EURL-SRM – Residue Findings Report

concerning the following…

- **Compounds**: Difluoroacetic acid (DFA), Trifluoroacetic acid (TFA)
- **Commodities**: various plant materials
- **Extraction Method**: QuPPe
- **Instrumental analysis**: ESI (neg.), LC-MS/MS, differential ion mobility spectrometry (SelexIon®)

Residues of DFA and TFA in Samples of Plant Origin

Version 1 (last update: 5.06.2017)

Short description:

This report gives an overview of the residue findings of trifluoroacetic acid (TFA) and difluoroacetic acid (DFA) in more than 1600 food samples of plant origin analyzed within 2016 and in the first quarter of 2017. The samples were extracted via the QuPPe method (Quick Polar Pesticides Method) and measured simultaneously by LC-MS/MS using DMS (differential mobility spectrometry) for better selectivity [1]. The residue situation in organic and conventional commodities is being compared.

Background information:

Trifluoroacetic acid (TFA) is a known breakdown product of numerous pesticides and of many other industrial chemicals including hydrochlorofluorocarbons and hydrofluorocarbons (HCFCs and HFCs, which are used as propellants in various sprays and as cooling agents), fluoropolymers (such as teflon) and medicinal products (such as the anaesthetic halothane). Based on the structural formula of pesticides EFSA has identified ca. 140 compounds containing the trifluoromethyl-group that could potentially lead to the formation of TFA - 39 thereof are currently approved under Regulation (EC) No 1107/2009/EC [2]. TFA has been reported to occur in the environment such as in chemical waste, rain, ocean water, surface water, tap water as well as in plants and animals [3-8]. According to a study by Wang et al. the origin of TFA in rainwater is predominantly due to the use of HCFCs and HFCs and to a smaller extend related to the thermolysis of fluoropolymers [7].

Difluoroacetic acid (DFA) is a known metabolite of the pesticide flupyradifurone and can occur in plant matrices (food and feed items) and also in matrices of animal origin. Flupyradifurone is approved in the EU and NAFTA, but formulations are yet to be registered within the EU. In addition to MRLs for flupyradifurone separate MRLs have been established for DFA in 2016 to account for DFA-residues in primary crops as well as for potential levels in succeeding crops [9]. In soil, DFA is formed as a major flupyradifurone metabolite and is preferentially taken up by plants forming the main residue in succeeding crops. Environmental background levels of DFA, which is also reported to be a minor thermolysis product of fluoropolymers, are much lower than those of TFA [8]. In the case of food of animal origin, especially in poultry, DFA is reported to be a major marker of the residues of flupyradifurone, although the absolute residue levels are low [10]. Due to the lack of detailed toxicological data for DFA EFSA has indicated that the toxicological reference values of flupyradifurone are also applicable to its metabolite DFA [10].

The multiple sources of TFA (including numerous pesticides) and the ubiquitous presence of TFA in the environment [3-8], make the establishment of separate MRLs virtually impossible, as the knowledge about the background levels in various crops is still limited. Based on existing data and applying a conservative approach, EFSA concluded that TFA does not raise any intake concerns. The calculated TFA exposure accounted for up to 5 % of the ADI and up to approximately 25 % of the ARfD (for potatoes). For TFA the toxicological reference values were only tentatively set as follows: ADI = 0.05 mg/kg bw per day; ARfD = 0.05 mg/kg bw. To verify the results of the dietary exposure assessment EFSA indicated that it would be desirable to generate more experimental data in the framework of a monitoring program on TFA in food [2]. The introduction of a method for TFA and the subsequent analysis of food samples from the market presented in this report were initiated as a reaction to this call by EFSA. DFA was also included in the work to gain the ability to control and enforce the recently established MRLs for DFA. As DFA it is structurally related to TFA it was expected that the two compounds would be analyzed simultaneously.

