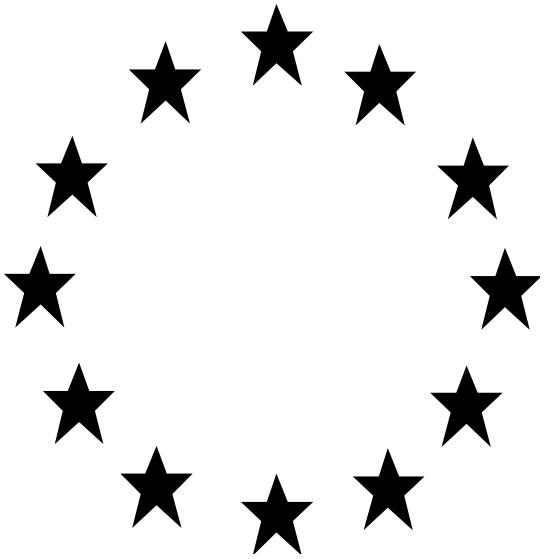

Final addendum to the

Draft Assessment Report (DAR)

- public version -

Initial risk assessment provided by the rapporteur Member State Italy for the existing active substance

FOLPET

of the second stage of the review programme referred to in Article 8(2) of Council Directive 91/414/EEC

Part 1

November 2005

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Folpet

Addendum to Draft Assessment Report:

Environmental fate and behaviour

Rapporteur Member State: Italy

EU review under Directive 91/414 EEC

Relating to Annex B (Volume 3) of the DAR

March 2005

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B.8 Environmental fate and behaviour

Introduction

This document is an Addendum to the Draft Assessment Report (DAR) for the EU review of **folpet**. The aim of this Addendum is to address 'Open points' and 'Data requirements' as raised in the official Reporting Table (dated 22.12.04) and Evaluation Table (dated 18.01.05) in the area of **Environmental fate and behaviour**.

This Addendum includes summarisation and evaluation of new assessments submitted by Makhteshim Chemical Works Ltd.

Section numbering in this Addendum is in line with Annex B (Volume 3) of the DAR.

The Good Agricultural Practice (GAP) uses proposed by the Notifier for consideration under the review are specified in Table 1.

(Please note that in the original DAR, use on North EU winter wheat at 2 x 1.6 kg a.s./ha was included. This use was subsequently removed from the EU review GAP by the Notifier, with the use on South EU winter wheat at 2 x 0.75 kg a.s./ha remaining).

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Table 1: Critical Good Agricultural Practice for folpet in the EU

Crop	Member state or country	Product name	F, G or I[a]	Pests or group of pests controlled	Formulation		Application			Application rate per treatment			PHI (days)	Remarks:
					Type	Conc. of a.s.	method kind	growth stage	number[b] (max.)	kg a.s./hL (max.)	water L/ha	kg a.s./ha (max.)		
Winter wheat	South EU	'Folpan' 80 WDG	F	*Septoria* Brown rust	WG	800 g/kg	Foliar spray; down-ward	Up to Z65	2	0.375	200	0.75	42	
Tomatoes	South EU	'Folpan' 80 WDG	F	various [c]	WG	800 g/kg	Foliar spray; down-ward	From beginning of fruit set	4	0.125	1000	1.25	7	
	South EU	'Folpan' 80 WDG	G	various [c]	WG	800 g/kg	Foliar spray; down-ward	From beginning of fruit set	3	0.16	1000 - 1300	1.6	7	
Grapes	North and south EU	'Folpan' 80 WDG	F	various [d]	WG	800 g/kg	Airblast foliar spray; upwards/ sideways	Shoot emergence to veraison	10	0.75	200 - 400	1.5	28	

[a] F= field; G = greenhouse.
[b] Sprays on all crops are applied typically at intervals of 7 to 28 days.
[c] *Alternaria solanum, Cladospora, Colletotrichum, Septoria, Botrytis*
[d] Black rot, *Botrytis cinerea phomosis*. *Plasmopara viticola*.

B.8.1 Route and rate of degradation in soil (Annex IIA 7.1.1; Annex IIIA 9.1.1)

B.8.1.1 Aerobic and anaerobic studies

The following is stated in the Evaluation Table:

Open point 4.7: RMS to revise to 1st order DT50 values for phthalimide in an addendum to be discussed in an expert meeting. *Reporting table comment 4(26)*

Open point 4.8: RMS to clarify amount of bound residues taking into account fulvic and humic acid in an addendum to be discussed in an expert meeting. *Reporting table comment 4(27)*

Open point 4.9: RMS to clarify which aerobic/anaerobic studies are acceptable and essential for the assessment in an addendum to be discussed in an expert meeting. *Reporting table comment 4(23) and 4(28)*

Comment 4(25) FR: in Table B.8.1.1.2, bound residues seem to have been underestimated (for example on day 14 fulvic acid fraction =14.6 % in text and bound residues = 9.2 % in table). Could this point be clarified.

RMS comment:
On closer inspection of the study report it appears that the sodium hydroxide extraction of the soil to generate the fulvic and humic acid fractions had been included as a soil extraction and the fulvic and humic acid fractions had then not been included as part of the bound residues. It is agreed that this approach is not valid and that the fulvic and humic acid components should be included as part of the bound residues. Therefore, Table B.8.1.1.2 has been amended to include the fulvic and humic acid fractions as part of the bound residues:

Table B.8.1.1.2: Distribution of radioactivity in aerobic soil metabolism of [U-phenyl - ^{14}C] folpet

Time	% of applied radioactivity (AR)						
	Folpet	$^{14}CO_2$	Other volatiles	Bound residues	Phthalimide	Phthalic acid	Total recovery
Day 0	86.9	0.0	0.0	0.7	7.7	3.6	100.0
Day 1	79.6	0.0	0.0	1.5	17.6	2.6	103.2
Day 2	67.5	0.0	0.0	2.4	31.1	3.1	107.0
Day 3	35.5	0.2	0.0	3.3	49.4	3.0	95.1
Day 4	22.2	0.5	0.0	3.8	57.2	3.7	91.7
Day 5	20.9	2.3	0.0	5.5	64.9	5.7	103.6
Day 7	16.2	5.7	0.1	8.4	58.3	2.4	96.6
Day 14	10.0	35.4	0.1	24.4	10.1	1.4	89.7
1 month	6.8	48.5	0.1	19.9	6.2	2.4	91.6
2 months	4.9	56.3	0.1	16.4	4.1	2.6	92.3
3 months	4.1	60.2	0.1	15.9	3.6	2.2	91.4
4 months	3.3	62.5	0.1	15.5	2.4	2.0	91.5
6 months	2.8	65.3	0.1	14.7	2.1	1.8	91.1
9 months	2.1	68.0	0.1	13.7	1.6	1.4	92.0
12 months	2.0	69.8	0.1	11.9	1.3	1.4	91.1

Open point 4.7, and Comment 4(26) FR: from Table B.8.1.1.2, the apparent DT50 for phthalimide is 7.3 d using linear 1st order for the 5-30 d period (R^2 0.81) at 25°C or 10.6 d at 20°C (1st order should be preferred instead of square root 1st order).

The Notifier has submitted the following (ref: Terry, A. 2005a. Responses to questions raised in the Reporting Table on fate and behaviour of folpet):

The degradation of phthalimide can be calculated from the data reported in study 7.1.1.1.1/01 (Daly, D. 1991a), in which the degradation of folpet was investigated. A first order degradation rate for phthalimide was calculated for the purpose of calculating FOCUS PEC_{GW} values and reported (in Mackay, N. 2002). The data from day 5 to day 120 was analysed and a rate of degradation of 28.2 days derived (with an r^2 value of 0.83), at 25°C. It was evident that this value was an over-estimation because the formation and decline of phthalimide was not taken into account, but it was the best fit value that could be obtained.

Open point 4.8, and Comment 4(27) FR: in table B.8.1.1.9 it is not clear why fulvic acid and humic acid fractions were excluded from bound residues. Could this point be clarified.

RMS comments:

In the relevant report, the fulvic and humic acid fractions were reported in a way which implied they were equivalent to a standard extraction. However, the RMS agrees that this is not the case and that fulvic and humic acid components should be regarded as part of the non-extractable fraction. The Table B.8.1.1.9 has been revised to include the fulvic and humic acid fractions as part of the 'bound residues' fraction:

Table B.8.1.1.9: Distribution of radioactivity (%AR) in anaerobic soil metabolism of [U-phenyl-^{14}C] folpet

Time (days)	% of applied radioactivity (AR)					
	Folpet	$^{14}CO_2$	Bound Residues	Phthalimide[a]	Phthalic acid[a]	Total Recovery
Aerobic						
0	88.0	0.0	1.4	8.7	0.0	100.0
1	77.3	0.0	1.9	19.1	0.0	102.4
2	63.8	0.1	2.5	27.7	3.8	102.4
3	41.6	1.8	3.4	41.7	5.4	103.7
4	28.0	6.1	5.9	46.4	4.9	99.3
Anaerobic						
0	27.4	6.1	2.5	50.6	5.0	97.1
3	20.2	6.4	6.6	47.5	6.4	96.4
15	10.9	14.3	8.6	50.2	3.8	96.2
30	7.3	21.6	3.6	46.4	9.2	94.4
45	5.1	25.0	7.1	46.0	5.6	97.2
60	3.5	26.3	6.3	36.3	13.3	92.7

[a]Totals from soil and water layers.

Open point 4.9, and Comment 4(28) FR: the second aerobic/anaerobic study should not be used (significant deviation from guideline). The first study suggests that anaerobic degradation could be similar to aerobic degradation but would occur at slower rate.

The Notifier has submitted the following (ref: Terry, A. 2005a. Responses to questions raised in the Reporting Table on fate and behaviour of folpet):

Folpet is only used in the spring and summer and not in the autumn and winter. In addition, folpet and its major soil metabolites degrade with laboratory DT_{50} values of between 0.8 and 28.2 days. Therefore, it is very unlikely that significant amounts of these substances will be present in soil during times when anaerobic conditions might be experienced (autumn/winter) following use according to the GAP. Therefore, the anaerobic degradation studies are not required for risk assessment purposes. Given the lack of need to evaluate fate of captan under anaerobic conditions, it is also unnecessary to address potential concerns arising from the submitted anaerobic degradation studies. However, of the two anaerobic studies submitted the recovery of radioactivity in the second study (7.1.1.1.2/02; Pack, D.E., 1980) was variable and, as a consequence, the first study (7.1.1.1.2/01; Daly, D. 1991b) should be considered as more reliable.

There were three aerobic soil degradation studies submitted and aerobic phases in one of the anaerobic studies (7.1.1.1.2/01; Daly, D. 1991b). To determine the fate and behaviour in soil, one route of metabolism study is required and the rate of degradation is required in three other soils, of diverse characteristics. The study 7.1.1.1.1/01 (Daly, D. 1991a) was conducted in a sandy loam soil (pH 5.4) with [U-phenyl-^{14}C] labelled folpet at 25°C and 75-80% of FC. The fate of folpet and its major soil metabolites was determined. In the more recently conducted study 7.1.1.1.1/03 (Crowe, A. 2001) the degradation of [U-phenyl-^{14}C] labelled folpet was investigated in three soils; loamy sand, silty loam and clay loam (pH 4.8, 6.2 and 7.5) at 20°C (and one soil at 10°C), and 40% WHC. The rate of degradation of folpet, phthalimide, phthalic acid and phthalamic acid was calculated. Together then, these two studies provide sufficient information to characterise the fate and behaviour of folpet in soil under aerobic conditions. These two studies were also sufficient to derive representative normalised (to pF 2.0 and 20°C,

according to FOCUS guidance) rates of degradation for folpet and its major degradation metabolites (see Mackay, N. 2002).

As such, it is proposed that these two studies (Daly, D. 1991a, and Crowe, A. 2001) are the only soil degradation studies submitted that are necessary for assessment purposes.

RMS comments: The RMS agrees with the position presented by the Notifier. The two studies, Daly, D. 1991a, and Crowe, A. 2001, are sufficient for assessment purposes.

B.8.1.4 . Summary and assessment.

Open point 4.10: RMS to provide r^2 for each determination and normalised DT_{50} in an addendum to be discussed in an expert meeting. *Reporting table comment 4(30)*

Open point 4.11: RMS to provide an addendum with a summary of studies that address the fate of side chain of folpet. Formation of thiophosgen should be addressed. Addendum to be discussed in an expert meeting. *Reporting table comment 4(31)*

Open point 4.18: RMS to clarify which studies of captan are used in the assessement of folpet and if these studies have actually been submitted in the folpet dossier. *Reporting table comment 4(48)*

Open point 4.10, and Comment 4(30) EFSA: R^2 should be indicated for each determination. Normalised DT_{50} to 1okPa of pF2, 20°C with Q10 of 2.2 should be calculated for FOCUS ground water modelling. (Table B.8.1.4.1)

The Notifier has submitted the following (ref: Terry, A. 2005a. Responses to questions raised in the Reporting Table on fate and behaviour of folpet):

Following consideration of open points 4(7), 4(9) and 4(10), Table B.8.1.4.1 has been revised to include r^2 values (taken from the relevant reports) and re-calculated first order DT_{50} values (taken from Mackay, N. 2002). The summary of rate of aerobic degradation presented in the table has been restricted to those studies considered essential for the assessment (see response to comment 4(28), above) and the summary of anaerobic degradation rates has been removed.

Table B.8.1.4.1 Summary of soil degradation rates of folpet and metabolites

First order DT_{50} (days)	First order DT_{90} (days)	Coefficient of fit (r^2)	Study (temperature of incubation)	DT_{50} normalised to pF 2.0 and 20°C (days)**
Folpet				
0.2	0.7	0.999	Crowe, A. 2001 (20°C)	0.12
0.8	2.8	0.986	Crowe, A. 2001 (20°C)	0.49
3.8	12.8	0.995	Crowe, A. 2001 (20°C)	2.92
3.8	12.6	0.998	Crowe, A. 2001 (10°C)	1.05
16.2*	53.8*	0.80	Daly, D. 1991a (25°C)	15.2
			Mean:	**4.0**
			Median:	**1.05**
Phthalimide				
0.5	1.7	0.984	Crowe, A. 2001 (20°C)	0.29
1.7	5.8	0.992	Crowe, A. 2001 (20°C)	1.04
4.8	16.1	0.876	Crowe, A. 2001 (20°C)	3.69
3.2	10.6	0.977	Crowe, A. 2001 (10°C)	0.89
28.2*	93.7*	0.83	Daly, D. 1991a (25°C)	26.5
			Mean:	**6.4**
			Median:	**1.04**
Phthalic acid				
0.6	2.1	0.999	Crowe, A. 2001 (20°C)	0.35
1.0	3.2	0.954	Crowe, A. 2001 (20°C)	0.61
4.1	13.7	0.892	Crowe, A. 2001 (20°C)	3.15
1.8	5.9	0.855	Crowe, A. 2001 (10°C)	0.50
			Mean:	**1.15**
			Median:	**0.56**
Phthalamic acid				
0.4	1.3	0.999	Crowe, A. 2001 (20°C)	0.24
0.8	2.7	0.973	Crowe, A. 2001 (10°C)	0.22
			Mean:	**NR**
			Median:	**NR**

*biphasic kinetics – these are worst case first order DT_{50}/DT_{90} generated for purposes of $FOCUS_{GW}$ modelling; $DT_{90}=DT_{50}$ x ln(10)/ln(2); from Mackay, N. (2002)

** from Mackay, N. (2002)

Open point 4.18 and 4.11, and Comment 4(31) EFSA: Degradation of the thio(trichloromethyl) side chain is addressed with some studies of the active substance captan. These studies should be properly summarised and included in the list of references relied on. Formation of thiophosgene should be assessed.

RMS comment:
Summaries of the two studies on captan, as they appear in the captan DAR, which indicate the likely fate of the side chain are included below. For clarity, the whole of the relevant section has been repeated here:

No laboratory degradation studies have yet been carried out using the labelled thio(trichloromethyl) sidechain of folpet, but an estimate of degradation may be made from

studies on the closely related compound, captan, which has an identical sidechain. These studies, which are summarised below, indicated that the sidechain was likely to be degraded rapidly, with mineralisation to carbon dioxide. In non-sterile soils**,** [trichloromethyl-^{14}C] captan was rapidly degraded under aerobic conditions. The experimentally observed DT_{50} was less than 4 hours, although the calculated DT_{50} using linear regression was 2.5 days if eight data points through 28 days were utilised, or 1.0 days if five earlier data points up to 7 days were utilised. The experimental mineralisation half-life was less than 3 days. The [trichloromethyl-^{14}C] label indicated conversion to thiocarbonic acid and then to CO_2 (without thiophosgene as an intermediate). Three minor unknown degradates were detected at low levels (less than 1% of the applied radioactivity). During the course of the study, the radioactive residues bound to soil averaged 10% of the applied dose. In sterile soil, some mineralisation to CO_2 was observed at a lower rate. Captan was rapidly degraded in soil, under aerobic conditions, with an estimated DT_{50} of less than 1 day. The estimated mineralisation half-life was less than 3 days. Bound residues levels reached a plateau after 4 days at about 14% AR. The [trichloromethyl-^{14}C] label indicated extensive mineralisation to CO_2.

(a) Aerobic metabolism of [trichloromethyl -^{14}C] captan in soil. (Diaz, D. and Lay, M.M. 1992; IIA, 7.1.1.1.1/04)

The aerobic metabolism of [trichloromethyl - ^{14}C] captan (radiochemical purity 99.0%, specific activity 40.4 mCi/mmole) was investigated in a sandy loam soil in the dark in accordance with USEPA Guidelines in a 1992 study. The soil characteristics are summarised in Table B.8.1.4.2. The concentration of the test substance in soil was equivalent to 8.76 mg/kg. The incubation conditions were 75% of field capacity at a temperature of 25 ± 2°C, for periods of 4 hours to 28 days under O_2 atmosphere. A further experiment was performed with a sterile soil sample under the same conditions. Duplicate samples were taken for each sample point.

The study met the essential criteria of USEPA 162-1. It was conducted according to Good Laboratory Practice.

Table B.8.1.4.2: Soil characteristics for aerobic metabolism study of [trichloromethyl -^{14}C] captan

Characteristic	Soil
Soil name	Visalia
Location	California, USA
pH	7.70
Sand (%)[1]	54.8[1]
Silt (%)	33.4
Clay (%)	11.8
Texture[2]	Sandy loam
Organic matter (%)	0.70
Organic carbon (%)[3]	0.41
Cation exchange capacity meq/100 g)	9.1
Half saturation (approximate field capacity) (%)	16.0

[1] Combination of very coarse sand (1.4%), coarse sand (3.8%), and medium to fine sand (49.6%).
[2] USDA classification.
[3] Organic carbon = organic matter/1.72

The results obtained, in terms of distribution of radioactivity and metabolites at different sampling dates in the non-sterile soil are summarised in Table B.8.1.4.3. Average total recoveries were in the range 84-96 % applied radioactivity (AR). The amount of $^{14}CO_2$ evolved increased during the study to 80.8% AR after the incubation period of 28 days. The level of bound residue increased to 13.3% during that period, reaching a maximum of 14.8% after 14 days. Low levels of other volatiles of about 0.2% AR were detected between 3 and 28 days.

Levels of captan declined from 86% to 0.1% AR during the 28 days of the study, and thiocarbonic acid was detected between 7 and 28 days at levels of 0.6 - 1.1% AR. Three minor unknowns (unk-0, unk-1 and unk-2) were detected, each individually at a level of <1% AR, and these appeared to be declining towards the end of the study.

Table B.8.1.4.3: Distribution of radioactivity in non-sterile aerobic soil (mean of duplicates)

Time	(% of applied radiolabel)[1]								
	Captan	$^{14}CO_2$	Volatiles	Bound residues	Thio-carbonic acid	Unk-0	Unk-1	Unk-2	Total
0 hour	86.07	0	-	0.15	0	0	0.46	0	92.53
4 hours	45.05	25.27	-	7.96	0	0.29	0.45	0.89	83.99
8 hours	49.85	30.62[2]	-	10.71	0	0.19	0.47	0.49	96.67
1 day	35.74	37.51	-	8.36	0	0.15	0.39	0.47	89.89
3 days	14.13	58.66	0.17	13.97	0	0	0.16	0.12	92.77
7 days	9.57	65.27	0.21	11.90	0.82	0	0	0.11	90.23
14 days	1.15	76.73	0.18	14.81	1.12	0	0	0.13	95.80
28 days	0.1	80.82	0.19	13.33	0.63	0	0.02	0.04	95.38

[1]Levels at origins of TLC systems used to separate metabolites were 0.03 - 1.21% (aqueous extracts) and 0.16 - 2.24% (organic extracts).
[2]Value from one sample only.

Results were obtained for the sterile soil at 3 days and 24 days. Total recoveries were 79.5 - 85.6% AR. $^{14}CO_2$ evolution was increased from 25.9 to 39.1% AR, while other volatiles were detected at slightly higher levels than for non-sterile soil, at 1.7-1.9% AR. Bound residues increased from 15.2 - 22.4%. In non-sterile soils**,** captan was rapidly degraded under aerobic conditions. The experimentally observed DT50 was less than 4 hours, although the calculated DT50 using linear regression was 2.5 days if eight data points through 28 days were utilised, or 1.0 days if five earlier data points up to 7 days were utilised. The experimental mineralisation half life was less than 3 days.

The [trichloromethyl - ^{14}C] label indicated conversion to thiocarbonic acid and then to CO_2. Three minor unknown degradates were detected at low levels (less than 1% of the applied radioactivity). During the course of the study, the radioactive residues bound to soil averaged 10% of the applied dose. In sterile soil, some mineralisation to CO_2 was observed at a lower rate.
Captan degraded very rapidly in non-sterile soil under aerobic conditions. The experimentally observed DT_{50} was less than 4 hours, although the calculated DT_{50} using linear regression was 2.5 days if eight data points through 28 days were utilised, or 1.0 days if five earlier data points up to 7 days were utilised.

Captan was rapidly degraded in soil under aerobic conditions.

(b) Aerobic soil metabolism of [trichloromethyl -^{14}C] captan. (Pack, D.E. and Verrips, I.S. 1988; IIA, 7.1.1.1.1/05)

The aerobic metabolism of [trichloromethyl - ^{14}C] captan (radiochemical purity 99.9 %, specific activity 40.4 mCi/mmoles) was investigated in a sandy loam soil in the dark in accordance with USEPA Guidelines. The soil characteristics are summarised in Table B.8.1.4.4. The concentration of the test substance in soil was 4.6 to 6.1 mg/kg, equivalent to an exaggerated field application rate of 23.2 to 30.7 kg a.s./ha, assuming 50% captan reaches the soil, and that it penetrates the top 15 cm of soil. The soil samples were incubated at 80% of

field capacity and a temperature of 25 ± 0.1°C, for a period of 30 days maximum under oxygen atmosphere.

The study met the essential criteria of USEPA 162-1. It was conducted according to Good Laboratory Practice.

Table B.8.1.4.4: Soil characteristics aerobic metabolism study of [trichloromethyl - ^{14}C] captan

Characteristic	Soil
Soil name	Greenville
Location	Mississippi, USA
pH	7.2
Sand (%)	58
Silt (%)	30
Clay (%)	12
Texture[1]	Sandy loam
Organic matter (%)	1.2
Organic carbon (%)[2]	0.7
Cation exchange capacity meq/100 g)	7.7
Capacity at 1/3 bar (%)	14.8

[1]USDA classification.
[2]Organic carbon = organic matter/1.72.

The results obtained, in terms of distribution of radioactivity and metabolites at different sampling dates are summarised in Table B.8.1.4.5. Average total recoveries were in the range 83-113% AR. The amount of $^{14}CO_2$ evolved increased during the study to 77.4 - 91.6 % AR after the incubation period of 30 days and low levels of other volatiles of about 0.7 - 1.2% AR were detected between 1 and 30 days. The level of bound residue increased to 16.7% AR after 1 day, and remained at a similar level during the rest of the study (14.3% AR at day 30). No non-volatile metabolites were detected during this study.

Table B.8.1.4.5: Distribution of radioactivity in soil (mean of duplicates at 1, 3, 14 and 30 days, and quadruplicates at 0 and 7 days)

Days	(% of applied radiolabel)			
	Captan	$^{14}CO_2$/volatiles[1]	Bound residues	Total
0	105.3	0	0.5	105.8
1	19.4	47.2	16.7	83.2
3	9.7	60.3	18.7	88.7
7	9.6	69.7	14.8	94.1
14	5.5	81.8	14.4	101.7
30	7.6	90.8	14.3	112.7

[1] $^{14}CO_2$ and volatile results recorded separately at 1, 2, 4, 5, 7, 12, 14, 22 and 30 days.

Captan was rapidly degraded in soil under aerobic conditions with an estimated DT_{50} of less than 1 day. The estimated mineralisation half-life was less than 3 days. Bound residues levels plateaued after four days at about 14% AR. The [trichloromethyl - ^{14}C] label indicated extensive mineralization to CO_2.

Captan degraded very rapidly under aerobic conditions, with an estimated DT_{50} of less than 1 day.

RMS assessment: It is clear from the two studies conducted with trichloromethyl - ^{14}C captan that the main route of degradation of the side chain was rapid conversion to thiocarbonic acid

(without thiophosgene as an intermediate) and then to CO_2. Therefore, thiophosgene would not be expected to be a significant folpet degradation product in soil.

B.8.2 Adsorption, desorption and mobility in soil (Annex IIA 7.1.2 and 7.1.3; Annex IIIA 9.1.2)

B.8.2.1 Adsorption and desorption

Open point 4.12: RMS to provide an addendum with Koc estimation of phthalamic acid and an assessment of its reliability to be discussed in an expert meeting. *Reporting table comment 4(32), 4(33)*

Open point 4.13: Acceptability of Koc for soils loam EUROSOIL 3 and sand soil LUFA2.1 to be discussed in an expert meeting. *Reporting table comment 4(34)*

Open point 4.12, and Comment 4(32) FR: Koc for phthalamic acid and phthalic acid has been estimated by means of the EPIWIN program but this is not described in the monograph. This point should be completed.

The Notifier has submitted the following (ref: Terry, A. 2005a. Responses to questions raised in the Reporting Table on fate and behaviour of folpet):

No sorption/desorption studies have been conducted with phthalamic and phthalic acid. As these degradation products only occurred briefly above 10% in soil degradation studies they were considered to be transient. The rapid formation and degradation of these secondary degradation products suggested that it was appropriate to employ estimates of sorption characteristics in order to assess potential mobility. The PCKOC programme (within the EPIWIN suite of programs) was used to estimate the K_{OC} values for phthalic acid (73.06) and phthalamic acid (10) (Mackay, N. 2002). The following description of the estimation program was obtained from the SRC website (http://www.syrres.com/esc/pckoc.htm).

"The PCKOC program (within EPIWIN) calculates the soil or sediment adsorption coefficient (K_{OC}, the ratio of the chemical adsorbed per unit weight of organic carbon in the soil or sediment to the concentration of the chemical in solution at equilibrium). SMILES notation structural input is used to calculate the K_{OC} from a correlation to the molecular connectivity indices and correction factors for certain chemical classes.

The newest version of PCKOC runs under Windows (3.1, 95, 98, NT) making the estimation of soil adsorption coefficients more convenient and accurate. Methods for chemical structure input now support SMILES notations as well as chemical structures produced in popular chemical drawing programs. Enhanced features of the Windows version include batch-mode data entry and user functions.

The method was developed with EPA's Office of Pollution Prevention and Toxics using a training set of 189 chemicals and evaluated with a validation set of 131 chemicals. The correlation coefficient for the validation set was 0.92 [Environmental Science and Technology, Volume 26, pp. 1560-67 (1992)]. The following is the article abstract:

"The first-order molecular connectivity index (MCI) has been successfully used to predict soil sorption coefficients (K_{OC}) for nonpolar organics, but extension of the model to polar compounds has been problematic. To address this, we developed a new estimation method based on MCI and series of statistically derived fragment contribution factors for polar compounds. After developing an extensive database of measured K_{OC} values, we divided the dataset into a training set of 189 chemicals and an independent validation set of 205 chemicals. Two linear regressions were then performed. First, measured log K_{OC} values for nonpolar compounds in the training set were correlated with MCI. The second regression was developed by using the deviations between measured log K_{OC} and the log K_{OC} estimated with the nonpolar equation and the number of certain structural fragments in the polar compounds. The final equation for predicting log K_{OC} accounts for 96% and 86% of the variation in the measured values for the training and validation sets, respectively. Results also show that the model outperforms and covers a wider range of chemical structures than do models based on octanol-water partition coefficients (Kow) or water solubility." [*Meylan, W., P.H. Howard and R.S. Boethling, "Molecular Topology/Fragment Contribution Method for Predicting Soil Sorption Coefficients", Environ. Sci. Technol. 26: 1560-7 (1992).*]."

Open point 4.13, and Comment 4(34) SI: The value of 1/n is too low for the loam soil (EUROSOIL 3) and sand soil (LUFA 2.1) in the study of Geffke, 2000. This means that adsorption/desorption behaviour is not adequately described by the Freundlich theory. Corresponding Koc values should not be further considered in the risk assessment.

RMS comment: The acceptability of the K_{OC} data derived for the two soils LUFA 2.1 (pH 6.0, 1/n 0.5171) and LUFA 2.2 (pH 5.8, 1/n 0.5794) for use in arriving at a representative K_{OC} value for phthalimide for calculating FOCUS PEC_{GW} was addressed in the report, Mackay, N. (2002). The following is taken from that report:

"In two of the soils tested there was evidence of a significant deviation from a linear sorption isotherm (1/n = 0.52, 1/n = 0.58). It is likely that this significant deviation from linearity is related to pH. The pH dependence of sorption (for phthalimide), and the practical experimental difficulties that are associated with it also suggest that these values may be a less reliable basis for defining sorption. It is, therefore, proposed that simulations are based upon a median sorption coefficient and coefficient (1/n) defining the Freundlich isotherm taking all of the data into account. If the two assessments conducted with the more alkali LUFA soils are removed from considerations (due to concerns related to reliability), the median value, by default, becomes a more reliable worst case. This is proposed as a pragmatic approach for circumventing the difficulties associated with reliability and availability of alkali sorption data. The median K_{OC} value reported is 142.5 cm^3/g. The median coefficient (1/n) defining the Freundlich isotherm is 0.83. On this basis, phthalimide is then classified as "Moderately mobile"."

RMS assessment: The approach set out above appears pragmatic and reasonable. The RMS agrees with the classification of phthalimide as moderately mobile and the appropriateness of selecting the K_{OC} value and the 1/n value for use in FOCUS PEC_{GW} calculations.

B.8.3 Predicted environmental concentrations in soil (PECS) (Annex IIIA 9.1.3)

The Notifier has submitted the following (ref: Terry, A. 2005a. Responses to questions raised in the Reporting Table on fate and behaviour of folpet):

To carry out earthworm risk assessments it was necessary to calculate PEC_{soil} values for folpet following application to winter wheat, tomatoes and vines. These were calculated according to the EU-review GAP assuming distribution into the top 5 cm of soil (density 1.5 g/cm^3), application intervals of 7 days and a first order degradation rate for folpet of 4.3 days. Crop interception rates were chosen according to FOCUS groundwater guidance. For vines, the applications span a number of different interception rates and as such a simple average of these values was calculated. Maximum PEC_{soil} values were calculated using an Excel spreadsheet (see Table B.8.3.2).

Table B.8.3.2: Maximum PEC_{soil} values for folpet

Crop	Selected crop interception (%)	Maximum PECsoil (mg/kg)
Winter Wheat	70	0.40
Tomatoes	80	0.49
Vines	66.3	1.00

RMS comment: The DT50 of 4.3 days is derived from Daly, 1991a (7.1.1.1.1/01) was observed during the first 14 days of the study. Hence, the value is considered relevant for PECsoil derivation as the minimum spray interval is 7 days. The degradation in this study was biphasic. In the context of the FOCUSgw assessment the Notifier has also derived a DT50 from this study based on first order kinetics (Table B.8.6.4), which is appropriate.

B.8.4 Fate and behaviour in water (Annex IIA 7.2.1; Annex IIIA 9.2.1, 9.2.3)

B.8.4.4 Water sediment studies

Comment 4(36) DE: The first sentence is not complete ("The degradation… was investigated… in accordance…" with?).

RMS: the complete sentence is given below (addition in bold):

"Folpet. Degradability in the water/sediment system. (Crowe, A. 1999; IIA, 7.2.1.3.2/01)
The degradation of [U-phenyl-^{14}C] folpet (radiochemical purity > 99.3%), was investigated in two sediment/water systems (one silty clay sediment and one sandy loam sediment) at 20°C in the dark in accordance **with SETAC/BBA guidelines in a 1999 study**. The system characteristics are summarised in Table 8.4.4.1. The test substance was applied to each sediment/water system at a nominal application rate of 1.6 kg a.s./ha (equivalent to 0.533 mg/L)."

Data requirement 4.1 *Reporting table comment 4(18)*	Notifier to give more details on bound residues and on identity of the absorbed residue in the sediment.

Open point 4.2:RMS to clarify if folpet or metabolites are found in the sediment in an addendum.
Reporting table comment 4(4)

Open point 4.14: RMS to provide an addendum to clarify and assess kinetic models employed to evaluate water sediment studies to be discussed in an expert meeting.
Reporting table comment 4(35)

Open point 4.2, and Comment 4(4) EFSA: It should be stated clearly if folpet is found in the sediment compartment.

RMS comment: Folpet was not found in the sediment at any time point. The following summary table (Table B.8.4.4.5) is taken from Terry, A. 2005b.

Table B.8.4.4.5: Summary of sediment/water systems and key findings

	System:	**Row Pond**	**Emperor Lake**
Water phase	pH	8.1	7.1
Sediment phase	pH	6.8	5.9
Sediment	texture class	Silty clay	Sandy loam
Sediment	Organic Carbon (%)	4.4	1.2
Water Phase	Maximum Phthalimide (%)	20.4	26.0
Water Phase	Maximum Phthalamic acid (%)	5.9	13.3
Water Phase	Maximum Benzamide (%)	10.2	0.0
Water Phase	Maximum Phthalic acid (%)	26.3	37.5
Water Phase	Maximum 2-cyanobenzoic acid (%)	39.7	0.0
Sediment Phase	Maximum Phthalimide (%)	3.0	5.9
Sediment Phase	Maximum Phthalic acid (%)	0.9	3.8

Data requirement 4.1, and Comment 4(18) DE: Considerable amounts of bound sediment residues of approx. 25% AR were detected 7 and 14 days after application of folpet. After 100 days, the residues decreased to approx. 10 %. Since folpet (1.5 kg a.s./ha) might be applied up to 10 times with weekly intervals, it is assumed that the bound residues will accumulate due to multiple application. This issue should be addressed in the discussion to this chapter and might also be of relevance in the risk assessment for aquatic compartment including the sediment dwelling organisms.

The importance of this comment might increase, if it would be demonstrated that a large portion of the bound residues is still related to the parent compound that can be mobilised and/or taken up by sediment dwelling organisms

The Notifier has submitted the following (ref: Terry, A. 2005a. Responses to questions raised in the Reporting Table on fate and behaviour of folpet):

The degradation of folpet was investigated in two contrasting sediment/water systems (*Folpet. Degradability in the water/sediment system. (Crowe, A. 1999; IIA, 7.2.1.3.2/01*)). It was evident that folpet and its degradation products were all rapidly dissipated from the whole

systems with DT_{50} values of between 0.014 and 6.45 days. These results were fully consistent with findings from hydrolysis studies conducted with folpet and phthalimide which demonstrated that both hydrolysed very rapidly (folpet DT_{50} 1.1 hours at pH 7 and 25°C; phthalimide DT_{50} 7.45 hours at pH 7 at 40°C which corresponds to 21.2 hours at 25°C). This suggests that the residues in the sediment (see Table B.8.4.4.6) were unlikely to be structurally similar to either folpet or phthalimide.

Table B.8.4.4.6: Summary of non-extractable residues and evolved carbon dioxide in two sediment/water systems

Incubation time	Silty clay system		Sandy loam system	
	Non-extractable residues (%)	Evolved CO_2	Non-extractable residues (%)	Evolved CO_2
0	2.1	0.0	3.7	0.0
1 hr	1.5	0.0	2.3	0.0
4 hr	3.3	0.0	6.0	0.0
1 day	14.0	0.5	11.6	1.0
2 days	24.0	6.4	19.5	4.1
7 days	24.7	12.4	25.1	9.7
14 days	26.3	20.8	25.5	19.2
30 days	20.4	42.8	22.0	39.2
62 days	17.2	50.5	18.0	50.5
100 days	12.5	58.1	19.3	54.6

In fact, the sediment phases in the study were exhaustively extracted. Following separation of the water and sediment phases, the latter was then extracted with acetonitrile/acetic acid (98:2, v/v) by shaking for 1 hour. The extracted sediment was then further extracted by refluxing in glacial acetic acid for 16 hours. This second extraction should be regarded as extraction under harsh conditions. The extracted sediment samples from the 100 day sampling point were further processed to estimate fulvic acid, humic acid and humin fractions. The results of these analyses are given in Table B.8.4.4.7.

Table B.8.4.4.7: Summary of further processing of extracted sediment samples from 100 day sampling point

Fraction	Silty clay system	Sandy loam system
Fulvic acid	2.2	1.1
Humic acid	0.0	0.0
Humin	12.6	9.0

It is evident from this last fractionation that the unextracted residue was mostly associated with the humin fraction. Given the severity of the sequential extraction procedures employed it is reasonable to conclude that the vast majority of the non-extracted sediment residue was covalently associated with the sediment (rather than being simply adsorbed) and that this residue was not readily released from the sediment, except as carbon dioxide (as the sediment non-extracted residue decreased towards the end of the study, the amount of carbon dioxide evolved increased).

It is possible to postulate that the non-extracted sediment residue arose from the covalent binding of phthalic acid type moieties to the organic carbon content of the sediment. During the course of the study the total radioactive recovery declined. It was suggested in the study report that this could have been due to the production of radio-labelled methane which would not have been trapped. It is known that one of the products of the *anaerobic* degradation of

phthalic acid in sludge is methane (Shelton, D.R., Boyd, S.A., Tiedje, J.M., 1984). Consequently, it appears likely that the non-extracted residue in the sediment/water systems did consist of phthalic acid type moieties which were then more slowly partially degraded in the anaerobic layers of the sediments to release radioactivity from the systems as methane which was not then trapped.

Overall, it would appear that the non-extractable sediment residue was covalently bound to the sediment and was not released except as carbon dioxide or methane. As such, there would not appear to be any concern with respect to the bioavailability of the residue over time, regardless of the number of successive applications of folpet.

RMS comment: It is agreed that the nature of the non-extracted sediment residue appears not to constitute a risk to sediment dwelling organisms.

Open point 4.14, and Comment 4(35) EFSA: The underlaying kinetics under the "computerized statistical model" used to calculate the degradation parameters should be given.

RMS comment: The following description of the kinetic analysis carried out on the sediment/water study results are reproduced from the study report (*Folpet. Degradability in the water/sediment system. (Crowe, A. 1999; IIA, 7.2.1.3.2/01)* page 33):

The DT_{50} and DT_{90} values were determined using the computerised statistical models of SAS 6.11 (SAS Institute, 1989). Simple exponential models (y = m x exp(-b(t-tm)), where tm is the starting time for the selected data) were fitted to the data for each sediment/water system and aqueous phase separately. The maximum was identified as the highest value except for phthalamic acid in the sandy loam sediment/water system where the 1 day results were used. The DT_{50} and DT_{90} values were defined as the time at which the concentration decayed to 50% and 10% of the 100% level respectively. These values were derived b y interpolation using the fitted models.

B.8.6 Predicted environmental concentrations in surface water and in ground water (PEC_{SW}, PEC_{GW}) (Annex IIIA 9.2.1, 9.2.3)

Groundwater

Open point 4.15: RMS to provide an addendum with an expanded summary of FOCUS gw modelling and recalculations if necessary to be discussed in an expert meeting. *Reporting table comment 4(37) and 4(38)*

Open point 4.15, and Comment 4(37) EFSA: The input parameters used for FOCUS ground water simulations and the rationale for their selection should be given in the DAR.

A fuller summary of the PEC_{GW} report (Mackay, N. 2002) is presented, in which the justification for the selection of parameters is also given:

Report: *Mackay, N. (2002). Predicted Environmental Concentrations of folpet and its degradation products in groundwater in the European Union using the FOCUS groundwater scenarios. CEA, unpublished report March 2002.*

Groundwater modelling of folpet has been undertaken with the FOCUS groundwater scenarios using the PELMO model (FOCUS version 1.1.1). The modelling undertaken was based on the use of the 80 WDG formulation. Simulations were conducted with applications to vines based on an application rate of 1.5 kg a.s./ha. Simulations were also carried out for Northern and Southern Europe winter wheat usages at 1.6 and 0.75 kg a.s./ha, respectively. Simulations included the evaluation of three degradation products, phthalimide, phthalamic acid and phthalic acid. *It should be noted that the North European use in wheat is no longer included in the EU-review GAP.*

A worst-case treatment to vines of 10 applications of 1.5 kg a.s./ha, at 7 day intervals, in northern and southern EU countries was simulated. Initial application dates ranged from 20th March to 1st May with applications continuing until 22nd May to 3rdJuly depending on scenario location. Crop interception factors ranged from 50% for applications carried out early season to 85% for applications carried out at fruit ripening. It should be noted that this application scenario is more worst case than use in tomatoes, which is therefore addressed in the vines simulation.

A worst-case treatment to winter wheat of 2 applications at 14 day intervals, in Southern EU countries at a rate of 0.75 kg a.s./ha was simulated and 2 applications at 14 day intervals in Northern EU countries at a treatment rate of 1.6 kg a.s./ha was also simulated. Initial applications dates ranged from 1st February to 15th April. Crop interception factors were 70% for all applications.

Due to the rapid hydrolysis of folpet, its adsorption coefficient, K_{OC}, could not be directly measured and was, therefore, estimated using a series of estimation methods. The worst case value of those generated, **304** cm^3/g, was selected to be used in the simulations. A default coefficient defining the Freundlich sorption isotherm (1/n = **0.9**) was also selected (FOCUS 2001).

For phthalimide, adsorption and desorption was measured in five soils. However, due to the instability of phthalimide under neutral and alkaline conditions the soils selected all had pH values less than or equal to 6, to enable the study to be carried out.

In two of the soils tested there was evidence of a significant deviation from a linear sorption isotherm (1/n = 0.52, 1/n = 0.58). It is likely that this significant deviation from linearity is related to pH. The pH dependence of sorption (for phthalimide), and the practical experimental difficulties that are associated with it also suggest that these values may be a less reliable basis for defining sorption. It was, therefore, proposed that simulations be based upon a median sorption coefficient and coefficient (1/n) defining the Freundlich isotherm taking all of the data into account. If the two assessments conducted with the more alkali LUFA soils were removed from consideration (due to concerns related to reliability), the median value, by default, became a more reliable worst case. This was proposed as a pragmatic approach for circumventing the difficulties associated with reliability and availability of alkali sorption data. The median K_{OC} value reported was **142.5** cm^3/g. The median coefficient (1/n) defining the Freundlich isotherm was **0.83**.

No sorption/desorption studies have been conducted with phthalamic and phthalic acid. As these degradation products only occurred briefly above 10% in soil degradation studies they were considered to be transient. The rapid formation and degradation of these secondary degradation products suggested that it was appropriate to employ estimates of sorption characteristics in order to assess potential mobility.

K_{OC} values for phthalamic acid and phthalic acid have been estimated by the PCKOC program within the EPIWIN suite (v 1.66, USEPA, 2000). Using this software the values were estimated to be **10** and **73.06** for phthalamic acid and phthalic acid, respectively. FOCUS default values of **0.9** were selected for the 1/n values.

For the derivation of degradation in soil parameters, two studies (Daly, D. 1991a, and Crowe, A. 2001) were found to be appropriate and sufficient for the assessment (see B.8.1.1 above).

In the first study (Daly, D. 1991a) the degradation assessment was conducted in a sandy loam (pH 5.4) with incubation conditions of 75 to 80% field capacity at a temperature of 25°C. One significant degradation product was found; phthalimide. The degradation of folpet proceeded *via* bi-phasic kinetics with a first order DT_{50} of 4.7 days relevant to the first 14 days of the study, followed by much slower degradation thereafter. As such, for modelling purposes (and following FOCUS guidance) the first order DT_{50} value was estimated over the first two months of the study. This yielded a DT_{50} of 16.2 days ($r^2 = 0.80$). This value was then normalised to pF 2.0 and 20°C, according to FOCUS guidance, giving a normalised value of 15.2 days. It was also possible to estimate the first order DT_{50} value for phthalimide in the same manner. This gave a value of 28.2 days ($r^2 = 0.83$), which normalised to 26.5 days.

In the second study (Crowe, A. 2001) the degradation assessment was conducted in a clay loam, a silty loam and loamy sand with incubation conditions of 40% of maximum holding capacity and temperatures of 10 and 20°C over 30 days. Three significant degradation products were found; phthalimide, phthalamic acid and phthalic acid. The first order DT_{50} values reported in this study were normalised according to FOCUS guidance.

