)				
1	< LOD	< LOD	30.1 ± 24.9	9.9 ± 2.6
2	< LOD	< LOD	2.3 ± 0.1	7.4 ± 0.5
3	< LOD	< LOD	1.2 ± 0.5	5.9 ± 0.6
4	< LOD	< LOD	0.6 ± 0.2	5.5 ± 1.4
5	< LOD	< LOD	0.6 ± 0.3	3.9 ± 1.2
6	< LOD	< LOD	0.5 ± 0.2	2.5 ± 1.0
7	< LOD	< LOD	1.5 ± 2.2	1.8 ± 0.4
8	< LOD	< LOD	0.2 ± 0.0	< LOQ
9	< LOD	< LOD	0.2 ± 0.0	< LOQ
10	< LOD	< LOD	0.2 ± 0.0	< LOQ
11	< LOD	< LOD	0.2 ± 0.0	< LOQ
12	< LOD	< LOD	0.3 ± 0.0	< LOQ
13	< LOD	< LOD	0.3 ± 0.0	< LOQ
14	< LOD	< LOD	0.3 ± 0.0	< LOQ
15	< LOD	< LOD	0.3 ± 0.1	< LOQ
16	< LOD	< LOD	0.3 ± 0.1	< LOQ
17	< LOD	< LOD	0.3 ± 0.0	< LOD
18	< LOD	< LOD	0.3 ± 0.0	< LOD
19	< LOD	< LOD	n.a.	< LOD
20	< LOD	< LOD	n.a.	< LOD

LOD (compound feed) = 8 mg / kg; LOQ (compound feed) = 32 mg / kg
LOD (yolk) = 5 µg / kg; LOQ (yolk) = 10 µg / kg
n.a. not analyzed

LOD (egg white) = 0.4 µg / kg; LOQ (egg white) = 1.1 µg / kg
LOD limit of detection, *LOQ* limit of quantification
* Mean feed intake per hen per day amounts to 120 g feed