Measurement Data and Discussion:

To account for the ubiquitous presence of TFA and the resulting background levels, the reporting limit for TFA in fruit and vegetables was set at 0.04 mg/kg. For DFA, where no noteworthy background levels were observed, the reporting limit was set at 0.01 mg/kg (0.02 mg/kg for commodities with reduced analytical portion weight).
TFA findings at levels between 0.02 mg/kg (50 % of the reporting limit) and < 0.04 mg/kg (reporting limit) were also tracked and evaluated in order to obtain a better picture of the overall residue situation of TFA. At such levels, TFA is normally well detectable, but as the initial validation did not meet the SANCO criteria in all cases (e.g. mean recovery in gooseberry was 63 % and thus outside the required 70-120% range) these findings are referred to as semi-quantitative.

Altogether 1681 samples were analyzed for DFA and TFA. All samples were sampled from local markets in Baden Württemberg and originated from 55 countries, thereof 15 EU member states. Whereas TFA was found in traces in almost every sample, DFA was not encountered above the reporting limit in any of the samples. An overview of the observed TFA residues in conventionally produced products of plant origin is shown in Table 1. The respective results of organically produced products are shown in Table 2. A comparison of the results of cultivated and wild mushrooms is shown in Table 3. An overview of the countries of origin of the various samples is shown in Figure 1. Figures 2 and 3 show the frequency of TFA findings above the reporting limit and the mean concentrations in selected commodity groups of conventional and organic production.

In the analyzed samples, the frequency of TFA findings at levels at or above the reporting limit of 0.04 mg/kg was 18 % in organic and 17 % in conventional products. The frequency of positives within the semi-quantitative range (between 0.02 mg/kg and 0.04 mg/kg) was 17 % in organic and 15 % in conventional samples. It should be noted that these overall figures very much depend on the share of different commodity groups with high or low frequencies of TFA findings in the total number of analyzed samples. For example, the share of commodities with a very low frequency of findings (e.g. pome fruit, stone fruit and citrus fruit) among the conventional products was 23 % compared to only 13 % among the organic products. In **pome fruit** of both organic and conventional origin there were no findings recorded. In **citrus fruit** and **stone fruit** there were only very few notable TFA findings in the tested conventional products (1.2 % and 3.2 % respectively) and no notable findings in the tested organic products.

TFA-findings in food from local markets previously generated by BASF confirm the low levels encountered in fruit of unknown TFA-related prehistory. According to their study TFA did not exceed 0.01 mg/kg in any of the tested apples, peaches, cherries and grapes. In oranges the TFA levels ranged between 0.012 and 0.021 mg/kg [2, 11].

Looking at commodity groups with a high incidence of TFA findings (e.g. sprout vegetables, root vegetables, cereals, fruiting vegetables and leafy vegetables) we observed remarkable differences in the detection frequency between conventional and organic products, especially as regards the residues above the reporting limit of 0.04 mg/kg.

In the case of **sprout vegetables** 18 % of the conventional samples contained residues ≥ 0.04 mg/kg (33 % if the semi-quantitative findings are included) whereas none of the 11 organic samples was found to contain residues within the quantitative or semi-quantitative range.

In the case of **root vegetables** 21 % of the conventional samples (carrots: 33 %) were found to contain residues ≥ 0.04 mg/kg. The respective figures in organic samples were 5 % (carrots: 9 %).

In the case of **cereals** the frequency of findings ≥ 0.04 mg/kg was 69 % in conventional samples (82 % if the semi-quantitative findings are included) and 27 % in organic samples (63 % if the semi-quantitative findings are included).

In the case of **fruiting vegetables** the frequency of findings ≥ 0.04 mg/kg was 11 % in conventional samples (29 % if the semi-quantitative findings are included) and 7 % in organic samples (21 % if the semi-quantitative findings are included).

In the case of **leafy vegetables** the frequency of findings ≥ 0.04 mg/kg was 31 % in conventional samples (52 % if the semi-quantitative findings are included) and 12 % in organic samples (31 % if the semi-quantitative findings are included).

In all the above cases excluding sprout vegetables (no findings in organic products) the median levels (based only on the samples containing residues ≥ 0.04 mg/kg) of conventional products were comparable to organic products.