A summary of the derived DT_{50} values are presented in Table B.8.6.4.

Table B.8.6.4: Summary of soil degradation rates of folpet and metabolites

First order DT_{50} (days)	Coefficient of fit (r^2)	Study (temperature of incubation)	DT_{50} normalised to pF 2.0 and 20°C (days)
Folpet			
0.2	0.999	Crowe, A. 2001 (20°C)	0.12
0.8	0.986	Crowe, A. 2001 (20°C)	0.49
3.8	0.995	Crowe, A. 2001 (20°C)	2.92
3.8	0.998	Crowe, A. 2001 (10°C)	1.05
16.2	0.80	Daly, D. 1991a (25°C)	15.2
		Mean:	**4.0**
		Median:	**1.05**
Phthalimide			
0.5	0.984	Crowe, A. 2001 (20°C)	0.29
1.7	0.992	Crowe, A. 2001 (20°C)	1.04
4.8	0.876	Crowe, A. 2001 (20°C)	3.69
3.2	0.977	Crowe, A. 2001 (10°C)	0.89
28.2	0.83	Daly, D. 1991a (25°C)	26.5
		Mean:	**6.4**
		Median:	**1.04**
Phthalic acid			
0.6	0.999	Crowe, A. 2001 (20°C)	0.35
1.0	0.954	Crowe, A. 2001 (20°C)	0.61
4.1	0.892	Crowe, A. 2001 (20°C)	3.15
1.8	0.855	Crowe, A. 2001 (10°C)	0.50
		Mean:	**1.15**
		Median:	**0.56**
Phthalamic acid			
0.4	0.999	Crowe, A. 2001 (20°C)	0.24
0.8	0.973	Crowe, A. 2001 (10°C)	0.22
		Mean:	**NR**
		Median:	**NR**

An examination of the normalised DT_{50} values revealed that the values generated in the Daly, D. 1991a study were significantly greater than those obtained in the Crowe, A. 2001 study. As such, it was considered appropriate to select the median DT_{50} values for folpet and phthalimide. In the case of phthalamic acid and phthalic acid it was considered appropriate to select the worst case DT_{50} values, particularly given the uncertainty associated with the estimated K_{OC} values for these two metabolites. These selections are summarised here:

- Folpet: **1.05** days (median of five measurements in four soils)
- Phthalimide: **1.04** days (median of five measurements in four soils)
- Phthalamic acid: **0.24** days (worst case of two measurements in two soils)
- Phthalic acid: **3.15** days (worst case of four measurements in three soils)

The results demonstrated that the predicted 80th percentile concentrations for folpet, phthalimide, phthalamic acid and phthalic acid were all <0.001 µg/L at 1 m depth in all scenarios as simulated by FOCUS PELMO.

Open point 4.15, and Comment 4(38) FR: for phthalimide, the lower Kdoc (56) was used for PECgw calculation. However Kfoc was available and was < Kdoc. Could this choice be explained. For phthalamic acid and phthalic acid it is stated that PECgw are not expected to exceed 0.001 µg/L but the input parameters have not been specified so it is not possible to conclude (even if low risk is expected with regard to fast degradation). This point should be completed.

RMS comments: In Table B.8.2.1.4 some of the headings were labelled incorrectly (see amended Table B.8.2.1.4). Instead of log K_f, the second and fifth headings were labelled as K_f. This would have a very significant effect and strongly imply that, indeed, the K_{fOC} values were very much lower than the K_{OC} values. However, in Table B.8.2.1.5 the K_{OC} and K_{fOC} values are given (from the relevant study report).

Table B.8.2.1.4: Freundlich adsorption and desorption coefficients ($logK_f^{ads}$ and $logK_f^{des}$)

Soil	Adsorption			1st Desorption		
	$logK_f^{ads}$	1/n	R^2	$logK_f^{des}$	1/n	R^2
EURO-Soil 1	0.7013	0.8900	0.9965	1.0925	0.7149	0.9909
EURO-Soil 3	0.3965	0.8846	0.9999	1.2873	0.4814	0.9934
EURO-Soil 5	1.1931	0.8373	0.9939	1.2868	0.8616	0.9969
LUFA 2.1	0.0749	0.5171	0.9719	0.4951	0.2426	0.9452
LUFA 2.2	0.4385	0.5794	0.9793	-	-	-

Table B.8.2.1.5: K_{OC} and K_{fOC} values for Phthalimide

Soil	**K_{OC}**	**K_{fOC}**
Euro-soil 1	293.1	385
LUFA 2.1	210.7	214
LUFA 2.2	142.5	123
Euro-soil 3	55.7	72
Euro-soil 5	140.3	169
Mean:	**168**	**193**

It is clear that the mean K_{fOC} value is higher than the mean K_{OC} value, which means that using the K_{OC} values (which has been the case in this assessment) is a more conservative approach, in this case. Putting this specific assessment aside, it appears, generally, that use of K_{OC} rather than K_{fOC} is a more common practice.

Surface Water

Open point 4.5: The need for PEC sw and PEC sediment taking into account run-off and drainage to be discussed in an expert meeting. *Reporting table comment 4(19)*

Data requirement 4.2 *Reporting table comment 4(39), 4(40)*	Notifier to submit PEC surface water for the metabolites.
Data requirement 4.3 *Reporting table comment 4(41)*	Notifier to submit PEC sediment calculations.

Open point 4.5, and Comment 4(19) DE: PEC calculations for surface water and sediment according to the Guidance Document on Aquatic Ecotoxicology (Sanco/3268/2001 rev.4

(final)) from October 17th, 2002, i.e. using the current FOCUS surface water modelling tools might yield more reliable data on the concentrations in sediment.

Loading to surface water via spray drift was calculated using the spray drift tables of Ganzelmeier et al. (1995). PEC sediment values were not reported due to the rapid degradation of folpet in surface water. For the same reason, runoff and drainage were not considered for the parent compound. PEC surface water values for metabolites were calculated assuming a runoff event of 0.5 % of the applied product entering a standard water body 3 days after application.

Since some essential input parameters and assumptions are different in the FOCUS models, the use of the current FOCUS software (FOCUS Steps 1-2 and FOCUS SWASH) would lead to different PEC values. At least at FOCUS-Step1/2 level, the PEC values are expected to be higher than those presented in the DAR.

MS should discuss, whether the available information on the fate of the compound in water/sediment might justify the additional use of FOCUSsw steps 1-2 for PEC calculation, even though they are usually not applied to second list compounds.

RMS comment:

In the Reporting Table, requests were made for PEC_{SW} and PEC_{sed} calculations to be carried out for folpet metabolites. It was suggested that these PEC values be calculated using the FOCUS SW modelling package. However, the Notifier maintains that as FOCUS SW was not required when the dossier was submitted a decision on annex1 listing can be made without FOCUS SW. Given the short soil DT_{50} for folpet there is unlikely to be any significant movement to surface water through run-off or drainage. Unrealistic worst case PECsw values for metabolites from run-off have already been calculated and included in the DAR. Given the GAP for folpet uses (spring/summer applications) drainage will not be a significant exposure route for metabolites either.

Data requirement 4 .2, and Comment 4(39) EFSA: PEC surface water for the metabolites should be provided.

Data requirement 4.3, and Comment 4(41) EFSA: PEC sed should be provided for the metabolites.

The Notifier has submitted a new report detailing PEC_{SW} and PEC_{sed} calculations for folpet metabolites following multiple applications:

Report: *Terry, A. (2005b). Predicted Environmental Concentrations of Metabolites of Folpet in Surface Water and Sediment arising from Spray Drift, in the European Union. CEA, unpublished report February 2005.*

PEC_{SW} and PEC_{sed} values have been calculated for three of the EU GAPs for folpet products using standard assumptions with respect to water depth (30 cm), mixing depth in sediment (5 cm), sediment density (1.5 g/cm^3) and spray drift factors (Rautmann, 2001). PEC_{SW} and PEC_{sed} values were calculated for Phthalimide and Phthalic acid, whilst only PEC_{SW} values were calculated for Phthalamic acid, benzamide and 2-cyanobenzoic acid, simulating multiple applications of folpet with resulting multiple drift into a water body.

PEC_{SW} and PEC_{sed} values were calculated for vines and tomatoes in Southern Europe and for winter wheat in Southern Europe. The applications have been taken to correspond to late applications to vines, with respect to the Rautmann, 2001 spray drift tables.

The degradation of folpet in sediment/water systems was investigated in the laboratory in two contrasting sediment/water systems at 20°C, using [U-phenyl-^{14}C]-folpet (Crowe, A. 1999).

Folpet was found to degrade extremely rapidly (DT_{50} <<1 day). In the water phase, phthalimide, phthalamic acid, phthalic acid, benzamide and 2-cyanobenzoic acid were found to be major metabolites. In sediment, there were no major metabolites, but phthalimide reached 5.9% and phthalic acid 3.8% in the Emperor Lake sediment. These findings are also summarised in Table B.8.4.4.5, above.

An analysis of the decline of the folpet metabolites in the two sediment/water systems was reported (Crowe, A. 1999, see Table B.8.6.5), but a full kinetic analysis of the data was not undertaken.

Table B.8.6.5: Summary of derived DT_{50} values for folpet metabolites in two sediment/water systems

Substance	DT_{50} (days)			
	Row Pond		Virginia Water System	
	Aqueous phase dissipation	Total system dissipation	Aqueous phase dissipation	Total system dissipation
Phthalimide	0.543	0.583	0.594	0.645
Phthalamic acid	3.546	3.978	5.499	6.087
Phthalic acid	1.381	1.409	6.359	6.453
Benzamide	1.625	1.625	-	-
2-cyanobenzoic acid	0.334	0.357	0.666	0.716

- DT_{50} not derivable

Phthalimide, phthalamic acid, phthalic acid, benzamide and 2-cyanobenzoic acid were found to be major metabolites in the water phase. As such PEC_{SW} values were calculated for each of these substances. Although no metabolite reached 10% in the sediment of either system at any timepoint, PEC_{SED} values have been calculated for phthalimide and phthalic acid, which were the only metabolites to reach or approach 5% in the sediment phase.

The calculation of PEC values was carried out according to the assumption that a percentage of the applied folpet drifts into a 30 cm deep water body with a 5 cm layer of sediment (with a density of 1.5 g/cm^3). The spray drift rate was taken from the Rautmann (2001) tables of values, using the values for late applications to vines and the values for field crops (winter wheat and tomatoes). The appropriate spray drift values for multiple applications were selected, according to the GAP. In addition, because most of the metabolites were very rapidly degraded, the spray drift values for a single application were also used to derive PEC values (see Table B.8.6.6 for a summary of the drift values used). Following calculation of the initial concentration of folpet in the water phase following each application it was assumed that the metabolites were formed instantaneously in both water and sediment phases at the maximum percentage found in the sediment/water systems, with initial PEC values accounting for the molecular weight differences between parent and metabolites.

The decline of each metabolite was simulated in an EXCEL spreadsheet according to first order exponential decay with the appropriate DT_{50} value (see Table B.8.6.7 for a summary of

the parameters selected for calculations of the PEC values). In all cases the GAP calls for multiple applications with a minimum application interval of 7 days. This was simulated in the EXCEL spreadsheet by adding each additional application as a concentration to that remaining from the previous application(s) and then allowing the new concentration to decay as previously described. These values constituted the instantaneous PEC value at any given time following the first or last application.

The TWA PEC values were calculated by first calculating average concentrations for each day of the simulation and then averaging the required number of days' averages with a sliding window to arrive at a maximum TWA PEC for the use.

Table B.8.6.6: Summary of Drift values used to calculate PEC values for folpet water/ sediment metabolites

Crop	Drift distance	% drift single application (90^{th}-percentile)	% drift multiple applications	
			(77^{th}-percentile)	(82^{nd}-percentile)
Winter Wheat	1 m	2.77	NR	2.38
Tomatoes	1 m	2.77	NR	2.38
Vines	3 m	8.02	6.90	NR

NR: not relevant for usage

Table B.8.6.7. Summary of Parameters Selected for Use in the Calculation of PEC values for folpet metabolites

Compound:	Folpet	Phthalimide	Phthalamic acid	Phthalic acid	benzamide	2-cyanobenzoic acid
Molecular Weight (g/mol)	296.59	147.13	165.15	166.13	121.14	147.13
Water depth (cm)	30	30	30	30	30	30
Sediment depth (cm)	5	5	5	5	5	5
Sediment density (g/cm^3)	1.5	1.5	1.5	1.5	1.5	1.5
Maximum % formed in water phase	NR	26.0	13.3	37.5	10.2	39.7
Maximum % formed in sediment phase	NR	5.9	NR	3.8	NR	NR
DT_{50} in water phase (days)	NR	0.594	5.499	6.359	1.625	0.666
DT_{50} in sediment phase (days)*	NR	0.645	6.087	6.453	1.625	0.716

NR: not relevant for these calculations

*: whole system DT_{50} values selected

PEC_{SW} values for phthalimide, phthalamic acid, phthalic acid, benzamide and 2-cyanobenzoic acid (both instantaneous and TWA) have been calculated for three uses of folpet. PEC_{SED} values for phthalimide and phthalic acid (both instantaneous and TWA) have been calculated for three uses of folpet.

The worst case calculated PEC_{SW} and PEC_{sed} values are summarised in Tables B.8.6.8-B.8.6.9.

Table B.8.6.8: Summary of maximum PEC_{SW} values for folpet metabolites

Substance	PEC type	PEC_{SW} value; single application (µg/L)	PEC_{SW} value; multiple application (µg/L)	GAP
Phthalimide	maximum instantaneous	5.17	4.45	Vines Southern Europe
	maximum 1 day TWA	3.39	2.92	
	maximum 2 day TWA	2.22	1.91	
	maximum 4 day TWA	1.22	1.05	
	maximum 7 day TWA	0.70	0.61	
	maximum 14 day TWA	0.35	0.61	
	maximum 21 day TWA	0.23	0.61	
	maximum 28 day TWA	0.18	0.61	
	maximum 42 day TWA	0.12	0.61	
	maximum 100 day TWA	0.05	0.42	
Phthalamic acid	maximum instantaneous	2.97	4.36	Vines Southern Europe
	maximum 1 day TWA	2.79	4.10	
	maximum 2 day TWA	2.63	3.86	
	maximum 4 day TWA	2.34	3.43	
	maximum 7 day TWA	1.98	2.90	
	maximum 14 day TWA	1.40	2.90	
	maximum 21 day TWA	1.04	2.90	
	maximum 28 day TWA	0.82	2.90	
	maximum 42 day TWA	0.56	2.89	
	maximum 100 day TWA	0.24	2.03	
Phthalic acid	maximum instantaneous	8.42	13.57	Vines Southern Europe
	maximum 1 day TWA	7.99	12.87	
	maximum 2 day TWA	7.58	12.21	
	maximum 4 day TWA	6.83	11.01	
	maximum 7 day TWA	5.90	9.50	
	maximum 14 day TWA	4.32	9.50	
	maximum 21 day TWA	3.31	9.50	
	maximum 28 day TWA	2.63	9.49	
	maximum 42 day TWA	1.82	9.44	
	maximum 100 day TWA	0.77	6.63	
Benzamide	maximum instantaneous	1.67	1.51	Vines Southern Europe
	maximum 1 day TWA	1.38	1.25	
	maximum 2 day TWA	1.14	1.03	
	maximum 4 day TWA	0.81	0.74	
	maximum 7 day TWA	0.54	0.49	
	maximum 14 day TWA	0.28	0.49	
	maximum 21 day TWA	0.19	0.49	
	maximum 28 day TWA	0.14	0.49	
	maximum 42 day TWA	0.09	0.49	
	maximum 100 day TWA	0.04	0.34	

Table B.8.6.8: Summary of maximum PEC_{SW} values for folpet metabolites (continued)

Substance	PEC type	PEC_{SW} value; single application (µg/L)	PEC_{SW} value; multiple application (µg/L)	GAP
2-cyanobenzoic acid	maximum instantaneous	7.90	6.80	Vines Southern Europe
	maximum 1 day TWA	5.34	4.60	
	maximum 2 day TWA	3.62	3.11	
	maximum 4 day TWA	2.03	1.75	
	maximum 7 day TWA	1.18	1.02	
	maximum 14 day TWA	0.59	1.02	
	maximum 21 day TWA	0.39	1.02	
	maximum 28 day TWA	0.30	1.02	
	maximum 42 day TWA	0.20	1.02	
	maximum 100 day TWA	0.08	0.71	

Table B.8.6.9: Summary of maximum PEC_{SED} values for folpet metabolites

Substance	PEC type	PEC_{SED} value; single application (µg/kg)	PEC_{SED} value; multiple application (µg/kg)	GAP
Phthalimide	maximum instantaneous	4.70	4.04	Vines Southern Europe
	maximum 1 day TWA	3.15	2.71	
	maximum 2 day TWA	2.11	1.82	
	maximum 4 day TWA	1.18	1.02	
	maximum 7 day TWA	0.68	0.59	
	maximum 14 day TWA	0.34	0.59	
	maximum 21 day TWA	0.23	0.59	
	maximum 28 day TWA	0.17	0.59	
	maximum 42 day TWA	0.11	0.59	
	maximum 100 day TWA	0.05	0.41	
Phthalic acid	maximum instantaneous	3.41	5.55	Vines Southern Europe
	maximum 1 day TWA	3.24	5.27	
	maximum 2 day TWA	3.08	5.00	
	maximum 4 day TWA	2.78	4.52	
	maximum 7 day TWA	2.40	3.91	
	maximum 14 day TWA	1.79	3.91	
	maximum 21 day TWA	1.36	3.91	
	maximum 28 day TWA	1.08	3.90	
	maximum 42 day TWA	0.75	3.88	
	maximum 100 day TWA	0.32	2.73	

B.8.7 Fate and behaviour in air (Annex IIA 7.2.2; Annex IIIA 9.3)

Data requirement 4.4 *Reporting table comment 4(43)*	Notifier to assess potential relevance of thiophosgene in the air compartment.

Data requirement 4.4, and Comment 4(43) EFSA: Thiophosgene should be considered for the residue definition in air.

The Notifier has submitted the following (ref: Terry, A. 2005a. Responses to questions raised in the Reporting Table on fate and behaviour of folpet):

No laboratory degradation studies have been carried out using the labelled thio(trichloromethyl) sidechain of folpet, but an estimate of degradation may be made from studies on the closely related compound, captan, which has an identical sidechain. These studies (see B.8.1.1, above) indicated that the sidechain was likely to be degraded rapidly, with mineralisation to carbon dioxide. It is clear from the two studies conducted with trichloromethyl - ^{14}C captan that the main route of degradation of the side chain was rapid conversion to thiocarbonic acid and then to CO_2 (without thiophosgene as an intermediate). Therefore, thiophosgene would not be expected to be a significant folpet degradation product in soil and would, therefore, not be expected to have a significant presence in air.

Therefore, it is not believed that thiophosgene is relevant for the air compartment.

RMS comment: It is agreed that thiophosgene should not be regarded as relevant for the air compartment.

B.8.9 Definition of the residue (Annex IIA 7.3)

Open point 4.17: MS to discuss the residues definition in an expert meeting. *Reporting table comment 4(44) and 4(47)*

Open point 4.17, and Comment 4(44) SE: We agree to include only folpet in the definition of residues in soil and in aquatic systems. However, as justification for excluding the metabolites, please also refer to the ecotoxicological studies available.
Before concluding on the definition of the residues in groundwater, the input values used for metabolites needs to be clarified.

Comment from RMS: The Notifier has clarified the input parameters for metabolites (see B.8.6) and the PEC_{GW} calculations indicate that neither folpet nor any of its degradation products are likely to exceed 0.1 μg/L. As such, it is proposed that the residue in groundwater should be considered to be folpet only (although based on the modelling folpet would not occur in groundwater).

In terms of surface water, metabolites are all of low toxicity to aquatic organisms. Hence, they should not be included in the residue definition. Studies on earthworms for folpet would have included exposure to major soil metabolites. Low toxicity was observed in these studies. Hence, metabolites should not be included in the residue definition.

B.8.11 References relied on

B.8.11.1Active ingredient

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company, Report No. GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N	Owner
IIA, 7.1.1.1.1/04	Diaz, D., Lay, M.M.	1992	Aerobic metabolism of [trichloromethyl -^{14}C] captan in soil. ICI Americas Inc. Western Research Center, Report No. PMS-320 (Company file: R 9280/TMN-0323). GLP, Unpublished.	Y	Makhteshim/ Calliope
IIA, 7.1.1.1.1/05	Pack, D.E., Verrips, I.S.	1988	Aerobic soil metabolism of [trichloromethyl -^{14}C] captan. Chevron Chemical Company, Report No. MEF 0060/8809887 (Company file: R-4994/TMN-0324). GLP, Unpublished.	Y	Makhteshim/ Calliope
IIA 7.2.1.3.2/02	Shelton, D.R., Boyd, S.A., Tiedje, J.M.	1984	Anaerobic biodegradation of phthalic acid esters in sludge. Environ. Sci. Technol., 18, 93-97 Non-GLP, Published	N	
IIA, 7.	Terry, A	2005a	Responses to questions raised in the Reporting Table on fate and behaviour of folpet CEA report CEA.053 Non-GLP, Unpublished.	Y	Makhteshim

B.8.11.2 Formulation

Folpan 80 WDG

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company, Report No. GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N	Owner
IIIA, 9.2.1/01	Mackay, N.	2002	Predicted Environmental Concentrations of Folpet and its Degradation Products in Groundwater in the European Union using the FOCUS Groundwater Scenarios. CEA report No.XA1105. Not GLP, Unpublished	Y	Makhteshim
IIIA, 9.2.3/01	Terry, A.	2005b	Predicted Environmental Concentrations of Metabolites of Folpet in Surface Water and Sediment arising from Spray Drift, in the European Union. CEA report CEA.056 Not GLP, Unpublished	Y	Makhteshim

Folpet

Addendum to Draft Assessment Report:

Ecotoxicology

Rapporteur Member State: Italy

EU review under Directive 91/414/EEC

Relating to Annex B (Volume 3) of the DAR

March 2005

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B.8 Ecotoxicology

Introduction

This document is an Addendum to the Draft Assessment Report (DAR) for the EU review of **folpet**. The aim of this Addendum is to address comments, 'Open points' and 'Data requirements' as raised in the official Reporting Table (dated 22.12.04) and Evaluation Table (dated 18.01.05) in the area of **Ecotoxicology**.

This Addendum includes summarisation and evaluation of new studies and risk assessments submitted by Makhteshim Chemical Works Ltd.

Section numbering in this Addendum is in line with Annex B (Volume 3) of the DAR.

The Good Agricultural Practice (GAP) uses proposed by the Notifier for consideration under the review are specified in Table 1. Please note that the GAP was changed after the original submission of the dossier. This change was the removal of North EU cereals from the Notified GAP, and also an adjustment to the pre-harvest interval for tomato.

Table 1: Critical Good Agricultural Practice for folpet in the EU

Crop	Member state or country	Product name	F, G or I[a]	Pests or group of pests controlled	Formulation		Application			Application rate per treatment			PHI (days)	Remarks:
					Type	Conc. of a.s.	method kind	growth stage	number[b] (max.)	kg a.s./hL (max.)	water L/ha	kg a.s./ha (max.)		
Winter wheat	South EU	'Folpan' 80 WDG	F	*Septoria* Brown rust	WG	800 g/kg	Foliar spray; down-ward	Up to Z65	2	0.375	200	0.75	42	
Tomatoes	South EU	'Folpan' 80 WDG	F	various [c]	WG	800 g/kg	Foliar spray; down-ward	From beginning of fruit set	4	0.125	1000	1.25	7	
	South EU	'Folpan' 80 WDG	G	various [c]	WG	800 g/kg	Foliar spray; down-ward	From beginning of fruit set	3	0.16	1000 - 1300	1.6	7	
Grapes	North and south EU	'Folpan' 80 WDG	F	various [d]	WG	800 g/kg	Airblast foliar spray; upwards/ sideways	Shoot emergence to veraison	10	0.75	200 - 400	1.5	28	

[a] F= field; G = greenhouse.
[b] Sprays on all crops are applied typically at intervals of 7 to 28 days.
[c] *Alternaria solanum, Cladospora, Colletotrichum, Septoria, Botrytis*
[d] Black rot, *Botrytis cinerea phomosis*. *Plasmopara viticola*.

B.9.1 Effects on birds (Annex IIA 8.1; Annex IIIA 10.1)
B.9.3 Effects on other terrestrial vertebrates (Annex IIIA 10.3)

As the issues are closely linked, and to avoid duplication, the risk to birds and mammals is evaluated together in this Addendum.

Open point 5.1:
RMS to prepare an addendum with an evaluation of the revised risk assessment for birds and mammals presented by the notifier. (see reporting table 5(1))

In response to the above, the Notifier has submitted a risk assessment for birds and mammals according to the Guidance document on risk assessment for birds and mammals (SANCO/4145/2000). This is summarised below.

Report: *Norman, S. and Wyness, L. (2003). Folpet. Response to Rapporteur Member State request for a revised avian and mammalian risk assessment in accordance with EU guidance document on risk assessment for birds and mammals (SANCO/4145/2000. Makhteshim Agan and TSGE, unpublished report 11 September 2003.*

Guidance: Guidance Document on Risk Assessment for Birds and Mammals under Council Directive 91/414/EEC' (SANCO/4145/2000; 25 September 2002.

The results of toxicity studies on folpet for birds and mammals are summarised below in Table 2. Overall, folpet is of low toxicity.

In accordance with the EU guidance document on risk assessment for birds and mammals, toxicity:exposure ratios (TERs) are based on intake in terms of daily dose (mg/kg body weight/day). Therefore, Tables 3 and 4 present the conversions from dietary concentration to daily dose which are required for the short and long-term dietary endpoints for birds.

Table 2. Folpet: Endpoints from toxicity studies on birds

Study	Species	Endpoint	Result
Acute oral	Bobwhite quail	LD_{50}	> 2510 mg/kg bw*
Short-term dietary	Bobwhite quail	LC_{50}	> 5000 ppm in diet*
	Mallard duck	LC_{50}	> 5000 ppm in diet*
Screening reproduction	Bobwhite quail	NOEC (reproduction) NOEC (adults)	4640 ppm in diet** 4640 ppm in diet**
One generation reproduction	Bobwhite quail	NOEC (reproduction) NOEC (adults)	1000 ppm in diet** 1000 ppm in diet**
	Mallard duck	NOEC (reproduction) NOEC (adults)	1000 ppm in diet** 1000 ppm in diet**

*For acute and short term studies, there were no treatment-related mortalities or overt signs of toxicity at any treatment level.
**For long-term studies there was no overall effect on adults or reproductive performance at any treatment level. NOECs are the highest concentrations tested.

Table 3. Conversion of short-term dietary endpoints to daily doses

Species	LC_{50} (ppm in diet)	Mean[a, b] food intake (g/bird/day)	Mean[a, c] bodyweight (g/bird)	Mean daily dose (mg/kg bw)
Bobwhite	> 5000	8	35.5[d]	> 1127
Mallard	> 5000	50	335[e]	> 746

[a] Birds of the LC_{50} dose group.
[b] Averaged over days 1 to 5.
[c] Averaged over days 1 to 8 (no data available for the exposure phase (days 1 to 5) only).
[d] Mean group bodyweights 28 and 43 g on days 1 and 8, respectively.
[e] Mean group bodyweights 243 and 427 g on days 1 and 8, respectively.

Table 4. Conversion of long-term dietary endpoints to daily doses

Species	NOEC (ppm in diet)	Mean[a] food intake (g/bird/day)	Mean[a] bodyweight (g/bird)	Mean daily dose (mg/kg bw)
Bobwhite	4640	31[b]	187[e]	769
Bobwhite	1000	17[c]	217[f]	78.3
Mallard	1000	105[d]	1167[g]	90.0

[a] Birds of the NOEC dose group.
[b] Based on 8 × weekly values (15, 34, 32, 26, 32, 36, 32, 38 g).
[c] Based on 9 × weekly values (16, 12, 16, 16, 14, 16, 21, 19, 20 g).
[d] Based on 10 × weekly values (94, 103, 88, 111, 91, 111, 121, 124, 102, 107 g).
[e] Mean group bodyweights of 173 and 201 g at start and termination, respectively.
[f] Mean group bodyweights of 203 and 231 g at start and termination, respectively.
[g] Mean group bodyweights of 1147 and 1186 g at start and termination, respectively.

For the avian risk assessment the following endpoints are selected:

Acute risk: > 2510 mg folpet/kg bw (limit value)**;**
Short-term risk: > 746 mg folpet/kg bw (lowest daily dose between two species)**;**
Long-term risk: 90.0 mg folpet/kg bw (lowest daily dose between two species).

Endpoints from mammalian toxicity studies are summarised in Tables 5 and 6. The justification for the no observed adverse effect levels (NOAEL) from the reproduction studies is contained in the text after Table 6.

Table 5. Summary of mammalian acute toxicity studies with folpet and 'Folpan' 80 WDG

Substance	Study	Species	Results
Folpet	Acute oral LD_{50}	Rat	> 2000 mg/kg bw[a]
	Acute dermal LD_{50}	Rabbit	> 2000 mg/kg bw[a]
		Rat	> 2000 mg/kg bw[a]
	Acute inhalation LC_{50} (4 hour nose only)	Rat	1.89 mg/L[b]
	Acute intraperitoneal LD_{50}	Rat	40.0 mg/kg bw males 36.0 mg/kg bw females
'Folpan' 80 WDG	Acute oral LD_{50}	Rat	> 2000 mg/kg bw[a]
	Acute dermal LD_{50}	Rabbit	> 1000 mg/kg bw[a]

[a] Results for male and female were the same.
[b] Result calculated for male and female combined.

Table 6. Summary of the reproductive toxicity of folpet to mammals

Study type	Animal species	Dietary concentrations	NOEL
Two generation reproduction, dietary	Rat	250, 1500, 5000 ppm	Parental: 250 ppm[a] Reproduction 5000 ppm
Two generation (two litter) reproduction, dietary	Rat	200, 800, 3600 ppm	Parental: 800 ppm[b] Reproduction: 3600 ppm
NOAEL for long-term risk assessment: 5000 ppm (548.6 mg/kg bw/day)[c]			

[a] Based on histopathological findings at 1500 ppm.
[b] Based on reduced bodyweight due to reduced food consumption at 3600 ppm.
[c] Explanatory text follows this table.

Mammalian multigeneration studies are conducted for the purpose of determining hazard and risk to humans. Therefore, the study designs (endpoints, selection of treatment levels) are usually inappropriate for ecological risk assessments. However, from such studies, ecologically meaningful information has to be gained in order to quantify long-term risk to mammals. As stated in the EU guidance document '*The usual approach is based on the consideration that effects on populations will not occur if the survival rate, reproduction rate and development of individuals are not affected. Therefore, in principle, only endpoints in toxicity tests which are related to these key factors of population dynamics are ecotoxicologically relevant.*'

An appropriate endpoint for long-term risk in mammals should be related to the survival of a mammalian population at risk in the field. Such parameters may be reduced survival rate, reproduction rate or development of individuals (as stated above). The two two-generation reproduction studies in the rat have been evaluated on this basis in order to derive an appropriate endpoint for use in the long-term risk assessment.

Two-generation reproduction study in the rat:

Folpet was administered in the diet of rats at 250, 1500 or 5000 ppm. Food consumption was reduced compared with the control at the highest treatment level, throughout the study. This was probably related to reduced palatability of the test diet due to the high concentration of folpet. In the F_0 generation, before the first pairing, the reduction was 8 to 9% compared with the control. This was accompanied by 7 to 10% lower body weights than in the control in males and females, respectively. Thus the effect on food consumption and bodyweight were of a similar magnitude.

The initial mean body weights of the F_1 animals as weanlings in the 5000 ppm treatment group were approximately 3% lower than in the control treatment. By the end of lactation (day 21 post-partum), the mean F_1 weanling body weight in the 5000 ppm group was significantly less than the controls (by approximately 10%), and was related to reduced food intake of the F_0 parents. At 5000 ppm, in the F_1 generation food consumption and body weight were lower than the controls, to the same degree as observed in the F_0 generation.

There were a few occasions at 1500 ppm, generally in the pre-pairing periods, when food consumption was slightly reduced but was not statistically significant (no greater than a 4% reduction from the control treatment). There was no effect at 250 ppm.

The effect on food consumption and body weight at 5000 ppm had no influence on reproductive performance (including litter size) compared with the control in either generation. There were also no effects on reproduction at 250 and 1500 ppm.

In terms of histopathological findings, hyperkeratosis (thickening of skin) of the non-glandular gastric mucosa and oesophagus was observed at 1500 ppm and to a greater degree at 5000 ppm. This was related to these high concentrations of folpet irritating the mucal membranes, which may have been the explanation for the reduced palatability of the feed.

The intake of folpet (mg/kg/day) during the study was calculated as follows:

Males, F_0	low dose	31.5 to 13.7 mg/kg bw/day
	intermediate dose	174.3 to 83.1 mg/kg bw/day
	high dose	535.5 to 281.7 mg/kg bw/day
Females, F_0	low dose	32.1 to 18.3 mg/kg bw/day
	intermediate dose	188.1 to 109.6 mg/kg bw/day
	high dose	577.5 to 359.5 mg/kg bw/day
Males, F_1	low dose	51.8 to 15.4 mg/kg bw/day
	intermediate dose	280.3 to 97.9 mg/kg bw/day
	high dose	973.1 to 328.3 mg/kg bw/day
Females, F_1	low dose	51.1 to 20.0 mg/kg bw/day
	intermediate dose	294.6 to 118.0 mg/kg bw/day
	high dose	940.5 to 411.2 mg/kg bw/day

The mean intakes at the highest treatment ranged from 340.4 to 756.7 mg/kg bw/day with an overall mean of 548.6 mg/kg bw/day.

The minor effects on food consumption and bodyweight, summarised above, are not considered to have ecological significance with respect to the risks to wild mammal populations potentially exposed to folpet. The effect on food consumption at 5000ppm is likely to be related to the reduced palatability of feed containing a high concentration of folpet, and the lack of an alternative untreated food source. This concentration would be much higher that would be found on sprayed insects or foliage following actual use. Hence, the marginally reduced food consumption (and consequent effect on bodyweight) at 5000 ppm is an artefact of the high treatment concentration and would not occur in the field.

Two-generation reproduction study in the rat (two litters per generation):

In a two-generation study, with each generation producing two litters, folpet was given to rats in the diet at 200, 800 and 3600 ppm. At 3600 ppm there were slightly lower body weights in F_0 parental males from week 11 onwards (mean body weight approximately 4% to 5% less than in the control treatment, significant only at one sampling point on day 92). For F_0 females in the 3600 ppm group, the difference in body weight, compared to the control treatment ranged from 2% to 10%, but was not significant at any sampling point. The bodyweights of the male F_1 generation were also reduced at 3600 ppm (8% reduction compared to the control treatment from day 1 to day 106), although the F_1 female weight gain was similar to the controls. These bodyweight effects corresponded closely to a reduction in food consumption (other than day 1, the F_0 male and female reduction in food consumption ranged from 4% to 12%). At 3600 ppm, mean pup weights were lower than the controls (up to a maximum of 17% reduction by Day 21 for the F_1 a and b litters and up to a maximum of 19% on Day 21 for F_2 a and b litters). Effect on pup weight was caused by reduced feeding of parents. There were

no effects on reproduction, litter sizes or pup survival at 3600 ppm. There were no treatment-related histopathological findings. Overall, there were no effects at 200 or 800 ppm.

Effects on food consumption and bodyweight at 3600 ppm were consistent with those observed in the first multigeneration study. As with the first study, it is considered that reduced food intake was related to the reduced palatability of diets containing a high concentration of folpet (possibly linked to irritation of mucal membranes). The minor effects on food consumption and bodyweight, summarised above, are not considered to have ecological significance with respect to the risks to wild mammal populations potentially exposed to folpet. In any case, 3600 ppm is much greater than potential concentrations on food items in the field. Hence, the reduced feeding response would not occur.

Conclusion on long-term endpoint for mammals:

Based on these two studies an appropriate long-term daily dose endpoint (NOAEL) for assessing long-term risk is 548.6 mg/kg bw/day (from the first two-generation study).

For the mammalian risk assessment the following endpoints are selected:

Acute risk: > 2000 mg folpet/kg bw (lowest limit value available)**;**
Long-term risk: 548.6 mg folpet/kg bw/day (mean of male and female daily dose from the highest dose group (5000 ppm) of first reproduction study discussed above)**.**

Tier 1 Exposure Scenarios

The estimated theoretical exposure (ETE) is based on the following:

ETE = (FIR/bw) x C x AV x PT x PD (mg/kg bw/d)

where:

FIR: Food intake rate of selected indicator species (g fresh wt. food/day);
bw: body weight (g);
C: concentration of folpet in fresh diet (mg folpet/kg);
AV: avoidance factor (1 = no avoidance);
PT: fraction of diet obtained from treated area (assume 1 for first tier evaluation);
PD: fraction of food type in diet (assume 1 for first tier evaluation).

C_0 is a function of the application rate (kg folpet/ha), the residue per unit dose (RUD), and the multiple application factor (MAF), taking into account crop interception, where appropriate.

For multiple application products, the concentration C, is expressed as $C = C_0 \times MAF \times f_{twa}$

where:

C_0: initial concentration in food after a single application;
MAF: multiple application factor (concentration immediately after the last application relative to the first application);
f_{twa}: time-weighted average factor.

Table 7. Tier 1 acute exposure scenarios for birds and mammals

Crop (scenario)	Crop stage[a]	Indicator species	FIR/bw[b]	Food
Winter wheat ('Cereals')	Late	Insectivorous mammal	0.63	large insects
		Insectivorous bird	1.04	small insects
Grapes ('Orchard/ vine/ hops')	Early/late	Small herbivorous mammal	1.39	short grass
		Insectivorous bird	1.04	small insects
Tomatoes ('Leafy crops')	Early/late	Medium herbivorous mammal	0.28	leafy crops
		Medium herbivorous bird	0.76	leafy crops
		Insectivorous bird	1.04	small insects

[a] Application of folpet to cereals is at late growth stages when the crop is not palatable to birds and mammals.
[b] Food intake rate based on food type, energy contents of foods, assimilation efficiencies and moisture contents.

If grass is present beneath grapevines this will be exposed to a fraction of the applied spray deposit as a result of crop interception. From shoot emergence to veraison (ripening), the interception factors (from the EU guidance document) range from 50% to 85%. For the acute and short term assessment, the worst case (50% interception) will be used. Acute ETE values are presented in Table 8.

Table 8. Folpet acute ETE values for birds and mammals

Crop (scenario)	Indicator species	App. rate (kg folpet/ha)	RUD (90%)[a]	Crop interception	MAF[b]	C (mg folpet/kg diet)[c]	ETE (mg folpet/kg bw/day)[d]
Winter wheat ('Cereals')	Insectivorous mammal	0.75	14	n/a	n/a	10.5	6.6
	Insectivorous bird	0.75	52	n/a	n/a	39.0	40.6
Grapes ('Orchard/ vine/ hops')	Small herbivorous mammal	1.50	142	50% (deposition 0.5)	2.0	213	296.1
	Insectivorous bird	1.50	52	n/a	n/a	78.0	81.1
Tomatoes ('Leafy crops')	Medium herbivorous mammal	1.25	87	n/a	1.8	195.8	54.8
	Medium herbivorous bird	1.25	87	n/a	1.8	195.8	148.8
	Insectivorous bird	1.25	52	n/a	n/a	65.0	67.6

[a] Residue per unit dose (90^{th} percentile).
[b] MAF based on 10 and 4 applications for grapes and tomatoes, respectively, with a minimum interval of 7 days. MAF not required for estimation of residues on insects.
[c] Concentration of folpet in fresh diet (application rate x RUD x deposition x MAF).
[d] ETE = C x FIR/bw.

Short term exposure scenarios for birds are stated in Table 9.

Table 9. Tier 1 short-term exposure scenarios for birds

Crop (scenario)	Crop stage[a]	Indicator species	FIR/bw[b]	Food
Winter wheat ('Cereals')	Late	Insectivorous bird	1.04	small insects
Grapes ('Orchard/ vine/ hops')	Early/late	Insectivorous bird	1.04	small insects
Tomatoes ('Leafy crops')	Early/late	Medium herbivorous bird	0.76	leafy crops
		Insectivorous bird	1.04	small insects

[a] Application of folpet to cereals is at late growth stages when the crop is not palatable to birds and mammals.
[b] Food intake rate based on food type, energy contents of foods, assimilation efficiencies and moisture contents.

Short term ETE values for the above scenarios are presented in Table 10.

Table 10. Folpet short-term ETE values for birds

Crop (scenario)	Indicator species	App. rate (kg folpet/ha)	RUD (mean)[a]	Crop interception	MAF[b]	C (mg folpet/kg diet)[c]	ETE (mg folpet/kg bw/day)
Winter wheat ('Cereals')	Insectivorous bird	0.75	29	n/a	n/a	21.8	22.6
Grapes ('Orchard/ vine/ hops')	Insectivorous bird	1.50	29	n/a	n/a	43.5	45.2
Tomatoes ('Leafy crops')	Medium herbivorous bird	1.25	40	n/a	2.2	110	83.6
	Insectivorous bird	1.25	29	n/a	n/a	36.3	37.7

Long-term exposure scenarios for birds and mammals are stated in Table 11.

Table 11. Tier 1 long-term exposure scenarios for birds and mammals

Crop (scenario)	Crop stage[a]	Indicator species	FIR/bw[b]	Food
Winter wheat ('Cereals')	Late	Insectivorous mammal	0.63	large insects
		Insectivorous bird	1.04	small insects
Grapes ('Orchard/ vine/ hops')	Early/late	Small herbivorous mammal	1.39	short grass
		Insectivorous bird	1.04	small insects
Tomatoes ('Leafy crops')	Early/late	Medium herbivorous mammal	0.28	leafy crops
		Medium herbivorous bird	0.76	leafy crops
		Insectivorous bird	1.04	small insects

[a] Application of folpet to cereals is at late growth stages when the crop is not palatable to birds and mammals.
[b] Food intake rate based on food type, energy contents of foods, assimilation efficiencies and moisture contents.

Long term ETE values are presented in Table 12, including the use of the standard time-weighted average factor of 0.53 for foliar residues. As *long-term* exposure is being assessed, crop interception for grapes is based on a *mean* of the relevant interception values (from the EU guidance document) for the intended period of use (shoot emergence to veraison). Hence, interception is taken as a mean of 50% ('first leaves'), 60% ('leaf development'), 70% ('flowering') and 85% ('ripening', i.e. veraison), which is 66.3%.

Table 12. Folpet long-term ETE values for birds and mammals

Crop (scenario)	Indicator species	App. rate (kg folpet/ha)	RUD (mean)[a]	Crop interception	MAF[b]	C (mg folpet/kg diet)[c]	ETE (mg folpet/kg bw/day)
Winter wheat ('Cereals')	Insectivorous mammal	0.75	5.1	n/a	n/a	3.8	2.4
	Insectivorous bird	0.75	29	n/a	n/a	21.8	22.6
Grapes ('Orchard/ vine/ hops')	Small herbivorous mammal	1.5	76	66.3% (depositions 33.7%)	2.5	50.9*	70.8
	Insectivorous bird	1.5	29	n/a	n/a	43.5	45.2
Tomatoes ('Leafy crops')	Medium herbivorous mammal	1.25	40	n/a	2.2	58.3*	16.3
	Medium herbivorous bird	1.25	40	n/a	2.2	58.3*	44.3
	Insectivorous bird	1.25	29	n/a	n/a	36.3	37.7

***** Including standard time-weighted average factor of 0.53 from the EU guidance document.

Risk Assessment for Birds and Mammals

Tier 1 Toxicity exposure ratios (TERs) are presented in Tables 13, 14 and 15.

Tier 1 Acute Risk Assessment

Table 13. Tier 1 acute TER values for birds and mammals following application of folpet to cereals, vines and tomatoes

Crop	Indicator species	ETE (mg folpet/kg bw/day)	LD_{50} (mg folpet/kg bw)	TER_a
Winter wheat	Insectivorous mammal	6.6	> 2000	> 303
	Insectivorous bird	40.6	> 2510	> 61.8
Grapes	Small herbivorous mammal	296.1	> 2000	> 6.8
	Insectivorous bird	81.1	> 2510	> 30.9
Tomatoes	Medium herbivorous mammal	54.8	> 2000	> 36.5*
	Medium herbivorous bird	148.8	> 2510	> 16.9*
	Insectivorous bird	67.6	> 2510	> 37.1

* TER is >10, indication a low risk. In any case, tomato foliage is not an attractive food source for birds and mammals.