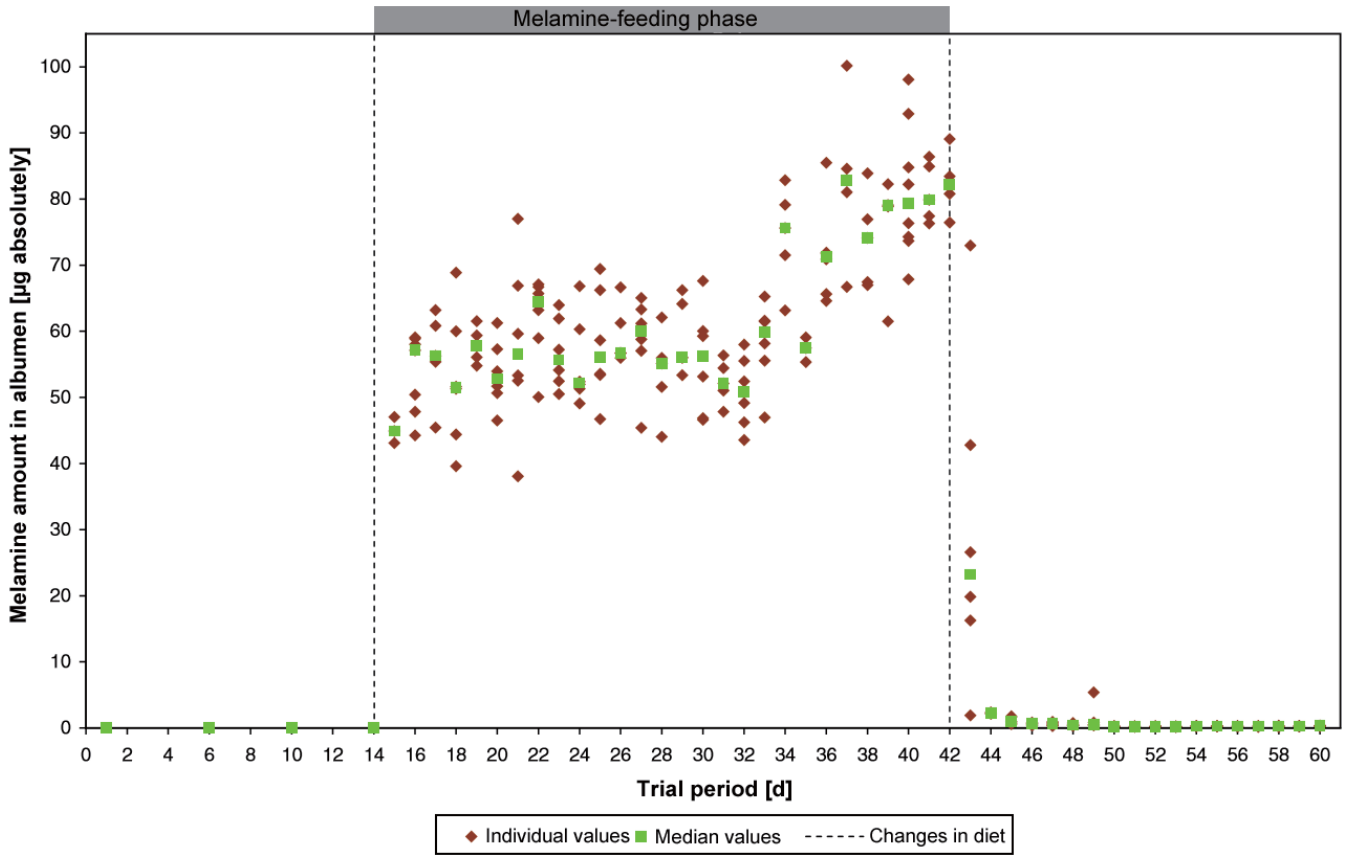

Figure 1. Individual values (brown diamonds) and median values (green squares) of melamine amounts [µg absolutely] in egg whites of eggs of Group C after feeding the daily ration per animal of 120 g of feed, either supplemented with melamine (feeding phase) or without melamine additive (pre- and after-period)

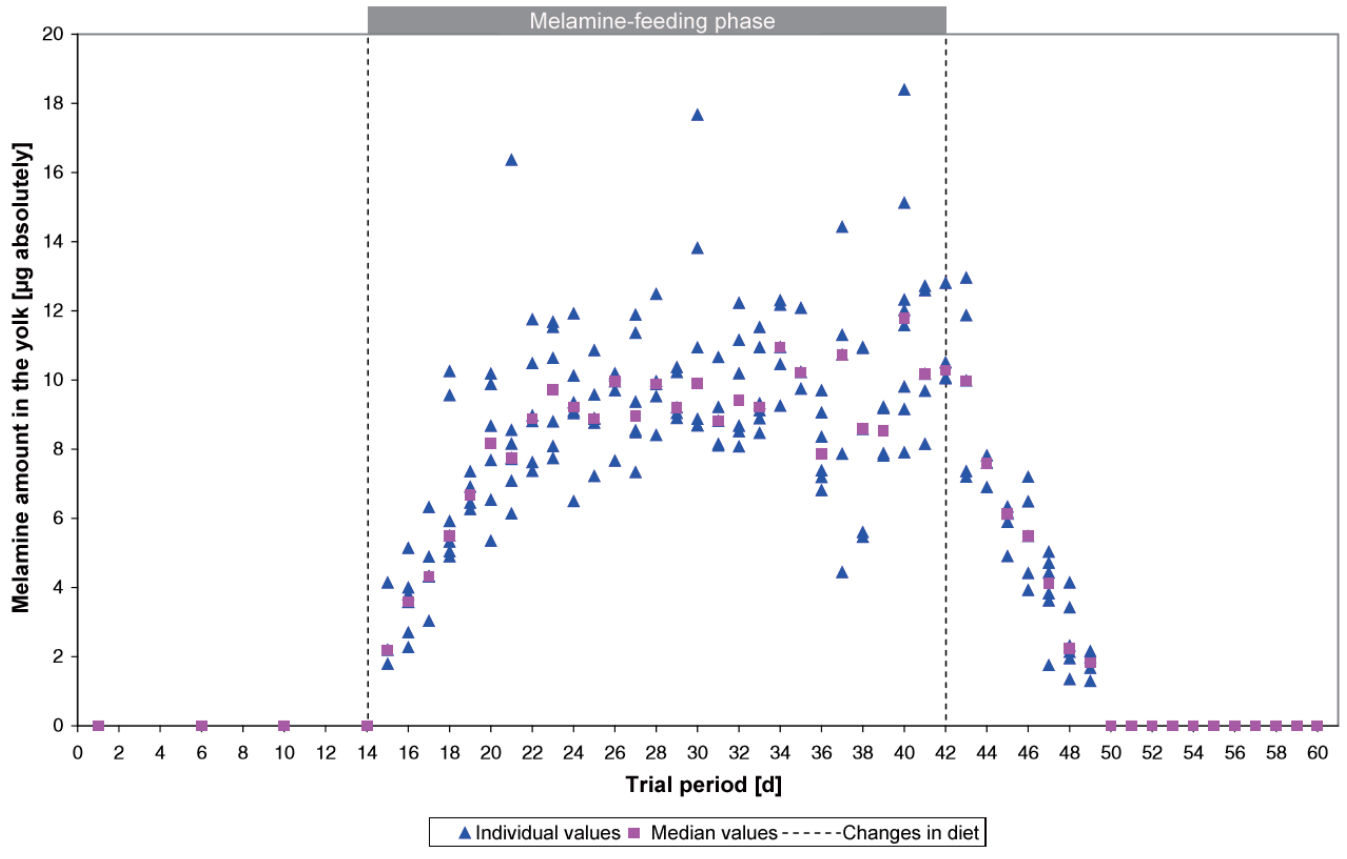

Figure 2. Individual values (blue triangles) and median values (pink squares) of melamine amounts [µg absolutely] in the yolk of the eggs of Group C after feeding a daily ration per animal of 120 g of feed, either supplemented with melamine-Feeding phase or without melamine additive (pre- and after-period)

applicable at the time of the testing level for melamine in feed of 2.5 mg of melamine per kg feed according to Regulation (EU). To the compliance with the regulations of food according to Regulation (EU) stood at the test series C No. 594/2012 in the foreground. Here, the working hypothesis should be checked based on experimentally obtained data whether there may be a transgression of the currently valid Ho¨chstgehalts for melamine in eggs of laying hens by 2.5 mg of melamine per kg egg at a supplementation of 100 mg of melamine per kg of food.