In the case of **dried pulses** the frequency of findings ≥ 0.04 mg/kg was very high, both in conventional and organic products (57 % and 41 % respectively). The detection frequencies increase to 78 % and 68 % respectively, if the semi-quantitative findings are included. This high frequency of findings is surely also related to the concentration of TFA in dry pulses due to the drying process. Shifting the reporting limit to 0.1 mg/kg we see 50 % of the conventional and only 18 % of the organic products exceeding this level. Based only on samples containing residues ≥ 0.04 mg/kg, the median concentration in conventional dry pulses (0.26 mg/kg) was ca. 3-fold higher than in organic pulses (0.085 mg/kg).

The examples above show that the overall frequency of TFA findings exceeding the reporting limit is clearly higher in conventionally produced products compared to organic ones. This suggests that there is, as expected, a link between the frequency of TFA findings and conventional farming. This is particularly evident in the case of vegetables. At lower levels TFA findings are distributed more evenly between conventional and organic products, reaching high frequencies of findings in both. This clearly suggests that there are also other important pathways of contamination, which go beyond the current or previous use of pesticides in the concerned fields affecting organic and conventional products alike. The ubiquitous presence and persistence of TFA in the environment has been subject of many publications [3-8]. The influence of environmental contamination sources is also reflected in the fact that 38 % of the wild mushrooms were found to contain TFA at levels ≥ 0.04 mg/kg (71 % if the semi-quantitative findings are included). Interestingly, there were no positives in any of the cultivated samples. This was somewhat surprising because, although cultivated indoors, they are typically grown on plant based substrates (such as straw), which are also expected to be TFA-contaminated. The findings in mushrooms are summarized in Table 3.

Looking at the rare findings of TFA in fruit on the one hand and the high frequency of findings in juices, wine and wine products on the other hand, this could be an indication of a contamination during processing of the raw material, possibly by contact with fluoropolymers.

For the assessment of the chronic exposure it is also important to view the median values of all data. Based on the data available so far the overall median is expected to be < 0.04 mg/kg for most commodity groups. Exceptions among the conventional products are cereals and cereal products (median = 0.048 mg/kg), dry pulses including soybeans (median = 0.093 mg/kg), fresh legumes (median = 0.043 mg/kg) as well as wine and wine products (median = 0.050 mg/kg). In the case of fresh herbs, juices, leafy vegetables, potatoes and other root vegetables the overall median is expected to fall between 0.02 and 0.04 mg/kg. Among the organic products the overall median of wine and wine products, dry pulses including soybeans, fresh herbs, and cereals was found to fall between 0.02 and 0.04 mg/kg. The overall median of wild mushrooms was also found to be within the range of 0.02 mg/kg and 0.04 mg/kg.