Folpet has a low acute toxicity to birds and mammals with LD_{50}s of >2000 and >2510 mg/kg bw. Generally, around 2000 mg/kg bw is the highest dose which is tested in acute toxicity studies. There were no treatment-related mortalities in the acute toxicity studies. Hence, all TERs are '*greater than*' values. One of the TERs in Table 13 is below the Annex VI trigger of 10 (>6.8). However, given that there were no mortalities at 2000 or 2510 mg/kg bw, the true LD_{50} would be much higher than these values. Hence, the true Tier 1 TER is likely to

be greater than 10. All other TERs are greater than the Annex VI trigger of 10 indicating a low risk. Overall, there is a low acute risk to birds and mammals.

Tier 1 Short-Term Risk

Table 14. Tier 1 short-term TER values for birds following application of folpet to cereals, vines and tomatoes

Crop	Indicator species	ETE (mg folpet/kg bw/day)	LC_{50} (mg folpet/kg bw/day)	TER_{st}
Winter wheat	Insectivorous bird	22.6	> 746	> 33.0
Grapes	Insectivorous bird	45.2	> 746	> 16.5
Tomatoes	Medium herbivorous bird	83.6	> 746	> 8.9
	Insectivorous bird	37.7	> 746	> 19.8

The short-term dietary toxicity of folpet is low, with an LC_{50} of >5000 ppm in the diet (>746 mg/kg bw if converted to daily dose). Generally, 5000 ppm is the highest concentration tested in short-term dietary studies. There were no treatment-related mortalities at this treatment level. Hence, all TERs in Table 14 are '*greater than*' values. The TER for medium herbivorous birds of > 8.9 for the use in tomatoes is slightly below the trigger of 10. However, the fact that there were no mortalities at 5000 ppm indicates that the true LC_{50} would be much greater than 5000 ppm. In turn, the true TER for medium herbivorous mammals would be >10. In any case, tomato foliage is not an attractive food source for birds or mammals and is unlikely to be grazed. All other TERs are greater than the trigger of 10. Overall, there is a low short-term risk to birds.

Tier 1 Long-Term Risk

Table 15. Tier 1 long-term TER values for birds and mammals following application of folpet to cereals, vines and tomatoes

Crop	Indicator species	ETE (mg folpet/kg bw/day)	NOEC (mg folpet/kg bw/day)	TER_{lt}
Winter wheat	Insectivorous mammal	2.4	548.6	229
	Insectivorous bird	22.6	90.0	4.0
Grapes	Small herbivorous mammal	70.8	548.6	7.7
	Insectivorous bird	45.2	90.0	2.0
Tomatoes	Medium herbivorous mammal	16.3	548.6	33.7
	Medium herbivorous bird	44.3	90.0	2.0
	Insectivorous bird	37.7	90.0	2.4

The long term TERs for insectivorous mammals in cereals, and herbivorous mammals in grapes and tomatoes are all greater than the Annex VI trigger of 5, indicating a low risk. In any case, tomato foliage is not an attractive food source for birds or mammals so is unlikely to be grazed. For this reason the TER of 2 for a medium herbivorous bird foraging in tomatoes, is not relevant to the risk assessment. Overall, there is a low long-term risk to mammals.

Long term TERs for birds in Table 15 are below the trigger of 5, which means further assessment is required. A refined risk assessment is presented below.

Refined assessment of long term risk to birds

General:

The Tier 1 scenarios as presented in the EU guidance document on risk assessment for birds and mammals have a tendency to combine worst case elements (exposure concentration; extent and duration of exposure; daily food consumption) leading to high predicted intakes, but with a low probability of occurrence. Together with the long-term TER trigger of 5, the scenarios provide a very conservative first tier screen to identify low risk situations (TER >5), and indicate where refinement is needed (TER <5).

Folpet undergoes rapid hydrolysis, with a DT_{50} of 1.1 hours at pH 7 and 25 °C in sterile water. (Ref: Ruzo and Ewing, 1988). In the presence of moisture on leaf surfaces (dew and rain), this property would limit the potential duration and magnitude of exposure of grazing birds. For example, in a plant metabolism study with vines, 88% of the folpet residue was removed by leaf washing (Annex II, Section 4, Point IIA 6.1).

No adverse effects on adults or reproduction: Folpet has a low long-term toxicity to birds, with no effects on adult birds or reproduction at 1000 ppm in the diet for both bobwhite quail and mallard duck (the highest concentration tested in these two studies). Hence, Tier 1 TERs which are below 5 are not a result of any adverse effects. They are simply a numerical artefact of the highest dietary concentration tested.

Refinement of long term toxicity endpoint for birds: In the reproduction studies with bobwhite quail and mallard duck, the highest dietary concentration tested was 1000 ppm, resulting in two daily dose endpoints which were similar at 78.3 and 90.0 mg folpet/kg bw/day, respectively. Therefore, there is no reason to assume that these two bird species differ in their sensitivity to folpet. In a third reproduction study (on bobwhite quail), folpet was tested at higher dietary concentrations, with a highest treatment level of 4640 ppm. This study involved constant exposure for 8 weeks, which is equivalent to the 6 weeks exposure period recommended in the new draft OECD guideline for avian reproduction studies (for Japanese quail). There were no significant effects on adults at 4650 ppm, and no effects on reproduction (total number of eggs produced was the same as the control). Converted to daily dose this NOEC is equivalent to 769 mg/kg bw/day. Given that there is no evidence for a difference in species sensitivity, 769 mg/kg bw/day is a reasonable overall NOEL for long-term/reproductive toxicity. Refined TERs using this endpoint are presented in Table 16. For completeness, TERs based on the NOEL of 90 mg/kg bw are also included Table 16.

Concentrations on insects ('C'): Exposure predictions in the Tier 1 risk assessment for small insects are based on the published paper by Hoerger and Kenaga (1972), and are extrapolated from generic measurements of residues on small seeds (residue per unit dose, i.e. RUD, of 29). The EU guidance document clearly states that this residue estimate for small insects 'appears unsatisfactory…'. In Tier 1, it is also assumed that birds feed constantly (and exclusively) on insects carrying initial concentrations. The EU guidance document on risk assessment for birds and mammals (Appendix 2) provides a comprehensive review of available data on residues on food items, including data on insects. For long-term exposure it is suggested that an arithmetic mean residue value is used. For foliar insects, this value is stated to be 5.1 mg/kg (normalised for an application rate of 1 kg a.s./ha), and is derived from the generic database collected by Fischer and Bowers (1997). It was commented by the Scientific Committee on Plants (SCP) that these data should be used for large insects only, due to a bias in the sampling methods. However, in comparison with a Tier 1 extrapolation based on small *seeds*, these data for measured levels on *insects* provide a useful basis for refining the risk assessment. Hence, an RUD of 5.1 mg/kg has been used for refined exposure estimates. It should be noted that no generic estimates of dissipation of residues on insects are currently available. Hence, at

present, assessments have to be based on initial residues. In any case, this fully addresses the exposure from the proposed multiple applications. An RUD of 5.1 mg/kg has been used in the refined TERs for insectivorous birds stated in Table 16.

Proportion of diet obtained from the treated area ('PT'): Research by the Central Science Laboratory in the UK has studied the behaviour of insectivorous birds in orchards. The results of this research are quoted (on p31) in the EU guidance document. The data indicate that for blue tits (a common example of a small insectivore, and one of the two species used in the standard scenario) 95% of the local population spent less than 61% of potential foraging time among orchard trees. Hence, as a conservative (95^{th} percentile) refinement option PT may be adjusted to 0.61 for applications in orchards. As this PT value is conservative (95^{th} percentile), it is proposed to extrapolate to insectivores in vineyards, to provide a general indication of how PT can be refined. Hence, a PT of 0.61 has been used in the derivation of the refined TERs in Table 16 for insectivorous birds.

Table 16. Refined long-term TER values for birds following application of folpet to cereals, vines and tomatoes

Crop	**Indicator species**	**ETE (mg/kg bw/day)**	**NOEL (mg/kg bw/day)**	**TER_{lt}**	**Refined NOEL (mg/kg bwday)**	**TER_{lt}**
Winter wheat	Insectivorous bird	22.6 x 5.1/29 x 0.61= 2.4	90	37.5	769	318
Grapes	Insectivorous bird	45.2 x 5.1/29 x 0.61= 4.8	90	18.8	769	160
Tomato	Insectivorous bird	37.7 x 5.1/29 x 0.61= 4.0	90	22.5	769	192

All refined long-term TERs are greater than the Annex VI trigger of 5, indicating a low risk.

Conclusion

There is a low risk to birds and mammals from the notified uses.

Comments from RMS on birds and mammals risk assessment submitted by the Notifier:

Folpet is of relatively low toxicity to birds and mammals. Where TER values are low, this is a result of the relatively high application rates and number of applications, rather than inherent toxicity. The RMS supports the risk assessment submitted by the Notifier. One query would be whether an RUD of 5.1 covers the worst case of the consumption of *small* insects by birds. However, it is recognised that this value is used in the *long*-term assessment and over this period the diet of an insectivorous bird is likely to consist of a mixture of small and large insects. In addition, the long term endpoints for birds are derived from the highest treatment levels in the reproduction studies, with no indications of reproductive effects in the studies. The long term risk to insectivorous birds is considered to be acceptable.

The assessment as presented above is considered to have addressed Open point 5.1 and the following comments as presented in the Reporting Table: 5(1), 5(10), 5(15), 5(21), 5(22), 5(23), 5,(25), 5(26), 5(27), 5(28), 5(37), 5(38), (37 and 38 are also addressed by RMS response in Reporting Table), 5(39), 5(40), 5(42)(also addressed by RMS response in Reporting Table).

Comment 5 (19)(NL):

'NL would like to know where the assumption comes from that earthworms will contain 30% of PECsoil. Based on the logPow of 3.017 and the worst case Koc of 304, a BCFworm of 1.8 can be calculated, which is a factor 6 higher than the assumed 0.30.'

Response from RMS:

Based on the formula in the EU guidance document on risk assessment for birds and mammals (SANCO/4145/2000), using a log Pow of 3.017 and a Koc of 304, the BCFworm is 1.8 (as concluded by NL). For the use in grapevines (10 applications), assuming 70% foliar interception, and 14 day time-weighted average PECsoil after the final application (0.35 mg/kg soil), the TERlt for earthworm-eating birds is 130. This is greater than the trigger of 5, indicating low risk. The calculation is stated below:

From p 20 of Guidance document on birds and mammals the equation for calculation of BCFworm is:

BCFworm = (0.84 + 0.01 Kow) / foc Koc

Koc = organic carbon adsorption coefficient
foc = organic carbon content of soil (0.02 is default value)

Hence, for captan:

BCFworm = (0.84 + (0.01 x 1039.9)) / (0.02 x 304) = 11.239 / 6.08 = **1.8**

PECworm = PECsoil x BCF

PECworm = 0.35* x 1.8 = **0.63 mg/kg worm**

ETE (daily dose) for earthworm-eating birds = 1.1 (default value) x 0.63 = **0.693 mg/kg bw**

TERlt for birds = NOEL / ETE = 90 / 0.693 = **130**

* From Section B.8 of the DAR (Table B.8.3.1) the 14 day twa PEC following the final of 10 applications in grapevines at 1.5 kg/ha is 0.596 mg/kg. This is based on 50% interception (50% deposition). For this assessment 70% interception (30% deposition) is relevant. Hence, the PEC is adjusted to 3/5 x 0.586 = 0.35 mg/kg.

Comment 5(24)(FR):

Regarding risk to birds: *'Folpet is intended to be used for a period ranging from 2 weeks to up to 10 weeks in some crops (e.g. vineyards). It is not sure that the risk arising from repeated exposure over a 2-month and a half period is addressed by the proposed calculations.'*

Response from RMS:

For all notified uses (late season cereals, tomatoes, grapevines) the treated foliage is unlikely to be an attractive food source for birds. Hence, this route of exposure is not relevant. There is potential for repeated exposure of birds through feeding on sprayed insects, and this could occur over a two and a half month period for the use in grapevines (10 applications, 7 day interval) as raised in the comment. Such extended exposure is already addressed in the risk assessment as the avian reproduction studies (mallard and bobwhite quail) included continuous exposure for 18 weeks (no effects at 1000 ppm, the highest dietary concentration tested).

Comment 5(29)(DE):

Regarding risk to birds: *'It might helpful, if the ERA for birds would be presented according to the Working Document SANCO/4145/2000. The use of the interception factor should be justified. This is of particular importance since the interception factor for fungicides is 0.4 according to SANCO/4145/2000. Furthermore, not only secondary poisoning from fish to fish eating birds but also from earthworm to earthworm eating birds should be presented.'*

Response from RMS:

First part of comment is addressed as a new risk assessment has been presented according to SANCO/4145/2000. In this new risk assessment, the foliar interception values used are taken from FOCUS groundwater guidance (hence, they are agreed at EU level). Risk from consumption of earthworms is addressed by the response to Comment 5(19) above. Regarding risk to fish-eating birds, folpet has a DT50 of 24 minutes in the water phase from the sediment water fate study, and in a fish bioconcentration study the highest whole fish BCF was 61. Hence, bioconcentration of residues in fish in the field is very unlikely. In turn, there is a low risk to fish-eating birds.

Comment 5(41)(FR):

Regarding risk assessment for mammals: *'folpet is intended to be used for a period ranging from 2 weeks to up to 10 weeks in some crops (e.g. vineyards). It is not sure that the risk arising from repeated exposure over a 2-month and a half period is addressed by the proposed calculations.'*

Response from Notifier (ref: Norman, 2005):

For all uses (late season cereals, tomatoes, grapevines) treated foliage is unlikely to be an attractive food source for birds. Hence, this route of exposure is not relevant. There is potential for repeated exposure of mammals through feeding on sprayed insects. This route is only relevant (according to birds and mammals guidance) for the use in wheat. For this use there are only two applications, so the potential for repeat exposure is limited. For application in grapevine, there is a potential for small herbivorous mammals to be exposed through consumption of grass under the vines. This could occur over a two and a half month period (10 applications, 7 day interval) as raised in the comment. Such extended exposure is already addressed in the risk assessment (Norman and Wyness, 2003) as the two generation rat study (which was used in the long term assessment) included an exposure period of 38 weeks, and pairs were exposed for 14 weeks prior to mating. In terms of exposure concentration, the use of a 7 day twa residue (instead of standard 21 day value) can be used in this case to estimate exposure during the spray program as the minimum spray

interval is 7 days. The 7 day ftwa can be calculated as follows (using the formula on p 27 of the EU guidance document on birds and mammals):

ftwa = $(1-e^{-kt})/kt$ k = ln2/DT50 t = averaging time

k = 0.6931471/10 = 0.0693147 t = 7

ftwa = $(1 - e^{-0.485203})/0.485203$ = (1 – 0.6155722)/0.485203 = **0.792303**

With reference to Table 15, for herbivorous small mammals in grapevines (feeding on 'short grass' under the vines) this ftwa can be used to adjust the TER of 7.7 to give a TER of (0.53/0.79) x 7.7 = 5.2. This TER is marginally greater than trigger of 5, indicating low risk.

The RMS agrees with the above response.

For other comments in the Reporting Table on the bird and mammal assessments, responses from the RMS are provided in the Reporting Table itself. These are: 5(20), 5(37), 5(38), 5(42) (the latter three comments are also covered by the new risk assessment),

Overall conclusion of the RMS:

It is concluded that there is a low risk to birds and mammals from the proposed uses.

B.9.2 Effects on aquatic organisms (Annex IIA 8.2; Annex IIIA 10.2)

Open point 5.9:
MS to discuss the risk to aquatic organisms in an expert meeting. (see reporting table 5(30))

Reference should be made to the existing evaluation and risk assessment for aquatic organisms in Volume 3 of the DAR (p291 to 346) as a basis for the discussion at the expert meeting.

In addition, reference should be made to the responses provided by the RMS which are included in the Reporting Table. Additional responses have also been provided below.

In line with the conclusions of the aquatic risk assessment for captan (ref: Ecotoxicology Addendum for captan), it is also proposed by the RMS that the following statement be added to the conclusions for folpet:

> For Member States which accept the use of Species Sensitivity Distributions (SSD) in aquatic risk assessment at the national level, the HC_5 of 26.2 µg/L may be used as an alternative EAC for fish.

Additional responses to address comments from the Reporting Table on the aquatic risk assessment:

Comment 5(30) (SI) in Reporting Table:

'According to the summaries the lower test concentrations were below the limit of quantification (102 µg/L). This has to be clarified. It is impossible to conclude on an end point if test concentrations cannot be adequately measured. It is not clear if initial concentrations in these media were >80% of nominal'.

Response from RMS:

The following response is included in the Reporting Table. An additional statement from the Notifier is also included:

> The series of acute static toxicity tests with fish are those of greatest significance in the risk assessment. The limitations of the analytical method and the very rapid dissipation of folpet in water precluded the determination of recovery of folpet in water in some cases. To take account of this, stock solutions were analysed. Where possible initial concentrations in the test media were analysed. Based on all of the suite of static studies (all conducted at the same time in the same laboratory), the measured concentrations of folpet in the stock solutions were 78 to 121% of nominal with an average of 90 to 105% of nominal. This provides confidence that the initial test media were prepared according to the expected nominal concentration. In addition, where it was possible to measure initial concentrations in the test media the recoveries were 67 to 103% (139% for carp but this species was particularly insensitive relative to the other species) of nominal [*the 139% value is actually for brown trout not carp*].
>
> Therefore, based on the rapid dissipation and analytical method limitations the reported endpoints, based on nominal concentrations, are considered to be a reasonable approach given that stock solutions were within acceptable limits and that where possible measured initial concentrations were reasonably consistent with nominal concentrations.

Additional statement from Notifier in response to Comment 5(30)(Ref: Norman, 2005):

Justification of validity of acute toxicity studies on 6 fish species: Table 17 summarises the analytical results for the stock solutions and test media, in relation to the LC50 derived from each study.

Table 17: Folpet: acute fish toxicity studies on 6 species. Results of analytical measurements in relation to the derived LC50 from each study.

Species	Analysis of stocks compared to intended conc. (%)	Range of nominal test concs.	Analysis of test media		LC50 (nominal)	Is LC50 in range where nominals confirmed by analysis?	Ref:
			Measured concs in test media compared to nominal	Nominal tests concs. for which % nominal range applies			
rainbow trout	78 – 96%	24.3 - 568 µg/L	87 – 103%	117 – 568 µg/L	233 µg/L	YES	Jenkins, 2002a
brown trout	91 – 100%	13.7 – 320 µg/L	139%	320 µg/L	98 µg/L	NO	Jenkins, 2002b
common carp	85 – 94%	64 – 1500 µg/L	67 – 76%	320 – 1500 µg/L	1012 µg/L	YES	Jenkins, 2002c
3-spined stickleback	97 – 121%	42.7 – 1000 µg/L	86 – 92%	207 – 1000 µg/L	229 µg/L	YES	Jenkins, 2002d
roach	93 – 101%	42.7 – 1000 µg/L	71 – 83%	207 – 1000 µg/L	211 µg/L	YES	Jenkins, 2002e
bream	97 – 121%	19.4 – 456 µg/L	92 – 101%	207 – 456 µg/L	114 µg/L	NO*	Jenkins, 2002f

* LC50 was outside the range where analytical measurement of test media was possible. However, from the concentration-response at nominal 207 µg/L (71% mortality) and 465 µg/L (100% mortality) it is possible to judge that an LC50 of 114 µg/L is likely to be accurate.

With reference to Table 17 above it can be seen that for 4 out of 6 of the above studies, the analysis of the test media confirms the nominal concentrations which bracket the LC50. For bream, although the LC50 was outside the nominal range for which there was confirmation, the analytical data still indicate the accuracy of the LC50.

Only for brown trout is the LC50 clearly outside the range of nominal concentrations for which there is analytical confirmation for the test media. It is noted that all the 6 studies used the same test method and were conducted at the same laboratory at around the same time. Hence, the fact that 5 out of 6 studies had acceptable analytical results for the test media can also be taken as an indication that all 6 studies in the series are valid. Also, the study on brown trout has confirmation of the applied stock solution, indicating the correct loading of the test system. Finally, there was a clear concentration-response in the brown trout study across the range of test levels, which suggests that exposure was at the intended levels. Overall, these studies are considered valid for use in the risk assessment.

The RMS agrees with the above statement from the Notifier.

Comment 5(35)(FR) in the Reporting Table:

A response from the RMS to this comment is provided in the Reporting Table. This comment also mentions possible need for PECsw values for metabolites. To satisfy Data Requirement 4.2 in the Evaluation Table the Notifier has submitted PECsw values for metabolites, taking into account multiple applications (these PEC values are summarised in the Addendum on fate and behaviour). These enable the calculation of TERs for aquatic organisms for these compounds. TER values are stated in Table 18 (it is only necessary to derive TER values for the worst case use on grapevines).

Table 18: Acute Toxicity Exposure Ratios for aquatic organisms for metabolites of folpet (input route: spray drift of folpet)

Species	Endpoint	value (µg/l)	PECmax*	TER
phthalimide				
bluegill	96 h LC50	38000	5.17**	7350
D. magna	48 h EC50	39000	5.17**	7544
Phthalic acid				
rainbow trout	96 h LC50	>100000	13.57	>7369
D. magna	48 h EC50	>100000	13.57	>7369
Selenastrum capricornutum	72 h EbC50	>100000	13.57	>7369
Phthalamic acid				
rainbow trout	96 h LC50	>100000	4.36	>22936
D. magna	48 h EC50	>100000	4.36	>22936
Selenastrum capricornutum	72 h EbC50	>100000	4.36	>22936
Benzamide				
rainbow trout	96 h LC50	>100000	1.67**	>59880
D. magna	48 h EC50	>102000	1.67**	>61078
Selenastrum capricornutum	72 h ErC50	>96000	1.67**	>57485
2-cyanobenzoic acid				
rainbow trout	96 h LC50	>100000	7.9**	>12658
D. magna	48 h EC50	>100000	7.9**	>12658
Selenastrum capricornutum	72 h EbC50	>100000	7.9**	>12658

*Following the final application. These PEC values are for worst case use of 10 applications in grapevines, with spray drift at a distance of 3 m.
** In these cases the PECsw for a single application (90^{th} percentile drift value) is greater than following multiple applications (77^{th} percentile drift for each application). Hence, the PEC for a single application is given.

All TER values in Table 18 are greater than the relevant Annex VI trigger (acute: 100, algae: 10). Hence, there is a low risk.

Responses from the RMS to the following comments are provided in the Reporting Table: 5(31), 5(32), 5(33), 5(34), 5(35)(with additional response above), 5(36).

B.9.4 Effects on bees (Annex IIA 8.3.1; Annex IIIA 10.4)

Open point 5.11:
RMS to summarise and evaluate the study by Nengel 1996c on bees in an addendum and revise the risk assessment for bees accordingly. (see reporting table 5(44))

The missing summary is included below. This replaces the incorrect summary on p355-356 of the DAR (Volume 3):

Assessment of side effects of Folpan 80 WDG to the honey bee Apis mellifera L. in the laboratory following the EPPO Guideline No. 170. (Nengel, 1996 c 10.4.1/03):

In the laboratory the oral and contact toxicity of the test substance was assessed according to EPPO guideline 170 and GLP. Bees were exposed to five dose rates of Folpan 80 WDG by

feeding (oral route) or by topical application (contact route). The tested doses were 12.5, 25, 50, 100 and 200 µg product/bee (in the oral test the actual intake was 12.72, 21.99, 43.05, 108.01 and 223.87 µg/bee). There were also untreated controls and a toxic reference (dimethoate). There were 10 bees per replicate (cage), and 3 replicates for each dose level and control. In both oral and contact tests the bees were kept in small cages.

For the oral test, the test item was suspended in water to produce a stock. Appropriate volumes of stock were then added to a 50% sucrose solution. Before feeding, the bees were not fed for 2 hours. A volume of 250 µL was offered to each cage of 10 bees. After the test substance was taken up, cages were supplied with 50% sucrose solution *ad libitum* (without the test material).

For the contact test, the test item was suspended in water. After bees had been anaesthetized with carbon dioxide, they were treated individually with a microapplicator. 4 µL of test substance suspension was added to the ventral side of the thorax of each bee. After application the bees were returned to the test cages and fed 50% sucrose solution, ad *libitum*.

Bees were observed after 2, 4, 24 and 48 hours.

In terms of results, there were no mortalities in the oral test with Folpan 80 WDG (or in the untreated controls). Mortality in the toxic reference was 100% after 48 h. In the contact test, 48 h mortality was: control: 3.3%, 12.5 µg/bee: 0%, 25 µg/bee: 3.3%, 50 µg/bee: 10%, 100 µg/bee: 3.3%, 200 µg/bee: 3.3%. Toxic reference gave 100% mortality at 48 h.

The oral LD50 for Folpan 80 WDG was >223.87 µg product/bee (>179 µg a.s./bee). The contact LD50 was >200 µg product/bee (>160 µg a.s./bee).

Risk to bees:

Based on the highest application rate of 1500 g a.s./ha HQ values are <8.4 (oral) and <9.4 (contact). These are below the trigger of 50, confirming the previous conclusion of low risk.

B.9.5 Effects on other arthropod species (Annex IIA 8.3.2; Annex IIIA 10.5)

Open point 5.5:
RMS to revise the risk assessment for NTA in an addendum to be discussed in an expert meeting. (see reporting table 5(11)

Comments in the Reporting Table (5(45), 5(50), 5(51)) express concern over the application rates in non-target arthropod studies being too low to address the uses notified in the review. In response to these comments, the Notifier has submitted four new extended laboratory studies, together with a revised risk assessment. Hence, data requirements 5.1, 5.2, 5.2, 5.4 and 5.5 in the Evaluation Table have been satisfied. These are summarised below:

Extended laboratory study on Aphidius rhopalosiphi (parasitoid wasp), Moll, M 2004a:

The formulation Folpan 80 WDG was applied once as a foliar spray to bean plants (*Phaseolus vulgaris*) which were grown outdoors. There were three application rates of 1.64, 3.38 and 5.25 kg folpet/ha, together with a control (sprayed with water) and a reference item (dimethoate). Rates for the test item were calculated based on ESCORT 2. Bean plants were selected for use

in the test because they provide a three dimensional leaf matrix which can be extrapolated to other plants.

Leaves were removed from the sprayed plants when the spray deposit had dried (30-40 minutes after application) and 14 days after application (aged residues). During the 14 day aging period plants were kept outside (protected from rain). The detached leaves were used as a substrate in laboratory bioassays. The bioassays followed the established published method (Mead-Briggs *et al* 2000, published in Candolfi *et al* 2000), modified to use a leaf substrate. Exposure units comprised of two glass plates (13 cm x 13 cm) which were held 1.5 cm apart by an aluminium frame. The entire lower glass plate was covered with 4 - 5 leaves (upper surface facing upwards) from the treated plants. Ten adult *A. rhopalosiphi* were introduced to each exposure unit. There were 4 replicates for each application rate and control (and reference item). The exposure period was 48 hours, after which the surviving wasps were placed on pots of 18-25 aphid-infested barley seedlings (1 wasp per pot), enclosed in a clear plastic cylinder. Wasps were allowed to parasitise the aphids for a period of 24 h. The number of aphid mummies was counted 11 days later (fresh residues bioassay) or 12 days later (aged residues bioassay). Results of the study are presented in Table 19.

Table 19: Results of an extended laboratory study on *Aphidius rhopalosiphi* for Folpan 80 WDG

Treatment group (kg a.s./ha)	Mortality %	Corrected mortality %	Reproduction mummies/female	Reduction of parasitisation efficiency %
Fresh residues:				
control	7.5 #	-	38.0 #	-
1.64	10.0 ns	2.7	27.8 ns	26.7
3.38	27.5 *	21.6	25.6 ns	32.5
5.25	77.5 *	75.7	-	-
toxic standard (dimethoate)	100 *	100	-	-
14 day aged residues:				
control	0 #		31.2 #	-
1.64	0 ns	0	24.6 ns	21.1
3.38	0 ns	0	12.0 *	61.5
5.25	0 ns	0	28.8 ns	7.7

* = statistically significant ($\alpha = 0.05$) ns= not statistically significant
# = meets validity criteria for control of ≤ 13% mortality, and ≥ 5 mummies/female

For fresh dry residues, at 1.64 and 3.38 kg a.s./ha effects were below the ESCORT 2 trigger of 50%. At 5.25 kg a.s./ha, the effect on survival was greater than 50% indicating the need for testing of aged residues.

For 14 day aged residues, there was no mortality at any treatment level. Reduction in parasitisation at 5.25 kg a.s./ha was below the trigger of 50%. The 61.5% reduction in parasitisation at 3.38 kg a.s./ha is not considered to be treatment related as there were no effects for aged residues at 5.25 kg a.s./ha, and fresh residues at 3.38 kg a.s./ha had a lower effect (32.5%).

Overall, effects were less than the ESCORT 2 trigger of 50% for fresh residues at 1.64 and 3.38 kg a.s./ha, and for 14 day aged residues at 5.25 kg a.s./ha.

Extended laboratory study on Typhlodromus pyri (predatory mite) Rosenkranz, 2004a:

The formulation Folpan 80 WDG was applied once as a foliar spray to bean plants (*Phaseolus vulgaris*) which were grown outdoors. There were three application rates of 1.64, 3.38 and 5.25 kg folpet/ha, together with a control (sprayed with water) and a reference item (dimethoate). Rates for the test item were calculated based on ESCORT 2. Bean plants were selected for use in the test because they provide a three dimensional leaf matrix which can be extrapolated to other plants.

Leaves were removed from the sprayed plants when the spray deposit had dried (45-100 minutes after application). Further tests on aged residues were not necessary based on the results for fresh residues. The detached leaves were used as a substrate in laboratory bioassays. The bioassays followed the established published method (Blumel et al *et al* 2000, published in Candolfi *et al* 2000), modified to use a leaf substrate.

The detached leaves were cut to discs with a diameter of approximately 45 mm. Mites were exposed to the upper surface of the leaf. A glue barrier was added to the upper surface to prevent escapes. To provide a water supply a hole was pierced in the disc and the test unit was placed on wet cotton wool pad (treated side upward) in a petri dish. Ten mites were added to each test unit at the start of the test. There were 10 replicate test units per treatment and control. Number of living, dead, and escaped mites was assessed after 2 and 7 days. If corrected mortality was less than or equal to 50% the exposure period was prolonged for another 7 days. Number of eggs laid, and number of live and dead juveniles were counted on days 7, 10, 13 and 14. Results are presented in Table 20.

Table 20: Results of an extended laboratory study on *Typhlodromus pyri* for Folpan 80 WDG

Treatment group (kg a.s./ha)	Mortality after 7 days %	Corrected mortality %	Reproduction eggs/female	Effect on reproduction %
Fresh residues:				
control	17.8	-	4.5	-
1.64	6.0 *	0[b]	8.1 ns	-80 [a]
3.38	12.0 ns	0[b]	9.8 ns	-118.7 [a]
5.25	4.0 *	0[b]	9.2 ns	-105.1 [a]
toxic standard (dimethoate)	91.0 *	89.1	-	-

* = statistically significant (α = 0.05) ns= not statistically significant
# = meets validity criteria for control of ≤ 20% mortality, and ≥4 eggs/female
a = a negative value denotes a greater reproductive performance than the control.
b = negative values for corrected mortality have been stated as zero.

There were no significant effects on survival or reproduction including at the highest rate tested (5.25 kg a.s./ha). Hence, the ESCORT 2 trigger of 50% is satisfied.

Extended laboratory study on Coccinella septempunctata (ladybird larvae): Moll, M. 2004b:

The formulation Folpan 80 WDG was applied once as a foliar spray to bean plants *(Phaseolus vulgaris)* which were grown outdoors. There were 4 application rates of 0.31, 1.64, 3.38 and 5.25 kg folpet/ha, together with a control (sprayed with water) and a reference item (dimethoate). Rates for the test item were calculated based on ESCORT 2 guidance. Bean plants were selected for use in the test because they provide a three dimensional leaf matrix which can be extrapolated to other plants.

Leaves were removed from the sprayed plants when the spray deposit had dried (40-65 minutes after application). Further tests on aged residues were not necessary based on the results for fresh residues.

The detached leaves were used in laboratory bioassays. These bioassays were conducted according to the established published method (Schmuck *et al* 2000, published in Candolfi *et al* 2000), modified to use a leaf substrate. For each treatment 40 leaves were cut to discs with a diameter of 50 mm. One leaf disc was used for each exposure unit. The disc was placed (upper surface facing upwards) on a wet cotton wool pad in a petri dish (60 mm diameter) with a hole in the petri dish for a wick. A cylinder (30 mm high, 40 mm diameter) was fixed on each leaf. The exposure units were placed in a bowl. At the start of the test one 2-3 day old larva of *C. septempunctata* was placed in each exposure unit. The larvae were then allowed to develop in the exposure unit through to pupation. Aphids were provided as food. At the end of the exposure period when the larvae had pupated, the cylinders were covered with a lid to prevent the adults escaping. The exposure period (i.e. development from larva to adult) ranged from 12 to 20 days.

Surviving adults were used in a reproduction assessment. Adults from an individual treatment level were placed all together in a single insect rearing cage (40 cm x 40 cm x 40 cm) containing broad bean plants (*Vicia faba*) infested with aphids. Number of eggs produced and larvae hatched, were counted daily (except weekends) during a two week period. Results are presented in Table 21.

Table 21: Results of an extended lab. study on *Coccinella septempunctata* for Folpan 80 WDG)

Treatment group (kg a.s./ha)	Mortality %	Corrected mortality %	eggs per female per day	fertile eggs per female per day	larval hatching rate %
Fresh residues:					
control	15 #		4.1	3.7 #	91.1 ns
0.31	5.0 ns	0[a]	6.8 *	5.4 ns	79.4 *
1.64	5.0 ns	0[a]	10.1 *	8.6 *	86.4 *
3.38	15.0 ns	0	8.2 *	6.7 ns	84.6 *
5.25	25.0 ns	11.8	8.4 ns	7.5 *	86.1 *
toxic standard (dimethoate)	100 *	100	-	-	-

* = statistically significant ($\alpha = 0.05$) ns= not statistically significant
# = meets validity criteria for control of ≤ 30% mortality, and ≥2 fertile eggs/female/day
a = negative values for corrected mortality have been stated as zero.

For fresh dried residues corrected mortality was less than the ESCORT 2 trigger of 50% at all treatment levels. There was no adverse effect on reproduction (fertile eggs per female) at any treatment level. Also, there were >2 fertile eggs/female in all treatment levels indicating no effects according to the assessment criteria for this published method (Schmuck *et al*, 2000). Overall, there were no negative effects >50% for fresh residues including for the highest application rate of 5.25 kg a.s./ha.

Extended laboratory study on Chrysoperla carnea (lacewing larvae): Rosenkranz, 2004b:

The formulation Folpan 80 WDG was applied once as a foliar spray to bean plants *(Phaseolus vulgaris)* which were grown outdoors. There were three application rates of 1.64, 3.38 and 5.25 kg folpet/ha, together with a control (sprayed with water) and a reference item (dimethoate). Rates for the test item were calculated based on ESCORT 2 guidance. Bean plants were selected for use in the test because they provide a three dimensional leaf matrix which can be extrapolated to other plants.

Leaves were removed from the sprayed plants when the spray deposit had dried (60-65 minutes after application). Further tests on aged residues were not necessary based on the results for fresh residues.

The detached leaves were used in laboratory bioassays. These bioassays were conducted according to the established published method (Vogt *et al* 2000, published in Candolfi *et al* 2000), modified to use a leaf substrate. For each treatment 50 leaf discs with a diameter of approximately 55 mm were cut. One leaf disc was used for each exposure unit. The disc was placed (upper surface facing upwards) on a wet cotton wool pad in a petri dish with a hole in the petri dish for a wick. A cylinder (15 mm high, 46 mm diameter) was fixed on each leaf. The exposure units were placed in a container filled with tap water (which is transported up the wick to the leaf surface). At the start of the test one larva of *C. carnea* was placed in each exposure unit (50 per treatment level). The larvae were then allowed to develop in the exposure unit through to pupation. At the end of the exposure period cocoons were transferred to untreated plastic boxes. The exposure period (i.e. development from larva to cocoon) ranged from 14 to 22 days. After hatching, the adults were sexed and transferred to oviposition cages. First assessment of egg laying was done 7 days after first egg laying was observed. Numbers of eggs were counted after 24 hour egg laying periods ('checks'). Two 'checks' were done within one week. Eggs were incubated in separate plastic boxes, and hatching of larvae was assessed. Results are presented in Table 22.

Table 22: Results of an extended lab. study on *Chrysoperla carnea* for Folpan 80 WDG)

Treatment group (kg a.s./ha)	Mortality %	Corrected mortality %	eggs per female per day	larval hatching rate %
Fresh residues:				
control	20.0	-	36.8	88.1
1.64	36.0 ns	20.0	31.8	87.3
3.38	28.0 ns	10.0	33.3	84.3
5.25	34.0 ns	17.5	34.1	86.5
toxic standard (dimethoate)	70.0 *	62.5	-	-

* = statistically significant ($\alpha = 0.05$) ns= not statistically significant
# = meets validity criteria for control of ≤ 20% mortality, and ≥ 15 eggs/female/day

There were no significant effects on survival or reproduction, including at the highest rate tested (5.25 kg a.s./ha). Hence, the ESCORT 2 trigger of 50% is satisfied.

Revised risk assessment submitted by the Notifier:

EU Review of Folpet: Non-target arthropods: Updated risk assessment incorporating new extended laboratory studies at higher application rates than previously tested: Norman, 2004:

Summary: **Non-target arthropods**: Folpet is in list 2 of the EU review programme. Data have been reviewed by the Rapporteur Member State (Italy) on toxicity to non-target arthropods as part of the ecotoxicology assessment. These studies indicated a general low toxicity. The application rates tested in the laboratory and extended laboratory studies do not cover the highest rates notified in the EU review. Hence, additional extended laboratory studies have been undertaken on *Aphidius rhopalosiphi*, *Typhlodromus pyri*, *Coccinella septempunctata* and *Chrysoperla carnea* which cover the proposed rates, and also the ESCORT 2 multiple application factor (MAF). Testing on these four species represents a complete dataset under ESCORT 2. From the proposed uses, the worst case is use on grapevines with a maximum of 10 applications at 1.5 kg a.s./ha. The highest rate in the new studies (5.25 kg a.s/ha, including

MAF) was selected to cover the grapevine use. At this rate, there were no significant effects on *T. pyri*, *C. septempunctata* or *C. carnea*.
A. rhopalosiphi gave 76% mortality at 5.25 kg/ha for fresh residues (i.e. greater than ESCORT 2 trigger of 50%). Effects for fresh residues were less than 50% for 3.38 kg a.s./ha (to cover proposed use on tomato). For 14 day aged residues at 5.25 kg/ha, there were no effects on *A. rhopalosiphi*. Hence, the ESCORT 2 criterion for potential for recovery/recolonisation within 1 year is satisfied. Overall, it can be concluded that there is a low risk to non-target arthropods in-field and off-field.

Introduction:

In order to support the non-target arthropod risk assessment (in accordance with ESCORT 2), four additional extended laboratory studies have been conducted. These were undertaken as previously submitted laboratory and extended laboratory studies did not cover the highest notified application rates under the review. The test species in the new studies are *Aphidius rhopalosiphi* (parasitoid), *Typhlodromus pyri* (predatory mite), *Coccinella septempunctata* (foliage dwelling predator) and *Chrysoperla carnea* (foliage dwelling predator). The rates selected in these studies also take account of the proposed multiple applications through the use of the ESCORT 2 multiple application factor (MAF). The study reports are submitted together with this paper.

Risk assessment:

Available laboratory and extended laboratory studies on the toxicity of folpet (formulated) to non-target arthropods are summarised in Table 23 (including the four new extended laboratory studies).

Table 23 Folpet: Summary of laboratory and extended laboratory studies on non-target arthropods (including four new extended laboratory studies)

Species	formulation	Folpet (kg/ha)	Test substrate	Endpoint	Conclusion	Reference as in DAR
*Typhlodromus pyri**	'Folpan' 500 SC	0.49	Residues on glass surfaces	*Mortality:* control: 13% 0.49 kg/ha: 14% *Reproduction: offspring/female* control: 8.9 0.49 kg/ha: 9.3	No effects	Kühner, C. (1994b)
*Typhlodromus pyri**	'Folpan' 80 WDG	1.64 3.38 5.25	Extended laboratory, bean leaves, whole plants sprayed	*Corrected mortality:* 1.64kg/ha:0% 3.38kg/ha:0% 5.25kg/ha:0% *Eggs/female:* control:4.5 1.64kg/ha:8.1 3.38kg/ha:9.8 5.25kg/ha:9.2	No effects	**NEW STUDY** Rosenkranz (2004a)
*Aphidius rhopalosiphi**	'Folpan' 500 SC	0.1 - 2.0	Extended laboratory Residues on apple leaves	*Corrected mortality%:* 0.1kg/ha:2.5 0.5kg/ha:10 1.2kg/ha:2.5 1.5kg/ha:7.5 2.0kg/ha:32.5 *Reduction in parasitisation:* 0.1kg/ha:32% 0.5kg/ha:33% 1.2kg/ha:23% 1.5kg/ha:68% 2.0kg/ha:75%	Effects on survival lower than ESCORT 2 trigger of 50%. Effects on reproduction lower than ESCORT 2 trigger of 50% at 1.2 kg/ha and below, and greater than 50% at 1.5 and 2.0 kg/ha.	Schuld, M.S. (1999)
*Aphidius rhopalosiphi**	'Folpan' 80 WDG	1.64 3.38 5.25	Extended laboratory, bean leaves, whole plants sprayed. Fresh residues, and 14 day aged residues	Fresh residues: *Corrected mortality%:* 1.64kg/ha:2.7 3.38kg/ha:21.6 5.25kg/ha:75.7 *mummies/ female:* control:38.0 1.64kg/ha:27.8 3.38kg/ha:25.6 14 day aged residues: *mortality%:* 1.64kg/ha:0 3.38kg/ha:0 5.25kg/ha:0 *mummies/ female:* control:31.2 1.64kg/ha:24.6 3.38kg/ha:12.0 5.25kg/ha:28.8	Effects less than ESCORT 2 trigger of 50% for *fresh* residues for 3.38 kg/ha. Mortality >50% for fresh residues at 5.25 kg/ha. Effects less than ESCORT 2 trigger of 50% for *14 day aged* residues for 5.25 kg/ha.	**NEW STUDY** Moll, M (2004a)

*Recommended test species under ESCORT 2.

Table 23 continued

Species	formulation	Folpet (kg/ha)	Test substrate	Endpoint	Conclusion	Reference as in DAR
*Coccinella septempunctata**	'Folpan' 500 SC	0.48	Residues on glass surfaces	*Mortality:* control: 20% 0.48 kg/ha: 13% *Reproduction, fertile eggs/female:* control: 373 0.48 kg/ha: 206	No effect on survival. Effect (45%) on reproduction was less than ESCORT 2 trigger of 50%	Kühner, C. (1994a)
*Coccinella septempunctata**	'Folpan' 80 WDG	0.53	Residues on glass surfaces	*Mortality:* control: 22% 0.53 kg/ha: 16% *Reproduction: fertile eggs/female:* control: 419 0.53 kg/ha: 188	No effect on survival. Effect (55%) on reproduction was slightly higher than ESCORT 2 trigger of 50%	Kühner, C. (1996b)
*Coccinella septempunctata**	'Folpan' 80 WDG	0.31 1.64 3.38 5.25	Extended laboratory, bean leaves, whole plants sprayed	*Corrected mortality%:* 0.31kg/ha:0 1.64kg/ha:0 3.38kg/ha:0 5.25kg/ha:11.8 *Fertile eggs /female/day:* control:4.1 0.31kg/ha:6.8 1.64kg/ha:10.1 3.38kg/ha:8.2 5.25kg/ha:8.4	No statistically significant adverse effects	**NEW STUDY** Moll, M (2004b)
*Chrysoperla carnea**	'Folpan' 500 SC	0.49	Residues on glass surfaces	*Mortality:* control: 21.1% 0.49 kg/ha:7.7% *Reproduction, fertile eggs/female:* control: 610 0.49 kg/ha: 624	No effects	Kühner, C. (1993)
*Chrysoperla carnea**	'Folpan' 80 WDG	1.64 3.38 5.25	Extended laboratory, bean leaves, whole plants sprayed	*Corrected mortality%:* 1.64kg/ha:20 3.38kg/ha:10 5.25kg/ha:17.5 *eggs/female/day* control:36.8 1.64kg/ha:31.8 3.38kg/ha:33.3 5.25kg/ha:34.1	No statistically significant effects.	**NEW STUDY** Rosenkranz (2004b)
*Aleochara bilineata**	'Folpan' 500 SC	0.49	Residues on sand	*Parasitism:* control: 36% 0.49kg/ha: 29% 19% reduction	Effect lower than ESCORT 2 trigger of 50%	Ullrich, B. (1993)
Poecilus cupreus	'Folpan' 80 WDG	0.66	Residues on sand	*Mortality:*0% No effect on feeding	No effect	Kühner, C. (1996a)
Trichogramma cacoeciae	'Folpan' 500 SC	0.53	Residues on glass surfaces	*Parasitised eggs/wasp:* control: 7.7 0.53 kg/ha: 6.3 18.5% reduction	Effect on reproduction was lower than ESCORT 2 trigger of 50%	Kühner, C. (1996c)

*Recommended test species under ESCORT 2.