Im Folgenden kann nur ein Überblick über die Ergebnisse gegeben werden. Eine ausführliche Darstellung der Ergebnisse befindet sich im Supplement zu diesem Artikel, das elektronisch eingesehen werden kann.

3.1 Analysis of food samples

At the supplementation of mixed fodders contaminated with melamine, on inhomogeneous distribution of melamine showed up in the feed samples, so that the actual salary data partially significantly differed from the desired target levels (table 2). Therefore, the average content of melamine in the feed samples has been daily determined and when evaluating account (fig. 3).

The samples of control feed showed no melamine (melamine Gehalt\LOD).

3.2 Analysis of the egg white samples

Table 2 is to see that a transfer of melamine from the feed in the egg of laying hen eggs has occurred. While no melamine in the egg white has been detected in the previous period (food without the addition of melamine), the medium amount of melamine in the egg white has grown by leaps and bounds after the change on the melamine supplemented experimental feed within the first 48 h until a concentration plateau (steady state). During the course of the feeding phase, the Middle melamine levels in the egg white samples followed by the variations in the levels of melamine in the feed samples (due to the inhomogeneous distribution of melamine in the feed).

The median values of the melamine levels in the egg white have a similar order of magnitude as the average amounts of melamine. Thus, the distribution of the single values follows a normal distribution (fig. 1).

After discontinuation of the melamine-containing feed occured a sharp decrease in the melamine levels in the egg white samples in all test series. So, the average amount of melamine declined within the first 24 hours after discontinuation of the supplemented feed by over 80% and after 48 h by more than 90%. After three days, held only a very small decrease of average amounts of melamine. Traces of melamine in the egg white samples were detectable at the end of the follow-up period (max. 20 d).

3.3 Analysis of egg yolk samples

In the egg yolk samples the medium amounts of melamine at the beginning of the feeding of the Melaminsupplementierten rose Chuck, where the increase was much slower as in the egg white. From the sixth day of the main period, a steady state (fig. 2) occurred with the egg yolk samples.

The median values of the melamine amounts are comparable to the average amounts of melamine so that the samples of egg yolk samples are normally distributed.

Nach dem Absetzen des supplementierten Futters nahmen die Melamin-Mengen in den Eigelb-Proben ab. Dieser Prozess vollzog sich jedoch deutlich langsamer als in den Eiklar-Proben. So war bei der Analyse der Eigelb-Proben erst ab etwa dem siebten Tag der Nachperiode eine Verminderung der mittleren Melamin-Menge um 80 % zu beobachten (Tab. 2).

3.4 Determination of the transfer rates

To quantify the transition of melamine from the feed in the hens egg, the egg white, egg yolk and whole egg transfer rates were calculated as follows:

Transfer rate [%]=[(Melamine much egg test [µg/s])/(Middle melamine on name [µg/s])] ×100

While the average intake of melamine was calculated per animal per day from the Middle melamine content of mixed fodders and assuming an average daily feed intake of 120 g (table 3). Transfer rates resulted in transition of melamine from the feed in the egg of laying hen by 0.6 to 0.8% for egg white, 0.1% for yolks and 0.7 to 0.9% for whole egg (tab. 3).

3.5 Discussion

It could be shown that in the feeding of melamine a transition out of the Chuck in the egg of laying hen takes place, where the egg white samples compared to the egg yolk samples exhibit a by a factor of 5 to 8 higher Middle melamine amount. While egg white consists primarily of water (water accounted for 88%), egg yolk has only a water content of 50% and also includes an average 17% proteins and 33% lipids. Thus lipophilic compounds accumulate mainly in the yolk, while hydrophilic compounds are preferentially deposited in the egg white. Because melamine is a slightly hydrophilic molecule, the transition and thus also the transfer rate in the egg whites are higher than in the egg yolk (tab. 3).

Furthermore, a comparison of the history of the Middle melamine amounts or concentrations shows in the three studied matrices, egg white, egg yolk and feed during the feeding period (main period) the fluctuations in the amounts of melamine in feed due to the inhomogeneous distribution of melamine within the feed samples with a time delay in the egg samples to find again (fig. 3). When the egg white samples, the melamine amounts follow the fluctuations in the feed with a time interval of 24 hours while watching a time delay of several days with the egg yolk samples. These effects reflect the physiological processes of the margarines.

