

POSITION PAPER No. 16 - 03
**“Phthalimid: Metabolite of Folpet or
unavoidable Artefact ?”**

Version 2016/07/22

Abstract

This position paper is related to the new residue definition of the fungicide Folpet by regulation (EU) 2016/156 dated 2016/01/18. This regulation shall apply from 2016/08/26 on with the residue definition of Folpet changing to:

“**Folpet**: Sum of Folpet and Phthalimid, expressed as Folpet”.

Up to now, Phthalimid - the metabolite of Folpet – was not part of the residue definition. As Phthalimid (hereafter named “PI”) is often detected and measured in many food products without the simultaneous presence of Folpet, serious concerns rose up about other sources of PI than of Folpet only.

This paper describes and summarises the experiences and data of the relana® laboratory circle related to the presence of PI in different kind of foodstuff. A potential, likely source and the related pathway for the generation of PI is described. The consequences related to the new residue definition of Folpet are discussed and as a final conclusion a proposal is provided of how to deal with this new residue definition in practice.

Introduction

With regulation (EU) 2016/156 of 18. January 2016 the European Commission has set a new residue definition for Folpet, which then will include the main metabolite “Phthalimid” (PI). The regulation shall apply starting from 26 August 2016 on.

With regulation (EU) 2016/452 of 29. March 2016 the European Commission has set a new residue definition also for **Captan**, which includes the main metabolite “Tetrahydro-Phthalimid” (THPI). The regulation shall apply from 19 October 2016 on.

The metabolite of Captan, the “THPI”, is clearly linked to the Captan itself. If levels of THPI are detected in a sample, Captan is most often present, too at least in traces. Other formation processes of THPI than in combination with Captan or Captafol are not documented. During the gaschromatographic (GC) analysis of Captan and/or Captafol, THPI is often produced from Captan or Captafol within the injection system of the GC, if the injection system is not running under best conditions. A residue definition of Captan without including THPI, which might be produced from Captan or Captafol during the GC injection, bears the risk of relevant under-

estimations of the levels of Captan and/or Captafol in analytical samples. Therefore, it is meaningful to include THPI into the residue definition of Captan.

From a first point of view, the situation related to **Folpet** seems to be quite similar and comparable. During the gaschromatographic (GC) analysis of Folpet, the metabolite PI is often produced within the injection system of the GC, if the injection system is not running under best conditions. A residue definition of Folpet without including PI, which might be produced during the GC injection, bears the risk of relevant under-estimations of the levels of Folpet in analytical samples. Therefore, at a first glance it seems to be meaningful to include PI into the residue definition of Folpet. However, the data provided by the laboratories of the relana® circle **does not support this assumption**.

Discussion of the presence and formation of Phthalimid (PI)

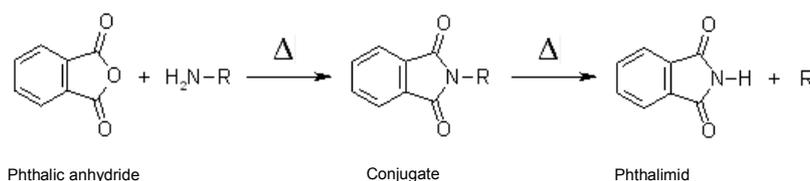
The strong difference compared to Captan/THPI is related to the presence and possible formation of PI not only from Folpet.

Investigations performed by the relana® member Labor Friedle GmbH resulted in the following hypothesis for an important pathway of the formation of PI under common environmental conditions:

In hundreds of house dust samples analysed by Labor Friedle, the presence of “**phthalic anhydride**” was evident. Phthalic anhydride can be classified as “ubiquitous”.

Phthalic anhydride (and the corresponding phthalic acid) is used in many different technical products like in resins, paintings, paper coverings, plastics (PVC) and newspaper printings. Phthalic acid is also used as a basic material of softeners and alkyd resins. Therefore, it is no surprise detecting phthalic anhydride especially in (house) dust samples.