Proposed field crop uses in the EU review are summarised in Table 1.

Standard species (Typhlodromus pyri and Aphidius rhopalosiphi):

ESCORT 2 requires testing on the two standard species, *T. pyri* and *A. rhopalosiphi*. The existing glass plate test on *T. pyri* at 0.49 kg/ha showed no effects. However, the application rate was not high enough to cover the proposed uses (in Table 1). Hence, a new extended laboratory study has been conducted at higher application rates (Rosenkranz, 2004a). For *A. rhopalosiphi* an extended laboratory dose response study had been conducted. The study did not cover high enough rates to address the multiple applications proposed in the EU review. Also, an effect on parasitisation (68% reduction at 1.5 kg/ha) indicated the need for aged residues testing. Hence, a new extended laboratory aged-residues study has been conducted, including higher application rates than previously tested (Moll, 2004a).

The worst case use in terms of the risk assessment is the proposed use on grapevines (see Table 1), with 10 applications at 1.5 kg a.s/ha. ESCORT 2 does not provide a multiple application factor (MAF) for 10 applications (8 is the maximum). Hence, the default MAF for *8* applications (3.5) has been used. Hence, the appropriate testing rate to address risk to non-target arthropods in-field for the worst case use is 3.5 x 1.5 kg a.s./ha = 5.25 kg a.s./ha. This was the highest application rate in the new extended laboratory studies on *T. pyri* and *A. rhopalosiphi*. In the study on *T. pyri*, there were no effects at 5.25 kg a.s./ha. Hence, there is a low risk to species represented by *T. pyri*. For *A. rhopalosiphi*, effects were less than the ESCORT 2 trigger of 50% at 3.38 kg a.s./ha. This is the rate to cover the proposed use on field grown tomatoes (4 x 1.25 kg/ha; MAF = 2.7; 2.7 x 1.25 =3.38 kg/ha). At 5.25 kg a.s./ha, exposure to fresh residues resulted in 76% mortality. Test plants were placed outdoors (protected from rain) for 14 days so the effects of aged residues could be tested. At 5.25 kg a.s./ha, there were no effects on *A. rhopalosiphi* for 14 day aged residues. Hence, if initial effects on species represented by the sensitivity of *A. rhopalosiphi* occur following the proposed worst case spray program on grapevines, recovery of the remaining population and immigration into the treated area are possible shortly after the final treatment. Hence, the ESCORT 2 criterion of demonstration of potential for recovery/recolonisation within one year has been satisfied. It should also be noted that there were no effects on *fresh* residues for 3.38 kg a.s./ha, which is over two times the maximum individual dose in grapes.

Given that a low risk has been demonstrated for in-field application rates, it can also be concluded that the risk off-field from spray drift is acceptable.

Two additional species as required under ESCORT 2:

Data should be provided on two additional species selected from the options provided in ESCORT 2. For folpet, data were already available for *three* additional ESCORT 2 recommended species (*Chrysoperla carnea*, *Coccinella septempunctata*, and *Aleochara bilineata*). These laboratory studies (on artificial substrates) had a single application rate of around 0.5 kg a.s./ha. This was not high enough to cover the proposed uses in the EU review. Only *C. septempunctata* gave an effect greater than 50% (55% reduction in reproduction, but no effect on survival). Hence,
C. septempuctata was selected for testing in a new extended laboratory study using a realistic substrate (bean leaves) and higher application rates than previously tested. The risk assessment is also supported by a new extended laboratory study on *C. carnea*. In both these new studies, there were no statistically significant effects for fresh residues at 5.25 kg a.s./ha. Hence, there is a low risk to species represented by the sensitivity of *C. septempunctata* and *C. carnea*.

Given that a low risk has been demonstrated for in-field application rates, it can also be concluded that the risk off-field from spray drift is acceptable.

Conclusions:

Four new extended laboratory studies (on *T. pyri, A. rhopalosiphi, C. septempunctata,* and *C. carnea)* at higher application rates than previously tested confirm that there is a low risk to non-target arthropods from the proposed uses.

Comments from RMS on new non-target arthropod studies and risk assessment:

The submitted studies are considered valid, and address the applications rates of the proposed uses in the review (including multiple applications, by the use of Multiple Application Factors). The RMS supports the risk assessment as presented above.

It is considered that the above studies and risk assessment address **Open point 5.5** and the following comments from the Reporting Table: 5(45), 5(48), 5(50), 5(51)(part of this comment is addressed in the Reporting Table).

Comment 5(46)(DE) in the Reporting Table:

'In Table B.9.5.1.9, in the control column at day 8, a CR is given of 42%. However, it is impossible by definition to give a CR here.'

Response from RMS:

This is a typographical error. The value of 42% should be deleted. The corrected Table is provided below:

Table B.9.5.1.9: Numbers of *T. pyri* on vine leaves after application of 'Folpan' 500 SC in western Germany

Assessment time	Assessment	Control	'Folpan' 500 SC (0.15%)	'Folpan' 500 SC (0.2%)	'Delan' SC 750
Pre-application, Day 0	N[a]	470	463	437	407
	CR%[b]	-	-	-	-
	E%[c]	-	-	-	-
7 days after 2nd application	N	266	291	343	282
	CR%	-	-9	-29	-6
	E%	-	-11	-39	-22
8 days after 5th application	N	147	114	107	123
	CR%	-	22	27	16
	E%	-	21	22	3
6 days after 8th application	N	59	34	29	42
	CR%	-	42	51	29
	E%	-	42	47	18
4 weeks after 8th application	N	61	37	31	39
	CR%	-	39	49	36
	E%	-	38	45	26

[a] N: number of mites/25 leaves.
[b] CR%: Abbots corrected formula applied to the number of mites/25 leaves.
[c] E%: Henderson and Tilton formula applied to the number of mites/25 leaves.

The remaining comments are addressed by the RMS responses stated in the reporting table. These are: 5(46), 5(47), 5(49), 5(51), 5(52)

Overall conclusion of the RMS on risk to non-target arthropods:

Low risk to non-target arthropods from the proposed uses.

B.9.6 Effects on earthworms (Annex IIA 8.4; Annex IIIA 10.6.1)

Open point 5.6: MS to discuss the risk to earthworms in an expert meeting.(see reporting table 5(12))
Open point 5.12: RMS to transfer the information on earthworms from column 3 of the reporting table to an addendum. (see reporting table 5(55))

As a basis for discussion in the Expert Meeting the risk assessment for earthworms has been revised and presented below (included transferring information from column 3 of the reporting table, i.e. **Open point 5.12**).

The Notifier has submitted a new earthworm reproduction study, which is summarised below. The study used artificial soil with a 50% reduced peat content (5% peat) compared with the standard approach (10% peat). This is to remove the need for the use of the correction factor of 2 when using the results in the risk assessment.

Effects of FOLPAN 80 WDG on Reproduction and Growth of Earthworms Eisenia fetida in Artificial Soil with 5% Peat (Ref: Goßmann, 2005):

An earthworm reproduction study was undertaken on Folpan 80 WDG (analysed a.s. content, 79.8% w/w folpet). The study was conducted according to BBA (1994) and ISO (1998) guidelines, and GLP. The test species was *Eisenia fetida*. Adult worms (8 - 9 months old) were used for the test (with clitellum and weight 300 - 500 mg). Test containers (plastic boxes: 18.3 x 13.6 x 6 cm deep) were filled with artificial soil (5 cm depth) with constituents according to OECD 207 (but with a reduced peat content: 5% peat rather than standard 10%). The soil also contained 10 g dry cattle manure/kg, as a food source (during the study additional food i.e. dry manure, was placed on the soil surface). After the filling of the test containers, the earthworms (10 per test container) were then introduced onto the soil surface. There were 4 replicate test containers for an untreated control and each treatment level (i.e. a total of 40 worms per treatment level). Worms were allowed to burrow into the soil, then an application of the test material was made by spraying onto the soil surface (at an application volume equivalent to 600 L/ha). Application rates were the same as in a previous earthworm reproduction study on Folpan 80 WDG which used a standard 10% peat content in the test soil (ref: Wachter 2000). Application rates were: 0 (control, sprayed with water), 1600, 3200, 4800, 6400 and 8000 g product/ha (equivalent to 1280, 2560, 3840, 5120 and 6400 g folpet/ha). Initial pH of the soil was 5.5 - 5.6 (5.9 to 6.1 at test end). Soil water content at test initiation was 25.8 - 25.9% and 26.6 - 30.0% at test termination. Temperature was 19 - 21 °C. Illumination schedule was 16 h light : 8 h dark.

Assessment of mortality, behavioural effects and measurement of weight change was carried out after 28 days exposure of adult worms (worms were not returned to the test containers). After an additional 28 days, determination of number of offspring was conducted. Amount of food added to eat test container (i.e. food consumption) was also monitored. Results are summarised in Table 24.

Table 24: Folpan 80 WDG earthworm reproduction study. Summary of results.

Test Item:	FOLPAN 80 WDG					
Test Species:	*Eisenia fetida*					
Exposure:	test item sprayed onto the soil					
Test Duration:	56 days					
	control	FOLPAN 80 WDG				
		1600 g/ha	3200 g/ha	4800 g/ha	6400 g/ha	8000 g/ha
mortality [%][1]	0.0 ± 0.0	0.0 - ± 0.0	2.5 n.s.[2] ± 5.0	5.0 n.s.[2] ± 5.8	0.0 - ± 0.0	0.0 - ± 0.0
body weight change [%][1]	56.9 ± 5.7	63.3 n.s.[3] ± 6.4	53.1 n.s.[3] ± 8.6	60.6 n.s.[3] ± 8.1	57.7 n.s.[3] ± 11.6	52.8 n.s.[3] ± 14.9
reproduction # of juveniles[1]	312 ± 35	303 n.s.[3] ± 39	323 n.s.[3] ± 39	276 n.s.[3] ± 69	309 n.s.[3] ± 24	251 n.s.[3] ± 86
% of control	-	97.0	103.6	88.3	99.1	80.3
amount of food added [g][1]	25.0 ± 0.0	25.0 ± 0.0	25.0 ± 0.0	25.0 ± 0.0	25.0 ± 0.0	24.8 ± 0.5

[1] mean ± standard deviation of 4 replicates; the results represent rounded values calculated on the exact raw data

- = not relevant

n.s. = not significantly different compared to the control

[2] = Fisher-exact test, $\alpha = 0.05$

[3] = Dunnett test, $\alpha = 0.05$, (two sided for weight changes, one-sided smaller for reproduction)

As shown in Table 24, there were no statistically significant effects on adult survival, feeding, growth or number of offspring at any treatment level. Hence, the NOEL was 8000 g product/ha (6400 g folpet/ha), *i.e.* the highest treatment level.

Available studies on earthworms are already summarised in Annex B.9.6 of the DAR. For ease of reference the endpoints are also presented in Table 25, together with the results from the new reproduction study.

Table 25: Summary of available toxicity data for earthworms (*Eisenia foetida*), for folpet

Test material	Study type	Exposure duration	Endpoint mg a.s./kg soil	'Corrected' Endpoints* mg a.s/kg soil	Ref:
folpet	acute	14 days	LC50: >1000	LC50: >500	Wuthrich, (1992)
Folpan 80 WDG	acute	14 days	LC50: >828	LC50: >414	Wachter (1996)
Folpan 80 WDG	reproduction	adults: 28 d total: 56 d	NOEC: 5.18 (3.88 kg a.s./ha)	NOEC: 2.59	Wachter (2000)
Folpan 80 WDG	reproduction	adults: 28 d total: 56 d	NOEC: 8.53** (6.4 kg a.s./ha)	Not applicable***	Goßmann (2005)

* According to the EU terrestrial guidance and EPPO earthworm scheme 2002, the endpoints have been divided by 2 (as the log Kow of folpet is >2) in order to account for the difference organic matter content of the test soil (10%) and field soils which lower organic matter content. If TER values are below their respective triggers, there is scope to refine this assumption based on the fate properties of folpet in soil (as stated in Reporting table in the answer to Comment 5(55)).
** Calculated assuming a test soil density of 1.5 g/cm^3 (as specified in EU guidance document on terrestrial ecotoxicology).
*** This study used artificial soil with a reduced peat (organic matter) content of 5%, compared with the standard content of 10%, i.e. the organic matter content was divided by 2. Therefore, the EPPO correction factor of 2 is not necessary, as this factor has already been taken into account in the study design (OM content of test soil).

Risk assessment:

PECsoil values are provided on in a separate Addendum to the DAR on fate and behaviour. Relevant PEC figures have been used to derive TER values for earthworms. The PEC and TER values are stated below in Table 26.

Table 26: Relevant PECsoil values and TER values for earthworms for the Notified uses of folpet.

Crop use and max. number of applications	timescale	Toxicity endpoint mg a.s./kg soil	Maximum PEC* mg a.s./kg soil	TER	Annex VI Trigger
winter wheat	acute	>414	0.40**	>1035	10
	long term	8.53	0.40**	21.3	5
tomato	acute	>414	0.49***	>845	10
	long term	8.53	0.49***	17.4	5
grapevines	acute	>414	1.00****	>414	10
	long term	8.53	1.00****	8.53	5

*PECsoil value directly after the final application.
** 70% foliar interception assumed for use on wheat in accordance with FOCUSgw guidance.
***80% foliar interception assumed for use on tomato in accordance with FOCUS gw guidance.
**** For use in grapevine, in the bird/mammal risk assessment the Notifier proposed an interception value which is a mean of the values provided in the FOCUSgw guidance for the range of growth stages which may be treated with folpet. For consistency, this value (66.3%) has also been used for the earthworm risk assessment.

All TER values in Table 26 are greater than their relevant triggers. Hence, there is a low risk to earthworms from the proposed uses.

Comment 5(55) in the Reporting Table (AT):

According to the GAP Folpet is applied up to 10 times per season in grapes. Sublethal effects on earthworms have to be tested if the number of applications is >6, regardless of persistence (GD Terrestrial Ecotoxicology). Although otherwise stated in Volume 1 of the DAR, an earthworm reproduction study was conducted (see Vol. 3 of DAR). In this study a NOEC of 5.2 mg ai/kg soil was determined. To account for potential toxicity in soils with lower amounts of organic matter than the artificial substrate used in toxicity studies, this number is divided by a factor 2 (EPPO). The PECmax was determined to be 1.478 mg ai/kg soil (50% interception) or 0.887 mg/kg (70% interception). NOECcorr. = 2.6. Thus TERlt is either 1.76 (assuming 50% interception) or 2.9 (assuming 70% interception). In both cases the Annex VI trigger of 5 is not met and save use for the application in vine not proven.

Response from RMS:

The following response from the RMS to the comments from AT is stated in the Reporting Table. It was requested that this also be provided in the Addendum to the DAR. Hence, it is repeated below:

> The logPow for folpet is >2, which means (according to EPPO, 2002, and EU terrestrial guidance document) that the earthworm endpoint should be divided by 2. The adjustment of an earthworm NOEC to account for the organic matter content of different substrates or soils is only valid if there is a relationship between the organic matter content of soil and toxicity. Toxicity will be determined by the adsorption properties of the substance (pore water concentration being a manifestation of adsorption). Regarding long-term exposure for earthworms, folpet rapidly degrades in soil to such an extent that the adsorption/desorption coefficients cannot be calculated. Therefore, a relationship between soil organic matter and possible hazard cannot be established. However, the soil adsorption/desorption of one of the significant soil metabolites of folpet, phthalimide, was measured. Phthalimide is structurally very similar to folpet. There was no relationship between soil organic matter and soil adsorption of phthalimide. Therefore, the NOEC does not need to be adjusted in this particular case. To support this argument the estimated Koc for folpet is relatively low 304 – 1167. Currently, without the use of the factor, the TER for use in vineyards is 5.8 (assuming 70% foliar interception).
>
> For use in wheat at 0.75 kg a.s./ha (2 applications) assuming 70% crop interception (PECsoil after 2 applications = 0.379 mg a.s./kg), the TER is 13 (without the factor of 2). For use in tomatoes at 1.25 kg a.s./ha (4 applications) assuming 80% crop interception (PEC soil after 4 applications: 0.487 mg a.s./kg), the TER is 10.6 (without the factor of 2). Hence, the use of the factor of 2 would not affect the conclusion of low risk (TER trigger = 5) (interception values from FOCUSgw guidance). In any case, the number of applications is less than 5, so the requirement for an earthworm reproduction test would not be triggered

Additional comments from Notifier responding to Comment 5(55)(ref: Norman, 2005):

The response to Comment 5(55) in the Reporting Table supports the position that the NOEC of 5.18 mg a.s./kg soil from the previous earthworm reproduction study (Wachter, 2000) can be used directly in the risk assessment without a correction factor of 2. This study was repeated with the same range of application rates, but with a 2x lower organic matter content in the test soil (Goßmann, 2005). The new study gave a NOEC of 8.53 mg a.s./kg soil. Hence, this is experimental evidence that the organic matter content of the soil does not influence the toxicity of folpet (and its degradation products) to earthworms. The risk assessment using the NOEC from the new study indicates a low long term risk to earthworms.

The RMS agrees with the above statement from the Notifier.

Comment 5(56)(SI) in the Reporting Table:

'We consider it more appropriate to use 50% interception as realistic worst case in grapes for the long-term risk assessment.'

Response from the Notifier (ref: Norman, 2005):

In the risk assessment in the DAR an interception value of 70% is used. It is now proposed that this be marginally adjusted to 66.3% which is the mean interception value (of those provided in FOCUSgw guidance) over the period when folpet could be applied to grapevines. This is proposed as an appropriate value for use in the risk assessment, and is consistent with the approach previously proposed in the bird/mammal risk assessment (Ref: Norman and Wyness, 2003).

The RMS agrees with the above statement from the Notifier.

The risk assessment in this section also is considered to have addressed Comment: 5(12) and 5(53).
Responses to the following Comments are provided in the Reporting Table: 5(53)(also covered in the above risk assessment)

Overall conclusion of RMS on risk to earthworms

There is a low risk to earthworms from the proposed uses.

New references, by Annex point

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company, Report No. GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N	Owner
IIA, 8.4 IIIA, 10.6	Goßmann, A	2005	Effects of FOLPAN 80 WDG on Reproduction and Growth of Earthworms Eisenia fetida in Artificial Soil with 5% Peat. IBACON, Project: 18205022 24 February 2005 GLP, Unpublished	Y	Makhteshim
IIA, 8.3.2	Moll, M	2004a	Effects of Folpan 80 WDG on the parasitoid *Aphidius rhopalosiphi*, extended laboratory study, aged residue test. IBACON project number 18201003. Date: 13 January 2004. GLP. Unpublished.	Y	Makhteshim
IIA, 8.3.2	Moll, M	2004b	Effects of Folpan 80 WDG on the ladybird beetle *Coccinella septempunctata*, extended laboratory study, aged residues test.. IBACON project number 18203013. Date: 13 January 2004. GLP. Unpublished.	Y	Makhteshim

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company, Report No. GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N	Owner
IIA, 8.3.2	Rosenkranz, B.	2004a	Effects of Folpan 80 WDG on the predatory mite *Typhlodromus pyri*, extended laboratory study, aged residues test. IBACON project number 18202060. Date: 27 January 2004. GLP. Unpublished.	Y	Makhteshim
IIA, 8.3.2	Rosenkranz, B.	2004b	Effects of Folpan 80 WDG on the lacewing *Chrysoperla carnea*, extended laboratory study, aged residues test. IBACON project number 18204048. Date: 27 January 2004. GLP. Unpublished.	Y	Makhteshim
IIIA, 10.4.1/03	Nengel, S	1996c	Assessment of side effects of Folpan 80 WDG to the honey bee *Apis mellifera* L. in the laboratory following the EPPO Guideline No. 170. Dated: 17.06.96 GLP. Unpublished.	Y	Makhteshim
IIIA, 11.1	Norman, S. and Wyness, L.	2003	Folpet. Response to Rapporteur Member State request for a revised avian and mammalian risk assessment in accordance with EU guidance document on risk assessment for birds and mammals (SANCO/4145/2000. Makhteshim Agan and TSGE, unpublished report 11 September 2003.	Y	Makhteshim
IIIA, 11.5	Norman, S	2004	EU Review of Folpet: Non-target arthropods: Updated risk assessment incorporating new extended laboratory studies at higher application rates than previously tested. Dated: 5 March 2004 Unpublished.	Y	Makhteshim
-	Norman, S	2005	EU Review of folpet: Notifier responses to various comments on ecotoxicology raised in the official Reporting Table. Dated: 4 March, 2005 Unpublished	Y	Makhteshim

Folpet

Dossier According to Directive 91/414/EEC

Summary Documentation

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Mammalian toxicology

Addendum to dossier

March 2005

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New references, by Annex point 161

Introduction

This document contains new information on mammalian toxicology submitted by Makhteshim Chemical Works Ltd to the RMS.

New information is presented here in the order of the Evaluation table for folpet, cross-referencing the Open point numbers and Reporting table numbers. New information is summarised under the dossier numbering system.

Document D1: Critical Good Agricultural Practice

The GAP is presented in the table below.

Critical Good Agricultural Practice for folpet in the EU

Crop	Member state or country	Product name	F, G or I[a]	Pests or group of pests controlled	Formulation		Application			Application rate per treatment			PHI (days)	Remarks:
					Type	Conc. of a.s.	method kind	growth stage	number[b] (max.)	kg a.s./hL (max.)	water L/ha	kg a.s./ha (max.)		
Winter wheat	South EU	'Folpan' 80 WDG	F	*Septoria* Brown rust	WG	800 g/kg	Foliar spray; down-ward	Up to Z65	2	0.375	200	0.75	42	
Tomatoes	South EU	'Folpan' 80 WDG	F	Various [c]	WG	800 g/kg	Foliar spray; down-ward	From beginning of fruit set	4	0.125	1000	1.25	7	
	South EU	'Folpan' 80 WDG	G	Various [c]	WG	800 g/kg	Foliar spray; down-ward	From beginning of fruit set	3	0.16	1000 - 1300	1.6	7	
Grapes	North and south EU	'Folpan' 80 WDG	F	Various [d]	WG	800 g/kg	Airblast foliar spray; upwards/ sideways	Shoot emergence to veraison	10	0.75	200 - 400	1.5	28	

[a] F= field; G = greenhouse.
[b] Sprays on all crops are applied typically at intervals of 7 to 28 days.
[c] *Alternaria solanum, Cladospora, Colletotrichum, Septoria, Botrytis*
[d] Black rot, *Botrytis cinerea phomosis*. *Plasmopara viticola*.

New information on mammalian toxicology

Evaluation table number	Reporting table number	Open Point number
-	**2(1)**	**2.1**

Conclusions of the EFSA Evaluation Meeting:

RMS to provide more detailed summary of short term oral toxicity for discussion of short term NOAEL at an expert meeting.

- **Point IIA, 5.10: Summary of short-term toxicity and derivation of the acceptable operator exposure level**

The Acceptable Operator Exposure Level (AOEL) is traditionally derived from the consideration of the critical end-points from short-term studies, taking the properties of the active substance into account. Where there are similar end-points in studies in more than one species, the precautionary principle dictates that the NOEL in the most sensitive species is used in deriving the AOEL, unless there are sound scientific reasons to disregard that species. Two 90-day studies were performed in rat and one 90-day study was performed in the dog. Two 52-week studies were also performed in the dog. Other studies which may be considered in the derivation of an AOEL are the teratology studies (rat and rabbit) and multigeneration studies in the rat.

One rat 90-day study cannot be considered, as effects were recorded at the lowest dose of 2000 ppm i.e. there was no NOEL. The second 90-day rat study has a NOEL of 1000 ppm (equivalent to 67 mg/kg/day in males and 56 mg/kg/day in females), based on slightly reduced bodyweight gains in male rats and a low incidence of stomach lesions at next highest dose of 3,000 ppm (equivalent to 169 mg/kg/day). The stomach lesions were similar to those seen at the highest dose level of 10,000 ppm (613 mg/kg/day in males and 718 mg/kg/day in females), and were considered treatment-related.

The 90-day dog study was not considered, as a NOEL was not established (<790 mg/kg bw/day; findings included reduced weight gain, clinical chemistry changes and intestinal mucosal congestion). Similarly, the first one-year dog study cannot be considered, as the lowest dose level of 325 mg/kg/day was associated with slight decreases in bodyweight gains in females, and an increased incidence of vomiting , diarrhoea and salivation also seen at higher dose levels and considered treatment-related. The second one-year dog study gave a NOEL of 10 mg/kg bw/day. This was based on initial reductions in bodyweight gain and food intake, and clinical chemistry changes at the intermediate dose level of 60 mg/kg/day. A four-week range-finding study in the dog showed reduced weight gains and food intake at the lowest dose level of 20 mg/kg/day.

Of the other studies available for consideration, one of the two rat multigeneration studies had a NOEL of 800 ppm (equivalent to 40 mg/kg/day, based on lower adult and mean pup weights at the highest dose of 3,600 ppm (equivalent to 180 mg/kg/day); and the second study NOEL of 12.5 mg/kg/day may be considered an artefact of the dosing regime (0, 250, 1,500 and 5,000 ppm, equivalent of 0, 12.5, 75 and 250 mg/kg/day) such that the NOEL of 40 mg/kg/day lies between the NOEL and LOEL of the second study).

One of the two rat teratology studies showed a NOEL of 150 mg/kg/day, based on reduced maternal weight gain and reduced mean foetal weight at 550 mg/kg/day. A second rat teratology study showed a NOEL of 10 mg/kg/day , based on maternal clinical signs and reduced bodyweight gains at 60 mg/kg/day. The rabbit teratology study showed a NOEL of 10 mg/kg/day, based on slight maternal bodyweight effects at 40 mg/kg/day.

Considering all of the above, it would appear that the most consistent effect at the LOELs was slight reduction in bodyweight gain, although other findings such as clinical chemistry changes, clinical signs and histopathology were also present. In the short-term studies, the dog was significantly more sensitive than the rat, with a NOEL of 10 mg/kg/day in the one-year study compared to approximately 40 – 50 mg/kg/day in the rat 90-day and 2-generation studies. A maternal (i.e. adult) NOEL of 10 mg/kg/day was also reported in the rabbit teratology study, and in one of the rat teratology studies. According to the precautionary principle, the NOEL of 10 mg/kg/day is the most appropriate for derivation of the AOEL.

A safety factor of 100 is considered appropriate in that there are no cumulative toxicity issues for folpet due to its extremely rapid transformation and elimination in mammals. There are no *in vivo* mutagenic or reproductive issues concerning folpet. The oncogenic effects of folpet found in one species (the mouse) in the G-I tract have been shown to be due to a non-genotoxic mechanism associated with clear threshold exposure related to chronic irritation with high oral exposures over the lifetime of the mice. A clear NOEL has been established. There were no adverse effects on fertility and general reproductive performance in either of two two-generation rat studies. Embryotoxicity studies in rat and rabbit showed no increase in incidence of foetal malformations. Minor foetal effects reported were only seen at maternally toxic dose levels. These effects were considered transient, and secondary to the maternal toxicity.

Based on a safety factor of 100 an AOEL of 0.1 mg/kg body weight/day is proposed; this is considered to provide an acceptable margin of safety.

Evaluation table number	Reporting table number	Open Point number
-	2(2)	2.2

Conclusions of the EFSA Evaluation Meeting:

MS to discuss the carcinogenic properties at an expert meeting.

Responses to comments by Member States are provided below (see also information provided in response to Open point 2.6 and 2.11 below):

Vol. 1, Level 2, 2.1.4, Classification and labelling

Sweden (SE) notes that Cancer Category 3* should be added, according to the list of classification and labelling (ref: Annex I of Directive 67/548/EEC).

> *Category 3: Substances, which cause concern for man owing to possible carcinogenic effects but in respect of which the available information is not adequate for making a satisfactory assessment. There is some evidence from appropriate animal studies, but this is insufficient to place the substance in Category 2.

Response

The risk phrase R-40, "Limited evidence of carcinogenicity" suggests that an uncertainty exists regarding the carcinogenic potential of folpet. There is no such uncertainty with folpet. Robust chemical/physical data, mechanistic data supporting a threshold MOA, and bioassays in rats, mice and dogs allow a judgment of no cancer risk to man with a high degree of certainty; accordingly, the risk phrase, R-40, is not required nor appropriate. Supporting this conclusion are the following:

1. Folpet is not carcinogenic to industrial or agricultural workers in that there is no systemic dose following dermal or inhalation exposure.

2. Folpet acts through a non-genotoxic threshold based mechanism. This MOA requires high oral doses that sustain a duodenal-specific proliferative response.

3. Persons ingesting folpet residues have a margin of exposure (MOE) well over one million.

4. Folpet is not carcinogenic in rats or dogs; the gastrointestinal tumors (primarily in the duodenum) that appear in mice may well be species specific.

Practically, folpet is not carcinogenic to industrial or agricultural workers in that it has been determined to act through a non-genotoxic threshold based mechanism that requires high oral doses that sustain a proliferative response of the duodenum. As the systemic exposure to folpet is essentially zero from dermal and inhalation routes (due to the rapid degradation of folpet and thiophosgene, half-life of folpet is 4.9 seconds and the half-life of thiophosgene is 0.6 seconds), there can be no adverse effects on the duodenum. Moreover, the mode of action is specific to irritation of the duodenal villi from the lumen side of the mucus membrane.

Weight of evidence analysis concludes that folpet is not a human carcinogen as it is used in agriculture and that the risk phrase, R-40, is inappropriate.

Vol. 3, B.6.5.3 (Long term toxicity)

Denmark suggests classification for carcinogenicity, based on the increased incidences of adenomas and carcinomas in the duodenum of male and female mice in two strains (CD-1 and B6C3F1). The highly reactive thiophosgene is most likely the metabolite responsible for duodenal tumor formation in mice. In rats, folpet was classified as a carcinogen in males based on an increase in the incidences of C-cell adenomas and carcinomas of the thyroid as well as interstitial cell tumors of the testes. There was no evidence of duodenal tumors in the rat; however, there was a dose related increase in incidence of severity of hyperkeratosis of the oesophagus and stomach, which may be due to thiophosgene.

The increase in the incidence of duodenal adenocarcinomas in the CD 1 mouse study occurred at relatively high doses. A similar response was observed in a 2-year feeding study with B6C3F1 mice.

Response

Ascribing the carcinogenic effect of folpet in the mouse duodenum to thiophosgene is not supported. Folpet, not thiophosgene, is administered to mice. It is folpet that initially reacts with thiol groups of tissue proteins and induces irritation (e.g., villi disruption). In the process of this initial chemical interaction, thiophosgene is generated. Thiophosgene is reactive not only with thiol groups but an array of other functional groups, thus extending the irritation effects. It is the collective actions of folpet and thiophosgene that most likely are responsible for the duodenal irritation, loss of villi, and eventual induction of tumors.

Folpet induces hyperkeratosis in the upper GI tract of rats but does not induce treatment related tumors. Folpet is not available systemically, regardless of the oral dose, due to the exponential degradation in blood (half-life of 4.9 seconds). There is no consistent pattern of tumors across studies (as there is with mice) and rat studies with captan, its sister fungicide with which it shares a common mechanism of toxicity do not show these same tumors (in contrast other non-treatment related tumors are seen).

Vol. 3, B.6.5.2, Long-term toxicity and carcinogenicity in the mouse

The United Kingdom (UK) notes the NOAEL in the chronic mouse study of East (1994) is considered to be 150 ppm as the histopathological findings in the gastrointestinal tract at 450 ppm are considered to be treatment –related.

Response
The NOAEL of 450 ppm is supported.

The study director cites hyperplasia (noted in the data below) as well as a benign squamous cell papilloma at 450 ppm but cited a reference supporting his conclusion that these findings were fortuitous as “between one and three tumours of the squamous epithelium of the non-glandular stomach will be found during the course of a carcinogenicity study” (Faccini et al., (1990) Mouse Histopathology, A glossary for use in toxicity and carcinogenicity studies. Elsevier, Publisher, Amsterdam, New York, Oxford).

Inspection of the data below show the nature and severity of effects on the gastrointestinal tract. In both cases where there was hyperplasia noted at 450 ppm, there was an absence of hyperplasia at the next higher dose, 1350 ppm. The lack of dose response, the expected background incidence (citation, above) and the absolute numbers involved support the study director's judgment that the NOAEL for this study is 450 ppm.

Data
Histopathology, non-neoplastic findings for all animals (Table 10H)

Dose	0	150	450	1350 ppm
Duodenum	**100**	**52**	**52**	**52 examined**
Hyperplasia,				
Lamina propria	0	0	0	2 male
	0	0	0	0 female
Mucosal dysplasia	0	0	0	0 male
	0	0	0	0 female
Villous hyperplasia	0	0	1	0 male
	0	0	0	3 female
Chronic inflammation	0	0	0	0 male
	1	0	0	0 female
Villous fusion	0	0	0	0 male
	0	0	0	1 female

Dose	0	150	450	1350 ppm
	100	**52**	**52**	**52 examined**
Jejunum				
Hyperplasia,				
Lamina propria	0	0	0	1 male
	0	0	0	0 female
Mucosal dysplasia	0	0	0	1 male
	0	0	0	0 female
Villous hyperplasia	0	0	1	0 male
	0	0	0	0 female
Chronic inflammation	0	0	0	0 male
	0	0	0	0 female
Villous fusion	0	0	0	1 male
	0	0	0	0 female

Dose	0	150	450	1350 ppm
	100	**52**	**52**	**52 examined**
Ileum				

Hyperplasia,				
Lamina propria	0	0	0	1 male
	0	0	0	0 female
Mucosal dysplasia	0	0	0	1 male
	0	0	0	0 female
Chronic inflammation	0	0	0	0 male
	0	0	0	0 female
Villous fusion	0	0	0	1 male
	0	0	0	0 female

Dose	0	150	450	1350 ppm
Stomach	**100**	**52**	**52**	**52 examined**
Keratoacanthosis	13	9	1	10 male
	7	6	5	13 female
Keratinised region				
Acute inflammation	0	0	0	1 male
	0	0	0	1 female
Glandular region				
Erosion	1	0	0	1 male
	1	1	0	0 female
Keratinised regions				
Mucosal/submucosal				
Oedema	2	1	1	0 male
	4	0	0	1 female
Glandular regions				
Acute inflammation	0	0	0	0 male
	0	0	1	0 female
keratinised region				
mucosal oedema	0	0	0	0 male
	1	0	0	0 female

Evaluation table number	Reporting table number	Open Point number
2.1	**2(4)**	**-**

Conclusions of the EFSA Evaluation Meeting:
Notifier to submit the position paper by Gordon E., 2004 and the study Moore and Creasey (2004).

The following new reports are submitted:

- **Point IIA, 5.10: Summary of mammalian toxicity and overall evaluation**

5.10/01

Report: Gordon, E. (2004). Folpet. A summary basis for why an acute reference dose (aRfD) is not needed. Submitted to the JMPR for the 2004 toxicological evaluation of folpet. Makhteshim-Agan, unpublished report.

Guidelines: Not applicable.

GLP: No.

Material and methods: An ARfD of 0.1 mg/kg bw is proposed in the DAR. Full and detailed comments on all aspects of the ARfD for folpet are presented in a position paper.

Findings:

The position paper concludes:
1) There is minimal irritation seen in the gastrointestinal tract after one day exposures to folpet at doses above 500 mg/kg.
2) There are minimal effects at doses above 500 mg/kg in a development study.
3) Gastrointestinal irritation following repeated folpet oral exposure is rapidly reversed upon cessation of treatment.
4) Folpet is not present in the systemic circulation and is not a systemic toxin.
5) Folpet will not induce adverse effects when residues are ingested continuously, even at the theoretical maximum residue values.
6) Folpet's oral toxicity is greater than 5 g/kg.

This position paper is supported by a new previously unsubmitted acute intestinal irritation study (see Point IIA 5.8.2/06).

Conclusions: Based on an evaluation of the toxicology database for folpet, an ARfD for folpet is not required.

Evaluation table number	Reporting table number	Open Point number
2.1	2(4)	2.3

Conclusions of the EFSA Evaluation Meeting:
RMS to provide more detailed summary of the studies which lead to the derivation of the ARfD for discussion at an expert meeting.

-	2(15)	2.7

Conclusions of the EFSA Evaluation Meeting:
MS to discuss the irritating properties, also in relation to classification, at an expert meeting.

- **Point IIA, 5.8.2: Supplementary studies on the active substance**

The following new study is submitted to support the contention that an ARfD is not applicable to folpet. The study is also relevant to the irritating properties of folpet.

5.8.2/06

Report: Moore, G.E. and Creasey, D. (2004). Intestinal irritation in CD-1 mice after a 24-hour exposure to folpet. [redacted] unpublished report number 13763 (Company file: R-16283).

Guidelines: In-house.
Deviations: Not applicable.

GLP: Yes (Study 1). Study 2 was not performed under GLP procedures but were performed under the same study director in a GLP compliant laboratory.

Material and methods: Test substance: folpet, batch number 8133181, purity 96.9%. Two studies were conducted. These were initially designed as pilot studies but, as the data were unambiguous with regard to minimal irritation, they were integrated into a final report. In the first study the test substance was administered to groups of 3 female ICR (CD-1 equivalent) mice as an 18% w/w suspension in a 1% w/w solution of CMC in distilled water by oral gavage at 900 mg/kg/bw (followed by untreated diet) or in the diet at 200 or 5000 ppm over a 24-hour period; a fourth group of 3 animals received untreated diet only. Animals were terminated after 24 hours.

In the second study, the test substance was administered to groups of 15 female ICR (CD-1 equivalent) mice as an 18% w/w suspension in a 1% w/w solution of CMC by oral gavage at 900 mg/kg/bw (followed by untreated diet) or in the diet at 50, 200, 500 or 5000 ppm over a 24-hour period; a control group of 5 animals received untreated diet only. Animals were exposed to the treated diet for 24 hours (Day 1). The 5000 ppm dietary dose was equivalent to approximately 1000 mg/kg bw/day. In treated groups, five animals in each group were killed

after 1 day, 3 days and 7 days, respectively. Of the five control animals, three were killed at 24 hours and one each at 3 and 7 days. Animals intended for sacrifice at 3 and 7 days were given control diet after the 24-hour exposure to test diets. These animals were designated recovery animals and were not evaluated histologically, as irritation was absent at Day 1.

Test concentrations in the suspension and diet were measured by HPLC. All animals were observed for mortality, signs of gross toxicity and behavioural changes. Food consumption was recorded during the first 24 hours (Day 1). Body weights were determined prior to administration (Study 1 and 2) and on Days 3 and 7 for animals on Study 2 maintained through Day 7.

At termination, mice in the first study were first injected with 2 mL/kg of 1% w/v Evans Blue Dye approximately 15 minutes before euthanasia with CO_2. This was done in an attempt to visualize areas of mucosal irritation. Following euthanasia, the stomach, and small intestine of each animal were removed and examined macroscopically using a binocular microscope. The number and size of lesions in the small and large intestines was recorded (large = > 2mm diameter, small = 1-2 mm diameter, punctiform = < 1 mm). For each tissue the severity of mucosal damage was estimated on a 0 to 5 scale, where 0 = no lesions, 1 = up to 5 punctiform lesions, 2 = more than 5 punctiform lesions, 3 = 1 to 5 small lesions, 4 = more than 5 small lesions or 1 large lesion and 5 = more than one large lesion. Lesions and samples taken from the forestomach, fundic and pyloric glandular mucosa, pyloric duodenum and distal duodenum were processed and examined histologically.

Mice from the second study were not injected with Evans Blue Dye, as this procedure proved to be of little value. Following euthanasia, the stomach with the duodenum attached were removed intact, food contents flushed from the stomach after the forestomach was cut along the greater curvature. Ten percent neutral buffered formalin was injected into the open end of the duodenum so that it flowed into the stomach. This procedure was instituted to reduce artefacts caused by mechanical manipulation (including the pinning out of the longitudinally cut duodenum on a board for the macroscopic evaluation) in the first study. Samples were taken from the glandular fundic mucosa, non-glandular forestomach, and proximal (pyloric) duodenum and examined histologically from all mice. Additional samples (eight step serial sections) from mice dosed orally at 900 mg/kg/bw and at 5000 ppm in the diet were examined microscopically for evidence of irritation. This was done to confirm the absence of irritation seen in the initial sections.

Findings:

The first study showed some indications of apparent irritation (Table 5.8.2-1), but these findings were absent in the expanded second study. Extensive examination of multiple sections (eight step-serial sections) from the second study showed no irritation in the duodenum and only two instances of focal erosion in the stomach in mice administered a bolus gavage dose of folpet (Table 5.8.2-2).

While a definitive cause for the findings noted in the first study was not identified, mechanical manipulation of the tissues during necropsy and macroscopic evaluation (longitudinal cutting and pinning of the duodenums) may have contributed to the apparent irritation.

Food consumption data were used to calculate the intake of folpet. Individual body weights were used to calculate the mg/kg bw/day dose for those mice receiving folpet admixed in the diet. The mean dose (of three animals) in Study 1 was 31 and 845 mg/kg/day for the 200 and 5000 ppm groups, respectively. The actual dose for the 900 mg/kg group was 1430 mg/kg.

In the second study, the mean dose (of five animals) was 10, 44, 123, and 1060 mg/kg/day for the 50, 200, 500 and 5000 ppm groups, respectively. The actual dose for the 900 mg/kg group was 815 mg/kg.

In the first study, mice treated with folpet at 900 mg/kg/bw by gavage or 5000 ppm in the diet showed apparent changes in the proximal region of the duodenum, close to the junction with the pyloric sphincter, and also in the stomach. These initial findings included minimal to moderate focal areas of epithelial loss (erosions) or degeneration/regeneration of the epithelium characterised by basophilia and reduced cell height. Loss of villous structure was associated with the more severe lesions and congestion of the mucosal vasculature was also seen, with mucosal damage in all animals treated with folpet at 900 mg/kg/bw by gavage or 5000 ppm in the diet. Similar findings in the fundic mucosa of the glandular stomach were also seen in two of the three mice receiving the 900 mg/kg/bw dose. There were no microscopic findings in the distal duodenum. Findings in the 200 ppm treated group were equivocal or of negligible significance.

In the expanded second study, there were no gross abnormalities and there were no degenerative changes in the duodenum. The instances of erosion in the fundic stomach of two mice administered 900 mg/kg were judged “minimal.”

In summary, these data show that a 24-hour exposure to folpet at 5000 ppm, equivalent to approximately 1000 mg/kg bw/day, does not cause irritation to the duodenum or stomach (although a bolus dose of folpet at 900 mg/kg did cause minimal erosion in the stomachs of two of five mice). The data from the first study, however, indicated some irritation might be occurring (although possibly artifactual) and thus the conclusion was drawn that folpet at 5000 ppm causes minimal (“borderline”) irritation in the duodenum of the mouse.

The duodenum is the site of tumor formation upon long-term dietary levels of 5000 ppm and has shown marked irritation after repetitive days dosing with a diet containing 5000 ppm folpet. This study shows that repetitive dosing is required for irritation in contrast to a single 24-hour period of dosing.

A single exposure of folpet at 5000 ppm over a 24-hour period (equivalent to approximately 1000 mg/kg bw/day) produces only minimal (“borderline”) irritation to the mouse duodenal mucosa.

Table 5.8.2-1: Macroscopic and microscopic findings in the stomach and duodenum of mice treated with folpet (Study 1)

Finding*	Dose level			
	0	200 ppm/diet	5000 ppm/diet	900 mg/kg/bw by gavage
Number of large (> 2mm) lesions in stomach (individual scores)**	0/3	1/3 (1)	0/3	1/3 (2)
Number of large (> 2mm) lesions in proximal duodenum/pylorus (individual scores)	1/3 (1)	1/3 (1)	1/3 (1)	3/3 (1, 1, 1)
Erosion/epithelial degeneration of stomach (individual scores)	0/3	0/3	0/3	3/3 (2)
Mucosal congestion of stomach (individual scores)	0/3	0/3	0/3	2/3 (1,2)
Erosion/epithelial degeneration of proximal duodenum (individual scores)	0/3	1/3 (1)	3/3 (1, 2, 3)	3/3 (2, 3, 3)
Loss of villi in proximal duodenum (individual scores)	0/3	0/3	1/3 (3)	3/3 (1, 3, 3)
Mucosal congestion of proximal duodenum	0/3	1/3 (1)	1/3 (2)	2/3 (1, 1)
Effects on distal duodenum	0/3	0/3	0/3	0/3

* Determined in a total of 3 animals
** 1 = minimal, 2 = slight, 3 = moderate

Table 5.8.2-2: Macroscopic and microscopic findings in the stomach and duodenum of mice treated with folpet (Study 2)

Finding*	Dose level					
	0	50 ppm	200 ppm	500 ppm	5000 ppm	900 mg/kg
Macroscopic	0/5	0/5	0/5	0/5	0/5	0/5
Stomach, focal erosion (individual scores)**	0/3	0/5	0/5	0/5	0/5	2/5 (1, 1)
Proximal duodenum abnormalities	0/3	0/5	0/5	0/5	0/5	0/5

* Determined in a total of 5 animals (three controls were examined microscopically). Microscopic evaluation included eight step serial sections of the duodenum for mice administered 5000 ppm or 900 mg/kg.
**1= minimal

Conclusions: Folpet administered by oral gavage at 900 mg/kg/bw or in the diet for 24 hours at 5000 ppm (as well as 500 ppm, 200 ppm, and 50 ppm) caused only minimal ("borderline") irritation of the proximal duodenum. The initial finding of apparent irritation in the first study was shown likely due to artefacts since a thorough (eight step serial section) examination of the expanded second study did not reveal significant irritation.