The emergence of a hen's egg begins in the ovary of the hen. After maturation of the egg yolks and the client was Eifollikels ovulation, the egg is based in several complex

Table 3. Transfer rates [%] of melamine from feed (compound feed) in the matrices white, yolk and whole egg laying hens of Group A, Group B and Group C

Group	Melamine content (MW) in the feed mg/kg = µg/g]	Medium melamine intake per hen per day [µg absolutely] ***	Melamine amount (MW) in the egg samples [µg absolutely]			Transfer rate [%]		
			Egg White	egg yolk	whole egg	Egg White	egg yolk	whole egg
A	380*	45600*	271*	56*	327*	0.6	0.1	0.7
B	2.6*	312*	2.4*	0.3*	2.7*	0.8	0.1	0.9
C	75*	9000*	62.1*	8.8*	70.9*	0.7	0.1	0.8
C	96**	11520*	78.4*	9.8*	88.2*	0.7	0.1	0.8

* Average (MW) over the entire main period (day 1-28)
** Average (MW) of 22-28 days the main period
*** Mean feed intake per hen per day amounts to 120 g feed

layers from the inside out. During this development phase, the egg travels through the fallopian tube. It comes to the rearrangement of the yolk with egg whites and final formation of the calcareous shell. For the process of egg white education are needed on average 24 hours. This period corresponds to the time lag between the feeding of melamine-feed and the detection of melamine in egg whites. In contrast, the yolk takes several days for the formation and maturation and thus provides an explanation that the melamine-amount increases in the yolk for several days before a plateau (steady state) is reached (Brade et al. 2008).

After discontinuation of the melamine supplemented feed using the same mechanism, the effects can be explained. So a decrease of amount of melamine of more than 90% (fig. 1) occurred in the egg white already in the first 48 hours after the change of feed, while the rate of elimination in the yolk less clearly and with a time lag of several days took place (fig. 2).

13 days after discontinuation of the melamine supplemented feed (after period) are still small traces of melamine were located in the various egg samples (tab. 2). A possible hypothesis would be the release of melamine, for example from the muscle tissue of laying hens, which could lead to the trace detection of melamine in egg samples. The training of a steady state in the egg sample during the main period suggests however, that melamine is accumulated after taking over the food not of the laying hen. The investigations described in the literature to the toxicokinetics also confirm a rapid elimination of melamine after oral ingestion ([90% within 24 h) (EFSA 2010).

In all three series of tests comparable results could be achieved, which are in the high-dose range as well as in the low dose range of validity and can be summarized as follows:

1) a transition of melamine from the feed in the hens egg takes place.

2) the transition in the egg white is greater than in the egg yolk by a factor of three to eight.

3) the transfer rate in the egg is a total (average B1%).

4) the transition in the egg white is faster than in the egg yolk.

5) variations in the levels of melamine in feed samples, which could be traced back to an inhomogeneous distribution of melamine in the experimental feed, are with a time delay in the egg white and yolk samples (fig. 3).

6) the amount of melamine reached a plateau (steady state) for a 28-day test period, both the egg white and the yolk.

7) the decrease in the amount of melamine after discontinuation of the melamine-containing feed is faster than in the egg yolk samples in the egg white samples.

8) 21 days after discontinuation of the melamine-containing feed still traces of melamine in the egg white - as well as in the egg yolk samples are detected.

The statements 1) to 4) and 7) are statistically significant (a\0, 05). The statements 5), 6) and 8) could not or only for individual experimental groups as statistically significant are confirmed.

So such as the spoken under 6 training of a plateau for the melamine levels in the Eiklarund egg yolk samples from Group C (100 mg of melamine per kg of food) but not for the melamine levels in the egg samples of from groups A and B is statistically significant. This can be (at least partially) on the special challenges in the analysis of melamine in the low dose range for Group B (2.5 mg of melamine per kg of food) as well as Group A (400 mg of melamine per kg of food) be attributed to the strong fluctuations in the levels of melamine in the feed and should be further studied for a statistically significant protection.

The following shall be based on the food regulations entered and a comparison of generated data with literature data are drawn.

3.6 Legislation provisions

When planning the test series C (feeding of 100 mg of melamine per kg of food), the issue came after the requirements for compliance with food regulations in the foreground. Regulation (EU) No. 594/2012 set a maximum level for melamine in foodstuffs of 2.5 mg / kg. This maximum level also applies to eggs from laying hens (whole egg).