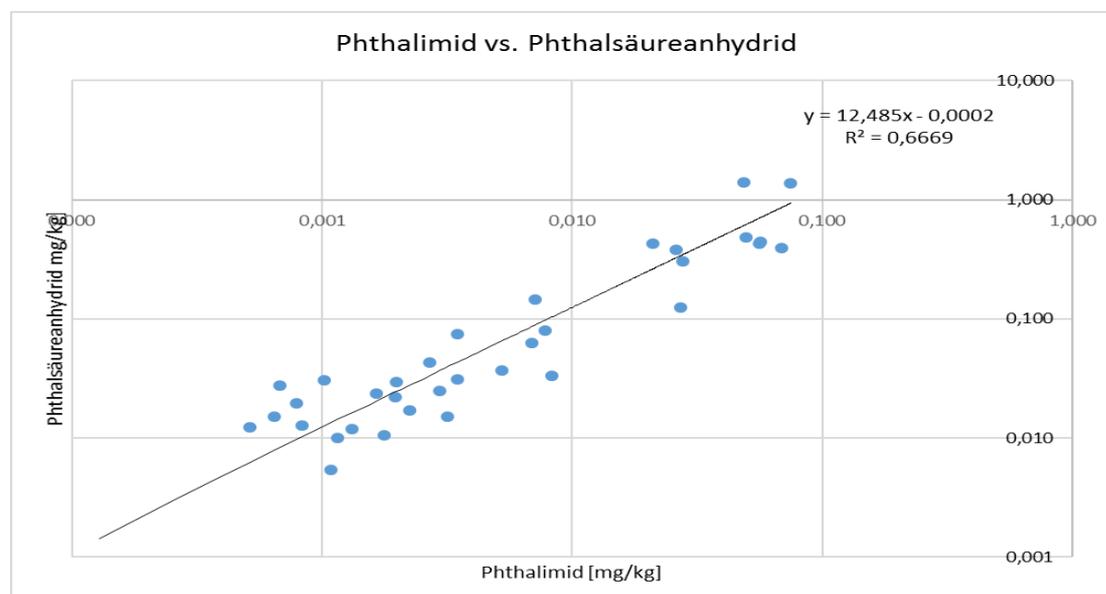
The correlation between this widely used industrial chemical and the presence of Phthalimid in food products can be explained by the reaction equation of phthalic anhydride with compounds of the food matrix containing primary amino-groups (like amino acids, peptides, etc.) under heating conditions:



Results provided by Labor Friedle indicate a strong correlation of the levels of Phthalimid (PI) and Phthalic anhydride in food products: Levels of Phthalic anhydride are approximately 10 times higher compared to the levels of Phthalimid (PI). The table below show some examples out of in total ca. 60 samples:

Sample	Phthalimid [ppm]	Phthalic anhydride [ppm]
bio parsley	0,002	0,024
bio melissa	0,002	0,022
bio salvia	0,008	0,080
bio nectarines	0,001	0,012
bio pineapples	0,003	0,043
bio onions	0,002	0,017
bio caraway	0,028	0,303
bio coriander powder	0,021	0,428
bio dill weed	0,026	0,378
bio apples (pieces, dried)	0,008	0,034
bio almonds	0,000	0,000
bio corn	0,027	0,124
blueberries	0,001	0,028
potatoes	0,003	0,015
peperoni	0,001	0,010
peperoni	0,002	0,011
herbs, dried	0,075	1,368
herbs, dried	0,049	1,401
chilli, dried	0,056	0,445
caraway, dried	0,007	0,062
mixed herbs, dried	0,056	0,428
mixed herbs, dried	0,049	0,482
parsley	0,003	0,025
mint	0,003	0,074
olives	0,003	0,031

The correlation is visualised in the graphic below (including more samples compared to the table above):



A second pathway of the formation of PI is already known and published by EFSA within a reasoned opinion document (EFSA Journal 2013; 11(12):3510 p. 15) related to phosmet:

