Residue findings of melamine and its structural analogues in food using LC-MS/MS

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Introduction

Melamine and its structural analogue cyanuric acid entered the spotlight of food control labs in 2007 following severe adulterations cases of animal feed, milk powder and infant formulae, which aimed at fraudulently increasing the apparent protein content in those products. Whereas food of animal origin has been regularly monitored for melamine and cyromazine following these incidences, food of plant origin initially remained out of the focus.

Numerous pathways exist through which food of plant origin may be contaminated with melamine and its downstream hydrolysis products ammeline, ammelide and cyanuric acid (Figure 1). Ammeline, ammelide and cyanuric acid are reported as metabolites of the triazine insecticide cyromazine, the triazine fungicide anilazine and numerous triazine herbicides, including terbuthylazine, prometryn, simazine, atrazine, ametryn and cyanazine. Cyromazine first leads to melamine which is then stepwise hydrolyzed to cyanuric acid. Among the above compounds, only terbuthylazine and cyromazine are currently still in use within the EU. Another source of food contamination with melamine, cyanuric acid and the intermediates is the use of cyanamide-based fertilizers [2]. Cyanamide is known to form melamine through trimerization. Another source of cyanuric acid are di- and trichloroisocyanurates, which are contained in cleansing agents, algicides and disinfectants as they ensure a slow release of chlorine and prolong its activity.

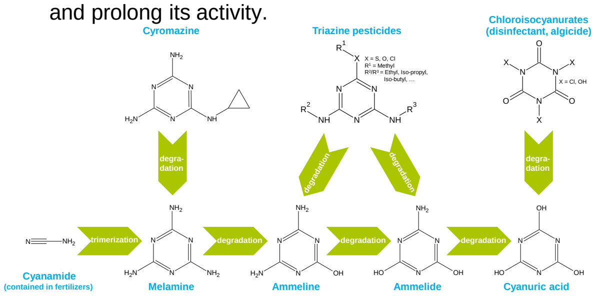

Figure 1: Degradation pathway of melamine and its downstream hydrolysis products as well as their possible sources.

Residues of triazine pesticides are regulated by Reg. 396/2005/EC whereas melamine is regulated by Reg. 1881/2006/EC as a contaminant. For the structural analogues (ammeline, ammelide and cyanuric acid) however, no current regulation is in place. Apart from controlling the legal limits, there is also interest in monitoring the consumer intake of these substances through various pathways. Therefore, melamine and its structural analogues are routinely targeted by the pesticide lab of the CVUA Stuttgart. They are all covered by the QuPPe method, which entails extraction with acidified methanol, filtration, dilution and LC-MS/MS measurement [1]. Ammeline, ammelide and cyanuric acid are covered in the ESI negative mode (Hypercarb column), whereas cyromazine and melamine are covered in the ESI positive mode (BEH Amide column).

References

[1] Quick Method for the Analysis of numerous Highly Polar Pesticides in Foods of Plant Origin via LC-MS/MS involving Simultaneous Extraction with Methanol (QuPPe-Method), Version 11, February 2020

[2] Adam, S., Wieland, M., Bauer, N., Scherbaum, E., Anastassiades, M., Residue Findings of Melamine and Cyanuric Acid in Food, Poster presented at the EPRW 2016, Cyprus.

Results

Between July 2013 and May 2020 CVUA Stuttgart analyzed more than 15,000 samples of plant origin for the presence of melamine, cyanuric acid and various triazine pesticides (Table 1). Ammeline and Ammelide were included to the scope only recently (approx. 300 total samples analyzed).

Table 1: Findings of cyromazine, other triazine pesticides and their possible degradation products melamine, ammeline, ammelide and cyanuric acid in conventional and organic samples (07/2013-05/2020).
***Maximum Residue Limit (MRL) exceedances according to Reg. 396/2005/EC and Reg. 1881/2006/EC (Melamine) after deducting the measurement uncertainty of ±50 % according to SANTE/12682/2019.**

Conventional samples										
		Findings (above limit of Quantitation)								
	Samples analyzed	Total Findings		<0.01 ppm		>0.01 ppm		MRL exceedances*	
Cyromazine	13320	76	0.6 %	31	0.2 %	45	0.3 %	5	0.04 %
Other triazine pesticides	14367	201	1.4 %	179	1.2 %	22	0.2 %	4	0.03 %
Melamine	13283	2112	15.9 %	543	4.1 %	1569	11.8 %	6	0.05 %
Ammeline	258	0	0.0 %	0	0.0 %	0	0.0 %	currently not regulated	
Ammelide	258	14	5.4 %	11	4.3 %	3	1.2 %		
Cyanuric acid	13192	4340	32.9 %	2133	16.2 %	2207	16.7 %		

Organic samples									
		Findings (above limit of Quantitation)							
	Samples analyzed	Total Findings		<0.01 ppm		>0.01 ppm		MRL exceedances*	
Cyromazine	2154	0	0.0 %	0	0.0 %	0	0.0 %	0	0.0 %
Other triazine pesticides	2401	41	1,7 %	35	1.5 %	6	0.2 %	4	0.2 %
Melamine	2148	221	10.3 %	57	2.7 %	164	7.6 %	5	0.2 %
Ammeline	54	0	0.0 %	0	0.0 %	0	0.0 %	currently not regulated	
Ammelide	54	4	7.4 %	2	3.7 %	2	3.7 %		
Cyanuric acid	2128	792	37.2 %	292	13.7 %	500	23.5 %		

Melamine and its degradation products ammelide and cyanuric acid were frequently detected both in conventional and organic samples, whereas ammeline was not found in any samples. While cyromazine was exclusively found in conventional samples, other triazine herbicides, especially terbuthylazine and its metabolite terbuthylazine-desethyl, were occasionally found in conventional and organic samples at comparable rates.

However, no clear correlation was found between the occurrence of melamine and/or its degradation products and findings of cyromazine or other triazine pesticides (data not shown). The frequent findings of melamine and its degradation products seem to be rather related to other sources, possibly the use of cyanamide-containing fertilizers as previously stated, even though the latter are not permitted in organic farming [2]. The successive degradation of melamine via ammeline and ammelide to cyanuric acid in soil, could be an explanation for the frequent findings of melamine, ammelide and cyanuric acid in organic samples.

Summary

Melamine, ammelide and cyanuric acid are frequently found both in conventional and organic products. No clear correlation between an actual use of triazine pesticides and the presence of these compounds was found.

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