In table 4 are mean values and standard deviations both the melamine levels in the whole egg in the unit micrograms absolutely (lg abs) as also the melamine levels in the whole egg in the unit milligrams per kilogram (mg/kg) over the entire trial period (pre-, primary and subsequent period) collectively represented. The amount of melamine in the whole egg, specified in the unit micrograms absolutely (lg abs), equal to the sum of the corresponding Middle melamine amounts of egg

white and yolk samples (tab. 2). The melamine content in the whole egg, expressed in the unit milligrams per kilogram (mg/kg) was calculated as follows:

Melamine whole egg [mg/kg =µg/kg]=(Melamine lot of whole egg [µg/s])/(Weight of whole egg [g])

In Fig. 4, the time course of the Middle melamine levels in the whole egg while the main and subsequent period as well as a comparison of salary data with the applicable maximum level for melamine is graphically represented in eggs of 2.5 mg / kg.

Abb. 4 ist zu entnehmen, dass bei Verfütterung eines Futters, das einen maximalen Melamin-Gehalt von 104 mg/kg aufweist, der geltende Höchstgehalt für Melamin in Lebensmitteln gemäß Verordnung (EU) Nr. 594/2012 von 2,5 mg/kg in Eiern nicht überschritten wird.

Die mittleren Melamin-Gehalte im Vollei bewegen sich in einem Bereich von 1,2 bis 2,0 mg/kg. Der maximale mittlere Melamin-Gehalt im Vollei von 2,0 mg/kg wurde am Ende der Fütterungsphase (Hauptperiode) gemessen und damit zu einem Zeitpunkt, als der mittlere Melamin-Gehalt im Futter einen Wert von ca. 100 mg/kg aufwies. Dieser Wert soll im Folgenden für einen Vergleich mit Daten aus der Literatur herangezogen werden (Tab. 5).

3.7 Comparing of the results with data from the literature

Three studies on laying hens are suitable for comparing the results of Group C (feeding of 100 mg of melamine per kg feed) (Chen et al. 2010;) Dong et al. 2010; Valat et al. 2011) as well as a study of quail (Zhang et al. 2012).

The salary data collected in the present study for melamine in the respective egg samples (egg white, egg yolk and whole egg) are moving on a similar level of concentration as the data described in the literature (table 5). However the melamine content in the whole egg samples has not been determined in all literature studies between the melamine content in the egg whites and yolks separately, but only (Chen et al. 2010;) Zhang et al. 2012). Moreover, Melamine was used in the study by Valat et al. (2011) only feed with 50 or 500 mg per kg.

A comparison of transfer rate is limited to the results of the study by Dong et al. (2010), because only in this study, a transfer rate for the transition of melamine from the feed in the whole egg was found. No transfer rates can be derived from all other studies.

The transfer rates from the study by Dong et al. (2010) and from the present study amounted to values of 1.2% and 0.8% respectively. The different findings agree mo¨glicherweise by the fact that in the study by Dong et al. (2010) a daily feed intake was placed 100 g per chicken to reason, while a daily food intake of 120 g per chicken was determined in the present study. Otherwise comparable values, a lower transfer rate in the present study arises from the different amount of feed intake calculated. Zumanderen were used not the Middle melamine levels of egg samples in the study by Dong et al. (2010) to determine of the transfer rate, but only a maximum content of melamine in the whole egg by 2.34 mg / kg. This maximum levels for melamine in the whole egg exceeds the maximum melamine content of the present study to 0.34 mg / kg.

Summary, it is to notice that the data collected in this study and the resulting conclusions in accordance with the published literature. The present study provides a comprehensive analysis of the transfers of melamine from the feed in the egg of laying hen, where each was an analysis of egg samples differentiated according to egg white and egg yolk.

3.8 Assessment of outcomes in terms of the health consumer protection

In the final part of this study, the results of an evaluation in terms of the health consumer protection are subjected. Therefore a model calculation for the intake of melamine the consumption of eggs is taken first.

3.8.1 model costing for establishing melamine consumption of eggs

The consumption data used for the calculation of the model comes from the national consumption study II (NVS II) the Max Rubner Institute (MRI 2008). The NVS II is the currently most recent representative study for the consumption of the adult German population. The study, some 20,000 persons aged between 14 and 80 years of age using three different survey methods (dietary history, 24 h recall and weighing Protocol) were interviewed on their eating habits, found between 2005 and 2006 in whole Germany instead (MRI 2008). The presented evaluations of data for eggs based on data of dietary history "-interviews, where was 15.371 persons interviewed and retrospectively her u¨blicher recorded consumption of the last four weeks. The food and dishes were back then-calculated under BERU¨cksichtigung of processing factors on raw foods.

Women take 17 g egg at an average consumption of eggs a day, while men daily consume 21 g egg. High (95th percentile) consumption of eggs women daily record 55 g egg, while men consume daily 78 g egg. While the consumption of eggs as well as the consumption of dishes made from eggs, such as scrambled eggs or fried egg, were taken into account in determining the amounts of consumed. However, more food containing eggs, such as baked goods and desserts, are not incorporated into the calculations.