Tab. 1: Overview of TFA Residues in CONVENTIONAL products of plant origin

Commodity Group	Noticeable Examples	No. of samples analyzed	Samples with TFA levels ≥ 0.04 mg/kg		Between 0.02 and 0.04 mg/kg		MEAN value (of samples with levels ≥ 0.04 mg/kg)	MEDIAN value (of samples with levels ≥ 0.04 mg/kg)	MAX. level detected in mg/kg and country of origin
			No.	Percent	No.	Percent			
Berries		**201**	**14**	**7 %**	**24**	**12 %**	**0.089**	**0.063**	**0.23 (Grapes/IT)**
	Grapes	69	12	17 %	15	22 %	0.092	0.063	0.23 (IT)
Cereals and Cereal Products		**16**	**11**	**69 %**	**2**	**13 %**	**0.090**	**0.058**	**0.28 (Rye/Unknown)**
Citrus Fruit		**83**	**1**	**1 %**	**4**	**5 %**	**-**	**-**	**0.11 (Orange/IT)**
Dried Fruit		**5**	**0**	**0 %**	**0**	**0 %**	**-**	**-**	**-**
Exotic Fruit		**93**	**14**	**15 %**	**18**	**19 %**	**0.088**	**0.069**	**0.24 (Mango/ES)**
	Bananas	5	0	n.c.	2	n.c.	-	-	-
	Kiwis	11	4	36 %	2	18 %	0.069	0.069	0.087 (NZ)
	Passion fruit	4	3	n.c.	0	n.c.	0.070	0.061	0.10 (ZA)
Fresh Legumes		**34**	**19**	**56 %**	**7**	**21 %**	**0.183**	**0.110**	**0.65 (Green Beans/DE)**
	Green beans	30	17	57 %	6	20 %	0.19	0.11	0.65 (DE)
Fruiting Vegetables		**177**	**20**	**11 %**	**31**	**18 %**	**0.099**	**0.087**	**0.27 (Tomato/DE)**
	Melons / Waterm.	26	8	31 %	8	31 %	0.080	0.082	0.12 (ES)
Herbal Infusions and Tea (from leaves and seeds)		**7**	**7**	n.c.	**0**	n.c.	**0.58**	**0.51**	**1.2 (Fennel inf./unknown)**
Herbs, fresh		**39**	**11**	28 %	**12**	31 %	**0.50**	**0.061**	**4.8 (Rosemary/IL)**
Juices		**11**	**3**	27 %	**3**	27 %	**0.078**	n.c.	**0.098 (Grape Juice/unknown)**
Leafy Vegetables		**177**	**55**	**31 %**	**37**	**21 %**	**0.085**	**0.073**	**0.29 (Endive lettuce/DE)**
	Lettuce varieties	81	31	38 %	24	30 %	0.098	0.077	0.29 (DE)
Oil seeds		**8**	**0**	**n.c.**	**2**	**n.c.**	**-**	**-**	**-**
Pome Fruit		**109**	**0**	**0 %**	**0**	**0 %**	**-**	**-**	**-**
Potatoes and Starchy Veg.		**36**	**13**	**36 %**	**12**	**33 %**	**0.07**	**0.063**	**0.15 (Potato/DE)**
Pulses, dry (incl. soy beans)		**14**	**8**	**57 %**	**3**	**21 %**	**0.29**	**0.26**	**0.64 (Soybeans/ DE)**
	Soybeans	4	3	n.c.	1	n.c.	0.37	0.34	0.64 (DE)
	Lentils	4	4	n.c.	0	n.c.	0.29	0.26	0.47 (unknown)
Root Vegetables		**52**	**11**	**21 %**	**17**	**33 %**	**0.086**	**0.098**	**0.15 (Carrots/DE)**
	Carrots	18	6	33 %	8	44 %	0.086	0.084	0.15 (DE)
Sprout Vegetables		**74**	**13**	**18 %**	**11**	**15 %**	**0.062**	**0.060**	**0.094 (Broccoli/DE)**
	Cauliflower	13	4	31 %	1	8 %	0.058	0.058	0.075 (DE)
	Broccoli	20	6	30 %	6	30 %	0.067	0.064	0.094 (DE)
Stone Fruit		**125**	**4**	**3 %**	**5**	**4 %**	**0.061**	**0.064**	**0.076 (Apricot/FR)**
Wine and Wine Products		**27**	**17**	**63%**	**5**	**19 %**	**0.077**	**0.070**	**0.12 (Wine/DE)**
Other		**26**	**6**	**23 %**	**3**	**12 %**	**0.267**	**0.245**	**1.3 (Mixed Veg./unknown)**
OVERALL		**1366**	**227**	**17 %**	**206**	**15 %**			