*“EFSA has already highlighted in a previous reasoned opinion (EFSA, 2012) that **phthalimide**, which is included in the residue definitions for enforcement and risk assessment of the active substance folpet as a relevant degradation product under processing conditions (EFSA, 2009), **may also be formed in relevant amounts in processed commodities after the use phosmet**. Thus, EFSA proposed to reconsider the residue definitions for folpet and phosmet with regard to a common metabolite phthalimide in the framework of Article 12 of Regulation (EC) No 396/2005 (EFSA, 2012). It would therefore be appropriate to provide more data on the actual concentration of phthalimide expected in processed products after the use of phosmet.”*

Interestingly, PI is not part of the residue definition of Phosmet, although it seems to be a relevant metabolite. This might be due to the aspect that the toxicity properties of PI seem to be low (EFSA Journal 2013; 11(12):3510 p. 9 foodnote 16):

“Phthalimide toxicological properties could not be fully characterize at the time of the peer review of folpet. During the PRAPeR 44 the experts agreed that phthalimide does not have the potential to induce critical effects (carcinogenic, reprotoxic effects) (EFSA, 2009). Phthalimide is of low acute toxicity: LD50 of 5000 mg/kg bw (Spain, 2004).”

If so, the inclusion of PI into the residue definition of Folpet should be re-evaluated also taking under consideration this aspect.

Analytical data related to the presence of Phthalimid in food samples

Within the last months (2015 to June 2016) the relana® laboratories analysed several hundreds of food products and collected the related data of Folpet and Phthalimid. All these data give evidence, that the presence of PI in food samples is not related to the use and presence of Folpet but due to other reasons.

Distinguishing between the several commodity groups as described in Annex 1 of this position paper is applied for interpretation of this data.

Group 1: Fresh fruits and vegetables

The median level of PI is typically at 0,001 mg/kg (just two exceptions of 0,003 resp. 0,008 mg/kg for two data sets of apples). As a conclusion, no problems related to such low PI findings have to be expected.

Nevertheless, some individual higher levels of PI are present with a maximum of 0,239 mg/kg on apples, 0,080 mg/kg on tomatoes, 0,061 mg/kg on grapes and 0,056 mg/kg on pears. As the related MRLs of reg. (EU) 2016/156 are significantly higher than the median levels and also higher than all maximum levels of PI, this should not cause any problems.

The situation is different related to organic products. Such levels - as reported by the relana® laboratories as highest levels - might provoke actions of the organic control bodies resp. control authorities, questioning the organic status of samples with levels of PI higher than 0,01 mg/kg.

Group 2: Dried food and vegetables

The median results of PI are at a higher level compared to fresh fruits and vegetables.

The concentration range is between 0,005 mg/kg (plums, dried) and 0,013 mg/kg (apples, dried). Because of the concentration process during drying, in almost every sample PI is detectable (RL at 0,001 mg/kg). Two possible reasons are assumed:

1. Increase due to concentration by drying;
2. The formation of PI during the heating process (as described earlier in this paper).

While applying a common processing factor of 5 for dried fruits, the related levels are still at the same range as the levels of fresh fruits and vegetables. Therefore, the consequences of reporting levels of PI without the presence of Folpet would be similar to the situation discussed for group 1 (fresh fruits and vegetables).

Group 3: Herbs and Spices

For this group the situation appears different. The median levels of PI in spices and dried herbs are typically at levels between 0,02 mg/kg up to 0,05 mg/kg, which corresponds to 20% resp. 50% of the related MRL of 0,1 mg/kg. Related to dried basil, the median levels are even higher at 0,063 (data set of dried bio basil) resp. 0,145 mg/kg. The highest levels are up to 0,742 mg/kg for dried basil.

Also some other products show significant high results of PI, f. ex. Cardamom at 0,288 mg/kg, chilli powder at 0,642 mg/kg, Cumin at 0,879 mg/kg, and black pepper at 1,302 mg/kg! These results of PI are always without detectable amounts of Folpet (RL at 0,02 mg/kg)!

Taking a closer look at the organic (bio) samples, the median levels exceed in every case the guideline value of 0,01 mg/kg. Of course, the highest levels are still higher. Even most of the minimum levels are above 0,01 mg/kg!. This will provoke a lot of complaints related to the organic status of such samples, although no Folpet is detectable!

Once again, the two possible reasons are assumed:

1. In case of dried herbs a concentration by drying and
2. the formation of PI during heating processes (as described earlier in this paper).