Table 6 shows the exhaustion of mg 0.2 / kg body weight (B.W.) per day (EFSA 2010) through the consumption of eggs that have a melamine content in Ho¨he of the current maximum level of 2.5 mg / kg the daily tolerable intake level (tolerable daily intake, TDI) of melamine. For male adults in Germany II is the daily intake of melamine at an average consumption of 21 g egg per day according to NVS 0.053 mg. At an assumed body weight of 60 kg, the TDI to 0.44% is thus exploited. Application

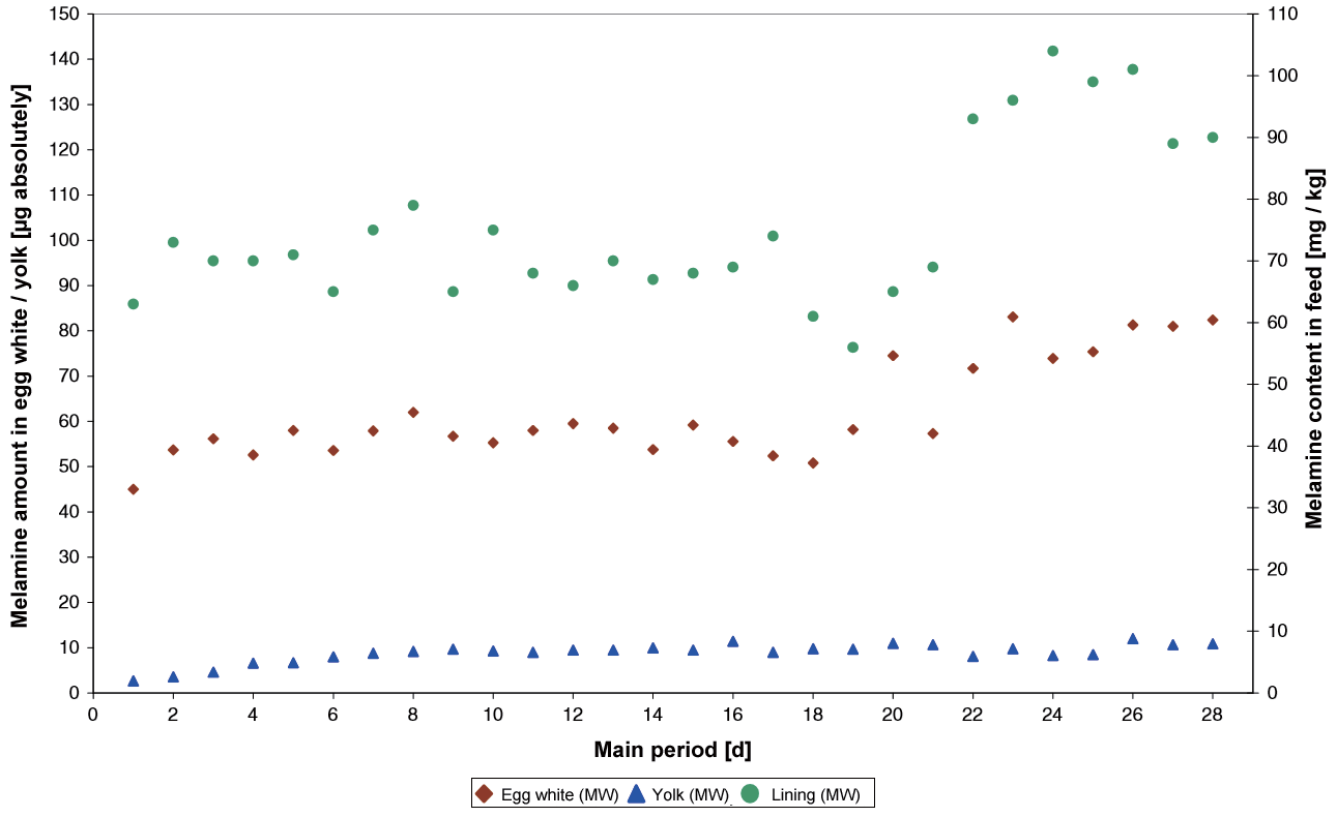

Figure 3. Overview of the middle melamine amounts [µg absolutely] in the matrices egg white (brown diamond) and yolk (blue triangle) the eggs of Group C after feeding the melamine-supplemented feed (Grune points) [mg / kg]