It was concluded that folpet was borderline for producing irritancy at 5000 ppm.

Evaluation table number	Reporting table number	Open Point number
2.2	2(5)	-

Conclusions of the EFSA Evaluation Meeting:

The notifier to send position paper regarding reproductive toxicity and teratogenicity of folpet to the RMS.

- **Point IIA, 5.6: Reproductive toxicity**

The following new report is submitted:

5.6/01

Report: Neal, B. (2004). Comments on folpet monograph Reproductive and developmental toxicity: section B 6.6 reproductive toxicity. The Weinberg Group Inc, unpublished report 18 October 2004.

Guidelines: Not applicable.

GLP: No.

Material and methods:

The existing data on reproduction toxicity and developmental toxicity were reviewed following comments in the Folpet DAR by the RMS that that new teratogenic studies in rat and rabbit were required with histopathological examination of the gastro-intestinal tract of the mothers.

Findings:

The findings are summarised as follows:

Reproductive toxicity studies

The NOEL for effects on pup body weight for folpet in reproductive toxicity studies is revised from 12.5 mg/kg bw/day to 40 mg/kg bw/day, based on a weight-of-the-evidence evaluation of the two studies. This dose level is equivalent to the parental NOEL, demonstrating a lack of unique susceptibility of the young to folpet toxicity. Using 12.5 mg/kg bw/day as the basis for the folpet AOEL as currently recommended provides a very conservative additional margin of safety for risk extrapolation.

Developmental toxicity studies

We concur with the RMS reviewer that the axial abnormalities observed at maternally toxic dose levels in several folpet developmental toxicity studies may be related to the maternotoxic effect elicited by folpet on the gastrointestinal tract. In addition to the noted irritant action of folpet on the gastrointestinal mucosae, high bolus gavage doses of folpet are likely to adversely affect the intestinal flora, leading to nutrient malabsorption or deficiencies.

The developmental NOAELs for folpet are 150 mg/kg bw/day and 40 mg/kg bw/day, for the rat and rabbit, respectively. There is no evidence of unique susceptibility of the foetus to folpet, and a weight-of-the-evidence evaluation does not support a conclusion that folpet is teratogenic.

Further, distribution of folpet to the foetus is considered unlikely because of the very short half-life of folpet in aqueous media, and the primary metabolite phthalimide produced no malformations in a supplementary teratogenicity evaluation in rabbits.

Response to the Requirement for Further Reproductive or Developmental Toxicity Studies of Folpet

The existing database provides adequate information regarding the reproductive and developmental toxicity of folpet to permit informed and conservative risk assessment.

For reproductive toxicity evaluation, we concur with the RMS reviewer that in cases where the studies are not congruent with existing guidelines, the absence of any evidence of reproductive toxicity in a study producing overt toxicity to the parental animals suggests no additional useful information would be obtained from further studies.

For developmental toxicity evaluation, we respectfully disagree with the reviewer that additional useful information would be obtained through replication of the rat and rabbit developmental toxicity studies, and that animals and resource expenditure in such an effort is therefore not justifiable. The basis for our conclusion is that:

- Existing studies comply with Guidelines in effect at the time the studies were performed, and provide information on the most critical elements in current Testing Guidelines.

- NOELs are available for all endpoints of concern,

- Folpet does not show unique evidence of developmental susceptibility, and a weight-of-the evidence evaluation does not support a concern for teratogenicity.

The one remaining question is that the postulated mechanism for maternotoxicity resulting in the axial respecifications observed in several developmental studies of folpet at maternally toxic dose levels has not been clearly demonstrated in the existing data. If this mechanism were confined to nutritional deficiencies resulting from gastrointestinal irritation, it could possibly be demonstrated through histopathological evaluation of the maternal gastrointestinal tract. However, it seems likely that the bacteriostatic action of folpet when administered in high gavage doses also plays a significant role in subsequent maternal nutrient deficiencies, contributing to the axial respecifications observed in some studies of folpet. Such a mechanism would not be possible to demonstrate in a conventional developmental toxicity study, and it is difficult to conceive of a study design to adequately test this mechanism. Folpet is used commercially as a bacteriostat in cosmetic formulations, and evidence of bacteriostatic action of captan (which is a closely structurally related chemical) is available in the published literature.

Based on these factors, we believe no useful information would be gained from further developmental toxicity studies of folpet.

Conclusions: The existing database provides adequate information regarding the reproductive and developmental toxicity of folpet to permit informed and conservative risk assessment. There is no evidence that there is any unique developmental susceptibility of the developing young to folpet. Further reproductive or developmental toxicity testing of folpet should not be required.

Evaluation table number	Reporting table number	Open Point number
-	**2(5)**	**2.4**

Conclusions of the EFSA Evaluation Meeting:
RMS to provide more detailed summary of the 2-generation reproduction toxicity study for derivation of NOAEL and discussion in an expert meeting.

-	**2(22)**	**2.11**

Conclusions of the EFSA Evaluation Meeting:
MS to confirm the NOAELs in the long term studies at an expert meeting.

This more detailed summary is presented in response to open point 2.4 and 2.11.

The following new summaries are presented in response to requests from the EFSA:

Vol. 3, B.6 General comment

The European Food Safety Authority (EFSA) notes the results in the studies are sometimes poorly described. There is a lack of informative tables and/or the effect as percent of control and if it as NOEL or a NOAEL value. [is] The concentrations of the compound is often presented in ppm without demonstrating the corresponding value in mg/kg bw/day. Furthermore, the conclusions are very brief and in some cases even lacking. The provision of an addendum where more information is provided, for instance for the studies being considered as crucial for setting of ADI, AOEL, and ARfD, would be appreciated in order to increase understanding and transparency.
Proposed studies are:
B.6.3. one year dog study (Daly 1986) – see additional information under Point 5.5 below
B.6.5 2-year rats study (Crown, 1989) - see additional information under Point 5.5 below
B.6.6 2-generation reproduction, rat (Rubin, 1986) – see below
B.6.6. Teratogenicity study, rabbit, Rubin 1985c) – see below.

Response

- **Report: Rubin, 1986, Folpan Two-generation reproduction study in the rat (MAK/052/FOL, R-4347).**

Experimental design:
0, 250, 1500, 5000 ppm, 25 male and 25 female F0 animals

Effects:

Reduced food intake and reduced body weight gain in F0 and F1 parents and reduced body weight gain of F1 and F2 offspring at 5000 ppm

Histology: hyperkeratosis of the non glandular gastric mucosa at 5000 and 1500 ppm in F0 and F1 generations; esophageal hyperkeratosis in the /F1 generation and a single case of ulceration of the non glandular gastric mucosa in an F1 5000 ppm male. There was also increased incidence of basophilic renal tubules at 5000 ppm in the F0 males.

Hyperkeratosis was seen also in the 90-day subchronic study and the two year chronic study with rats. This is judged due to the irritant action of folpet.

Males treated with 5000 ppm folpet showed an increase in the number of foci of basophilic tubules in the kidney. F1 animals were generally unaffected, but may not have developed this lesion due to their age difference at sacrifice.

Basophilic tubules in the kidney, F0 males

Dose:	0	250	1500	5000 ppm
slight, focal	3/25 (12%)	3/25 (12%)	2/25 (8%)	7/25 (28%)
moderate, focal	0/25	0/25	0/25	2/25 (8%)

No Effects:
Mating performance and reproductive success.

The no effect levels for reproductive toxicity are based on the collective data for folpet.This analysis shows the appropriate NOEL for both adults and pups is 800 ppm. The NOEL for reproductive toxicity is 5000 ppm, the highest dose tested in the Rubin (1986) study.

A summary of these NOELs and LOELs follow.

Weight of the Evidence Effect Levels

Study	**Rubin (1986)**	**Richter (1985)**	**Cox (1985)[1]**
Dose	0, 250, 1500, 5000	0, 200, 800, 3600	0, 200, 800, 3200
LOEL adult ↓ wt gain	1500 ppm	3600 ppm	3200 ppm (slight)
NOEL adults ↓ wt gain	250 ppm	800 ppm	800 ppm
LOEL adult Hyperkeratosis	1500 ppm	not evaluated	3200 ppm
LOEL pup ↓ wt gain	1500 ppm	3600 ppm	not evaluated
NOEL Reproductive	5000 ppm (HDT)	3600 ppm (HDT)	not evaluated

[1] Cox (1985) is a chronic study from which interim one-year results are considered for comparison.

Response

- **Report: Rubin, 1985, Folpan Teratology studying the rabbit (MAK/051/FOL, R-3684**).

0, 10, 40, 160 mg/kg bw/day, GD 7-19, 14 dams/group, HY/CR NZW rabbits.

The NOEL for maternal toxicity and developmental effects is 10 mg/kg bw/day.

The NOAEL for developmental effects is 40 mg/kg bw/day, based on the high incidence of 13th extra ribs in rabbits and the doubtful biological significance of this finding.

Dams treated with 160 mg/kg bw/day showed marked maternal toxicity as evidenced by decreased food intake and reduction in bodyweight gain. There was some reduction in bodyweight gain at 40 mg/kg bw/day, but this was not statistically significant (but judged treatment related). Some clinical signs, suggesting maternal toxicity, were also evident at the high dose.

Fetuses showed increased variations and delays in maturation (ossification) at 160 mg/kg bw/day and to a slight extent at 40 mg/kg bw/day.

The data and incidence or percent control values follow.

Maternal toxicity, clinical signs

Soft feces and yellow or orange discoloration of the urine.

0	10	40	160 mg/kg bw/day
0/14	1/14	2/14	5/14[a]

No or few feces

0	10	40	160 mg/kg bw/day
0/14	0/14	0/14	9/14[c]

White mucous excrement

0	10	40	160 mg/kg bw/day
1/14	0/14	2/14	3/14

Yellow/orange urine

0	10	40	160 mg/kg bw/day
0/14	0/14	0/14	3/14

Maternal toxicity, food intake, g/animal/day

Period	0	10	40	160 mg/kg bw/day
0-4	223	231	232	241
5-6	239	255	248	247
7-10	223	235	206	103[c]
11-14	207	212	204	87[c]
15-19	213	231	213	110[c]
20-23	197	211	215	200
24-26	166	181	201	207[a]
27-29	167	170	173	217[a]

Maternal toxicity, food intake, percent of control

Period	0	10	40	160 mg/kg bw/day
0-4	223	>100%	104%	108%
5-6	239	>100%	104%	103%
7-10	223	>100%	92%	46%[c]
11-14	207	>100%	99%	42%[c]
15-19	213	>100%	100%	52%[c]
20-23	197	>100%	109%	101%
24-26	166	>100%	121%	125%[a]
27-29	167	>100%	104%	130%[a]

Maternal toxicity, body weight, kg, group means (select data from Table 3, page C-3).

Day	0	10	40	160 mg/kg bw/day
0	3.4	3.4	3.4	3.5
3	3.5	3.6	3.5	3.6
7	3.6	3.7	3.7	3.7
10	3.6	3.7	3.6	3.5
13	3.7	3.7	3.7	3.5
16	3.7	3.8	3.8	3.6
19	3.8	3.9	3.8	3.6
29	4.0	4.0	4.0	3.9

Maternal toxicity, body weight, percent of control.

Day	0	10	40	160 mg/kg bw/day
0	3.4			>100%
3	3.5			>100%
7	3.6			>100%
10	3.6			97%
13	3.7			95%
16	3.7			97%
19	3.8			95%
29	4.0			98%

Maternal toxicity, body weight change, kg, group means (rounded)

Day	0	10	40	160 mg/kg bw/day
7-19	0.17	0.19	0.17	-0.09[c]
0-29	0.63	0.65	0.61	0.37[b]
7-29	0.43	0.40	0.36	0.17[c]

There is some weight gain depression at 40 mg/kg bw/day in addition to the high dose.

Post-implantation loss

0	10	40	160 mg/kg bw/day
14.4%	10.0%[b]	8.1%[c]	21.8%[c]

Small fetus (less than 30g),

0	10	40	160 mg/kg bw/day
3%	0%	2%	19%[c] percent of fetuses (rounded)
3%	0%	2%	15%[c] percent of litters (rounded)

Skeletal observations (percent of affected fetuses)

	0	10	40	160 mg/kg bw/day
Ossification[1]	0	1	2%	6% (rounded)
13th rib[2]	52	48	59%	84% (rounded)
Ossification[3]	1	1	7[a]%	10%[b]
Ossification[4]	21	16	25	43%[b]

[1]: Fewer than 16 caudal vertebral centra ossified
[2]: 13th (lumbar) rib present bilaterally
[3]: reduced/irregular ossification among sternebrae 1-4
[4]: Reduced ossification of long bone epiphyses

Evaluation table number	Reporting table number	Open Point number
-	**2(8)**	**2.6**

Conclusions of the EFSA Evaluation Meeting:
RMS to provide more detailed summary of studies leading to the derivation of the ADI value to be discussed at an expert meeting.

-	**2(22)**	**2.11**

Conclusions of the EFSA Evaluation Meeting:
MS to confirm the NOAELs in the long term studies at an expert meeting.

- **Point IIA, 5.5: Long term toxicity and carcinogenicity**

The following new summaries are presented in response to requests from the EFSA in relation to comments 2(4), 2(5), 2(6), 2(8), and to Open Points 2.4, 2.5 and 2.6.

In addition, the notifier's response to comments by the UK Member State on the conclusions from the 2-year rat study (Crown 1989) are given below.

Vol. 3, B.6 General comment

The European Food Safety Authority (EFSA) notes the results in the studies are sometimes poorly described. There is a lack of informative tables and/or the effect as percent of control and if it as NOEL or a NOAEL value. [is] The concentrations of the compound is often presented in ppm without demonstrating the corresponding value in mg/kg bw/day. Furthermore, the conclusions are very brief and in some cases even lacking. The provision of an addendum where more information is provided, for instance for the studies being considered as crucial for setting of ADI, AOEL, and ARfD, would be appreciated in order to increase understanding and transparency.
Proposed studies are:
B.6.3. one year dog study (Daly 1986) – see below
B.6.5 2-year rats study (Crown, 1989) - see below
B.6.6 2-generation reproduction, rat (Rubin, 1986) – see additional information under Point 5.6 above
B.6.6. Teratogenicity study, rabbit, Rubin 1985c) - see additional information under Point 5.6 above.

Response

- **Report: Daly, 1986, One year Dog study**

0, 10, 60, 140 mg/kg bw/day by capsule to 36 beagle dogs, 6/sex/group

Group IV, 140 mg/kg bw/day was changed to 120 mg/kg bw/day on Day 50 due to poor food intake and markedly depressed body weight gain among the high dose males. Controls received empty gelatin capsules.

No treatment-related effects:

Mortality
Physical observations
Macroscopic lesions
Microscopic lesions
Hematology
Urinalysis
Ophthalmology

Treatment-related effects:

Body weight

Dose	Mean bw gain (Kg), Males page 15 of report (over 52 week period)	
0	4.2	(control)
10	3.5	83% of control
60	2.1	50% of control
120	1.6	38% of control

Dose	Mean bw gain (Kg), Females page 16 of report (over 52 week period)	
0	2.6	(control)
10	2.4	92% of control
60	1.8	69% of control
120	1.5	58% of control

Food intake

There was a transient decrease in food intake in males at 60 and 160/120 mg/kg bw/day for the first three months of the study. Data were comparable to controls for the remainder of the study.

There was a transient decrease in food intake in females at 60 and 160 mg/kg bw/day for the first month of the study. Data were generally lower than controls for the remainder of the study but the variable nature of the data did not support a treatment relationship.

Cholesterol levels

Cholesterol, Month 12, Male			Female (pages 23, 24 of report)	
0	168	(control) mg/l	188	
10	155	92% of control	213	242% of control
60	142	85% of control	192	102% of control
120	154	92% of control	139	90% of control

Total Protein levels

Total Protein, Month 12, Male			Female	
0	6.7	(control) g/l	6.1	
10	6.5	97% of control	6.3	103% of control
60	5.7*	85% of control	5.9	97% of control
120	5.7*	85% of control	5.1**	84% of control

Albumin levels

Albumin, Month 12, Male			Female	
0	3.6	(control) g/l	3.5	
10	3.4	94% of control	3.4	97% of control
60	3.2*	88% of control	3.3	94% of control
120	3.2*	88% of control	3.2	91% of control

Globulin levels

Globulin, Month 12, Male			Female	
0	3.2	(control) g/l	2.6	
10	3.2	100% of control2.9	116% of control	
60	2.5	78% of control	2.6	100% of control
120	2.5*	78% of control	2.9	73% of control

* = $p<0.05$

Conclusion

1. Folpet is not carcinogenic in the dog when administered orally by capsule for one year at doses up to 120 mg/kg bw/day (initially 160 mg/kg bw/day to Day 50).

2. Folpet does not induce histologic changes in the dog when administered orally by capsule for one year at doses up to 120 mg/kg bw/day (initially 160 mg/kg bw/day to Day 50). In particular, irritation and/or hyperkeratosis of the esophagus, stomach or intestinal tract were absent.

3. Folpet causes an initial decrease in food intake in the dog when administered orally by capsule for one year at doses up to 120 mg/kg bw/day (initially 160 mg/kg bw/day to Day 50). This reduction is greater in males than females and is transient. By three months, male food intake is similar to controls; by one month, female food intake is similar to controls.

4. Folpet causes a reduction in body weight gain in the dog when administered orally by capsule for one year at doses up to 120 mg/kg bw/day (initially 160 mg/kg bw/day to Day 50). The effect is greater in males than females, with a clear dose-relationship in males and a decrease in the mid and high dose in females that are generally similar to one another. The low dose in females does not result in a decrease in body weight gain. While the low dose in males generally shows a body weight gain less than controls, at the conclusion of the study, the control and low dose are similar (mean body weight of low dose actually is greater than control mean body weight) and the study director noted this level, 10 mg/kg bw/day as a NOEL.

5. While mortality, physical observations, hematology, urinalysis, and ophthalmology in treated dogs were similar to control animals, there were changes in clinical chemistry parameters noted. These were cholesterol, total protein, albumin and globulin. The absolute values of these parameters generally decreased with dose level.

Cholesterol was lower at 160/120 mg/kg bw/day (-8% in males, -10% in females), however there was not a clear dose relationship in males and only the high dose in females appeared to be affected. These decreases were not statistically significant ($p>0.05$). The treatment relationship is judged tenuous at best.

Total protein decreased in males and females and this decrease was statistically significant (taking month 12 data) for mid and high dose in males and high dose in females (-15%, males; -9%, females).

Albumin was decreased in males at the mid and high dose (-12% for both groups), referencing month 12 data, but was not decreased in females in a significant manner (-6%, mid dose; -9%, high dose, $p>0.05$).

Globulin was decreased in high dose males (-22%, $p<0.05$), referencing month 12 data, but while the absolute value was decreased in high dose females it was not significant (-27%, $p>0.05$).

It is judged that cholesterol is not affected by treatment but that protein synthesis is. This affect is greater in males than females and may be reflective of the decreased bodyweight gain. The effect is considered secondary to local disruption of the gastrointestinal tract processes as folpet is not carried to the liver, in tact, due to its rapid degradation in blood (half-life of 4.9 seconds, measured in human blood).

- **Report: Crown et al., 1989 Two year Rat study**

Experimental design

0, 250, 1500, 5000 ppm by dietary admixture. Twenty animals per sex per group. Average concentrations, measured analytically and calculated by regression analysis were 190, 1,288 and 4,532 ppm.

No treatment-related effects included:

Mortality
Clinical signs including palpable masses
Organ weights
Hematology
Macroscopic lesions
Hematology
Ophthalmology

Treatment-related effects included:

Body weight, food intake and water intake were depressed in the high dose group.
At 5000 ppm male body weight and food intake was depressed 9-10% and female bodyweight was depressed 6% for most of the treatment period. This reduction was greater in the early part of the study. Water consumption was depressed in a similar way as food intake. Female reduction in water intake was greater than males and was approximately 20% compared to controls.

Microscopic lesions
There were no treatment related tumors noted.
Non-neoplastic lesions consisted of diffuse hyperkeratosis of the esophageal and gastric squamous epithelium, noted in rats administered 1500 and 5000 ppm (nominal doses). The gastric hyperkeratosis was sporadically associated with slight diffuse epithelial hyperplasia.

Clinical chemistry (data and percent control values are noted below)
Alkaline Phosphatase: Effect in males is greater than females; there is a general decrease throughout the study.

Alanine Aminotransferase: The effect in females is greater than males and is decreased at 5000 ppm. The effect in males is judged equivocal.

Aspartate aminotransferase: The effect on aspartate aminotransferase is judged equivocal to treatment.

Creatinine Phosphokinase: The effect on creatinine phosphokinase is judge equivocal to treatment.

Gamma-glutamyl transferase: It is judged that there is no treatment effect.

Cholesterol: was depressed in both males and females treated with 5000 ppm folpet. The effect was generally consistent throughout the study.

Total plasma protein: was reduced in male high dose group during the first year of treatment

Phosphate: was increased in the male high dose group at most time points.

Urea: was increased in the high dose group, females, at time points up to 18 months

Most males excreted a more concentrated urine (smaller volume) at Month 3 and Month 6.

Of the routine liver function tests, only serum albumin, bilirubin and prothrombin time provide useful information on how well the liver is functioning. The common markers of hepatocyte injury are:
Aspartate aminotransferase (AST, formerly SGOT)
Alanine aminotransferase (ALT, formerly SGPT)
ALT is cytosolic; AST is both cytosolic and mitochondrial. These markers are also found in skeletal muscle (AST and ALT were once used as markers of myocardial infarction)./

Albumin level in serum can serve a an index of liver synthetic capacity, but has a number of confounding factors that affect interpretation. It has a plasma half-life of three weeks, therefore serum albumin concentrations change slowly in response to alterations in synthesis; the liver can synthesize albumin at twice the healthy basal rate and thus partially compensate for decreased synthetic capacity or increased albumin losses; and, since 2/3 of the amount of body albumin is located in the extravascular, extracellular space, changes in distribution can alter the serum concentration.

Alkaline phosphatase has two main functions: removes 5’ phosphates from plasmid and bacteriophage vectors and removes 5’ phosphates from fragments of DNA prior to labeling with radioactive phosphate

Data

Week		0	250	1500	5000
0	M	88	88	87	87
	F	81	80	81	81
13	M	292	284	271	246[c] (84%)*
	F	177	173	177	166[b] (94%)
52	M	381	391	375	341[c] (90%
	F	226	229	225	211[c] (93%)
78	M	373	374	365	333[c] (89%)
	F	244	245	246	226[b] (93%)
104	M	336	339	337	312[a] (93%)
	F	248	239	251	227[b] (92%)

*percent control value

MONTH THREE

Dose	Sex	ALPH	ALT	AST	GGTP	CPK	CHOL
0	M	144	58	86	0.4	328	2.22
250	M	141	52	97	2.5	556[b]	2.04
1500	M	143	54	111[c]	1.4	478[a]	2.08
5000	M	116[c]	51	91	2.9	363	2.02[a]
0	F	103	57	113	2.2	490	2.65
250	F	96	46[a]	102	3.22	428	2.47
1500	F	85[c]	46[c]	107	4.2	490	2.47
5000	F	76[c]	34[c]	88[b]	4.0	345[a]	2.11[c]

a: $p<0.05$; b: $p<0.01$; c: $p<0.001$
ALPH: Alkaline phosphatase, IU/l
ALT: Alanine aminotransferase, IU/l
AST: Aspartate aminotransferase, IU/l
CPK: Creatinine Phosphokinase, IU/l
GGTP: Gamma-glutamyl transferase, IU/l
CHOL: Cholesterol, mmol/l

Alkaline phosphatase decreased at the high dose in males and in the mid and high dose in females.
Alanine aminotransferase was unaffected in males and decreased in females, the decrease was dose related.

Aspartate aminotransferase was not affected in males and decreased in the high dose in females.

Gamma-glutamyl transferase was not affected

Creatinine phosphokinase was increased, but not in a dose related manner in males and decreased in the high dose in females.

Cholesterol was decreased in the high dose of both males and females.

MONTH THREE, continued

Dose	Sex	Total protein	Albumin	Globulin
0	M	69.4	36.9	32.5
250	M	67.9	36.9	32.0
1500	M	66.7[b]	36.1	30.6[a]
5000	M	62.7[c]	34.5[c]	28.2[c]
0	F	58.1	35.6	22.5
250	F	58.8	34.8	23.9
1500	F	57.9	34.9	23.0
5000	F	56.2	33.4[c]	22.8

Data in g/l

Total protein decreased in males, mid and high dose, and was not affected in females.
Albumin was decreased in the high dose in males and females
Globulin was decreased in the mid and high dose in males but not affected in females.

MONTH SIX

Dose	Sex	ALPH	ALT	AST	GGTP	CPK	CHOL
0	M	102	113	184	2.79	277	1.36
250	M	96	89	129	2.65	93[c]	1.46
1500	M	89[a]	108	212	3.25	273	1.56
5000	M	65[c]	46[a]	82[a]	1.44	137[c]	1.09[a]
0	F	80	54	78	8.44	172	1.93
250	F	73	61	85	8.08	167	1.73
1500	F	78	52	82	15.5	115[b]	2.01
5000	F	49[b]	38[a]	69	3.89	121[a]	1.27[a]

a: $p<0.05$; b: $p<0.01$; c: $p<0.001$
ALPH: Alkaline phosphatase, IU/l
ALT: Alanine aminotransferase, IU/l
AST: Aspartate aminotransferase, IU/l
CPK: Creatinine Phosphokinase, IU/l
GGTP: Gamma-glutamyl transferase, IU/l
CHOL: Cholesterol, mmol/l

At 3 months alkaline phosphatase decreased at the high dose in males and in the mid and high dose in females.
At 6 months, depression continued: mid and high dose in males and high dose in females.

At 3 months alanine aminotransferase was unaffected in males and decreased in females, the decrease was dose related.
At 6 months males are now affected, mid and high dose, and only the high dose in females was decreased.

At 3 months aspartate aminotransferase was not affected in males and decreased in the high dose in females.
At 6 months was now decreased in the high dose in males but not affected in females

At 3 months gamma-glutamyl transferase was not affected
At 6 months, GGTP continues not to be affected

At 3 months creatinine phosphokinase was increased, but not in a dose related manner in males and decreased in the high dose in females.
At 6 months, CPK appeared decreased at the high dose but the large decrease in the low dose, males, confounds interpretation.

At 3 months cholesterol was decreased in the high dose of both males and females.
At 6 months, cholesterol was again decreased in both males and females at the high dose.

MONTH SIX, continued

Dose	Sex	Total protein	Albumin	Globulin
0	M	80.8	35.7	45.1
250	M	80.3	36.0	44.2
1500	M	77.3[c]	35.1	42.1
5000	M	74.0[c]	34.4	39.6[b]
0	F	58.1	35.6	22.5
250	F	58.8	34.8	23.9
1500	F	57.9	34.9	23.0
5000	F	56.2	33.4[c]	22.8

Data in g/l

At 3 months total protein decreased in males , mid and high dose, and was not affected in females.
At 6 months total protein decreased in males, mid and high dose but, again was not affected in females.

At 3 months albumin was decreased in the high dose in males and females
At 6 months albumin was not decreased in males and again decreased in high dose females.

At 3 months globulin was decreased in the mid and high dose in males but not affected in females.
At 6 months globulin was decreased only in the high dose males and again was not affected in females.

MONTH TWELVE

Dose	Sex	ALPH	ALT	AST	GGTP	CPK	CHOL
0	M	260	68	96	5.15	261	2.31
250	M	265	73	106	5.7	172	2.19
1500	M	218[a]	70	112	4.14	232	2.08
5000	M	165[c]	52[a]	115	2.41[c]	284	1.59[c]
0	F	159	57	78	5.79	129	2.68
250	F	145	64	80	4.5	93[a]	2.32[a]
1500	F	133	60	75	4.86	89[a]	2.41
5000	F	105[c]	35[a]	58	4.33	82[b]	1.86[c]

a: $p<0.05$; b: $p<0.01$; c: $p<0.001$
ALPH: Alkaline phosphatase, IU/l
ALT: Alanine aminotransferase, IU/l
AST: Aspartate aminotransferase, IU/l
CPK: Creatinine Phosphokinase, IU/l
GGTP: Gamma-glutamyl transferase, IU/l
CHOL: Cholesterol, mmol/l

At 3 months ALPH decreased at the high dose in males and in the mid and high dose in females.
At 6 months ALPH depression continued: mid and high dose in males and high dose in females.
At 12 months ALPH depression continue at the mid and high dose in males and high dose in females.

At 3 months alanine aminotransferase was unaffected in males and decreased in females, the decrease was dose related.
At 6 months males are now affected, mid and high dose, and only the high dose in females was decreased.
At 12 months only the high dose of both sexes were affected (decreased).

At 3 months aspartate aminotransferase was not affected in males and decreased in the high dose in females.
At 6 months was now decreased in the high dose in males but not affected in females
At 12 months neither males nor females were affected.

At 3 months gamma-glutamyl transferase was not affected
At 6 months, GGTP continues not to be affected
At 12 months the high dose males were decreased (first indication of effect)

At 3 months creatinine phosphokinase was increased, but not in a dose related manner in males and decreased in the high dose in females.
At 6 months, CPK appeared decreased at the high dose but the large decrease in the low dose, males, confounds interpretation.
At 12 months females, only, were decreased in a dose-related manner.
At 3 months cholesterol was decreased in the high dose of both males and females.
At 6 months, cholesterol was again decreased in both males and females at the high dose.
At 12 months cholesterol was again decreased in both males and females at the high dose.

MONTH TWELVE, continued

Dose	Sex	Total protein	Albumin	Globulin
0	M	80.1	35.4	44.6
250	M	80.1	34.5	45.5
1500	M	78.1	34.8	43.3
5000	M	75.8[c]	33.9[a]	41.9[b]
0	F	83.3	41.4	42.0
250	F	82.6	41.3	41.3
1500	F	81.6	40.7	40.9
5000	F	80.7	40.9	39.9[a]

Data in g/l

At 3 months total protein decreased in males , mid and high dose, and was not affected in females.
At 6 months total protein decreased in males, mid and high dose and, again, was not affected in females.
At 12 months total protein decreased in males at the high dose only and, again, was not affected in females.

At 3 months albumin was decreased in the high dose in males and females
At 6 months albumin was not decreased in males and again decreased in high dose females.
At 12 months albumin was decreased in high dose males but not high dose females.

At 3 months globulin was decreased in the mid and high dose in males but not affected in females.
At 6 months globulin was decreased only in the high dose males and again was not affected in females.
At 12 months globulin was now decreased in both males and females of the high dose

MONTH EIGHTEEN

Dose	Sex	ALPH	ALT	AST	GGTP	CPK	CHOL
0	M	226	47	58	2.98	131	2.31
250	M	206	42	56	2.97	98	2.34
1500	M	190[a]	41	69	3.13	152	2.31
5000	M	172[c]	39	65	3.14	128	1.72[c]
0	F	161	53	68	4.05	147	2.75
250	F	160	46	53	3.04	143	2.75
1500	F	153	48	63	4.15	167	2.75
5000	F	126	31[c]	58	2.96	96	1.93[a]

a: $p<0.05$; b: $p<0.01$; c: $p<0.001$
ALPH: Alkaline phosphatase, IU/l
ALT: Alanine aminotransferase, IU/l
AST: Aspartate aminotransferase, IU/l
CPK: Creatinine Phosphokinase, IU/l
GGTP: Gamma-glutamyl transferase, IU/l
CHOL: Cholesterol, mmol/l

At 3 months ALPH decreased at the high dose in males and in the mid and high dose in females.
At 6 months ALPH depression continued: mid and high dose in males and high dose in females.
At 12 months ALPH depression continue at the mid and high dose in males and high dose in females.
At 18 months ALPH depression continues in the mid and high dose males but is absent in females.

At 3 months alanine aminotransferase was unaffected in males and decreased in females, the decrease was dose related.
At 6 months males are now affected, mid and high dose, and only the high dose in females was decreased.
At 12 months only the high dose of both sexes were affected (decreased).
At 18 months only the high dose females are affected (decreased).

At 3 months aspartate aminotransferase was not affected in males and decreased in the high dose in females.
At 6 months was now decreased in the high dose in males but not affected in females
At 12 months neither males nor females were affected.
At 18 months neither males nor females were affected.

At 3 months gamma-glutamyl transferase was not affected
At 6 months, GGTP continues not to be affected
At 12 months the high dose males were decreased (first indication of effect)
At 18 months neither males nor females were affected.

At 3 months creatinine phosphokinase was increased, but not in a dose related manner in males and decreased in the high dose in females.
At 6 months, CPK appeared decreased at the high dose but the large decrease in the low dose confounds interpretation.
At 18 months neither males nor females were affected.

At 3 months cholesterol was decreased in the high dose of both males and females.
At 6 months, cholesterol was again decreased in both males and females at the high dose.

At 12 months cholesterol was again decreased in both males and females at the high dose.

MONTH EIGHTEEN, continued

Dose	Sex	Total protein	Albumin	Globulin
0	M	71.0	33.5	37.6
250	M	72.5	32.7	39.8[a]
1500	M	70.7	33.0	37.7
5000	M	69.4	33.7	35.7[a]
0	F	74.3	38.9	35.4
250	F	75.9	37.1	38.9
1500	F	75.7	39.4	36.3
5000	F	69.4	36.0	33.4

Data in g/l

At 3 months total protein decreased in males, mid and high dose, and was not affected in females.
At 6 months total protein decreased in males, mid and high dose and, again, was not affected in females.
At 12 months total protein decreased in males at the high dose only and, again, was not affected in females.
At 18 months total protein was not affected

At 3 months albumin was decreased in the high dose in males and females
At 6 months albumin was not decreased in males and again decreased in high dose females.
At 12 months albumin was decreased in high dose males but not high dose females.
At 18 months albumin was not affected.

At 3 months globulin was decreased in the mid and high dose in males but not affected in females.
At 6 months globulin was decreased only in the high dose males and again was not affected in females.
At 12 months globulin was now decreased in both males and females of the high dose
At 18 months globulin was decreased in the high dose males only.

MONTH TWENTY-FOUR

Dose	Sex	ALPH	ALT	AST	GGTP	CPK	CHOL
0	M	248	42	55	7.03	144	3.21
250	M	199	48	62	3.59	162	3.13
1500	M	198	39	61	7.45	105	2.91
5000	M	209	38	58	6.19	122	1.81[b]
0	F	214	53	61	4.84	123	3.19
250	F	173	43	48	3.15[a]	94	3.03
1500	F	172	48	66	2.41[b]	209[a]	2.97
5000	F	120[b]	28[b]	45	2.72[b]	110	1.92[b]

a: $p<0.05$; b: $p<0.01$; c: $p<0.001$
ALPH: Alkaline phosphatase, IU/l
ALT: Alanine aminotransferase, IU/l
AST: Aspartate aminotransferase, IU/l
CPK: Creatinine Phosphokinase, IU/l
GGTP: Gamma-glutamyl transferase, IU/l
CHOL: Cholesterol, mmol/l

At 3 months ALPH decreased at the high dose in males and in the mid and high dose in females.

At 6 months ALPH depression continued: mid and high dose in males and high dose in females.
At 12 months ALPH depression continue at the mid and high dose in males and high dose in females.
At 18 months ALPH depression continues in the mid and high dose males but is absent in females.
At 24 months ALPH depression is present in the high dose females only.

The percent control values, for instances that were statistically significant, $p<0.05$, $p<0.1$, or $p<0.01$, for alkaline phosphatase over the study are:

ALPH M	3	6	12	18	24
1500	-	87%	84%	84%	-
5000	81%	64%	63%	76%	-

ALPH F	3	6	12	18	24
1500	83%	-	-	-	-
5000	74%	61%	66%	-	56%

Males appear more sensitive than females, based on three time periods where the mid dose was depressed versus only on period in females where the mid dose was depressed. The effect seems to lessen with age, in that males are normal at 24 months and females are not depressed at 18 months but are at 24 months..

At 3 months alanine aminotransferase was unaffected in males and decreased in females, the decrease was dose related.
At 6 months males are now affected, mid and high dose, and only the high dose in females was decreased.
At 12 months only the high dose of both sexes were affected (decreased).
At 18 months only the high dose females are affected (decreased).
At 24 months only the high dose females are affected (decreased).

The percent control values, for instances that were statistically significant, $p<0.05$, $p<0.1$, or $p<0.01$, for alanine aminotransferase over the study are:

ALT M	3	6	12	18	24
1500	-	-	76%	-	-
5000	-	41%	63%	-	-

ALT F	3	6	12	18	24
250	81%	-			
1500	81%	-	-	-	-
5000	60%	70%	61%	58%	53%

Females appear more sensitive than males in that rats administered 5000 ppm have decreases at all time points whereas males are decreased only at month 6 and 12.

At 3 months aspartate aminotransferase was not affected in males and decreased in the high dose in females.
At 6 months was now decreased in the high dose in males but not affected in females
At 12 months neither males nor females were affected.
At 18 months neither males nor females were affected.

At 24 months neither males nor females were affected.

The percent control values, for instances that were statistically significant, $p<0.05$, $p<0.1$, or $p<0.01$, for aspartate aminotransferase over the study are:

AST	M	3	6	12	18	24
1500		-	-	-	-	-
5000		-	45%	-	-	-

AST	F	3	6	12	18	24
1500		-	-	-	-	-
5000		78%	-	-	-	-

There is only one instance in each sex in which aspartate aminotransferase was significantly decreased compared to controls. The effect of treatment is judged equivocal.

At 3 months gamma-glutamyl transferase was not affected
At 6 months, GGTP continues not to be affected
At 12 months the high dose males were decreased (first indication of effect)
At 18 months neither males nor females were affected.
At 24 months there was no effect in males but dose related decrease in females.

The percent control values, for instances that were statistically significant, $p<0.05$, $p<0.1$, or $p<0.01$, for aspartate aminotransferase over the study are:

GGTP	M	3	6	12	18	24
1500		-	-	-	-	-
5000		-	-	47%	-	-

GGTP	F	3	6	12	18	24
250		-	-	-	-	65%
1500		-	-	-	-	50%
5000		-	-	-	-	56%

These data do not show a consistent treatment effect. The 24-month decrease in females is unusual in that females at no other time point showed an effect.

At 3 months creatinine phosphokinase was increased, but not in a dose related manner in males and decreased in the high dose in females.
At 6 months, CPK appeared decreased at the high dose but the large decrease in the low dose confounds interpretation.
At 18 months neither males nor females were affected.
At 24 months neither the males nor females were

The percent control values, for instances that were statistically significant, $p<0.05$, $p<0.1$, or $p<0.01$, for creatinine phosphokinase over the study are:

CPK M	3	6	12	18	24
250	-	34%	-	-	-
1500	-	-	-	-	-
5000	-	49%	-	-	-

CPK F	3	6	12	18	24
250	-	-	72%	-	-
1500	-	67%	69%	-	170%
5000	70%	70%	64%	-	-

In males there is no consistent treatment effect; in females it appears that a transient treatment effect occurred between months 3 and 12. Overall the effect of treatment is equivocal.

At 3 months cholesterol was decreased in the high dose of both males and females.
At 6 months, cholesterol was again decreased in both males and females at the high dose.
At 12 months cholesterol was again decreased in both males and females at the high dose.

The percent control values, for instances that were statistically significant, $p<0.05$, $p<0.1$, or $p<0.01$, for cholesterol over the study are:

CHOL M	3	6	12	18	24
250	-	-	-	-	-
1500	-	-	-	-	-
5000	91%	80%	69%	74%	56%

CHOL F	3	6	12	18	24
250	-	-	-	-	-
1500	-	-	-	-	-
5000	80%	66%	69%	70%	60%

There is a treatment effect at 5000 ppm for both males and females. There is no consistent change in severity of effect with time.

MONTH TWENTY-FOUR, continued

Dose	Sex	Total protein	Albumin	Globulin
0	M	69.6	32.5	37.1
250	M	71.3	32.5	38.7
1500	M	69.9	31.6	38.2
5000	M	69.9	34.0	37.1
0	F	69.9	35.8	34.0
250	F	70.5	34.8	35.7
1500	F	71.1	36.6	34.5
5000	F	69.5	36.9	32.6

Data in g/l

At 3 months total protein decreased in males, mid and high dose, and was not affected in females.
At 6 months total protein decreased in males, mid and high dose and, again, was not affected in females.
At 12 months total protein decreased in males at the high dose only and, again, was not affected in females.
At 18 months total protein was not affected.
At 24 months total protein was not affected.

The percent control values, for instances that were statistically significant, $p<0.05$, $p<0.1$, or $p<0.01$, for total protein over the study are:

Protein M	3	6	12	18	24
250	-	-	-	-	-
1500	97%	95%	-	-	-
5000	90%	91%	95%	-	-

Protein F	3	6	12	18	24
250	-	-	-	-	-
1500	-	-	-	-	-
5000	-	-	-	-	-

There is a transient treatment related decrease in protein in males from month 3 – 12. Females are not affected.

At 3 months albumin was decreased in the high dose in males and females
At 6 months albumin was not decreased in males and again decreased in high dose females.
At 12 months albumin was decreased in high dose males but not high dose females.
At 18 months albumin was not affected.
At 24 months albumin was not affected.

The percent control values, for instances that were statistically significant, $p<0.05$, $p<0.1$, or $p<0.01$, for albumin over the study are:

AlbuminM	3	6	12	18	24
250	-	-	-	-	-
1500	-	-	-	-	-
5000	95%	-	97%	-	-

AlbuminF	3	6	12	18	24
250	-	-	-	-	-
1500	-	-	-	-	-
5000	92%	97%	-	-	-

There is an equivocal effect on albumin in both males and females. The fact that this decrease does not mirror the pattern in total protein well casts further doubt on the treatment relationship.

At 3 months globulin was decreased in the mid and high dose in males but not affected in females.
At 6 months globulin was decreased only in the high dose males and again was not affected in females.
At 12 months globulin was now decreased in both males and females of the high dose
At 18 months globulin was decreased in the high dose males only.
At 24 months globulin was not effected.

The percent control values, for instances that were statistically significant, $p<0.05$, $p<0.1$, or $p<0.01$, for globulin over the study are:

Globulin M	3	6	12	18	24
250	-	-	-	-	-
1500	94%	-	-	-	-
5000	85%	88%	93%	95%	-

Globulin F	3	6	12	18	24
250	-	-	-	-	-
1500	-	-	-	-	-
5000	-	-	95%	-	-

There is a consistent decrease in globulin in males treated with 5000ppm folpet, except for the 24-month time period. Females are judged not affected.

The percent control values, for instances that were statistically significant, $p<0.05$, $p<0.1$, or $p<0.01$, for Phosphate,(mmol/l) over the study are:

Phosphate M	3	6	12	18	24
250	-	-	-	-	-
1500	-	-	-	-	113%
5000	105%	107%	117%	103%	117%

Phosphate F	3	6	12	18	24
250	-	-	-	-	-
1500	-	-	-	-	-
5000	-	-	-	-	-

There is a increased amount of phosphate in males due to treatment.

Histology of the liver

Basophilic cell type focal or diffuse areas of cellular alteration were noted, consistent with findings of rats as they age. These were judged not precursors to tumors.

Hepatic nodular neoformation (hepatodiaphragmatic nodule) increased with dose but were judged incidental to treatment. It is considered a developmental malformation in the Fischer rat.

Slight subchronic hepatitis was noted, particularly in males at 5000 ppm (four of the five cases seen). The cause of this sporadic inflammatory lesion is not known.

Conclusion

Treatment with folpet at doses up to 5000 ppm for two years resulted in decreases in weight gain and some effects on clinical chemistry parameters. The most consistent finding was a decrease in cholesterol in the high dose animals. The effects on other clinical chemistry parameters often varied between measurement points, but it was judged that folpet caused a decrease in alkaline phosphatase (males>females), alanine aminotransferase (females>males) and a decrease in total protein in males (reflected by a decrease, also, in globulin at 5000 ppm, primarily in males). Phosphate was reduced to a small, but statistically significant, extent in males at 5000 ppm.

The NOAEL for this study was 250 ppm, based on weight gain depression at 1500 ppm and above as well as the incidence of hyperkeratosis in the esophagus and non-glandular portion of

the stomach. The authors judged this to be a NOEL, however, the overall weight gain depression in male rats suggests that NOAEL is more appropriate.