Group 4: Tea (black, green), herbal and fruit teas

Related to this group of commodities, the median levels of PI are at about 0,02 mg/kg, with highest levels at 0,572 mg/kg (tea, black or green) resp. 0,229 mg/kg (herbal and fruit teas). Such levels clearly exceed the new MRLs of reg. (EU) 2016/156 of 0,1 mg/kg. This also will cause complaints, although no Folpet is present at all. As discussed before, the heating (drying, roasting) of tea leaves resp. herbal and fruit ingredients might cause the formation of PI, if phthalic anhydride and primary amino groups are present.

Conclusions and demands

Based on the data provided in this **relana® position paper** and the discussed possible formation processes, considering the ubiquitous presence of phthalic acid and phthalic anhydride the following conclusions and recommendations resp. demands are defined:

1. Phthalimid is not only a metabolite of the fungicide Folpet, but might as well be formed as an artefact during processing (mainly drying) of food products. It is also known as a degradation product of the insecticide Phosmet.
2. Phthalic anhydride as a ubiquitous environmental chemical can easily react under specific conditions (esp. higher temperatures) with primary amino groups (like present in amino acids, peptides, etc.) to form Phthalimid (PI).
3. The presence / detection of Phthalimid in many commodities, without the simultaneous detection of Folpet confirms the assumption, that other sources (than of Folpet) of Phthalimid are evident.
4. As a consequence, the new residue definition of Folpet – as described and published in reg. (EU) 2016/156: “Folpet = Sum of Folpet and Phthalimid, expressed as Folpet” – is not suitable to supervise the related MRLs in case Folpet itself is not detected in the sample.
5. This new residue definition bears a high risk of false positive results related to the presence or application of the fungicide Folpet.
6. This risk is also evident if Folpet is detected as it can not be distinguished, whether the measured levels of Phthalimid are linked to the presence of Folpet or due to the formation from phthalic anhydride with primary amino groups of the food product or as a combination of both. As a consequence, the calculated level of Folpet might be too high.
7. The most important consequences are related to findings of Phthalimid in organic samples if no Folpet is detected simultaneously. In many samples, the organic guideline value of 0,01 mg/kg will be clearly exceeded if applying the new residue definition of Folpet. Consequently, organic control bodies and authorities, but also the clients of producers and traders of organic products might complain about the compliance of the related food products with the rules of organic farming as published by the regulations (EC) Nos. 834/2007 and 889/2008. This situation would hamper the trading of organic products, although they would have been produced and processed in compliance with the related organic regulations and rules.

Taking all these facts and aspects into consideration, it is highly recommended to re-evaluate the residue definition of Folpet!

In the meantime, the new residue definition should apply only if Folpet is detected and reported above the related reporting limit, too. Nevertheless, this still bears a high risk of overestimation, as the level of Phthalimid might be partly derived by the formation from phthalic anhydride.

The presence of Phthalimid only gives no indication of a possible non-conformity of organic food products with the related organic regulations!

Literature

- Regulation (EU) 2016/156 of 18.01.2016
- Regulation (EU) 2016/452 of 29.03.2016
- Regulation (EC) No 834/2007 of 28.06.2007
- Regulation (EC) No 889/2008 of 05.09.2008
- EFSA Journal 2013; 11(12):3510 (Reasoned opinion on the modification of existing MRLs for phosmet in citrus fruits, pome fruits and rape seed)
- EFSA Scientific Report (2009) 297, 1-80 (Conclusion on the peer review of folpet)
- EFSA Journal 2012; (10(6):2769 (reasoned opinion on the modification of the existing MRL for folpet on wine grapes)

relana[®] Position Papers

This Position Paper is a literary property of relana[®], based on the contribution and the knowledge of the members of the relana[®] laboratory quality circle. The aim of this publication is to increase knowledge and to provide expertise to all relevant and interested stakeholders in order to achieve best practices on analytical services related to food and feed testing. Everybody is invited to make use of this Position Paper and to circulate it wherever meaningful. While using this Position Paper please make the reference as:

relana[®] Position Paper No. 16-03 "Folpet/Phthalimid" version 2016/07/22.