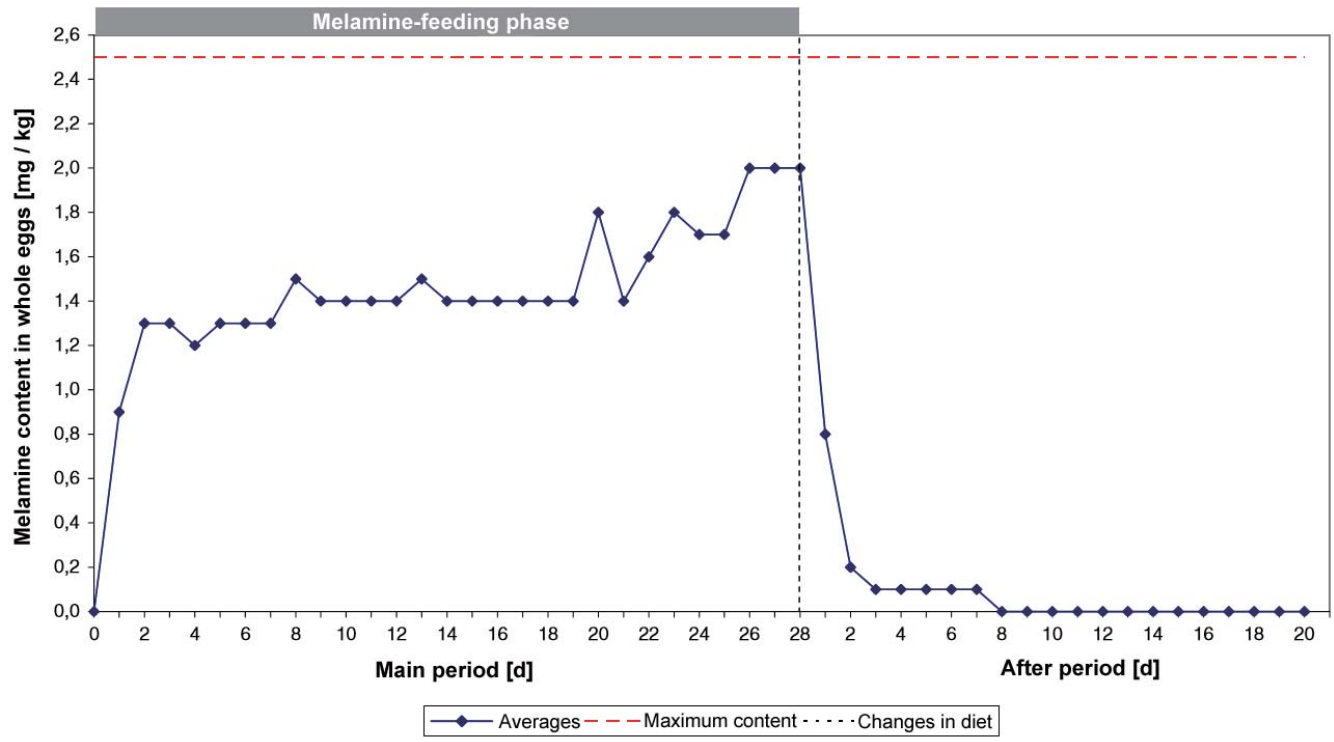

Figure 4. Medium melamine contents [mg / kg] in the whole egg of eggs of Group C after feeding the daily ration per animal of 120 g of feed, either supplemented with melamine (feeding phase) or without melamine additive (after-period). dashed red / kg is the current maximum level for melamine in eggs according to Regulation (EU) no. 594/2012 of 2.5 mg given.

of analog calculation results in a daily intake of melamine by 0.195 mg and thus an exhaustion of the TDI of 1.63% from a high consumption of 78 g egg per day.

Lower absorption rates arise due to the lower daily consumption for women.

It is assumed a unique high intake of 142 g egg per day for a worst case calculation (two or three equivalent to eggs per day; 95th percentile of the maximum consumption according to NVS II), the TDI to 2.96% is exhausted.

Thus is practically excluded from a high consumption of eggs (up to 142 g egg per day), which have a melamine content in the amount of the applicable of maximum level for melamine in eggs of 2.5 mg / kg, assure the safety of the consumer by eating these eggs.

3.8.2 Detection of structural analogues Ammelin, Amme eyelid and cyanuric acid and impact of the results on the health assessment

Also random investigations of egg samples, as well as the food samples the content of structural analogues of melamine (Ammelin, Amme eyelid and cyanuric acid) were carried in addition to the determination of melamine content in the context of the present study. However, the structure analogues were detected in any of the samples analysed. This result suggests that the melamine oral absorbed through the lining of the hen is not metabolized so that the structure analogues were not included in the health assessment of the results of the present study.

4 Views

In terms of the health consumer protection, tests for the presence of melamine in foods of animal origin should be extended to studies on the structural analogues of melamine (Ammelin, Amme lid and Cyanursa¨Ure). Work on assessing the health risk in domestic animals by the ingestion of melamine has shown so the food purchase, with simultaneous oral ingestion of melamine and cyanuric acid toxicity significantly increased compared to the conditions where only melamine is absorbed. While melamine is again excreted within 24 h to over 90% in the urine after sole application, kidney dysfunctions already at a simultaneous application of melamine and cyanuric acid at low concentrations to observe (EFSA 2010) (education of kidney stones and acute kidney failure).

The U.S. food and Drug Administration (U.S. FDA) has at the disposal of toxicological reference values the different effect of melamine and melamine in combination with the structure analogues (Ammelin, Amme eyelid and cyanuric acid) beru¨cksichtigt, and in 2007 a TDI for melamine by 0.63 mg / kg bw / day derived (USFDA 2007). For the toxicological evaluation of the combination of melamine and analogues of the structure, the U.S. FDA introduces an additional safety factor of 10 and led a TDI of 0.063 mg / kg bw / day for the combination of melamine and analogues of structure from (US FDA 2008) in 2008.