Response to questions from the UK Member State on the conclusions of this study

(1) United Kingdom (UK) notes the endpoint used to determine the NOAEL in the study of Crown (1989) is considered to be appropriate; however, the demonstrated decomposition of folpet in the diet should be taken into consideration. The NOAEL for this study is therefore calculated to be 190 ppm (equivalent to 12 an 16 mg/kg bw/day in males and females, respectively.

Response
We calculate the NOAEL 191 ppm, confirming the comment by the UK. The formula for determining the concentration of folpet in the diet is:

		Interpolated	
Nominal	Regression function	Day 4	Day 8
250 ppm	Y= 247 –14X	190	134 ppm

Since the diets were prepared weekly, a midpoint, 4 days, gives a decrease of 56 ppm, on average. Y therefore = 247 – (14*4) = 191 ppm.

Data:
0, 250, 1500, 5000 ppm
The decomposition appeared biphasic. There was a rapid decline of folpet in for days 1-8 then a slower degradation from days 8-18.
The following is noted in the report:

		Interpolated	
Nominal	Regression function	Day 4	Day 8
250 ppm	Y= 247 –14X	190	134 ppm
1500 ppm	Y= 1431 – 36X 1288	1144 ppm	
5000 ppm	Y= 5003 – 118X	4532	4062 ppm

where,
Y is the concentration in ppm
X is the day after preparation
Batches were prepared weekly

(2) The United Kingdom (UK) considers the NOAEL in the rat carcinogenicity study of Crown (1985) to be 500 ppm, based on hyperkeratosis of the forestomach epithelium at 1000 ppm.

Response
500 ppm appears to be the NOAEL.

Data:
0, 500, 1000, 2000 ppm with 60/sex/group for 104 weeks.
At 1000 and 2000 ppm, findings included hyperkeratosis of the esophagus and non-glandular keratin layers, ulcerations in the gastric non-glandular mucosa and foci or areas of cellular alteration (basophilic cell type) in the liver.

Evaluation table number	Reporting table number	Open Point number
2.3	**2(14)**	**-**

Conclusions of the EFSA Evaluation Meeting:
Notifier to submit the new toxicokinetic study Arndt and Dohn (2004).

- **Point IIA, 5.1: Studies on absorption, distribution, excretion and metabolism in animals**

The following report is submitted (previously submitted November 2004):

5.1/06

Report: Arndt, T. and Dohn, D. (2004). Measurement of the half-life of thiophosgene in human blood. PTRL West, Inc., unpublished report number 1146W (Company file: R-17121)

Guidelines: In-house.
Deviations: Not applicable.

GLP: Yes.

Material and methods: Test substance: thiophosgene, batch number 22123BO, purity 99.4%. Thiophosgene is an important degradate of folpet. Data indicate that thiophosgene is produced if folpet or its analogue captan is present in the blood. Thiophosgene itself reacts rapidly with blood nucleophiles, but the reaction is quenched with phosphoric acid in acetone. A method of detecting thiophosgene in the blood was developed. Thiophosgene reacts with cysteine to produce 2-thioxo-4-thiazolidinecarboxylic acid (TTCA), which is capable of detection with UV light (maximum absorbency at 271 nm) and quantification by HPLC at that wavelength. Human blood was collected under heparin from a single male volunteer, stored overnight at 10^{o}C and brought back to 37^{o}C before use. The experimental method involved adding 10 µL of 10 mg/mL thiophosgene to duplicate 1 mL samples of human whole blood at 37^{o}C, incubating for various times, quenching the reaction with chilled 1.5% phosphoric acid in acetone, and then adding cysteine buffer to produce TTCA, which was then quantified using HPLC. TTCA was shown to be produced in a linear relationship to the amount of thiophosgene added. Thiophosgene was added to blood and allowed to react for <3, 3, 7.5, 15 and 30 seconds (kinetic samples). Positive and negative control samples were also prepared. The negative controls were blood samples that were not fortified with thiophosgene. Positive controls were blood samples that were quenched and chilled (to prevent thiophosgene reaction with blood nucleophiles) before addition of thiophosgene, such that the thiophosgene was fully able to react with the cysteine without competition from the blood nucleophiles. Positive control samples were prepared with 10, 30 or 100 µL of 10 mg/mL thiophosgene added to the quenched, chilled blood.

Findings:

The positive controls showed an average recovery of 42% ± 8.6% SD (n = 6 from the three duplicate positive control samples at 10, 30 and 100 µL) calculated from the mass of thiophosgene added to quenched chilled blood samples and the mass of thiophosgene detected,

calculated as thiophosgene equivalents from a TTCA standard curve. The recovery was very consistent; all kinetics sample values were corrected for 42% recovery. An exponential decline equation of the form $y = a + b*exp^{(-kt)}$ was generated by plotting the reaction time (in seconds) versus normalised thiophosgene recovery data from <3 to 7.5 second timepoints. The half life was determined by inserting 50% as y and solving for t: where t = {Ln[50-a) ÷ -k.

No TTCA was detected in the negative control samples.

Thiophosgene rapidly disappeared from the whole blood samples. Samples from > 7.5 seconds showed a low, consistent, residual level of TTCA. This was considered to be the result of saturation of the active sites in the blood that react with thiophosgene. The sample recovery data were normalised for this residual level by subtracting the average corrected recovery from the 15 and 30 second samples from each of the corrected recovery kinetics samples. The resulting normalised % thiophosgene recovery data were fitted to the equation and a half-life of 0.6 seconds was derived.

Table 5.1-1: Kinetics sample summary

Kinetics Sample Identification	Actual Reaction Time (s)	Mass Thiophosgene Added (µg)	Mass Thiophosgene Detected (µg)	Corrected % Recovery[1]	Normalised % Recovery[2]
30 second rep.1	31.1	100	0.38	0.9	-0.2
30 second rep.2	30.7	100	0.46	1.1	0.1
15 second rep.1	16.3	100	0.40	1.0	0.0
15 second rep.2	15.8	100	0.50	1.2	0.2
7.5 second rep.1	7.3	100	0.54	1.2	0.2
7.5 second rep.2	7.3	100	0.51	1.2	0.2
3 second rep.1	4.4	100	0.74	1.7	0.7
3 second rep.2	3.7	100	2.55	6.2	5.2
<3 second rep.1	1.9	100	4.08	9.8	8.8
<3 second rep.2	2.4	100	5.58	13.3	12.3

[1] Corrected % Recovery = recovery based on TTCA standard curve divided by the average recovery value (42%) of positive control samples from initial blood test analysis

[2] Normalised % Recovery = Corrected % Recovery – average corrected recovery value (1.05%) of 15 and 30 second (nominal) samples.

Conclusions: Thiophosgene disappears rapidly when added in excess (100 µg/mL) to human whole blood *in vitro.* The half-life was calculated to be 0.6 seconds.

Evaluation table number	Reporting table number	Open Point number
-	**2(15)**	**2.7**

Conclusions of the EFSA Evaluation Meeting:

MS to discuss the irritating properties, also in relation to classification, at an expert meeting.

Vol. 3, B.6.2.3, Acute inhalation toxicity

United Kingdom (UK): Evidence of respiratory irritation was seen in this study (Cracknell, 1983); this finding is also consistent with the known mechanism of action of the breakdown product thiophosgene. Consideration should therefore be given to classification of folpet as R37: "irritating to respiratory system."

Response

The R37 risk phrase for folpet is not appropriate.
The active substance will be classified as Xn R20 Harmful by inhalation, based on deaths in an acute (4-hour) inhalation toxicity study. The Directive (67/548, as amended by 2001/59) is quite clear in defining the criteria for R37: there should be evidence that the substance or preparation can cause serious irritation to the respiratory system based on practical observations in humans, or positive results from appropriate animal tests. There are no recorded instances of inhalation irritation in humans, despite the active substance being manufactured and used in agriculture for several years. In further defining positive results from animal tests, the Directive cites as examples histopathological data from the respiratory system, and that data from the **measurement** of experimental bradypnea may also be used to assess airway irritation. In specifically defining measurement i.e. accurate quantification by experimental means, the Directive does not cite cage-side observations from acute studies (and therefore implies that cage-side observations, made in every acute inhalation study, are insufficient). There were no adverse findings in the lung histopathology from the long-term toxicity studies, in which the finely-ground test material was administered in a mixture with powdered diet, to indicate any irritant effects on the lungs, yet the fine nature of the dietary admixture inevitably results in some inadvertent inhalation of both diet and test material during feeding. It is important to recognise that there were also no irritance data from the buccal tissues in the chronic dietary studies. Secondly, during inhalation studies, irregular or slow respiration and gasping are standard responses to inhaling a harmful material: there were several deaths during and shortly following exposure.

Moreover, the International Programme on Chemical Safety does not list folpet as irritating to the respiratory tract. The mode of action (MOA) of folpet centers on the chemical reaction of these compounds with thiol groups on the surface of tissues (e.g., mucus membranes) that they contact. This MOA results in the transient irritation seen in Cracknell (1993). Since both folpet and captan degrade rapidly (half-life in blood is 4.9 for folpet ,the half-life for thiophosgene is 0.6 seconds), the irritation due to inhalation is restricted to the surface layers of epithelium only. The absence of treatment related findings in surviving animals are consistent with this MOA.

In conclusion, R37 is not appropriate because there is no evidence from humans, and no supporting scientific data from animal experiments. R20 should be sufficient to warn of the risks from inhalation.

Data:
Folpet micronized, Cracknell, 1993. Nose only. 0.80, 1.60 and 1.99 mg/L. Four hours. Three groups of five animals each. Observed for 14 days.

	Dose:	0.80	1.60	1.99 mg/L
Deaths	M	0/5	3/5	4/5
	F	05	1/5	1/5

Deaths occurred on days 1 and 2, only; no deaths after day 2. During exposure, irregular slow, deep or shallow respiration and gasping were recorded, with the numbers of animals affected related to the atmosphere concentration.
Macroscopic changes seen only in decedents: clear viscous fluid in the trachea, dark lungs, incomplete collapse of the lungs and occasional pale areas on the lungs. No treatment related findings in surviving animals to indicate irritation of the respiratory tract.

Acute median lethal concentration for four hours was 1.89 mg/L of air (95%: 1.47 – 2.31 mg/L). Folpet is not listed by International Chemical Safety Cards as respiratory irritants.

The notifier's conclusion is consistent with the conclusion of the RMS that R20 is appropriate for folpet but that R37 is not appropriate for folpet.

Vol. 3, B.6.2.5, Eye irritation

United Kingdom (UK) considers that the severity and irreversibility of the findings in the eye irritation study (Dreher, 19992c) warrant a R41 classification.

Response
The rabbit bioassay is a surrogate test system to assess human hazard. Experience with folpet and its sister fungicide, captan, shows that the rabbit study does not reflect the actual hazard of folpet and captan. Over 100 years of combined use (folpet and captan, taken together) does not support a R41 risk phrase. The mode of action (MOA) of these two fungicides centers on the rapid reaction with available thiol groups associated with mucus membranes. This chemical reaction is responsible for the severe eye irritation noted in rabbit studies. The collective eye irritation study data, however, do not support the "irreversible" nature of the adverse effects. The weight of evidence shows that eye damage is restricted to surface areas (including the cornea) but that these insults do recover.

Analysis of the collective data on captan, the sister fungicide to folpet based on their common mechanism of toxicity, show that folpet and captan are not corrosive chemicals and that irreversible damage to the eye does not occur.

The collective data both from non-clinical studies, where recovery from irritation (including corneal opacity) is always evident as well as clinical experience, where there is an absence of credible reports of eye injury argues against the issuance of R41.

By example, as noted in "Captan and Folpet," Gordon, E.B. (2001) In Handbook of Pesticide Toxicology (R. I. Krieger, ed., Volume 2, Agents, pp 1171-142, Academic Press, San Diego), a review of the literature for the years to 2001 did not indicate any reports of eye injury. Additionally, agricultural workers in California, USA who routinely reenter captan treated fields (e.g., strawberries) indicate there is not a problem with eye irritation (R. Krieger, personal communication).

The notifier's conclusion is consistent with the conclusion of the RMS that R36 is appropriate for folpet.

Evaluation table number	Reporting table number	Open Point number
-	**2(17)**	**2.8**

Conclusions of the EFSA Evaluation Meeting:
MS to agree on NOAEL in rat 90-day study at an expert meeting.

Vol. 3, B.6.3.2, Short-term toxicity studies in the rat.

United Kingdom (UK) considers it is not possible to determine a NOAEL for the 90-day rat study (Reno, 1981), as histopathology was not performed on the stomachs of rats from the lower dose groups.

Response
We agree that a NOAEL cannot be determined for the Reno (1981) study. As hyperkeratosis was absent in both females at 3000 ppm, as well as focal ulceration, there is evident a reduction of histologic changes with decreasing dose. It is likely that the NOAEL would be 300 ppm and perhaps as high as 1000 ppm. It is relevant to note that the histologic changes noted were transitory in that by 14 days after treatment, the stomachs were normal in all ten animals of each sex. The collective data on folpet and captan are consistent with the mode of action that is based on rapid chemical reaction with free thiol groups of mucus membranes such as the lining of the gastrointestinal tact.

Study data:
Dose levels: 0, 300, 1000, 3000, 10,000 ppm. 20/sex/group. 10/sex/group were killed at 13 weeks; 10/sex/group were held for 2 weeks and then killed.
No treatment related gross pathology was seen in any group.
Stomach lesions in non-glandular portion of stomach at 10,000 ppm but these cleared in 14 days.

The findings at 10,000 ppm	Males	Females
Pleocellular inflammatory infiltrate	9/10	10/10
Submucosal edema	10/10	10/10
Acanthosis	10/10	9/10
Hyperkeratosis	10/10	3/10
Focal erosion	2/10	3/10
Focal ulceration	1/10	2/10

Findings at 3000 ppm	Males	Females
Pleocellular inflammatory infiltrate	-	2/2
Submucosal edema	-	2/2
Acanthosis	-	1/2
Hyperkeratosis	-	0/2
Focal erosion	-	1/2
Focal ulceration	-	0/2

The findings at 10,000 ppm + 2 week recovery

	Males	Females
Pleocellular inflammatory infiltrate	0/10	0/10
Submucosal edema	0/10	0/10
Acanthosis	0/10	0/10
Hyperkeratosis	0/10	0/10
Focal erosion	0/10	0/10
Focal ulceration	0/10	0/10

In conclusion, this issue is not significant as this study is not used to derive any relevant end-point.

Evaluation table number	Reporting table number	Open Point number
-	**2(18)**	**2.9**

Conclusions of the EFSA Evaluation Meeting:
The RMS to summarize the study (Collins, 1972a) in an addendum.

The following new report is submitted. Responses to comments by a member state (UK) on this study are presented below.:

- **Point IIA, 5.4.3: In vivo studies in germ cells**

5.4.3/04

Report: Collins, T.F.X. (1972). Dominant lethal assay. II Folpet and difolatan. Division of toxicology FDA, published report *Fd Cosmet. Toxicol. Vol. 10, pp 363-371*. (Company file: R-545)

Guidelines: In-house.
Deviations: Not applicable.

GLP: No.

Material and methods: Test substance: folpet, batch number 5X121 (no. 42367), purity 100%; vehicle: carboxymethyl cellulose. Folpet as a suspension in the vehicle was administered by interperitoneal injection at 0, 2.5, 5.0 or 10.0 mg/kg/day for five days or by oral intubation at 0, 50, 100 or 200 mg/kg/day for five days to groups of 15 male rats. Each treated rat, 9 to 10 weeks old at treatment, was then mated each week for 10 weeks with one untreated female of the same age. A Caesarean section was performed on each female rat on day 13 of pregnancy and at autopsy females were scored for total implantations, early foetal deaths and late deaths.

Findings:

No male rats died during treatment. There were no adverse effects on mating performance or pregnancy rate following interperitoneal treatment with folpet, but there was a dose-related decrease in the number of pregnancies following oral intubation at the first week of mating, which was also observed at the high dose level up to five weeks after treatment. Mean total numbers of implantations per female were not affected by folpet at any dose level, at either route of administration.

Increases in mean early deaths per pregnancy occurred for the first seven weeks at folpet interperitoneal treatment at 10.0 mg/kg/day, with a statistically significant effect after three weeks and a dose-related response in weeks 3 and 4. Following intubation at 100 and 200 mg/kg/day, increases in mean early deaths occurred for the first six weeks. There was a slight increase at 50 mg/kg/day, particularly during the first four weeks.

Following interperitoneal treatment with folpet, there was only one statistically significant increase in the percentage of litters with one or more deaths and no statistically significant

increases in those with two or more deaths. Following intubation, there were statistically significant increases in the percentage of litters with two or more deaths at 100 and 200 mg/kg/day but no effect in the percentage of litters with one or more deaths. Significant linear trends were apparent in the number of litters with one or more early deaths (at 3 weeks) and two or more early deaths (at 1, 2 and 4 weeks) after interperitoneal treatment. Significant linear trends were apparent in the number of litters with two or more early deaths (at 2 and 3 weeks) after intubation.

The mean results are summarised in Table 5.4.3-1.

Table 5.4.3-1: Summary of results

Folpet dose (mg/kg/day)	Incidence of pregnancy (%)	Mean total implantations/ pregnancy	Mean early deaths/ pregnancy	Percentage of litters with one or more early deaths	Percentage of litters with two or more early deaths
Interperitoneal injection					
0	95.3	11.4	0.43	41.3	2.1
2.5	94.0	11.0	0.48	40.6	7.7
5.0	93.3	11.1	0.57	46.5	10.1
10.0	88.0	11.1	0.76	55.9	19.7
Oral intubation					
0	94.4	12.3	0.40	37.8	2.1
50	93.8	12.0	0.53	40.2	11.9
100	90.0	11.9	0.72	47.2	20.1
200	85.9	12.2	0.74	46.2	21.1

All results are the mean vales for 10 weeks.

Conclusions: Folpet did not adversely affect fertility or mean total implants per female following interperitoneal injection at up to 10 mg/kg/day or oral intubation at up to 200 mg/kg/day. Folpet caused a dose-related increase in mean early embryonic deaths per pregnancy and the mean percentage of litters with two or more deaths.

These data are in contrast with other dominant lethal studies that showed no treatment effect of folpet [Jacoby, 1985 #587;Esber, 1983 #590;Bootman, 1987 #628]. The interperitoneal route of administration is not appropriate for evaluating folpet as the normal route of exposure is oral or dermal. In both cases, folpet is not stable enough to reach the uterus or developing foetuses. Direct interperitoneal injection, in contrast, bypasses the means for rapid elimination of folpet and thiophosgene by bypassing the systemic circulation. Folpet has a half-life of 4.9 seconds in the systemic circulation and thiophosgene has a half-life of 0.6 seconds.

We conclude that folpet did not cause the apparent treatment-related effects seen in the Collins study.

Vol. 3, B.6.4.2.2, In vivo genotoxicity studies in germ cells.

United Kingdom (UK) notes an additional published study (Collins, 1972) reporting a positive result in a rat dominant lethal assay with folpet following oral and intraperitoneal dosing must be taken into consideration.

Response
Consideration of Collins (1972) in light of the collective data on folpet (and captan, its sister fungicide that shares a common mechanism of toxicity) shows that folpet is not mutagenic *in vivo*.

Collins reports one significant ($p<0.01$) instance where litters from week three (late spermatid phase) had two or more early deaths per litter. In contrast to these data other dominant lethal studies with folpet are negative. These include:

Jorgenson et al. (1976): negative in mice.
Kennedy et al. (1975): negative in mice
Bradfield (1980): negative in rats

In addition, dominant lethal assays with captan are negative except when studied by Collins (1975); thus both folpet and captan were reported positive using the experimental design and procedures of Collins (1972, 1975) but were negative when studied by other investigators. As folpet and captan share a common mechanism of toxicity, it is likely that whatever conditions that appear unique to the Collins studies, they affected the results with folpet and captan in a similar manner.

The mode of action of folpet and captan make dominant lethal effects highly unlikely:

1. No folpet or captan reaches the gonads. This follows from the rapid degradation in blood with a half-life of 4.9 seconds (captan half-life of 0.97 seconds) and a corresponding half-life of thiophosgene of 0.6 seconds (thiophosgene is common to both folpet and captan).
2. The relatively stable degrades of folpet (phthalimide) and captan (THPI) are not reactive compounds and are not mutagenic in bacterial system assays.

We conclude that the dominant lethal effects for the late spermatid phase in males is not biologically relevant (i.e., not treatment related).

Data:
0, 2.5, 5, 10 mg/kg bw/day for five days by intraperitoneal injection
0, 50, 100, 200 mg/kg be/day for five days by oral intubation to groups of 15 male rats
CMC was the control. Males were treated when 9 – 10 weeks of age then mated at week 10 for ten successive weeks. Females were killed on day 13 of gestation and scored for total implantation, early fetal deaths and late deaths.

Parameter	ip admin	oral admin
Incidence of pregnancy (%)	no effect	no effect
Mean total implantations/pregnancy	no effect	no effect
Mean early deaths/pregnancy	effect*	no effect

*Week 3 with males treated ip at 10 mg/kg bw/day showed 0.92 mean early deaths/pregnancy ($p<0.05$) compared to 0.29 in control. Week three control value, 0.29 is the lowest of all the control values (weeks 1 – 10), which averaged for the remaining nine weeks, 0.45 (range: 0.36 – 0.50). It is judged that this statistical significance is not biologically significant.

Percentage of litters with one or more and with two or more early deaths after treatment of males with folpet for five days (ip injection or oral intubation).

% of Litters with two or more early deaths

Week	0	50	100	200	slope
1	6.7	20	15.4	30.8	0.00106
2	0	18.8	33.3*	38.5*	0.00172*
3	0	20	28.6*	40*	0.00188**

4	6.7	12.5	30.8	23.1	0.00091
5	7.1	0	20	15.4	0.00064
6	0	6.7	20	6.2	0.00032
7	0	0	18.8	12.5	0.00075
8	0	20	7.7	18.8	0.00068
9	0	14.3	6.7	6.7	0.00015
10	0	6.7	20	18.8	0.00095

Number of Litters (of 15)	Percent
1	6.7%
2	13.3%
3	20%
4	26.7%
5	33.3%
6	40%

It is not clear why the percent litters do not follow this pattern; the actual numbers of litters examined is not provided.

The six stages of spermatogenesis:*

1 Sperm in vas deferens, 3-4 days
2 Epididymal sperm, from 3-4 days to 2 weeks
3 Late spermatids, 3 weeks
4 Mid to early spermatids, 4-5 weeks
5 Spermatocytes, 6-8 weeks
6 Spermatogonia, from 9 weeks

* Development stage of sperm occur this time prior to ejaculation; thus, adverse effects on reproduction (dominant lethal effects) that occur the first week after treatment would imply effects to the sperm in the vas deferens while effects seen at week 9 would suggest effects on the spermatogonia.

Evaluation table number	Reporting table number	Open Point number
-	2(19)	2.10

Conclusions of the EFSA Evaluation Meeting:
MS to discuss the genotoxicity, also in relation to classification, and the need of additional studies to be performed at an expert meeting.

- **Point IIA, 5.4: Genotoxicity testing**

The following new report is submitted. Responses to comments by members states and an overall conclusion on genotoxicity are presented below.:

5.4/01

Report: Clay, P. (2004). Folpet: in vivo mouse duodenum comet assay. [redacted] unpublished report number [redacted]SM1245/REG/REPT (Company file: R-17100)

Guidelines: In-house.
Deviations: Not applicable.

GLP: Yes.

Material and methods: Test substance: folpet, batch number 60798002, purity 98.6%; vehicle 1% aqueous carboxymethyl cellulose; positive control N-methyl-N-nitrosourea (MNU), administered in corn oil at 100 mg/kg bw. The *in vivo* mouse Comet assay is capable of detecting DNA damage in several tissues. In this study, cells in the crypt regions of the duodenum were of interest. Mouse carcinogenicity studies in this strain of mouse indicated that the female maybe more sensitive with regard to folpet toxicity. Female CD-1 mice 5-7 weeks old were obtained from a commercial supplier and acclimatised for at least 5 days. In Phase I of the study, folpet as a suspension in the vehicle was administered, by single oral dose, to a group of 3 female CD-1 mice at a dose of 2000 mg/kg in a volume of 10 mL/kg to determine the clinical effects of treatment over a 4-day observation period. Subsequently, three groups of 3 female mice were given vehicle control or folpet at 1250 or 2000 mg/kg and killed 24 hours later to determine the effects on the duodenum. In Phase II of the study, groups of 8 female mice were given vehicle control, folpet at 1000 or 2000 mg/kg or MNU at 100 mg/kg (two groups per treatment) and one group was sampled after two hours and the other after six hours to investigate effects in cells from the crypt region of the duodenum. No analyses for stability homogeneity or achieved concentration were performed in view of the short-term nature of the study. All test substance and positive control substance preparations were prepared as close to the time of dosing as possible.

At sampling, 4 cm sections of duodenum, starting immediately adjacent to the gastric pylorus, were removed, flushed and the villi scraped off and discarded. The remaining tissue was trimmed and cut into three sections. The two outer sections were suspended in medium and minced. The mixture was allowed to stand and the supernatant (forming the crypt cell suspension) was removed and stored. The middle section was fixed in formalin and processed for histology. For each animal the degree of villus removal was assessed. The comet assay was only performed on crypt cell samples from animals where the villi were mainly or partly

absent. Where the villi were either mainly present, or the crypt cells were also removed, the samples were classed as unacceptable and were discarded. Comet slides (3 per animal; 4 animals per group) were prepared from the crypt cell suspension, subject to electrophoresis for 30 minutes, stained and scored for comet formation using the Comet Assay III system. The intention was to have 50 cells per slide, 150 cells per animal, from at least 4 animals per group. The primary measure of DNA damage was tail moment, a combined measure of the fraction of migrated DNA multiplied by a measure of tail length. The positive control should show a clear increase in mean tail moment (greater than twice control values and statistically significant). Results were statistically analysed.

Findings:

In Phase I, animals treated at 2000 mg/kg showed no adverse clinical effects in the 4-day observation period. The degree of villus removal was unaffected by folpet.

In Phase II, sufficient animals showing optimal villus removal were available for analysis. 150 cells were analysed for most animals, though in some cases sufficient analysable cells could not be located. Folpet caused no statistically significant or biologically significant increase in mean tail moment compared to the vehicle only control at the 2 hour and 6 hour sampling times. MNU induced statistically significant increases in mean tail moment at both sampling times. The results are summarised in Table 5.4-1.

Table 5.4-1: Summary of results in the Comet Assay test

Treatment	Dose (mg/kg)	No. cells scored (4 animals per treatment)	Mean tail moment
2 hour sampling			
Vehicle only	--	435	0.19
Folpet	1000	600	0.15
Folpet	2000	568	0.32
MNU	100	538	16.51**
6 hour sampling			
Vehicle only	--	439	0.17
Folpet	1000	600	0.15
Folpet	2000	471	0.23
MNU	100	373	10.27**

** Significantly different from vehicle only, $p < 0.01$.

Conclusions: There was no DNA damage in the mouse duodenum following treatment with folpet at 1000 or 2000 mg/kg as measured by a Comet Assay test.

Responses to comments made by Member states

(1) The United Kingdom (UK) notes that a number of additional studies of the genotoxicity of folpet *in vivo* are available. These include a mouse spot test (negative), a mouse dominant lethal assay (negative, but concerns about the study quality) and the rat dominant lethal assay, discussed above. All studies should be considered. The relevance of the tissues investigated in each study should also be considered, given the known rapid degradation of the folpet molecules and the likely reactive species.

Response

The tissues that are relevant for investigation of folpet's mutagenicity *in vivo* are those tissues that come into direct contact with the intact molecule or the reactive degradate, thiophosgene. *In vivo*, these tissues are the cells of the gastrointestinal tract. The remainder of the mammalian system is "off limits" to folpet and thiophosgene due to their rapid degradation in blood (folpet: 4.9 second half-life, thiophosgene: 0.6 second half-life, respectively).

Further to the issue of relevant tissues, it is the permanent basal cells of the gastrointestinal tract that are the appropriate targets to investigate. The epithelial layer of the gastrointestinal tract that comprises the villi is replaced every three to four days; thus, any mutagenic events taking place in this compartment are of no consequence.

The appropriate tissue to investigate is the crypt cell compartment in the mouse, as this compartment gives rise to duodenal tumors that appear after oral exposure at doses of approximately 1000 ppm and higher in cancer bioassays.

This tissue compartment has been investigated, *in vivo*, using the single cell Comet assay (Clay, 2004). The negative results confirm that folpet is not mutagenic *in vivo*. This finding is consistent with that for captan with which it shares a common mechanism of toxicity.

(2) Denmark (DK) notes folpet induces a wide range of genotoxic events *in vitro* including gene mutations/DNA damage in bacteria and mammalian cells, chromosomal aberrations in mammalian cells and mitotic recombination in yeast (not present in DAR). Although folpet was active in both the +/-S9 activation, the response was generally more pronounced without S9 activation.

Response

S9 "activation" is not relevant to the mutagenic activity of folpet. The role S9 plays in bacterial assays is that of a supply of available thiol groups associated with the enzyme fractions. These thiols react chemically (not enzymatically) with folpet and result in its degradation. They also promote the degradation of folpet's reactive degradate, thiophosgene. The collective data on the mutagenicity of folpet supports the conclusion taken by other regulatory and expert bodies that evaluated the full data package and concluded that Folpet is not genotoxic (e.g., JMPR, USEPA, and Germany).

Overall conclusion on genotoxicity

Folpet has been tested extensively for its potential mutagenic effects. These data, collectively, characterise folpet as a chemical that can induce mutations in experimental settings that allow direct contact with unprotected cells or bacteria. In practical terms this means folpet, while having inherent reactivity with regard to DNA, does not act in living mammals.

This paradox is solved by understanding the mode of action of folpet and its short half-life in vivo. The trichloromethylthio side chain and its labile nitrogen-sulphur bond to the phthalimide ring is responsible for the chemical reactivity of folpet. Its reaction with thiol groups leading to the degradation of folpet and the concurrent generation of thiophosgene is the key step in both its fungicidal activity and its general mammalian toxicity. Once folpet and thiophosgene have been "neutralised" via reaction with thiols and, in the case of thiophosgene, reaction with a host of other functional groups, no mutagenic potential remains.

As the half-life of folpet in mammals is 4.9 seconds and the half-life of thiophosgene is 0.6 seconds, there is essentially no opportunity for folpet or thiophosgene to come into contact with potential target cells for mutagenic effects.

Experimentally, this translates into positive findings in such assays as the Ames bacterial mutagenicity assays and negative findings in such assays as the micronucelus, nuclear aberration, and comet assay.

In particular, it is relevant to the understanding of folpet's mode of action to note that adverse chromosomal events in the cancer target tissue in mice do not occur, even with direct attempts at folpet exposure.

The nuclear aberration assay used massive oral doses of folpet and looked for aberrations (mainly micronuclei) in the crypt cells of the mouse duodenum. None were found. The Comet assay further confirmed the absence of effects by harvesting individual crypt cells and showing normal DNA patterns after large doses of folpet. In both cases, positive control agents induced expected effects.

Absent exposure, there can be no adverse effects, regardless of the inherent toxicity of a chemical. The *in vivo* toxicity of folpet is an example that conforms to this rule. The experimental data and our understanding of the mode of action for folpet combine to provide absolute assurance that folpet does not pose a mutagenic or genotoxic risk to humans.
No further testing is required.

The notifier's conclusion is consistent with the conclusion of the RMS that folpet does not meet the EC classification criteria for mutagenicity.

Evaluation table number	Reporting table number	Open Point number
-	2(26) 2(27) 2(28)	2.12

Conclusions of the EFSA Evaluation Meeting:
Teratogenic properties, also in respect of classification and labelling, to be discussed at an expert meeting.

Responses to comments by Member States and EFSA are presented below.

Vol. 3, B.6.6.2 Developmental toxicity in the rabbit

The United Kingdom (UK) considers the maternal NOAEL in the rabbit developmental study (Rubin, 1995) to be 10 mg/kg bw/day based on the slight initial reduced body weight gain at 40 mg/kg bw/day. Developmental effects however are not serious enough to warrant further investigation in either rat or rabbit, and might be expected given the level of maternal toxicity seen.

Response

Folpet (and captan) exert their developmental toxicity through their primary irritancy effect on the gastrointestinal tract of the dams. In addition, these fungicides are bacteriostats and therefore are expected to disrupt the normal gastrointestinal flora present in the rabbit intestine. This flora is essential for proper nutrition in that rabbits rely on a fermentation process and coprophagia to obtain nutrients. To the extent that folpet (and captan) disrupt this natural cycle, nutritional deficiencies would occurs.

In this regard, the rabbit test system is not appropriate as a surrogate for human hazard identification.

Vol. 3, B.6.6.4 Reproductive toxicity

Denmark suggests classification for developmental toxicity.
Folpet caused an increase in the incidence of hydrocephaly in fetuses with associated domed skull and irregularly shaped fontanelles in NZW rabbits in the presence of maternal toxicity. Both fetal and litter incidences of this malformation were increased. There was also evidence of fetal effects (delayed ossification of the sternebrae) in rabbits at a lower dose than that causing maternal toxicity.

Response

Analysis of the collective rabbit data show that folpet does not cause an increase in hydrocephaly in rabbits. From an analysis of the folpet database (Gordon and Neal, 1997,):

At severely toxic or maternally lethal doses, folpet shows embryotoxicity in rabbits. A further developmental toxicity study showed a possible dose relationship with an increased incidence of hydrocephaly in New Zealand White rabbits only at a maternally toxic dose of 60 mg/kg

bw/day administered on days 6-28 (Feussner et al, 1984, "Teratology study in rabbits, [redacted]). This finding (hydrocephaly) has a variable incidence in the New Zealand White rabbit strain and tends to occur in non-dose-related clusters (Christian, 1985, "Variations in the incidence of hydrocephalus observed in caesarean-delivered control new Zealand White rabbit fetuses, Journal of the American College of Toxicology, 4(2): 218). Further, the findings were not replicated in a predictable manner on pulsed exposure to the same high dose of folpet in the same rabbit strain done by the same investigators (Feussner, 1985, 'Teratology study in rabbits with folpet technical using a 'pulse-dosing' regimen."[redacted]. Additionally other rabbit studies with folpet (e.g., Rubin, 1985, "Folpan: Teratology study in the Rabbit." [redacted] Report No. MAK/051/FOL) have not shown hydrocephaly associated with gestation exposure to folpet. On review of the complete developmental toxicity data on folpet, WHO-JMPR concluded that folpet is not teratogenic in rabbits, even at a dose that is clearly maternally toxic (WHO-FAO, 1986, cited in WHO-FAO Pesticide Residues in Food – 1990, folpet 51-62, JMPR 1986).

An additional confounding factor in interpreting rabbit developmental toxicity studies is the indirect action on maternal nutritional status caused by disruption of the intestinal flora from the bacteriostatic action of folpet. This adverse effect of bacteriostatic agents, such as folpet and captan, in rabbits may contribute to maternal toxicity and thus promote secondary effects in fetuses.

Vol. 3, B.6.6 Developmental toxicity

The European Food Safety Authority (EFSA) notes that there seems to be evidence of teratogenic potential of folpet at maternal non-toxic doses both in rat and rabbit. Thus, Classification of R63 is proposed.

Response

R63 ("possible risk of harm to the unborn child") is not appropriate. A weight of evidence analysis of the collective data for folpet and captan show that these compounds do not pose a rise to the unborn child:
(1) The uterus and developing fetus does not come into contact with folpet or captan due to their rapid disappearance in blood.
(2) Developmental studies show folpet and captan are not frank teratogens.
(3) Developmental effects in fetuses at doses that are maternally toxic, particularly in rabbits, does not warrant R63.
(4) Rabbits are less than optimal for studying folpet or captan's developmental effects because these two fungicides are bacteriostatic and disruption of the intestinal flora in rabbits may have a deleterious effect on the health of the dams and, secondarily, on the fetuses.

The conclusion of the notifier that R63 is not appropriate is consistent with the conclusion of the RMS.

Evaluation table number	Reporting table number	Open Point number
-	**2(30)**	**2.13**

Conclusions of the EFSA Evaluation Meeting:

MS to discuss the toxicity of the metabolites phthalimide and phthalic acid and their possible inclusion in the residue definition at an expert meeting.

-	**3(12)**	**3.2**

Conclusions of the EFSA Evaluation Meeting:

MS to discuss the residue definition for risk assessment in an expert meeting.

RMS to prepare an assessment of the toxicological relevance of metabolites (including their contribution to the toxicological burden).

- **Point IIA, 5.8.1: Toxicity studies of metabolites**

The proposed definition of the residue in plants and animals commodities is folpet alone.

The following new reports are submitted in support of the claim that is the relevant definition of the residue. These reports are also summarised in the new residues addendum under Point IIA 6.7.

5.8.1/01

Report: Seilfried, H.E. (2000). Review: Toxicological risk characterisation of potential folpet metabolites. The toxicity profiles of phthalic and phthalamic acids and phthalimide – is there a significant risk from metabolite exposure. Consultants, unpublished report dated August 1, 2000 (Company file: R-12331).

Guidelines: Not applicable.

GLP: Not applicable.

Material and methods: The position paper includes summaries the toxicity findings of the folpet metabolites.

Findings:

Phthalamic acid, a major degradate when folpet undergoes hydrolysis, is the main metabolite following oral administration to rats. Phthalic acid is a minor metabolite. Phthalamic acid is the main metabolite in goats and phthalic acid is not seen in the urine but is present in the kidney. Phthalamic acid is hydrolyses to phthalic acid at acid pH. TOPKAT was used to predict that phthalamic acid would have an acute oral rat LD_{50} of ~ 700 mg/kg bw, and would be negative in the Ames test. As a metabolite in the rat, animals are considered to have been

exposed during oral toxicity studies. It is not possible to establish a risk level due to the lack of toxicological data on the compound itself, but based on the low toxicity of phthalate and phthalimide, the level of toxicity of phthalamic acid is expected to be low.

Phthalimide is an intermediate metabolite, capable of being metabolised to phthalamic acid, phthalate and possibly methylphthalate. It is not mutagenic in the Ames test, in yeast, mouse lymphoma assay or in a cytogenetic assay in human lymphocytes. There is conflicting evidence of teratogenic activity (resorptions and malformation after i.p injection, but no indication of teratogenicity in rats, rabbits or hamsters following oral administration). The weight of evidence suggests a low level of risk. TOPKAT was used to predict that phthalimide would have an acute oral rat LD_{50} of ~ 980 mg/kg bw, and would be negative in the Ames test.

Phthalic acid is not mutagenic in Ames or other bacterial assays, but does act synergistically with some but not all heterocyclic amine mutagens. It is not carcinogenic based on negative rodent bioassays with phthalic anhydride (which converts to phthalic acid). Phthalic acid does not accumulate in the body and is essentially cleared by 48 hours after oral administration. Phthalic acid is not teratogenic in rats. The purported activity on male and female reproductive systems in some less-than-robust studies is not well supported when all results are taken into consideration and the weight of evidence for all folpet metabolites is considered. TOPKAT was used to predict that phthalic acid would have an acute oral rat LD_{50} of ~ 2500 mg/kg bw, and would be negative in the Ames test.

The related compounds phthalic anhydride (which converts to phthalic acid in aqueous media) and phthalamide have been tested for carcinogenicity in rats and mice under a US Government testing programme. Neither compound showed increased incidence of tumours.

Phthalic acid is ubiquitous in the environment from industrial sources (used as plasticizers and in the production of polyester) and can be formed from environmental phthalate esters via hydrolysis where they can be found widely distributed, generally at low levels in air, rain water, sediment, soil and biota, food samples, and human and animal tissues.

Conclusions: Folpet metabolites have a very low level of hazard to humans when exposed through the diet and to the environment compared to parent folpet. The appropriate residue expression for folpet is folpet per se.

5.8.1/02

Report: Gordon, E. (2005). Folpet. Toxicological significance of relevant degradates. Makhteshim, unpublished report dated March 21, 2005.

Guidelines: Not applicable.

GLP: Not applicable.

Material and methods: The discussion paper expands on the discussion of the toxicological significance of the degradates of folpet.

Findings:

The degradates of folpet should not be included in the residue expression, as defined by the Guideline:

-Their basic toxicology

The physical and chemical properties of a chemical determine the nature and severity of effects in mammals. Folpet has an active moiety that is responsible

both for its fungicidal properties and its toxicological effects in mammals. The degradates phthalimide, phthalamic acid and phthalic acid lack this active moiety and thus have a spectrum of effects distinct from their parent. These three degradates are not acutely toxic, are not developmental or reproductive toxins, are not mutagenic, are not carcinogenic, and do not exhibit any relevant systemic long term or sub-chronic toxicity.

The absence of significant toxicity is reflected in a Structure Activity Relationship (SAR) analysis for the three degradates of folpet (TOPKAT 2000). Where predictions could be made (i.e., where similar molecules/functional groups existed in the database with associated toxicity), low potential for mutagenicity and carcinogenicity were calculated. Interestingly, the SAR analysis did predict mutagenicity for folpet in the Ames Assay, providing some sense of validation for the analysis as folpet is an in vitro mutagen but not mutagenic in vivo (TOPKAT 2000).

-Their presence in significant amounts
The definition of "significant amounts" is not clear in the guideline; however, phthalimide is only present in the environment in a transient way, as it degrades further to phthalic acid via the intermediate phthalamic acid. Phthalamic acid has been shown not to be present in plants in "significant" amounts, based on laboratory studies.

> Phthalic acid is present in the environment at relatively high levels, compared to the contribution expected with the agricultural use of folpet (Neyroud and Schnitzer 1977; Schnitzer 1977). This background level of phthalic acid is due to the industrial production of both phthalic acid and its anhydride (Slooff, Bont et al. 1994; Kleerebezem, Pol et al. 1999).

Inclusion of phthalic acid in the residue expression would confound the understanding of folpet residues present as the majority of phthalic acid found would be from sources other than folpet.

Conclusions: The collective data on folpet degradates shows that the appropriate residue definition for folpet is the parent molecule, only, due to the lack of toxicity exhibited by these substances. This is in conformity with the conclusions of the JMPR and US EPA (FAO/WHO 1996; US-EPA 1999) This is in conformance with DG SANCO Guideline for Metabolism and Distribution in Plants (n° 7028/VI/95 rev.3, 22 July) note that:

> Residues are expressed as parent compound if there are no metabolites or if the metabolites are known to be of no toxicological significance.

> The metabolites present a significantly lower hazard to man than folpet, evidenced by the complete lack of systemic toxicity observed in the folpet long term and subchronic toxicity studies. In addition, direct comparisons of folpet and phthalimide and other metabolite aquatic toxicity further reinforces the differences due primarily to its mode of action as a primary irritant. Key to resolving the differences in toxicity between folpet, phthalimide and other systemically

circulating metabolites is the exceptionally rapid degradation of folpet in the presence of blood. As such, all systemic toxicity observed in folpet studies is attributed to the metabolites along with secondary effects of folpet's irritation of the GI tract.

The metabolites do not contribute to the overall toxicological burden.

Evaluation table number	Reporting table number	Open Point number
-	**2(34)**	**2.14**

Conclusions of the EFSA Evaluation Meeting:

MS to discuss the dermal absorption value at an expert meeting.

- **Point IIIA, 7.3: Dermal absorption**

Responses are given below to comments made by Member States:

(1) The Netherlands (NL), Austria and UK disagree with the value of 1% for dermal absorption based on the information in the DAR. RMS concludes that a dermal absorption of 1% is appropriate based n an *in vitro* study with rat and human skin and a publication of *in vivo* data in rats. The data are entirely based on the amount absorbed through the skin. No data are given for the amount of folpet in the treated skin (dermal depot) and its possible systemic availability. In the in vitro study the amount absorbed through the skin is much higher after 24 hours than after 8 hours exposure. This could (at least partly) be the result of dermal depot becoming systemically available. Without data on the dermal depot a higher value for dermal absorption should be considered. Since the study was done in a laboratory, which always gives data on the dermal depot in its report, a better estimation of dermal absorption should be possible.

Response

The study exposed human skin samples continuously to liquid formulations for 72 hours, with samples of receptor fluid being taken at 1, 2, 4, 8, 24, 28, 32 and 48 hours. The application site was covered with a glass slide, such that the applied formulation did not dry on the surface of the skin. After removal of the formulation, the skin samples were subject to histomorphological examination in case the test formulations had damaged the skin. It is not possible to derive the dermal delivery from the study data or to determine whether the difference between 8 and 24 hours was due to a dermal depot laid down in the 0-8 hour period becoming available or if it was due to continual diffusion through the skin during the whole 24 hour period.

The study was conducted in 1997 and does not fully meet the draft OECD guideline 428 and the 2002 Guidance Document (SANCO 222 rev. 6). Ideally, there would have been 8 hours of exposure followed by removal of test formulations and a further period of collecting receptor fluid with tape stripping of the skin samples followed by analysis for dermal depot. However, it is not acceptable to use the 24-hour data: 24 hours direct exposure to the liquid formulations is an excessive period; the 8-hour exposure to the liquid represents very much a worst-case as it represents continuous immersion of the skin in the spray solution whereas in reality there would be only occasional exposure following splashing of the skin during the working day prior to skin washing. The value of 1% represents a conservative estimation of the dermal absorption of folpet through human skin.