Tab. 2: Overview of TFA Residues in ORGANIC products of plant origin

Commodity Group	Noticeable Examples	No. of samples analyzed	Samples with TFA levels				MEAN value (of samples with levels ≥ 0.04 mg/kg)	MEDIAN value (of samples with levels ≥ 0.04 mg/kg)	MAX. level detected in mg/kg and country of origin
			≥ 0.04 mg/kg		Between 0.02 and 0.04 mg/kg				
			No.	Percent	No.	Percent			
Berries		**24**	**2**	**8 %**	**2**	**8 %**	**0.052**	**-**	**0.063 (Grapes/IT)**
	Grapes	13	2	15 %	2	15 %	0.052	-	0.063 (IT)
Cereals and Cereal Products		**22**	**6**	**27 %**	**8**	**36 %**	**0.061**	**0.069**	**0.083 (Buckwheat/CN)**
Citrus Fruit		**5**	**0**	**n.c.**	**0**	**n.c.**	**-**	**-**	**-**
Dried fruit		**1**	**0**	**-**	**0**	**-**	**-**	**-**	**-**
Exotic Fruit		**5**	**3**	**n.c.**	**1**	**n.c.**	**0.079**	**0.07**	**0.11 (Bananas/DO)**
	Bananas	4	3	n.c.	0	n.c.	0.079	0.057	0.11(DO)
	Kiwis	1	0	n.c.	1	n.c.	-	-	-
	Passion fruit	0	0	-	0	-	-	-	-
Fresh Legumes		**1**	**1**	n.c.	**0**	**0 %**	**0.13**	**-**	**0.13 (Green Beans/DE)**
Fruiting Vegetables		**43**	**3**	**7 %**	**6**	**14 %**	**0.098**	**0.098**	**0.098 (Cucumber/DE); 0.098 (Melon /FR)**
	Melons / Waterm.	3	2	n.c.	0	n.c.	0.070	-	0.098 (FR)
Herbal Infusions and Tea (from leaves and seeds)		**3**	**3**	**n.c.**	**0**	**n.c.**	**0.16**	**0.17**	**0.17 (Black Tea/unknown)**
Herbs, fresh		**10**	**1**	**10 %**	**6**	**60 %**	**0.088**	-	
Juices		**8**	**1**	**n.c.**	**2**	**n.c.**	**0.04**	-	
Leafy Vegetables		**26**	**3**	**12 %**	**5**	**19 %**	**0.068**	**0.072**	**0.088 (Parsley/DE)**
	Lettuce	15	0	0 %	3	20 %	-	-	-
Oils		**3**	**0**	**n.c.**	**0**	**n.c.**	**-**	**-**	**-**
Oil seeds		**10**	**1**	**10 %**	**2**	**20 %**	**0.046**	**-**	**0.046 (Hemp seed/DE)**
Pome Fruit		**15**	**0**	**0 %**	**0**	**0 %**	**-**	**-**	**-**
Potatoes and Starchy Vegetables		**2**	**1**	**n.c.**	**0**	**0 %**	**0.042**	**-**	**0.042 (Sweet Potato/ES)**
Pulses, dry (incl. soybeans)		**22**	**9**	**41 %**	**6**	**27 %**	**0.086**	**0.085**	**0.14 (Lentils/TR)**
	Soybeans	5	2	n.c.	0	n.c.	0.10	-	0.11 (China)
	Lentils	12	6	50 %	6	50 %	0.078	0.084	0.14 (TR)
Root Vegetables		**22**	**1**	**5 %**	**7**	**32 %**	**0.073**	**-**	**0.073 (Carrots/DE)**
	Carrots	11	1	9 %	5	45 %	0.073	-	0.073 (DE)
Sprout Vegetables		**11**	**0**	**0 %**	**0**	**0 %**	**-**	**-**	**-**
	Cauliflower	4	0	n.c.	0	n.c.	-	-	-
	Broccoli	1	0	n.c.	0	n.c.	-	-	-
Stone Fruit		**16**	**0**	**0 %**	**0**	**0 %**	**-**	**-**	**-**
Wine and wine products		**17**	**8**	**47 %**	**3**	**18 %**	**0.063**		**0.11 (Wine /DE)**
Other		**16**	**9**	**56 %**	**0**	**0 %**	**0.55**	**0.27**	**1.3 (Veg. product/unknown)**
OVERALL		**284**	**52**	**18 %**	**48**	**17 %**			