The European authority for food safety (EFSA) derived on the other has been merely a TDI for melamine. The TDI for melamine has been reduced in the year 2010 of 0.5 mg / kg bw / day to 0.2 mg / kg bw / day. Furthermore, EFSA opinion from the year 2010 indicates that an exposure estimate of the oral ingestion of cyanuric acid is so far impossible because no data on levels of cyanuric acid in food. In addition was also information about a possible, background levels "of food with melamine, which is also taken into account were the assessment (EFSA 2010).

Recently it published a review of Dorne et al. (2013) of Toxicology and risk assessment of melamine and cyanuric acid in pet food. A carry-over study on the transfer of melamine and cyanuric acid from the diet in food of animal origin would be useful for further studies.

References

1. Bai X, Bai F, Zhang K, Lv X, Qin Y, Li Y, Bai S, Lin S (2010) J Agric Food Chem 58(9):5414–5420.
2. BfR (2011) Stellungnahme Nr. 012/2011 des BfR vom 09.03.2011.
3. Brade W, Flachowsky G, Schrader L (Hrsg.) (2008) Landbauforschung vTI, Sonderheft 322.
4. Centre for Food Safety, Food and Environmental Hygiene Department, Hong Kong (2008). http://www.cfs.gov.hk/english/whatsnew/whatsnew_fstr/files/melamine_dec/List_of_unsatisfactory_food_samples.pdf
5. Chen Y, YangW, Wang Z, Peng Y, Li B, Zhang L, Gong L (2010) J Agric Food Chem 58(6):3512–3516.
6. Dong XF, Liu SY, Tong JM, Zhang Q (2010) Food Addit Contam Part A Chem Anal Control Expo Risk Assess 27(10):1372–1379.
7. Dorne JL, Doerge DR, Vandenbroeck M, Fink-Gremmels J, Mennes W, Knutsen HK, Vernazza F, Castle L, Edler L, Benford D (2013) Toxicol Appl Pharmacol 270(3):218–229
8. EFSA (2007) Question No EFSA-Q-2007-093.
9. EFSA (2008) Question No EFSA-Q-2008-695. EFSA J 807:1–10
10. EFSA (2010) Questions No EFSA-Q-2009-00234 and No EFSA-Q-2009-00235. EFSA J 8(4):1573.
11. Entscheidung der Kommission (2008/757/EG) vom 26. September 2008. Amtsblatt der Europa¨ischen Union L 259 vom 27.09.2008, 10–11.
12. Entscheidung der Kommission (2008/798/EG) vom 14. Oktober 2008. Amtsblatt der Europa¨ischen Union L 273 vom 15.10.2008, 18–20.
13. Gallo A, Bertuzzi T, Battaglia M, Masoero F, Piva G, Moschini M (2012) Animal 6(7):1163–1169
14. Gao CQ, Wu SG, Yue HY, Ji F, Zhang HJ, Liu QS, Fan ZY, Liu FZ, Qi GH (2010) J Agric Food Chem 58(8):5199–5205.
15. MRI (2008) Nationale Verzehrsstudie II. Ergebnisbericht 1:2 Richtlinie 2009/39/EG des europa¨ischen Parlaments und des Rates vom 6. Mai 2009. Amtsblatt der Europa¨ischen Union L 124 vom 20.05.2009, 21–29.
16. U.S. Food and Drug Administration (2007) http://www.fda.gov/ScienceResearch/SpecialTopics/PeerReviewofScientificInformationandAssessments/ucm155012.htm
17. U.S. Food and Drug Administration (2008) http://www.fda.gov/Food/FoodborneIllnessContaminants/ChemicalContaminants/ucm164520.htm
18. Valat C, Marchand P, Veyrand B, Amelot M, Burel C, Eterradossi N, Postollec G (2011) Poult Sci 90:1358–1366.
19. Verordnung (EG) Nr. 1135/2009 der Kommission vom 25. November 2009. Amtsblatt der Europa¨ischen Union L 311 vom 26.11.2009, 3–5.
20. Verordnung (EU) Nr. 574/2011 der Kommission vom 16. Juni 2011. Amtsblatt der Europa¨ischen Union L 159 vom 17.06.2011, 7–24.
21. Verordnung (EU) Nr. 594/2012 der Kommission vom 5. Juli 2012.
22. Amtsblatt der Europa¨ischen Union L 176 vom 06.07.2012, 43–45.
23. Wei Y, Liu D (2011) Toxicol Ind Health 28(7):579–582.
24. WHO (2008) Ottawa, Canada, 1–4 December 2008.
25. Yang T, Huangfu WG, Wu YL (2011) Poult Sci 90:701–704.
26. Zhang BK, Guo YM, Wang L (2012) Br Poult Sci 53(1):66–70. ■