<u>Bolus release of folpet from the skin depot.</u>

The amount of radioactivity in the receptor vessel corresponds, approximately, to the amount expected as determined by the steady state kinetics. By example, with a steady state flux of 0.25 for human skin, the amount expected at 24 hours after a lag time of 3 hours is 5.3 μg/cm^2. This compares with 4.6 μg/cm^2 measured.

Similarly, with rat skin, the amount expected with a flux of approximately 0.2 ug/cm2/hour, after a lag time of three hours, is 4.6 μg/cm^2 whereas the amount measured was 4.2 μg/cm^2. These numbers are essentially similar, given the expected variation in biological assays. Thus, there is no "bolus release" of material from the dermal depot.

Relevance of skin absorption studies with folpet

The receptor fluid concentration of folpet in the in vitro study was radioassayed (folpet was labeled with ^{14}C in the phenyl ring) and the in vivo assay (Shah et al., 1987) was labeled with ^{14}C in the side chain.

Both these assays measure primarily folpet degradates. Since folpet degrades in blood with a half-life of 4.9 seconds (as well as hydrolyzes at pH 7 with a half-life of 1.1 hours), there is essentially no systemic dose of folpet, regardless of the dermal exposure level or the dermal penetration rate.

Conclusion

Neither a higher value for dermal absorption should be considered nor should risk assessors ascribe any toxicologically relevant meaning to the absorption results obtained by these methods. In short, these data are moot; there is no systemic exposure to folpet from the dermal portal.

Nonetheless, should Experts of the European Food Safety Authority (EFSA) require a dermal penetration number, the 1% value outlined in the DAR is justified.

Data:

	80 WDG	50 SC	50 WP
8 hr			
Human	0.58%	0.67%	0.44% relative absorption
	1.58	1.69	1.14 μg/cm^2
Rat	0.34%	0.60%	0.45% relative absorption
	0.96	1.52	1.17 μg/cm^2
24 hr			
Human	2.08%	2.25%	1.75% relative absorption
	5.65	5.70	4.58 μg/cm^2
Rat	1.38%	2.13%	1.77% relative absorption
	3.96	5.43	4.64 μg/cm^2

Steady state conditions were reached after 2-3 hours and the steady state flux was 0.25, 0.25 and 0.22 μg/cm^2 human skin and 0.19, 0.25 and 0.22 μg/cm^2 rat skin. Kp values were 0.03, 0.03 and 0.03 cm/h 10-3, human skin and 0.02, 0.03 and 0.03 cm/h. 10-3 rat skin. Lag times ranges from 1.5 to 2.8 hours in human skin and 1.9 to 2.9 hours in rat skin.

Kp = permeability coefficients for tritium water.

(2) The United Kingdom (UK) considers the design of this study to be sub-optimal as full-thickness skin was used. Additionally, 24-hour absorption following an 8-hour skin wash was not measured; figures for residual skin radioactivity and total recovery are not reported. It is therefore not possible to propose dermal absorption values of 1% from this study.

Dermal absorption value is estimated from the collective data on folpet and captan. Suboptimal study design in one experiment may be bridged with supplemental data from other studies, both with folpet and its sister fungicide, captan, a compound that shares a common mechanism of toxicity with folpet.

In this case, there are two *in vivo* studies available for folpet and *in vivo* studies with captan that collectively show a low dermal absorption.

As noted in the response to the Netherlands (above), the question of dermal absorption for folpet with regard to systemic risk characterization is moot. Regardless of the dermal exposure to folpet, the systemic dose remains essentially at zero. This follows from the exponential degradation rate of folpet. The folpet remaining after t seconds is the starting concentration times ($e^{-0.141t}$). With a resulting in a half-life of 4.9 seconds less than 1% remains after 35 seconds In addition the nature and severity of effects with oral administration of folpet are dependent on its irritant properties. This irritancy follows from the chemical reaction of folpet and captan with available thiol groups in tissues of mucus membranes that it encounters. The target organ for folpet (and captan) is the gastrointestinal tract. The mode of action is irritancy of the epithelial lining from exposure to this fungicide from the lumen of the intestine. This mode of action is not possible from dermal exposure since, as already noted, there is essentially no folpet absorbed and the intestinal lining is not exposed from the lumen. As Folpet degrades very fast there will be no systemic exposure.

Evaluation table number	Reporting table number	Open Point number
2.4	2(35)	-

Conclusions of the EFSA Evaluation Meeting:
The notifier to submit the study Wilson, 1990 (dermal absorption).

The requested study is summarised below. In addition, a position paper with the notifier's interpretation of the study is provided below together with an overall conclusion on dermal absorption.

- **Point IIA, 5.8.2: Supplementary studies on the active substance**

The following new reports are submitted:

5.8.2/07

Report: Wilson, A.S. (1990). A study of dermal penetration of ^{14}C-folpet: in the rat. [redacted] unpublished report number MAG/1/PH (Company file: R-5470)

Guidelines: US EPA 85-2.
Deviations: None.

GLP: Yes.

Material and methods: Test substance: [U-phenyl-^{14}C] folpet, batch number 078F9213, specific activity 17.2 mCi/mmol, for dosing. Vehicle 1% aqueous carboxymethyl cellulose. Folpet was administered at a dose volume of 200 µL to the shaved backs of groups of 24 Sprague Dawley rats at 0.00053, 0.0053, 0.053 and 0.53 mg/cm^2, equivalent to 0.01, 0.1, 1 and 10 mg/animal, as a 0.05, 0.5, 5 and 50 mg/mL suspension in the vehicle. All dose preparations were adjusted to approximately pH 6.35 with 0.1% phosphoric acid. The dose was evenly spread over an area of 18.9 cm^2, dried with a hair drier and covered with a non-occlusive dressing. All animals were fitted with an Elizabethan collar and housed individually in metabolism cages. After 0.5, 1.0, 2.0, 4.0, 10.0 and 24 hours, four animals per group were injected with sodium pentobarbitone, the dressing was removed and the application site washed with Teepol (detergent) solution. The application site was dissected free from the main carcass and a blood sample removed by cardiac puncture and stored under heparin. Radioactivity was measured in the blood, urine, faeces, carcass, skin surface (not removed by washing), skin application device, washings and dressings.

Findings:

The actual quantities applied to the skin were less than nominal because of adhesion to the application devices and the non-occlusive cover. The nominal dose levels of 10, 1, 0.1 and 0.01 mg/animal yielded applied dose levels of 4.8, 0.46, 0.049 and 0.0064 mg/animal. At nominal doses of 1.0 and 10.0 mg/animal, 26% and 33%, respectively, of the applied radioactivity was washed off the skin after 10 hours. At 0.01 and 0.1 mg/animal, only 2.3% and 0.8%, respectively, was washed off after 10 hours. The majority of radioactivity remained

in the skin and the peak was reached after 10 hours at 0.01 and 0.1 mg/animal, and after 24 hours at 1.0 and 10.0 mg/animal. Very low levels of radioactivity were detected in the blood, urine and faeces. In blood, radioactivity was present after 0.5 hours, corresponding to 0.1% or less of the applied dose at 10 or 1 mg/animal. No radioactivity was detected in the blood of the two lower dose groups. In the urine, increasing quantities of radioactivity were collected over time, reaching a maximum of 1.8% after 10 hours and 13.2% after 24 hours (all doses). Radioactivity was detected in the faeces of the three higher doses, but not in the low dose o f 0.01 mg/animal. Radioactivity was initially quickly retained in the carcass. This may have been explained by seepage of radioactivity from te target skin area to the surrounding skin during the washing process. Residues in the carcass were generally lowest where residues in the skin at the dose site were highest.

Table 5.8.2-1: Recover of radioactivity after dermal application

Nominal dose mg/animal (actual dose)	Site	Mean % of actual dose recovered					
		0.5 hr	1 hr	2 hrs	4 hrs	10 hrs	24 hrs
0.01 (0.0064)	Blood	0	0	0	0	0	0
	Urine	0	0	0	0.6	1.4	10.9
	Faeces	0	0	0	0	0	0
	Carcass	44.7	0	35.6	0	0	0
	Skin	51.4	78.0	29.1	91.8	97.8	83.6
	Washed off	3.9	22.0	35.3	7.6	0.8	5.5
0.1 (0.049)	Blood	0	0	0	0	0	0
	Urine	0	0	0.1	1.4	1.8	13.2
	Faeces	0	0	0	0	0	0.6
	Carcass	24.8	7.6	14.6	10.9	3.3	6.6
	Skin	46.1	53.4	50.1	72.7	92.6	77.9
	Washed off	29.1	39.0	35.2	15.0	2.3	1.7
1.0 (0.46)	Blood	0	0.1	0.1	0	0	0
	Urine	0	0	0	0.5	1.3	3.5
	Faeces	0	0	0	0	0	0
	Carcass	10.8	11.2	7.6	8.2	9.6	7.0
	Skin	57.7	48.8	75.8	69.7	63.3	85.0
	Washed off	31.5	40.0	16.6	21.6	25.8	4.5
10.0 (4.8)	Blood	0	0.6	0	0	0	0
	Urine	0	0	0	0.7	0.9	1.3
	Faeces	0	0	0	0	0	0
	Carcass	15.1	10.6	14.2	12.8	21.0	7.9
	Skin	58.5	68.6	55.4	67.4	44.5	87.5
	Washed off	26.4	20.8	30.4	19.1	32.8	3.3

Conclusions: Following dermal application of [U-phenyl-^{14}C] folpet, the majority of radioactivity was absorbed into the treated skin and carcass. There was evidence that carcass levels were due to radioactivity seeping from the treated area during washing. Very low levels of radioactivity were found in the blood and faeces. Once absorbed, radioactivity was excreted via the urine (1.3 to 13.2% of applied radioactivity), with a higher rate of excretion at lower doses. The actual absorption of folpet per se, however, is essentially zero (see 5.8.2/08).

5.8.2/08

Report: Gordon, E. (2005). Folpet: The appropriate dermal penetration factor for use in occupational risk assessment is zero percent, unpublished Makhteshim report dated February 18, 2005.

Guidelines: Not applicable.

GLP: No.

Material and methods: The paper reviews the properties of folpet, the toxicity of phthalimide and its degradates, and methodology and results of the dermal absorption studies conducted by Shah (IIA 5.8.2/02) and Wilson (IIA 5.8.2/07).

Findings:

Dermal penetration studies are usually conducted using radiolabelled materials. ^{14}Carbon is the preferred radiolabel for most organic materials. In the case of folpet, the location of the ^{12}C (non-radioactive carbon), which is replaced by ^{14}C, determines the identity of the molecular species measured. Folpet labeled on the side chain (-trichloromethylthio group) will allow detection of thiophosgene-related degradates (e.g., CO_2, COS) and reaction products (and TTCA) whereas folpet labeled on the phthalimide ring (either on the aromatic portion or the carbonyl portion) will allow detection of phthalimide and its degradates. In both situations, however, neither allows an opportunity to measure folpet in the animal. The pathway showing the label on the –[trichloromethylthio] group is seen in Figure 1. The products labeled when the ^{14}C in on the ring are shown in Figure 2.

Figure 1. Degradation pathway of ^{14}C[-trichloromethylthio]folpet.

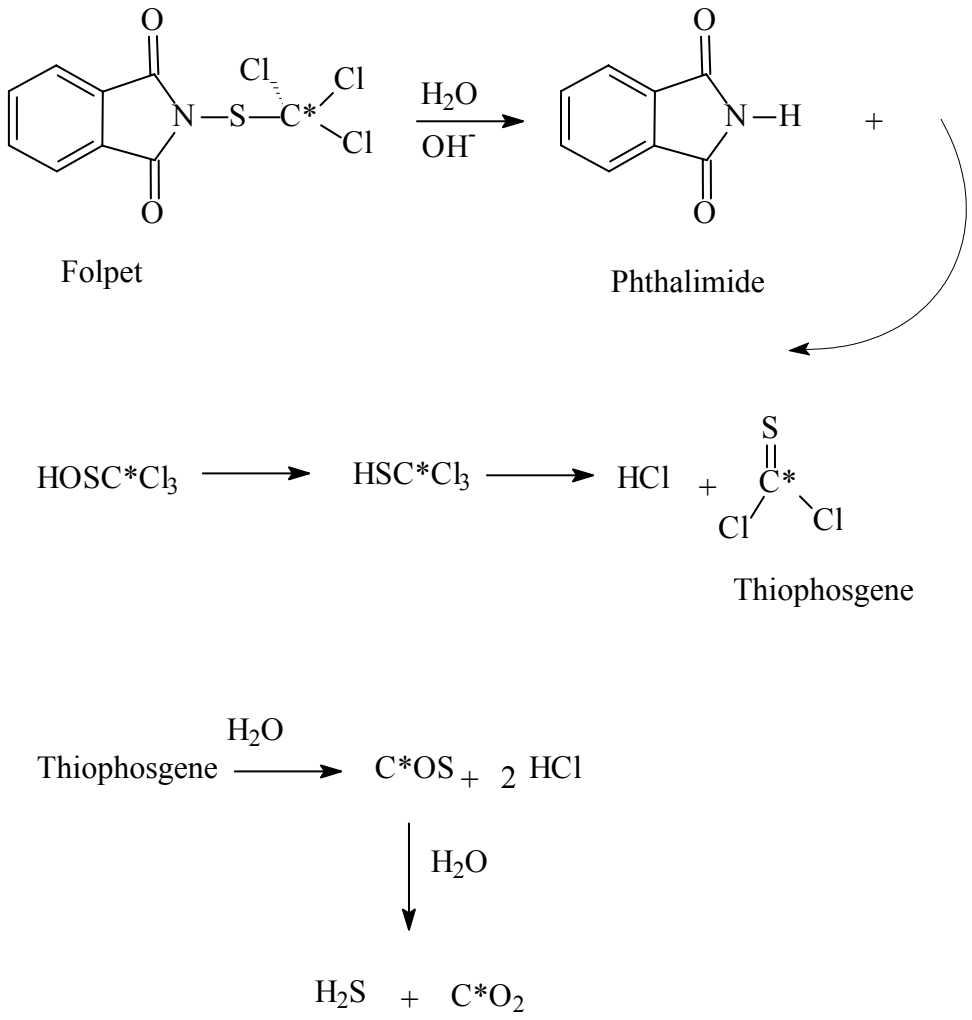
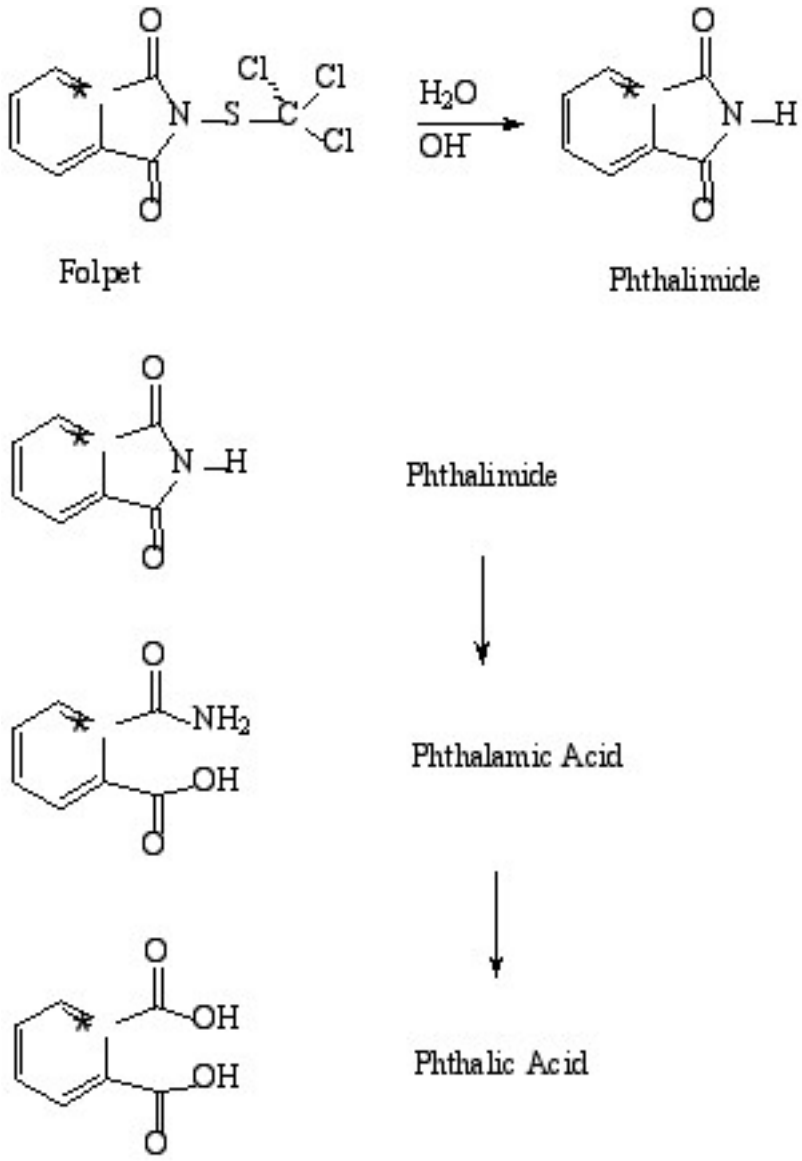


Figure 2. Degradation pathway of ^{14}C[-ring]folpet.



In a ring labelled rat dermal absorption study, nominal doses of folpet were 0.01, 0.1, 1.0 or 10.0 mg/rat (Wilson & Wright, 1990). The translocation of folpet was monitored by carbon-14 introduced to the phthalimide portion of the molecule.

The phthalimide ring of folpet "carries" the trichloromethylthio side chain. The ^{14}C-radiolabel reflects the presence of folpet only to the extent that folpet remains an intact molecule. Since folpet quickly degrades to phthalimide, the ^{14}C-label becomes a measure of the presence of phthalimide, not folpet.

To the extent that this degradation has progressed, the ^{14}C-moiety in the Wilson and Wright (1990) study reflects phthalimide and phthalimide degradates rather than folpet.

Since the half-life of folpet is approximately five seconds where thiols are available (e.g., in blood), it is reasonable to conclude that as folpet penetrates the skin, it quickly will be degraded to phthalimide.

A study that uses folpet labeled on the trichloromethylthio side-chain (Shah et al., 1987) has been used by EPA since "it is the thiophosgene moiety which is responsible for the majority of the toxicity seen with folpet administration" and "the phthalimide and related products are not

toxic metabolites of concern (Diwan, 1999, page 21). EPA has used this absorption factor in its occupational risk assessments (US-EPA, 1999, page 29).

In the study cited by EPA, both young (33 day old) and adult (82 day old) female Fischer 344 rats were administered [^{14}C-tichloromethyl]-folpet to their shaved backs (three rats/dose level). The specific activity of the test material was 10.6 μCi/mM. The treatment areas were 2.8 cm^2 for the young animals and 5.6 cm^2 for the adults. This was approximately 2.3% of the body area. The young animals received 1.5 and 7.5 μm as the medium and high dose and the adults received 3 and 15.0 μM, keeping the dose per unit area constant (0.535 and 2.679 μm/cm^2 for the medium and high dose, respectively). The low dose was 0.0839, μm/cm^2 for the young and 0.10 μm/cm^2 for the adults.

At 72 hours after application, the animals were killed and treated skin, perforated plastic blister,[2] urine, feces and carcasses were analyzed to determine absorption and recovery of radioactivity. Fractional absorption was calculated by dividing the radioactivity in the body plus excreta by the total radioactivity recovered.

The percent dermal penetration was inversely proportional to the dose applied (as is usual in dermal penetration studies). The mean fractional penetration of the recovered dose was:

	Low	Medium	High
Young	0.15	0.03	0.01[3]
Adult	0.12	0.03	0.01

The skin absorption did not vary between young and adult and was:

	Low	Medium	High
Absorption	14.8%	2.7%	1.1%[4]

The mid-dose was judged most appropriate for selection of the penetration factor.

Discussion

Risk characterizations are only as sound as the data upon which they are based. A confounding aspect of folpet risk characterization is the rapid degradation of this fungicide as soon as it confronts the systemic circulation.

The biological availability of folpet from dermal exposure is essentially zero.

Dermal penetration studies using carbon-14 as the means of detection, detect this radioactive carbon as a surrogate for the parent material. The interpretation of these studies must be made using the collective data from mammalian metabolism and other relevant mechanistic studies. The fate of the compound in question, once it enters the animal, is a key component of this risk characterization.

When ^{14}C is incorporated into the ring of folpet, the radio analysis measures phthalimide, not folpet. When ^{14}C is incorporated into the side-chain of folpet, the radio analysis measures thiophosgene reaction products such as TTCA[5] as well as ^{14}C-carbon dioxide. In addition, while the folate one-carbon pool derives carbon primarily from serine and glycine (transported by coenzyme tetrahdyrofolate), it is postulated that some $^{14}CO_2$ generated by the degradation

[2] The blisters were used on adults to protect the treated area.
[3] Data presented are rounded from four significant figures.
[4] Mean data taken from Diwan (1999).
[5] TTCA is thiazolidene-2-thione-4-carboxylic acid, a reaction product of thiophosgene and cysteine.

of folpet can enter this pool. Once apart of the C-1 pool, radioactivity will be introduced into a variety of compounds.

Dermal penetration studies using sulfur-35 as the means of detection,[6] detect this activity as a surrogate to folpet, similar to carbon-14. What is detected, however, can include thiophosgene-related reactants, H_2S, COS, and compounds that have undergone sulfur exchange. In each case, folpet is not measured.

Folpet degrades in aqueous solution at pH 7 with a half-life of 1.1 hours (Ruzo & Ewing, 1988). Since the skin is aqueous based environment (once the stratum corneum is passed), folpet will degrade and generate phthalimide (along with thiophosgene). As this degradation progresses, the measurements of residual radioactivity in the skin (with folpet labeled on the ring) will reflect phthalimide, not folpet.

Folpet degrades in blood with a half-life of 4.9 seconds (Gordon et al., 2001). Since systemic absorption requires folpet to enter the blood, it is essentially completely degraded within seconds, preventing transit to distant organs. Thiophosgene, folpet's reactive degradate, is lost at a rate over eight times faster than folpet; thus, both the parent and reactive degradate quickly disappear.

Occupational risk assessments are usually based on oral toxicity studies. Estimated dermal exposure is combined with a dermal penetration factor to derive the systemic dose. This dose is compared with effects seen in the oral studies.

In the case of folpet, little confidence exists in such occupational risk assessments, as folpet is completely degraded before it reaches targets upon which oral toxicity estimates are made. The effective dermal absorption of the intact folpet molecule is zero percent.

Nonetheless, if regulatory guidelines require the introduction of an absorption factor, one should not rely on a study that is ring labeled, as data from such a study reflect the absorption of phthalimide, not folpet.

Shah and co-workers (1987) use side chain ^{14}C-labeled folpet; while a similar argument could be made that this study measures thiophosgene related compounds and $^{14}CO_2$, it does represent the "active" molecular site as opposed to the relatively innocuous phthalimide ring.

Sound science dictates that any dermal penetration study with folpet that is based on radiolabels (either ^{14}C in the ring or side chain or ^{35}S in the side chain[7]) is not reliable for occupational risk assessment. The measurements taken do not reflect folpet presence but do reflect either phthalimide (from the ^{14}C ring label) or carbon dioxide, anabolic products derived from the C-1 pool (to which $^{14}CO_2$ has contributed to) or thiophosgene-reacted products such as TTCA for ^{14}C and $H_2{}^{35}S$ and products associated with sulfur exchange for ^{35}S. Direct measurement for folpet will show a complete absence of this compound in the systemic circulation.

Conclusions: The systemic dose to folpet or its reactive degradate, thiophosgene, following dermal exposure in workers is essentially zero. This results from the rapid loss of these materials as soon as they contact biological matrices that contain thiols (half-life of folpet is 4.9 seconds; half-life of thiophosgene is 0.6 seconds).

The remaining relatively stable moieties (phthalimide, phthalamic

[6] The use of ^{35}S is included for completeness; no dermal studies are known to have used this radiolabel.
[7] ^{35}S has a half-life of 87.2 days.

acid and phthalic acid) are considered not toxicologically relevant.

If regulatory guidelines mandate that a dermal penetration factor based on animal studies be obtained, data developed from studies with folpet labeled on the ring should not be used as they reflects the presence of phthalimide, not folpet. The study by Shah and co-workers use folpet labeled on the side-chain and, while still 'unreliable' for the reasons stated, may be considered more appropriate in that the side-chain is the "active" molecular site. The dermal absorption of the Shah study is low.

The appropriate dermal absorption factor for occupational risk assessment is 0%.

Overall conclusion on dermal absorption

Folpet absorption is approximately 1% based on traditional studies, but special mechanistic studies actually suggest this absorption is effectively much lower. For regulatory purposes, the notifier accepts a 1% absorption rate while this issue is further evaluated by EU scientists.

Evaluation table number	Reporting table number	Open Point number
-	**2(43)**	**2.16**

Conclusions of the EFSA Evaluation Meeting:

MS to discuss available residue decline data with respect to worker exposure at an expert meeting.

- **Point IIIA, 7.2.3.1: Estimation of worker exposure**

In the original dossier an assessment of worker exposure was presented using the German Model[8]. An additional assessment is requested based on multiple applications of 'Folpan' 80 WDG to grapes.

'Folpan' 80 WDG is recommended on grapes at 1.5 kg folpet/ha with up to 10 applications from shoot emergence to ripening. The minimum interval between sprays is 7 days and the minimum PHI is 28 days.

Residue studies in grapes, tomatoes and wheat (see Section 7) demonstrate that residues in fruit decline. In a number of studies, residues were measured immediately after the final application and at intervals thereafter. For each of the studies, DT_{50} values were calculated based on first-order kinetics. The results are shown in the Appendix below. Harvesting grapes involves contact with treated fruit and leaves and so consideration of decline data in a range of substrates (grapes fruit, tomato fruit, cereal leaves) provides a more accurate estimate of the decline of folpet in plants.

The DT_{50} values for all trials and all crops (n = 14) ranged from 5 to 32 days with a median decline rate of 15 days.

The German model assumes foliar dislodgeable residues of 1 $\mu g/cm^2$/kg a.s. Therefore, an application rate of 1.5 kg/ha results in foliar dislodgeable residues of 1.5 $\mu g/cm^2$. For 10 applications at 7 day intervals, based on the median DT_{50} of 15 days, the foliar dislodgeable residue value immediately after the final application is 5.214 $\mu g/cm^2$. Subsequent decline over 28 days to harvest will reduce the residue to 1.43 $\mu g/cm^2$.

In practice, applications occur over a long season (approximately 6 months) and so assuming ten applications at 7 day intervals immediately prior to harvest represents very much the worst-case.

An additional estimate of worker exposure based on the above using the German model is presented below:

[8] Hoernicke, E. *et al.*, 1998. Hinweise in der Gebrauchsanleitung zum Schutz von Personen bei Nachfolgearbeiten in mit Pflanzenschutzmitteln behandelten Kulturen. Nachrichtenbl.. Deut. Pflanzenschutzd. 50 (10) p. 267.

Estimate 1: Workers harvesting grapes treated with 'Folpan' 80 WDG 10 applications at 1.5 kg folpet/ha with a 7-day interval and 28 day PHI.

Dermal exposure

D (without protective gloves) = FDR x TF x R

where:
D = dermal exposure (mg/person/day)
FDR = foliar dislodgeable residues (1.43 μg/cm^2/kg a.s.)
TF = transfer factor (30,000 cm^2/person/hour)
R = working time (8 hours/day)

D (without protective gloves) = 1.43 x 30,000 x 8 ÷ 1000
= 343 mg/person/day

Systemic exposure

S = D ÷ bw x AF

where:
S = systemic exposure (mg/kg bw/day)
bw = worker body weight (60 kg)
AF = dermal absorption (1%)

S (without protective gloves) = 343 x 0.01 ÷ 60
= 0.057 mg/kg bw/day

Published data (Krieger *et al*., 1992[9]) provide alternative values for dislodgeable foliar residues (0.3 μg/cm^2) and transfer factors (18,000 cm^2/hour) for harvesters harvesting grapes treated with captan. Captan has a similar structure and properties to folpet and is used in a similar way and so these values are valid for folpet.

Based on these values a modified calculation of worker exposure can be made:

Estimate 2: Workers harvesting grapes treated with 'Folpan' 80 WDG 10 applications at 1.5 kg folpet/ha with a 7-day interval and 28 day PHI (based on data for captan from Krieger et al, 1992).

Dermal exposure

D (without protective gloves) = FDR x TF x R

where:
D = dermal exposure (mg/person/day)
FDR = foliar dislodgeable residues (0.3 x 1.43 μg/cm^2/kg a.s.)
TF = transfer factor (18,000 cm^2/person/hour)
R = working time (8 hours/day)

D (without protective gloves) = 0.3 x 1.43 x 18,000 x 8 ÷ 1000

[9] Krieger, R.I., Ross, J.H. and Thongsinthusak, T. (1992). Assessing human exposure to pesticides. *Reviews of Environmental Contamination and Toxicology, Vol. 128.*

= 61.8 mg/person/day

Systemic exposure

S = D ÷ bw x AF

where:
S = systemic exposure (mg/kg bw/day)
bw = worker body weight (60 kg)
AF = dermal absorption (1%)

S (without protective gloves) = 61.8 x 0.01 ÷ 60
= 0.010 mg/kg bw/day

The maximum exposure of workers in the worst-case calculation above (based on 10 applications to grapes at the maximum recommended rate) in the absence of protective gloves is 0.057 mg/kg bw/day (based on the German model) and 0.010 mg/kg bw/day (based on published data on published data on captan). Thus, exposure of workers is lower than the AOEL of 0.1 mg/kg bw/day. Consequently, the risk to workers is considered to be low and it is not necessary to set an additional re-entry period for workers harvesting treated grapes.

Appendix:

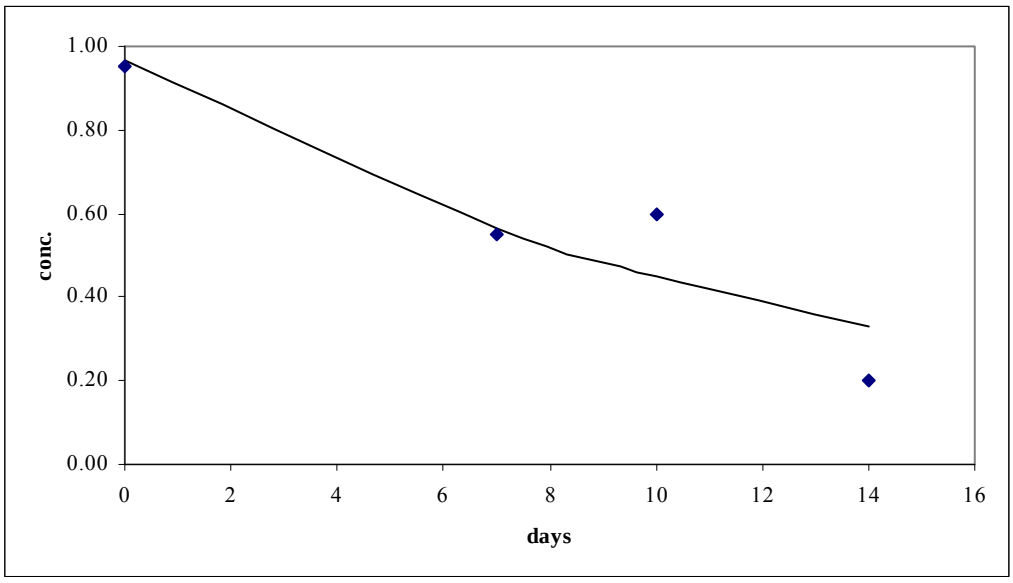
Folpet residue decline in plants kinetics.

Outdoor Tomatoes - Italy 1995 - De Paoli (1995)

Time	*Residue*	*Residue calc.*	*residuals sq.*	*(% calc)2*
0	0.95	0.97	0.0002	0.9318
7	0.55	0.57	0.0003	0.3202
10	0.60	0.45	0.0225	0.2026
14	0.20	0.33	0.0174	0.1100
	totals	**5.4**	**0.0403**	**1.6**

Solver function

Co	0.9653
k	0.0763
half-life	**9 days**
DT90	**30 days**
SSE =	0.0403
SST =	0.2272
R2 =	**0.823**

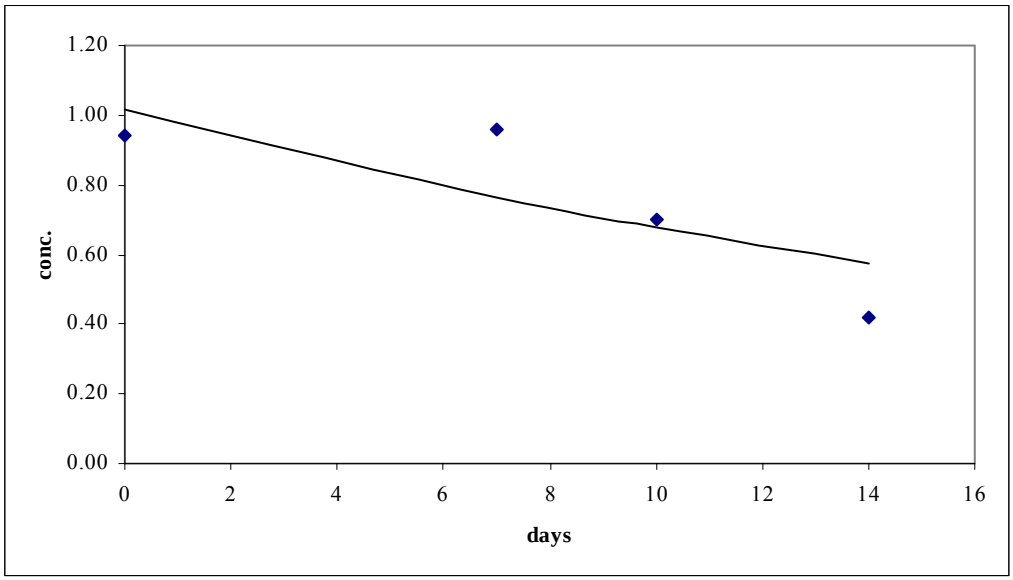


Outdoor Tomatoes - Italy 1996 (96IT30) - Baluff (1997b)

Time	*Residue*	*Residue calc.*	*residuals sq.*	*(% calc)2*
0	0.94	1.02	0.0062	1.0372
7	0.96	0.76	0.0386	0.5829
10	0.70	0.67	0.0006	0.4553
14	0.42	0.57	0.0232	0.3276
	totals	**9.2**	**0.0686**	**2.4**

Solver function

Co	1.0184
k	0.0412
half-life	**17 days**
DT90	**56 days**
SSE =	0.0686
SST =	0.1093
R2 =	**0.372**

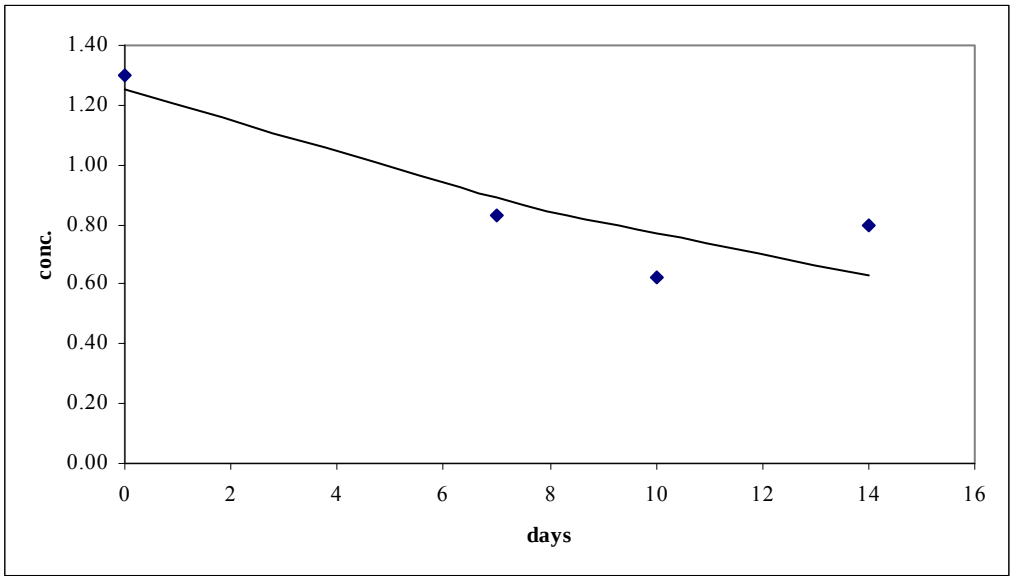


Outdoor Tomatoes - Italy 1996 (96IT31) - Baluff (1997b)

Time	*Residue*	*Residue calc.*	*residuals sq.*	*(% calc)2*
0	1.30	1.25	0.0022	1.5709
7	0.83	0.89	0.0035	0.7902
10	0.62	0.77	0.0217	0.5886
14	0.80	0.63	0.0287	0.3975
	totals	**12.5**	**0.0561**	**3.3**

Solver function

Co	1.2533
k	0.0491
half-life	**14 days**
DT90	**47 days**
SSE =	0.0561
SST =	0.2143
R2 =	**0.738**



Outdoor Tomatoes - Italy 1995 - Baluff (1995)

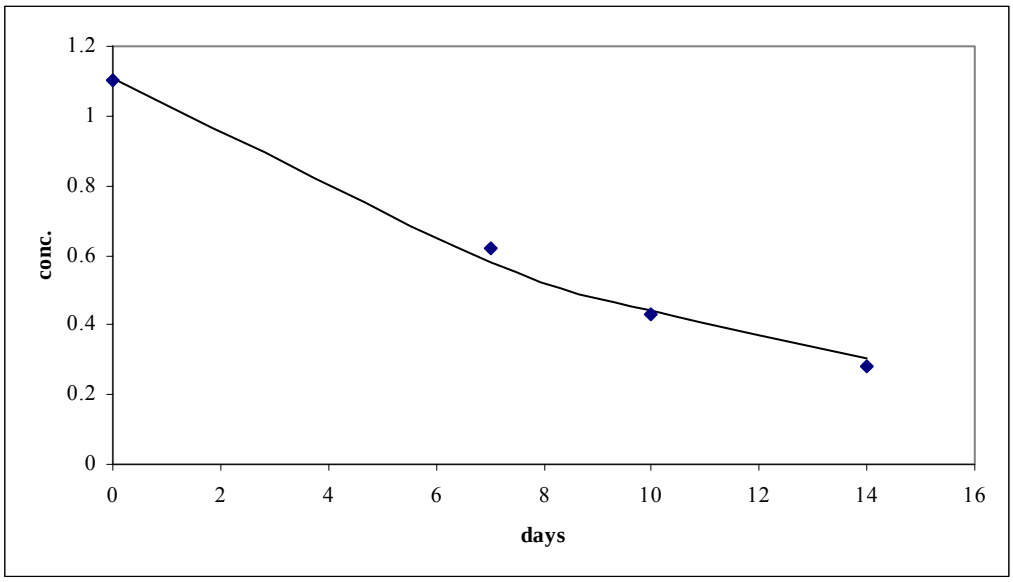
Time	*Residue*	*Residue calc.*	*residuals sq.*	*(% calc)2*
0	1.1	1.1	0.0001	1.2294
7	0.62	0.6	0.0015	0.3383
10	0.43	0.4	0.0001	0.1946
14	0.28	0.3	0.0006	0.0931
	totals	**5.9**	**0.0023**	**1.9**

Solver function

Co	1.1088
k	0.0922

half-life	**8**	**days**
DT90	**25**	**days**

SSE =	0.0023
SST =	0.6680
R2 =	**0.997**

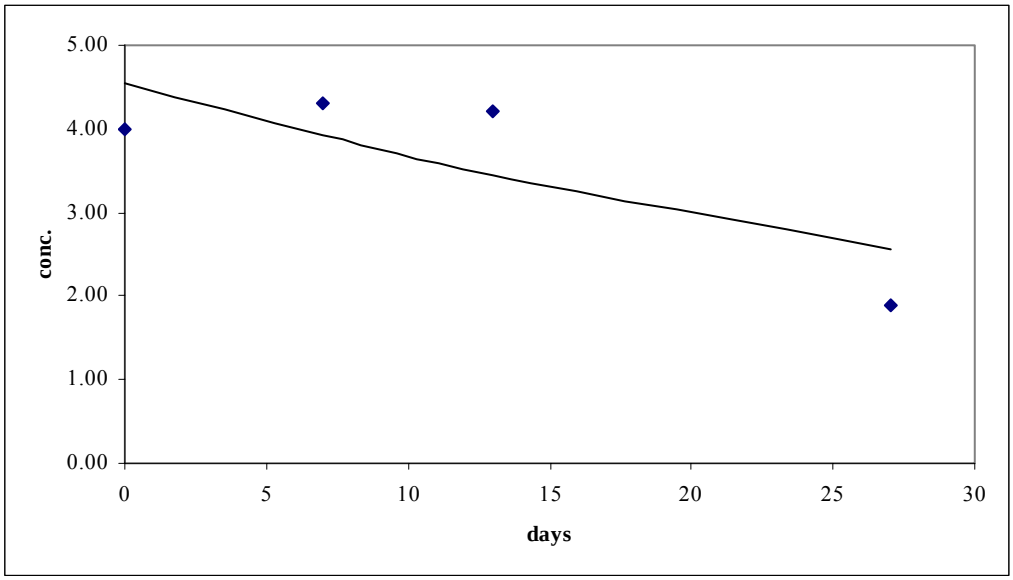


Grapes - Spain 2001 (PA2) - Simek and Perney (2002)

Time	*Residue*	*Residue calc.*	*residuals sq.*	*(% calc)2*
0	4.00	4.55	0.2975	20.6612
7	4.30	3.91	0.1504	15.3051
13	4.20	3.44	0.5775	11.8341
27	1.90	2.55	0.4203	6.4938
	totals	**208.7**	**1.4457**	**54.3**

Solver function

Co	4.5455	
k	0.0214	
half-life	**32**	**days**
DT90	**107**	**days**
SSE =	1.4457	
SST =	2.1225	
R2 =	**0.319**	

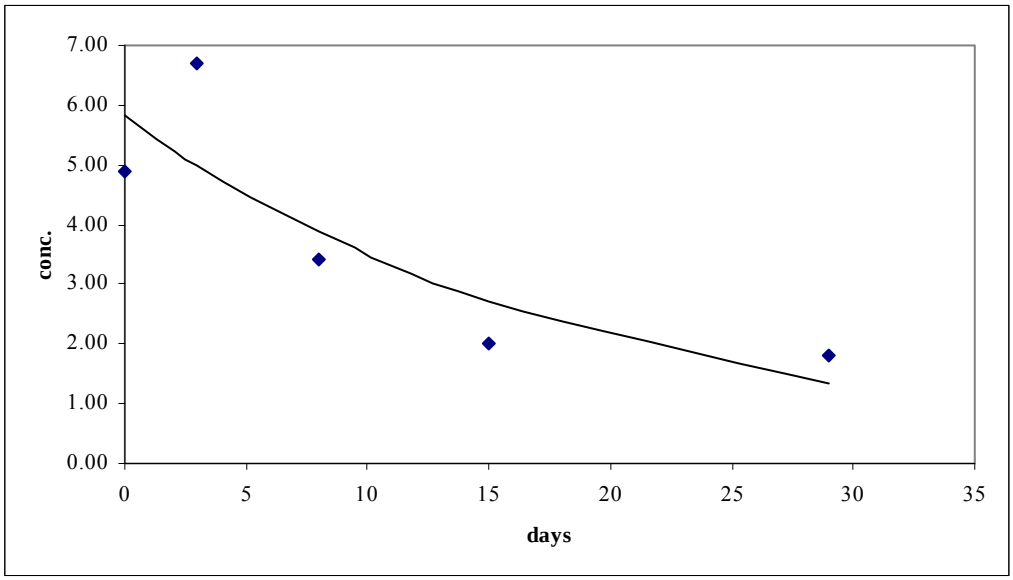


Grapes - Italy 2001 (IT1) - Simek and Perney (2002)

Time	*Residue*	*Residue calc.*	*residuals sq.*	*(% calc)2*
0	4.90	5.82	0.8499	33.8945
3	6.70	5.00	2.8998	24.9712
8	3.40	3.87	0.2245	15.0068
15	2.00	2.71	0.5074	7.3566
29	1.8	1.33	0.2213	1.7679
	totals	**351.0**	**4.7029**	**83.0**

Solver function

Co	5.8219	
k	0.0509	
half-life	**14**	**days**
DT90	**45**	**days**
SSE =	4.7029	
SST =	12.7984	
R2 =	**0.633**	