Annex 1

All data presented here include the analysis of **Folpet**, in any case with a result **“not detected”**. The related reporting limits (RL) depending on the commodities are between 0,02 mg/kg and 0,001 mg/kg for both Folpet and Phthalimid.

Exception: The data provided of group 4 for “tea (black, green)” did not include the analysis of Folpet, subsequently the absence of Folpet cannot be asserted.

Levels of Phthalimid in several commodities analysed by relana[®] laboratories within 2015 and 2016

Product	Number of samples	Median (mg/kg)	Max (mg/kg)	Min (mg/kg)
Group 1: Fresh food and vegetables				
Apples (dataset 1)	117	0,003	0,239	n.d. < 0,001
Apples (dataset 2)	104	0,001	0,063	n.d. < 0,001
Apples (dataset 3)	9	0,001	0,005	n.d. < 0,001
Apples (dataset 4)	4	0,008	0,016	0,006
Bananas (dataset 1)	27	< 0,001	0,035	n.d. < 0,001
Bananas (dataset 2)	10	0,001	0,005	n.d. < 0,001
Grapes (dataset 1)	129	< 0,001	0,061	n.d. < 0,001
Grapes (dataset 2)	35	< 0,001	0,043	n.d. < 0,001
Pears (dataset 1)	66	0,001	0,056	n.d. < 0,001
Pears (dataset 2)	54	< 0,001	0,010	n.d. < 0,001
Pears (dataset 3)	8	0,001	0,009	n.d. < 0,001
Tomatoes	179	< 0,001	0,080	n.d. < 0,001
Group 2: Dried food and vegetables				
Apples, dried	10	0,013	0,048	0,005
Bananas, dried	7	0,006	0,023	0,001
Blueberries, dried	6	0,008	0,130	0,002
Oranges, dried	7	0,008	0,020	0,003
Plums, dried	8	0,005	0,041	0,004
Raisins, Sultanas	5	0,008	0,009	0,001
Tomatoes, dried	46	0,011	0,247	n.d. < 0,001

Product	Number of samples	Median (mg/kg)	Max (mg/kg)	Min (mg/kg)
Group 3: Herbs and Spices				
Basil, dried	16	0,145	0,742	0,043
Basil, dried	8 (bio)	0,063	0,072	0,054
Blackberry leaves, dried	5	0,024	0,049	0,010
Blackberry leaves, dried	4 (bio)	0,035	0,040	0,014
Cardamom	13	0,032	0,288	0,011
Cassia (Cinnamon)	10	0,013	0,067	0,004
Cassia (Cinnamon)	3 (bio)	0,030	0,050	0,013
Chamomile, dried	14	0,029	0,154	0,013
Chamomile, dried	26 (bio)	0,020	0,068	0,005
Chilli powder (cayenne pepper)	84	0,027	0,642	0,004
Cumin	293	0,023	0,879	0,008
Dill	11 (2 bio)	0,016	0,033	0,006
Fennel seed	41 (4 bio)	0,018	0,154	0,005
Ginger	33	0,021	0,328	0,008
Hibiscus, dried	13 (2 bio)	0,013	0,070	0,009
Mix of spices	36	0,028	0,154	0,003
Oregano, dried	20 (3 bio)	0,054	0,171	0,009
Paprika powder	54	0,020	0,308	0,003
Paprika powder	7	0,023	0,030	0,012
Pepper, dried	274	0,034	1,302	0,003
Peppermint, dried	45	0,028	0,577	0,008
Peppermint, dried	5 (bio)	0,030	0,055	0,027
Rose hip, dried	13	0,014	0,158	0,006
Valerian root	14	0,018	0,050	0,010
Group 4: Tea and Herbal/Fruit Teas				
Tea (black, green)	1988	0,014	0,572	0,002
Fruit Tea	48	0,018	0,229	0,001
Herbal Tea	65	0,025	0,299	0,002

Remark: If no characteristic of the samples is mentioned, it is not known exactly, whether and how many of the samples are of conventional resp. organic origin.