Tab. 5: Overview of TFA Residues in Mushrooms

Commodity Group	Type	No. of samples analyzed	Samples with TFA levels …				MEAN value (of samples with levels > 0.04 mg/kg)	MEDIAN value (of samples with levels > 0.04 mg/kg)	MAX. level detected in mg/kg and country of origin
			> 0.04 mg/kg		Between 0.02 and 0.04 mg/kg				
			No.	Percent	No.	Percent			
Mushrooms		**55**	**9**	**16 %**	**8**	**15 %**	**0.086**	**0.063**	**0.22 (boletus/Romania)**
	Wild	24	9	38 %	8	33 %	0.086	0.063	0.22
	Cultivated	31	0	0 %	0	0 %	-	-	-

Fig. 1: Overview of the countries of origin of the samples analyzed for TFA findings in conventional and organic produce as regards the percentage of samples exceeding the reporting limit

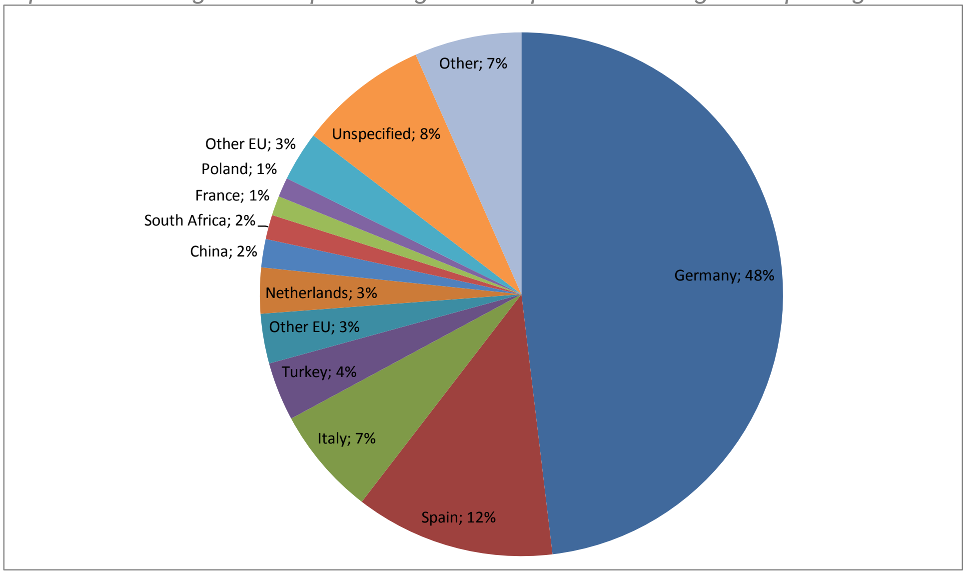


Fig. 2: Comparison of TFA findings in conventional and organic products as regards the percentage of samples exceeding the reporting limit (RL=0.04 mg/kg)

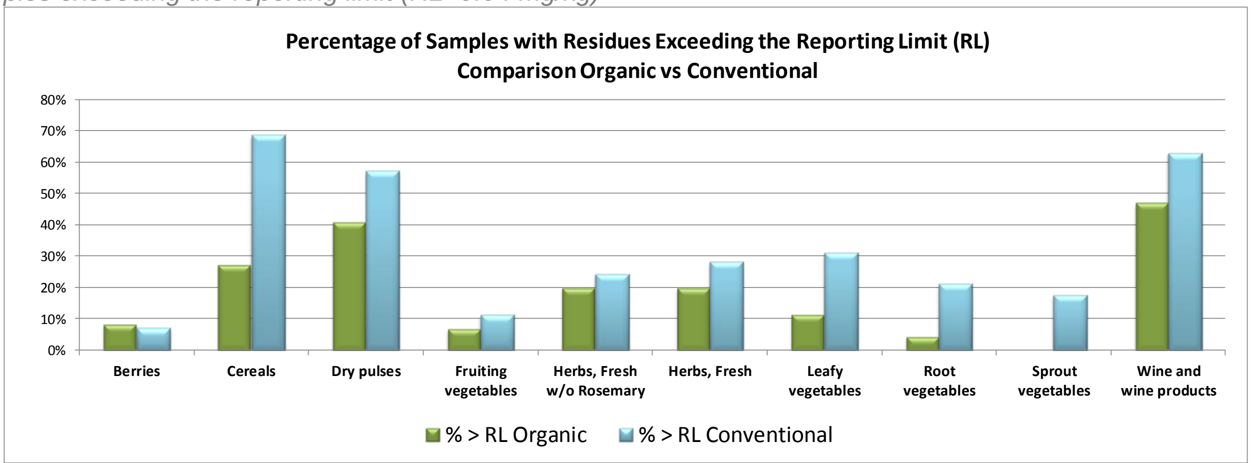
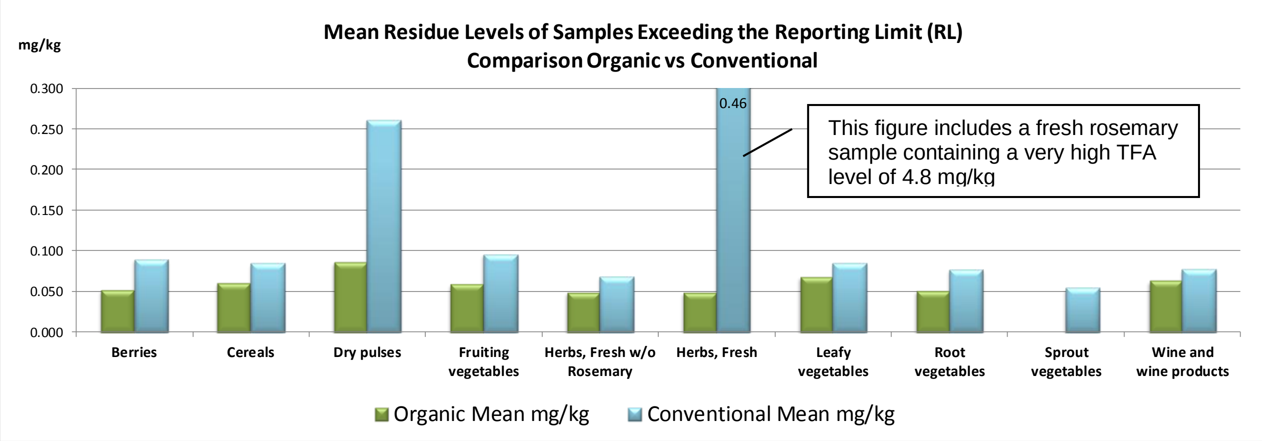


Fig. 3: Comparison of TFA findings in conventional and organic products as regards the mean residue levels of samples exceeding the reporting limit (RL=0.04 mg/kg)



Overview and Conclusions:

Unlike DFA, TFA was frequently found in many products of plant origin. On average approximately 15 % of the tested produce were found to contain TFA levels at or above the quantitative reporting limit of 0.04 mg/kg. Including semi-quantitative results in the concentration range between 50 and < 100 % of the reporting limit, the frequency of TFA findings doubles to approximately 30 % of the tested samples overall. Comparing the frequency of TFA-findings at or above the reporting limit in most conventional and organic products, a strong relationship between TFA-residues and conventional farming becomes evident. Although the frequency of high level findings in organic products is much lower than in conventional products of the same type, it still cannot be assumed that the residues result from pesticide applications alone, since a very large percentage of the tested organic products was found to contain TFA at lower levels (18 % above the reporting limit, 17 % within the semi-quantitative range between 50 and < 100 % of the reporting limit and many more at lower levels producing well detectable peaks which were not quantified). Frequent findings in wild mushrooms (mostly fresh chanterelles) also suggest a very significant pesticide-independent TFA presence within the environment resulting in considerable contamination. The intensive monitoring of agricultural products needs to be continued in order to gain the ability to draw more sound conclusions. Plant samples from areas with no pesticide application history should also be tested for comparison.

Literature

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History

Action	When	Document Version
Initial Experiments	April 2015 - February 2016	-
Data collecting	July 2016 - February 2017	1
Further Experiments	Planned for 2017	-
Document placed on-line	19.06.2017	1