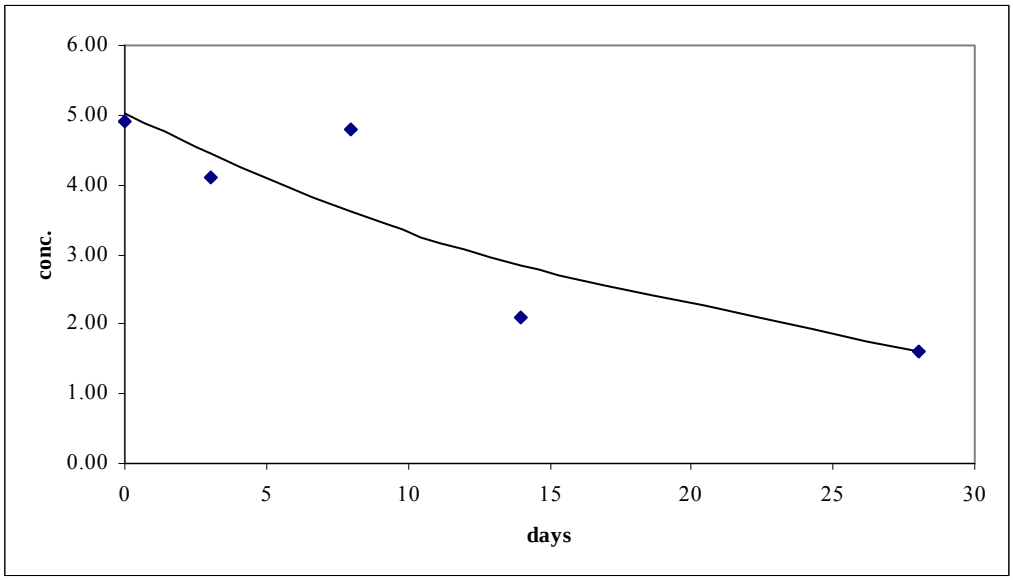


Grapes - Italy 2001 (IT2) - Simek and Perney (2002)

Time	*Residue*	*Residue calc.*	*residuals sq.*	*(% calc)2*
0	4.90	5.02	0.0154	25.2423
3	4.10	4.45	0.1193	19.7617
8	4.80	3.63	1.3803	13.1416
14	2.10	2.84	0.5447	8.0545
28	1.60	1.60	0.0000	2.5701
	totals	**307.5**	**2.0598**	**68.8**

Solver function

Co	5.0242	
k	0.0408	
half-life	**17**	**days**
DT90	**56**	**days**
SSE =	2.0598	
SST =	7.2685	
R2 =	**0.717**	

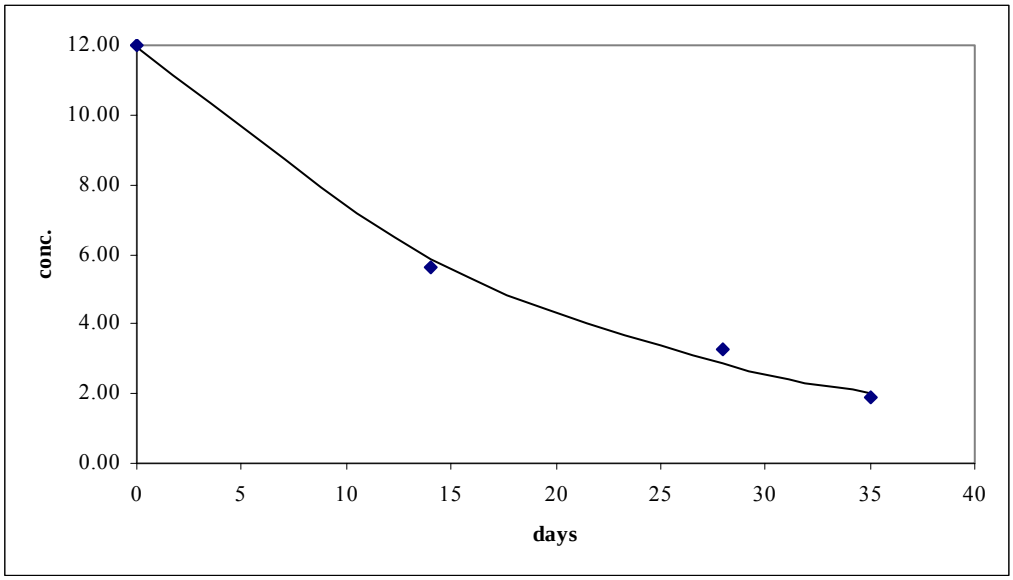


Grapes - Germany 1993 (UHL 13) - Fuchsbichler (1994)

Time	*Residue*	*Residue calc.*	*residuals sq.*	*(% calc)2*
0	12.00	11.94	0.0040	142.4794
14	5.60	5.88	0.0789	34.5857
28	3.30	2.90	0.1620	8.3954
35	1.90	2.03	0.0179	4.1363
	totals	**517.5**	**0.2629**	**189.6**

Solver function

Co	11.9365	
k	0.0506	
half-life	**14**	**days**
DT90	**46**	**days**
SSE =	0.2629	
SST =	60.2209	
R2 =	**0.996**	

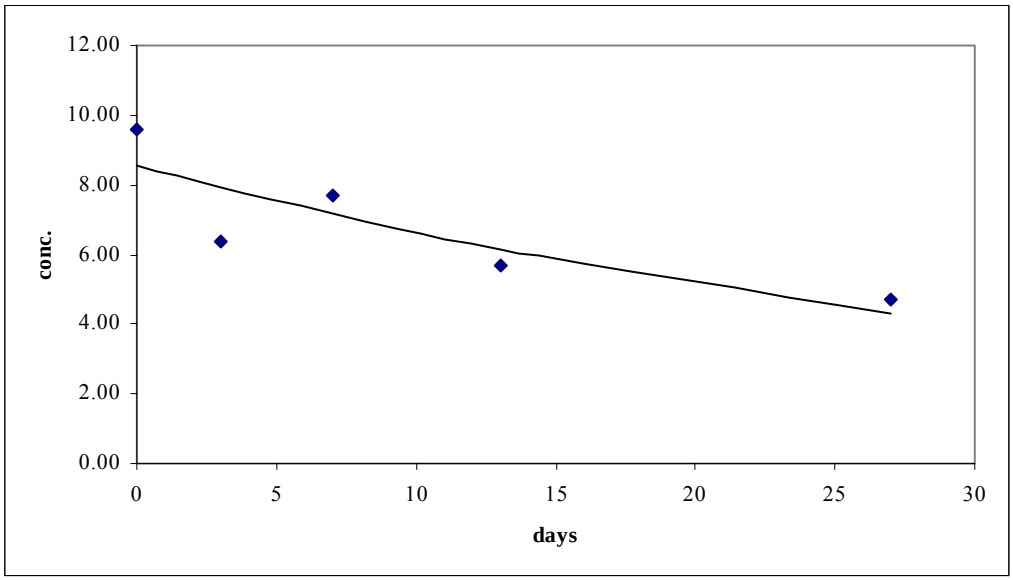


Grapes - Northern France 2001 (AN2) - Simek and Perney (2002)

Time	*Residue*	*Residue calc.*	*residuals sq.*	*(% calc)2*
0	9.60	8.53	1.1457	72.7543
3	6.40	7.91	2.2770	62.5517
7	7.70	7.15	0.3013	51.1383
13	5.70	6.15	0.2010	37.8013
27	4.70	4.32	0.1432	18.6759
	totals	**1160.1**	**4.0681**	**242.9**

Solver function

Co	8.5296	
k	0.0252	
half-life	**28**	**days**
DT90	**91**	**days**
SSE =	4.0681	
SST =	10.9113	
R2 =	**0.627**	



Grapes - Southern France 1992 - Laurent (1997)

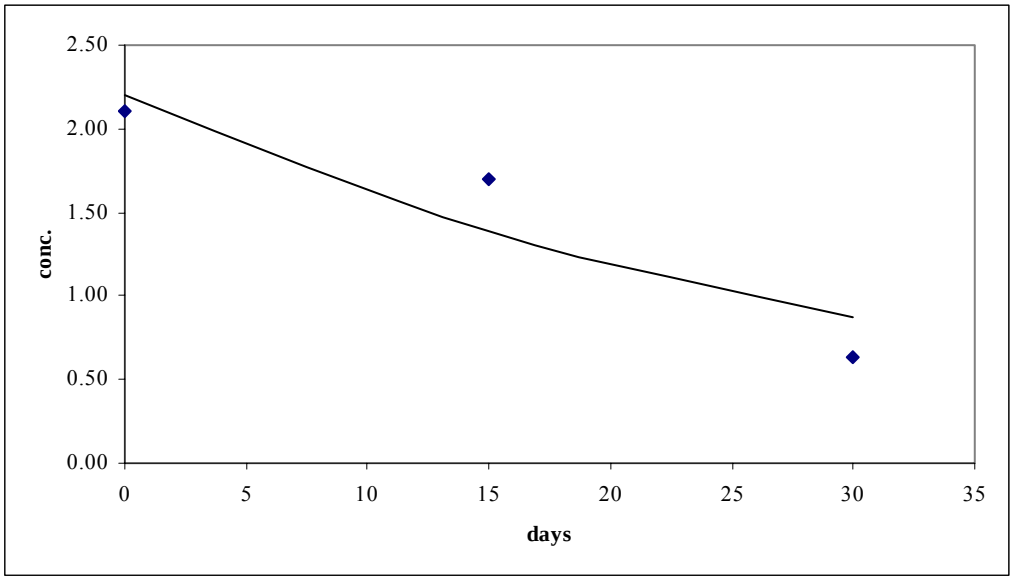
Time	*Residue*	*Residue calc.*	*residuals sq.*	*(% calc)2*
0	2.10	2.20	0.0097	4.8330
15	1.70	1.39	0.0971	1.9275
30	0.63	0.88	0.0609	0.7687
	totals	**19.9**	**0.1677**	**7.5**

Solver function

Co	2.1984
k	0.0306

half-life	**23**	**days**
DT90	**75**	**days**

SSE =	0.1677
SST =	0.8882
R2 =	**0.811**

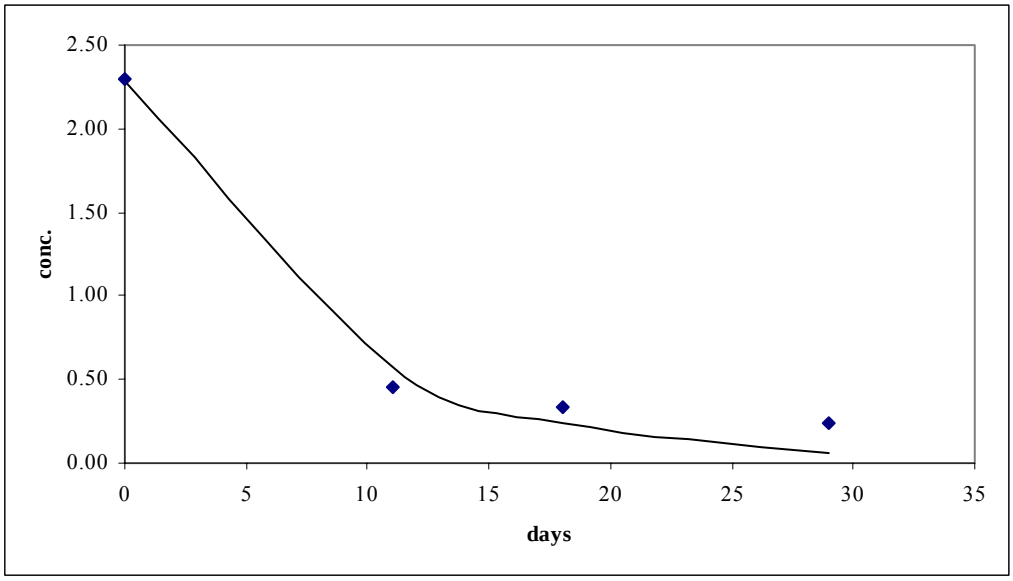


Wheat - Southern France 2001 (A1044 SA1) - Perney (2002a)

Time	*Residue*	*Residue calc.*	*residuals sq.*	*(% calc)2*
0	2.30	2.29	0.0002	5.2236
11	0.45	0.57	0.0144	0.3248
18	0.34	0.24	0.0109	0.0555
29	0.24	0.06	0.0329	0.0034
	totals	**9.9**	**0.0584**	**5.6**

Solver function

Co	2.2855	
k	0.1263	
half-life	**5**	**days**
DT90	**18**	**days**
SSE =	0.0584	
SST =	3.1273	
R2 =	**0.981**	

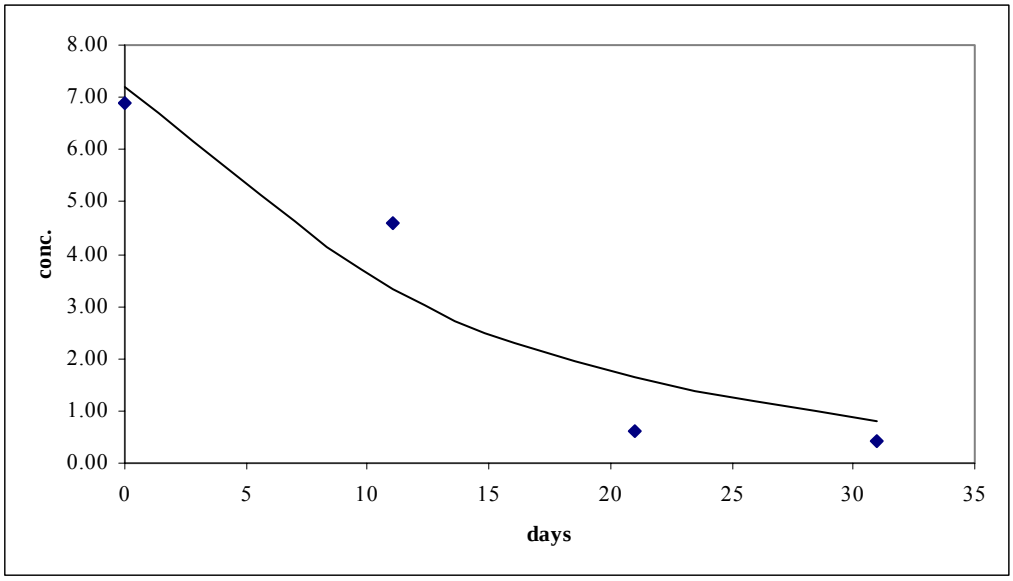


Wheat - Southern France 2001 (A1044 DR1) - Perney (2002a)

Time	*Residue*	*Residue calc.*	*residuals sq.*	*(% calc)2*
0	6.90	7.20	0.0902	51.8458
11	4.60	3.33	1.6009	11.1205
21	0.61	1.66	1.0949	2.7436
31	0.43	0.82	0.1542	0.6769
	totals	**169.4**	**2.9402**	**66.4**

Solver function

Co	7.2004	
k	0.0700	
half-life	**10**	**days**
DT90	**33**	**days**
SSE =	2.9402	
SST =	24.0442	
R2 =	**0.878**	



Wheat - Southern France 2001 (A1044 TL1) - Perney (2002a)

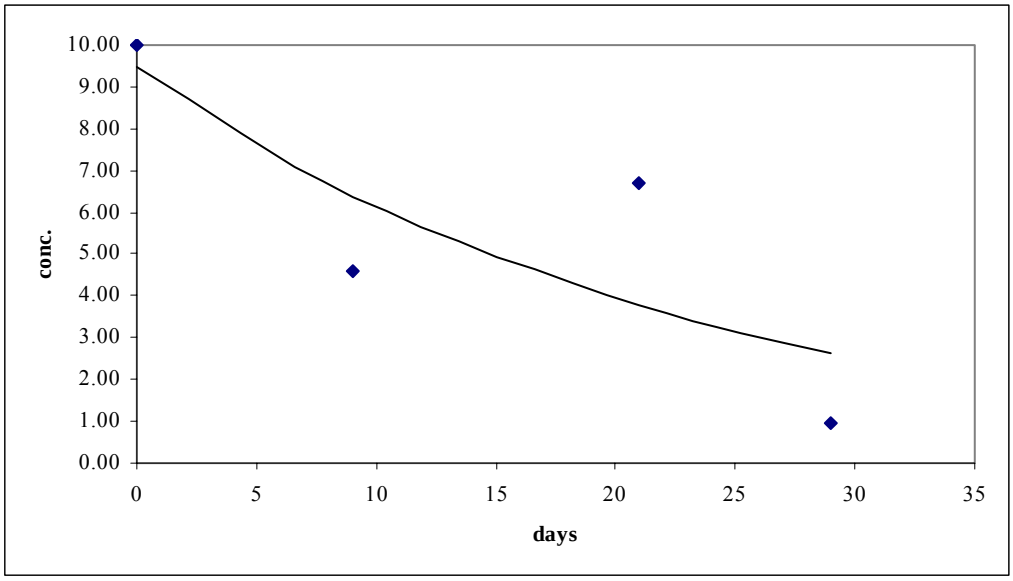
Time	*Residue*	*Residue calc.*	*residuals sq.*	*(% calc)2*
0	10.00	9.49	0.2559	90.1386
9	4.60	6.38	3.1812	40.7501
21	6.70	3.76	8.6425	14.1390
29	0.95	2.64	2.8637	6.9815
	totals	**496.4**	**14.9432**	**152.0**

Solver function

Co	9.4941
k	0.0441

half-life	**16**	**days**
DT90	**52**	**days**

SSE =	14.9432
SST =	27.9079
R2 =	**0.465**



Wheat - Southern France 2001 (A1044 TL2) - Perney (2002a)

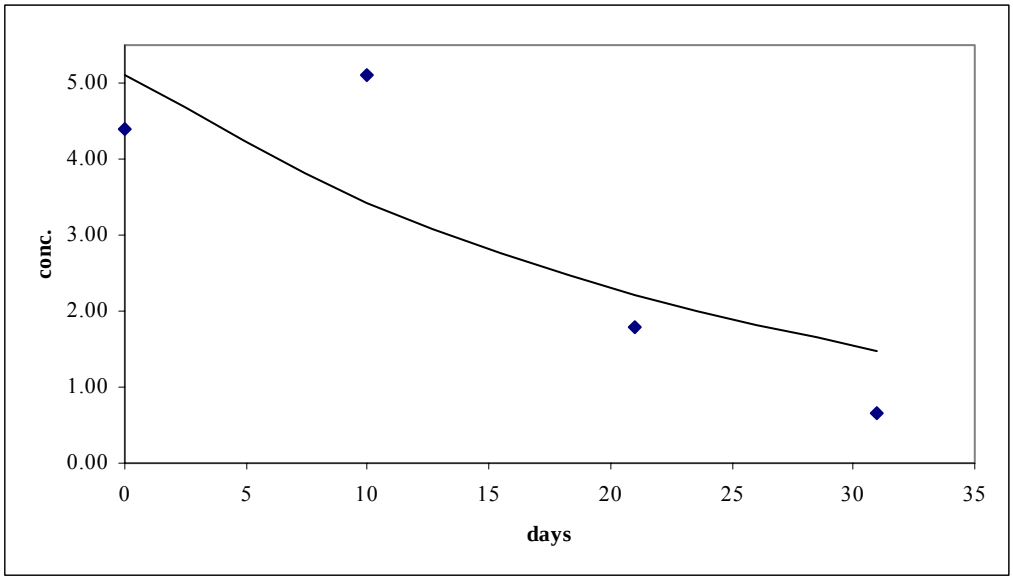
Time	*Residue*	*Residue calc.*	*residuals sq.*	*(% calc)2*
0	4.40	5.10	0.4942	26.0407
10	5.10	3.43	2.7950	11.7523
21	1.80	2.21	0.1707	4.8983
31	0.66	1.49	0.6836	2.2106
	totals	**149.6**	**4.1436**	**44.9**

Solver function

Co	5.1030
k	0.0398

half-life	**17**	**days**
DT90	**58**	**days**

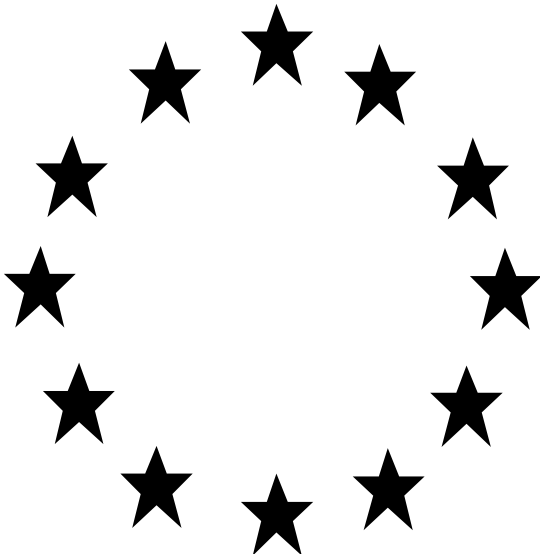
SSE =	4.1436
SST =	7.5014
R2 =	**0.448**



New references, by Annex point

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company, Report No. GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N	Owner
IIA, 5.1/06	Arndt, T., Dohn, D.	2004	Measurement of the half-life of thiophosgene in human blood. PTRL West, Inc., Report number 1146W (Company file: R-17121). GLP, Unpublished	Y	Makhteshim
IIA, 5.4/01	Clay, P.	2004	Folpet: in vivo mouse duodenum comet assay. [redacted] Report number [redacted]/SM1245/REG/REPT (Company file: R-17100). GLP, Unpublished.	Y	Makhteshim
IIA, 5.4.3/04	Collins, T.F.X	1972	Dominant lethal assay. II Folpet and difolatan. Division of toxicology FDA, *Fd Cosmet. Toxicol. Vol. 10, pp 363-371.* (Company file: R-545). Not GLP, Published.	N	Makhteshim
IIA, 5.6/01	Neal, B.	2004	Comments on folpet monograph Reproductive and developmental toxicity: section B 6.6 reproductive toxicity. [redacted] unpublished report 18 October 2004. Not GLP, Unpublished	N	Makhteshim
IIA, 5.8.1./01	Seilfried, H.E.	2000	Review: Toxicological risk characterisation of potential folpet metabolites. The toxicity profiles of phthalic and phthalamic acids and phthalimide – is there a significant risk from metabolite exposure. Consultants, report dated August 1, 2000 (Company file: R-12331). Not GLP, Unpublished.	Y	Makhteshim
IIA, 5.8.1./02	Gordon, E.	2005	Folpet. Toxicological significance of relevant degradates. Makhteshim, report dated March 21, 2005. Not GLP, Unpublished.	N	Makhteshim
IIA, 5.8.2./06	Moore, G.E., Creasey, D.	2004	Intestinal irritation in CD-1 mice after a 24-hour exposure to folpet. [redacted] Report number 13763 (Company file: R-16283). GLP, Unpublished.	Y	Makhteshim
IIA, 5.8.2./07	Wilson, A.S.	1990	A study of dermal penetration of ^{14}C-folpet: in the rat. [redacted] report number MAG/1/PH (Company file: R-5470). GLP, Unpublished.	Y	Makhteshim
IIA, 5.8.2./08	Gordon, E.	2005	Folpet: The appropriate dermal penetration factor for use in occupational risk assessment is zero percent, report February 18, 2005. Not GLP, Unpublished.	N	Makhteshim

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company, Report No. GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N	Owner
IIA, 5.10./01	Gordon, E.	2004	Folpet. A summary basis for why an acute reference dose (aRfD) is not needed. Submitted to the JMPR for the 2004 toxicological evaluation of folpet. Makhteshim-Agan . Not GLP, Unpublished.	N	Makhteshim



Final addendum to the

Draft Assessment Report (DAR)

- public version -

Initial risk assessment provided by the rapporteur Member State Italy for the existing active substance

FOLPET

of the second stage of the review programme referred to in Article 8(2) of Council Directive 91/414/EEC

Part 2

November 2005

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Folpet

Dossier According to Directive 91/414/EEC

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March 2005

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New references, by Annex point 17

Introduction

This document contains new information on residues submitted by Makhteshim Chemical Works Ltd to the RMS.

New information is presented here in the order of the Evaluation table for folpet, cross-referencing the Open point numbers and Reporting table numbers. New information is summarised under the dossier numbering system.

Document D1: Critical Good Agricultural Practice

The GAP is presented in the table below.

Critical Good Agricultural Practice for folpet in the EU

Crop	Member state or country	Product name	F, G or I[a]	Pests or group of pests controlled	Formulation		Application			Application rate per treatment			PHI (days)	Remarks:
					Type	Conc. of a.s.	method kind	growth stage	number[b] (max.)	kg a.s./hL (max.)	water L/ha	kg a.s./ha (max.)		
Winter wheat	South EU	'Folpan' 80 WDG	F	*Septoria* Brown rust	WG	800 g/kg	Foliar spray; down-ward	Up to Z65	2	0.375	200	0.75	42	
Tomatoes	South EU	'Folpan' 80 WDG	F	Various [c]	WG	800 g/kg	Foliar spray; down-ward	From beginning of fruit set	4	0.125	1000	1.25	7	
	South EU	'Folpan' 80 WDG	G	Various [c]	WG	800 g/kg	Foliar spray; down-ward	From beginning of fruit set	3	0.16	1000 - 1300	1.6	7	
Grapes	North and south EU	'Folpan' 80 WDG	F	Various [d]	WG	800 g/kg	Airblast foliar spray; upwards/ sideways	Shoot emergence to veraison	10	0.75	200 - 400	1.5	28	

[a] F= field; G = greenhouse.
[b] Sprays on all crops are applied typically at intervals of 7 to 28 days.
[c] *Alternaria solanum, Cladospora, Colletotrichum, Septoria, Botrytis*
[d] Black rot, *Botrytis cinerea phomosis*. *Plasmopara viticola*.

New information on residues

Evaluation table number	Reporting table number	Open Point number
3.1	**3(5)**	**-**

Conclusions of the EFSA Evaluation Meeting:
Notifier to provide hydrolysis studies in representative hydrolytic conditions

- **Point IIA, 6.5.1: Effects on the nature of the residue**

The following new report is submitted:

6.5.1/01

Report: Goodyear, A.P. (2004). Folpet. Position paper on effects on the nature of the residue. TSGE, unpublished report July 2004.

Guidelines: Not applicable.

GLP: No.

Material and methods: The DAR volume 1 concludes that a hydrolysis study in representative hydrolytic conditions is required. The requirement for a new study and the response to the data requirement are addressed in the position paper.

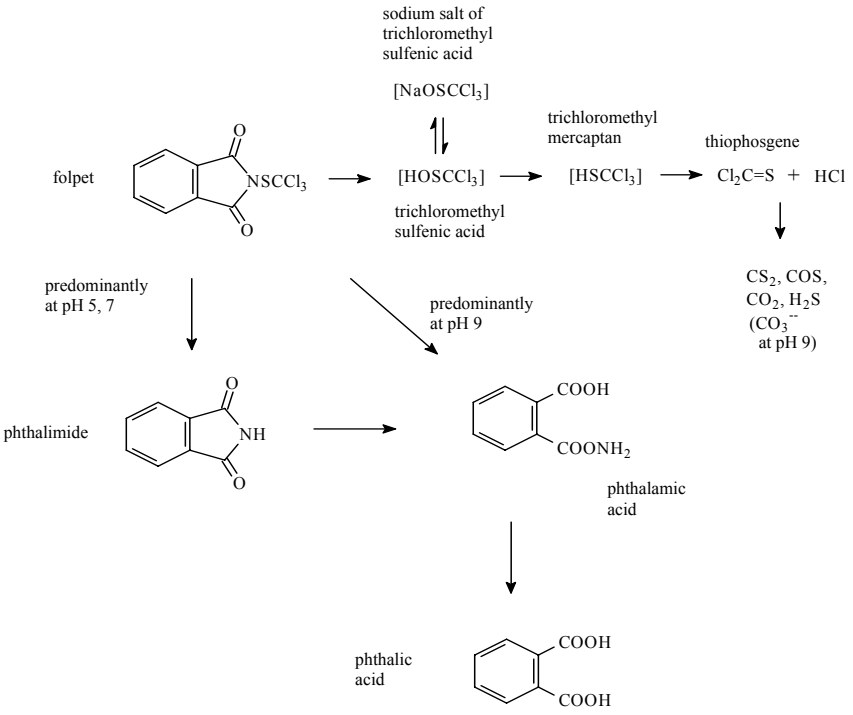
Findings:
The pathway of folpet hydrolytic degradation has been established in studies already presented.

In a study performed with [carbonyl-^{14}C] folpet (Point IIA, 7.2.1.1/01), the primary metabolite formed under acid conditions (pH5) was phthalimide, with phthalamic acid and phthalic acid also observed at lower levels. Under neutral conditions (pH7) the same metabolites were observed, but with the amounts formed shifted in favour of phthalic acid. No other significant degradation products were observed at either pH5 or PH7. In the study, phthalimide and phthalic acid were the only degradates accumulating at the end of the study indicating these compounds as the terminal hydrolysis products. Since phthalimide can be hydrolysed, via phthalamic acid, to phthalic acid it is concluded that phthalic acid is the stable end point of [carbonyl-^{14}C] folpet hydrolysis under acid and neutral conditions.

The metabolites phthalimide and phthalic acid are not considered to be of toxicological concern because they were found in both plants and animals and do not form part of the definition of the residue in crops.

In the study performed with [trichloromethyl-^{14}C] folpet (Point IIA, 7.2.1.1/02), the primary metabolite formed under acid and neutral conditions (pH5 and pH7) was carbon dioxide. Unidentified intermediate degradates were observed, but in biological systems these are expected to be readily converted to carbon dioxide. Therefore, it is concluded that carbon dioxide is the stable end point of [trichloromethyl-^{14}C] folpet hydrolysis under acid and neutral conditions. Therefore, no toxic metabolites will be formed.

The hydrolytic degradation of folpet is summarised as follows:



The pH conditions of the proposed simulated processing study (pH, 4, 5 and 6) would expose folpet residues to the same conditions as those described in the above tests. Therefore the stable hydrolytic end points (phthalic acid and carbon dioxide) are expected to be the same. The only effect of increased temperature in a simulated processing study will be to drive the hydrolytic reaction to its conclusion at a faster rate. Data are already available at pH4 and 100°C (Point IIA, 7.2.1.1/04) and these show that phthalimide degrades with a half-life of 5.5 hours, considerably longer than the incubation time required in the proposed tests. Therefore, studies under simulated processing conditions would only provide additional data on the rate of formation of the known degradation products and would not alter the route of degradation already established.

It is concluded that potentially toxic metabolites would not be formed during a simulated processing study and that a study of this type is therefore not considered necessary.

Conclusions: Sufficient data already exist to predict the effect of processing hydrolysis on the nature of the residue and therefore new studies are not required.

RMS comments

We feel that the studies on the nature of the residue are a key point to minimize consumer risks. The aim of the hydrolysis studies is to exclude that in "extreme" conditions potentially toxic metabolites of folpet are formed. Within the aim is to obtain information about unknown or unpredicted breakdown or reaction products which may require a separate risk assessment. This is therefore, by definition, to be addressed by specific studies.
Such specific studies are not available. **A hydrolysis study in representative hydrolytic conditions is therefore required**. This should be carried out with radiolabelled folpet exploring the following conditions:
90°C x 20 min (pH4), representative of pasteurization (i.e. for wine)
100°C x 60 min (pH5), representative of baking and boiling (i.e. for bread, and tomato paste)
120°C x 20 min (pH6), representative of sterilization (i.e. for tomato canned).

Evaluation table number	Reporting table number	Open Point number
3.2	**3(6)**	**-**

Conclusions of the EFSA Evaluation Meeting:
Notifier to provide a whole balance study for tomato washed, peeled and canned or used for juice, 3 follow-up studies in juice and canned tomato.

- **Point IIA, 6.5.2: Effects on the residue levels**

The following new report is submitted:

Tomatoes

6.5.2/07
Report: Pollmann, B. (2005). Determination of the residues of folpet in/on tomato and processed fractions after spraying of Folpan 80 WDG in Spain 2004. GAB, unpublished report 20044043/S1-FPTO.

Analytical methods

Residues of folpet were analysed by GC with ECD with a LOQ of 0.05 mg/kg.

Procedural recoveries

Mean procedural recoveries were within acceptable limits (70 to 110%).

Storage stability

Storage stability was tested as part of the study by spiking frozen untreated samples of tomato commodities (wet pomace, raw juice and canned fruit) with folpet at 1 mg/kg and storing deep frozen for 57 days. Residues of folpet were measured before and after storage. The results are summarised in **Table 6.5.2-1**.

Table 6.5.2-1 Stability of folpet in processed tomato commodities

Commodity	Storage period (days)	Residue[a] (mg/kg)	% of initial value
Wet pomace	0	0.828	100
	57	0.822	99
Raw juice	0	0.925	100
	57	0.731	79
Canned fruit	0	0.913	100
	57	0.733	80

[a] Mean of two samples

There no significant reduction in folpet residues in wet pomace, raw juice or canned fruit following freezer storage for 57 days (mean recovery following freezer storage was within 70% to 110% of Day 0 values). Folpet residues were therefore stable in tomato wet pomace, raw juice and canned fruit for at least 57 days following freezer storage.

In the study summarised here, the tomato samples were processed on the day of sampling and the processed commodities were stored deep frozen at approximately -20°C for up to 55 days prior to extraction/analysis. Therefore, no reduction in residue levels recorded in tomato commodities is expected to have occurred during storage.

Residue results

A study to investigate the effects on residue levels in tomato commodities after processing was carried out in Spain in 2004.

Residues in whole tomato fruit treated with folpet (4 sprays at 1.54 to 1.59 kg a.s./ha) were 0.63 mg/kg (PHI 10 days) (Table 6.5.2-2).

In the juice processing balance study, residues of folpet were reduced by washing (0.24 mg/kg). Residues of folpet were lower than the unwashed fruit in wet pomace (0.34 mg/kg) and raw juice (0.08 mg/kg). Residues were below the LOQ (< 0.05 mg/kg) in pasteurised juice.

In the canned fruit processing balance study, residues of folpet were reduced by washing (0.20 mg/kg). Residues of folpet were higher than the unwashed fruit in the peel plus peeling water (2.24 mg/kg) and below the LOQ in wet pomace, raw juice and canned fruit.

In the follow-up studies in pasteurised juice (three studies) and canned fruit (three studies), residues of folpet were less than the LOQ in all samples.

Table 6.5.2-2 Residues of folpet in processed tomatoes in Spain

Location Year Trial	Application				Portion analysed	PHI (days)	Folpet residue (mg/kg)	Ref.
	Formul-ation (type and a.s. content)	No.	kg a.s./ ha	kg a.s./ hL				
Spain 2004 S04W05 2R-A-002	WG, 800 g/kg	4	1.54-1.59	0.27	**Juice processing – balance study**			(IIA 6.5.2/ 07)
					whole fruit	10	0.63	
					washed fruit	10	0.24	
					washing water	10	0.15	
					wet pomace	10	0.34	
					raw juice	10	0.08	
					pasteurised juice	10	< 0.05	
					Juice processing – follow-up studies			
					pasteurised juice-1	10	< 0.05	
					pasteurised juice-2	10	< 0.05	
					pasteurised juice-3	10	< 0.05	
					Canned fruit processing – balance study			
					whole fruit	10	0.63	
					washed fruit	10	0.20	
					washing water	10	< 0.05	
					peeling water+peel	10	2.24	
					peeled fruit	10	< 0.05	
					wet pomace	10	< 0.05	
					raw juice	10	< 0.05	
					canned fruit	10	< 0.05	
					Canned fruit processing – follow-up studies			
					canned fruit-1	10	< 0.05	
					canned fruit-2	10	< 0.05	
					canned fruit-3	10	< 0.05	

Balance calculations

Balance calculations for processed tomato fractions based on folpet residues are presented in **Table 6.5.2-3**.

In the juice processing study, 38% of the residues remained in the washed fruit with 42% in the washing water. A total of 6% of the residue was distributed into the raw juice, 16% in the pomace and less than 2% in the pasteurised juice.

In the canned fruit processing study, 32% of the residues remained in the washed fruit. A total of 26% of the residue was distributed into the peel plus peeling water, with less than 6% in the peeled fruit and less than 2% in the wet pomace, raw juice and canned fruit.

Table 6.5.2-3 Balance calculations for processed tomato following applications of folpet

Process	Fraction	Residues folpet found (mg/kg)	Fraction weight (kg)	Total folpet residues (mg/kg)	Distribution of folpet residues (%)
Juice	whole fruit	0.63	4.51	2.84	100
	washed fruit	0.24	4.51	1.08	38
	washing water	0.15	8.00	1.20	42
	wet pomace	0.34	1.31	0.45	16
	raw juice	0.08	2.09	0.17	6
	pasteurised juice	< 0.05	1.13	< 0.06	< 2
Canned fruit	whole fruit	0.63	5.71	3.60	100
	washed fruit	0.20	5.71	1.14	32
	washing water	< 0.05	8.00	< 0.40	< 11
	peeling water+peel	2.24	0.41	0.92	26
	peeled fruit	< 0.05	4.03	< 0.20	< 6
	wet pomace	< 0.05	1.25	< 0.06	< 2
	raw juice	< 0.05	1.64	< 0.08	< 2
	canned fruit	< 0.05	1.10	< 0.06	< 2

Transfer factors

Transfer factors for tomato fruit to the human edible commodities pasteurised juice and canned fruit are summarised in Table 6.5.2-4. There was no concentration of residues in either processed tomato commodity.

Table 6.5.2-4 Transfer factor values for processed tomato following applications of folpet

Year	Study	Residue in whole fruit (mg/kg)	Pasteurised juice		Canned fruit	
			Residue (mg/kg)	Transfer factor	Residue (mg/kg)	Transfer factor
2004	Balance	0.63	< 0.05	< 0.1	< 0.05	< 0.1
	Follow-up 1	0.63	< 0.05	< 0.1	< 0.05	< 0.1
	Follow-up 2	0.63	< 0.05	< 0.1	< 0.05	< 0.1
	Follow-up 3	0.63	< 0.05	< 0.1	< 0.05	< 0.1

RMS comments

The studies are acceptable. Taking into account folpet, the transfer factor (TF) for tomato juice is <0.1, and the TF for canned tomatoes is <0.1.

Evaluation table number	Reporting table number	Open Point number
3.3	**3(7)**	**-**
Conclusions of the EFSA Evaluation Meeting: ***Notifier to provide 2 greenhouse residue trials for tomatoes.***		

- **Point IIA, 6.3: Residue trials**

Tomatoes

Folpet is recommended on tomatoes grown in the field (4 applications at 1.25 kg a.s./ha; PHI 7 days) and in greenhouses (3 applications at 1.6 kg a.s./ha; PHI 7 days). Residue trials were conducted in crops grown in both situations and greenhouse grown crops were identified as the 'worst-case' for residues, i.e. residues were higher in greenhouse grown crops compared to field grown crops following applications according to the EU GAP. This conclusion is presented in the DAR.

There were 10 trials in greenhouse grown tomatoes treated according to the EU GAP. Residue levels in fruit at harvest (PHI 7 days) ranged from 0.38 to 2.0 mg/kg. In four trials, samples were stored for periods longer than the period tested in a freezer storage stability study and so were not accepted by the RMS. Residue levels in the six trials accepted and summarised in the DAR also ranged from 0.38 to 2.0 mg/kg. The four greenhouse trials not accepted led to residue levels within the range of all the results.

A new freezer storage stability study in tomato fruit is underway to validate the residue studies in tomato which were not accepted by the RMS. This study will be available at the beginning of 2006.
The current EU MRL for tomato for folpet is 3 mg/kg. The results of new trials in field and greenhouse grown tomato conducted according to the EU GAP and summarised in the DAR support the existing MRL of 3 mg/kg and enable a risk assessment for consumers to be made.

Therefore, since a EU MRL for folpet in tomatoes already exists, and since the existing value of 3 mg/kg is supported by the results of 10 trials carried out under worst-case conditions for residues, i.e. under greenhouse conditions, (of which 6 are validated by freezer storage study), it is not necessary to set a new MRL for folpet in tomato as part of the EU review of folpet. Therefore, it is concluded that as sufficient information is available, additional residue trials in greenhouse grown tomatoes are not required for the EU review of folpet.

RMS comments

Ten trials in greenhouse grown tomatoes treated according to the EU GAP were originally presented. In four trials, samples were stored for periods longer (11 months) than the period tested in freezer storage stability studies (4 ½ months, with a recovery of the 53%) and so were not accepted (results relevant to the critical GAPs of the four trials were 0.55, 0.75, 1.2 and 1.4 mg/kg).
According to the applicant, new freezer storage stability study in tomato fruit is underway to validate the residue studies in tomato which were not accepted, and results will be available at the beginning of 2006.

The MRL for folpet in tomatoes of 3 mg/kg is therefore provisionally accepted, waiting for results of the above mentioned studies. In case stability is not confirmed, 2 greenhouse residue trials for tomatoes are still required.

Evaluation table number	Reporting table number	Open Point number
-	**3(12)**	**3.2**
Conclusions of the EFSA Evaluation Meeting: ***MS to discuss the residue definition for risk assessment in an expert meeting.*** ***RMS to prepare an assessment of the toxicological relevance of metabolites (including their contribution to the toxicological burden).***		
-	**3(13)**	**3.3**
Conclusions of the EFSA Evaluation Meeting: ***MS to discuss the residue definition for animal commodities, including the need for it, in an expert meeting.***		
-	**2(30)**	**2.13**
Conclusions of the EFSA Evaluation Meeting: ***MS to discuss the toxicity of the metabolites phthalimide and phthalic acid and their possible inclusion in the residue definition at an expert meeting.***		

- **Point IIA, 6.7: Proposed residue definition**

The proposed definition of the residue in plants and animals commodities is folpet alone.

The following new reports are submitted in support of the claim that is the relevant definition of the residue. These reports are also summarised in the new toxicological addendum under Point IIA 5.8.1.

- **Point IIA, 5.8.1: Toxicity studies of metabolites**

5.8.1/01

Report: Seilfried, H.E. (2000). Review: Toxicological risk characterisation of potential folpet metabolites. The toxicity profiles of phthalic and phthalamic acids and phthalimide – is there a significant risk from metabolite exposure. Consultants, unpublished report dated August 1, 2000 (Company file: R-12331).

Guidelines: Not applicable.

GLP: Not applicable.

Material and methods: The position paper includes summaries the toxicity findings of the folpet metabolites.

Findings:

Phthalamic acid, a major degradate when folpet undergoes hydrolysis, is the main metabolite following oral administration to rats. Phthalic acid is a minor metabolite. Phthalamic acid is the main metabolite in goats and phthalic acid is not seen in the urine but is present in the kidney. Phthalamic acid is hydrolyses to phthalic acid at acid pH. TOPKAT was used to

predict that phthalamic acid would have an acute oral rat LD_{50} of ~ 700 mg/kg bw, and would be negative in the Ames test. As a metabolite in the rat, animals are considered to have been exposed during oral toxicity studies. It is not possible to establish a risk level due to the lack of toxicological data on the compound itself, but based on the low toxicity of phthalate and phthalimide, the level of toxicity of phthalamic acid is expected to be low.

Phthalimide is an intermediate metabolite, capable of being metabolised to phthalamic acid, phthalate and possibly methylphthalate. It is not mutagenic in the Ames test, in yeast, mouse lymphoma assay or in a cytogenetic assay in human lymphocytes. There is conflicting evidence of teratogenic activity (resorptions and malformation after i.p injection, but no indication of teratogenicity in rats, rabbits or hamsters following oral administration). The weight of evidence suggests a low level of risk. TOPKAT was used to predict that phthalimide would have an acute oral rat LD_{50} of ~ 980 mg/kg bw, and would be negative in the Ames test.

Phthalic acid is not mutagenic in Ames or other bacterial assays, but does act synergistically with some but not all heterocyclic amine mutagens. It is not carcinogenic based on negative rodent bioassays with phthalic anhydride (which converts to phthalic acid). Phthalic acid does not accumulate in the body and is essentially cleared by 48 hours after oral administration. Phthalic acid is not teratogenic in rats. The purported activity on male and female reproductive systems in some less-than-robust studies is not well supported when all results are taken into consideration and the weight of evidence for all folpet metabolites is considered. TOPKAT was used to predict that phthalic acid would have an acute oral rat LD_{50} of ~ 2500 mg/kg bw, and would be negative in the Ames test.

The related compounds phthalic anhydride (which converts to phthalic acid in aqueous media) and phthalamide have been tested for carcinogenicity in rats and mice under a US Government testing programme. Neither compound showed increased incidence of tumours.

Phthalic acid is ubiquitous in the environment from industrial sources (used as plasticizers and in the production of polyester) and can be formed from environmental phthalate esters via hydrolysis where they can be found widely distributed, generally at low levels in air, rain water, sediment, soil and biota, food samples, and human and animal tissues.

Conclusions: Folpet metabolites have a very low level of hazard to humans when exposed through the diet and to the environment compared to parent folpet. The appropriate residue expression for folpet is folpet per se.

5.8.1/02

Report: Gordon, E. (2005). Folpet. Toxicological significance of relevant degradates. Makhteshim, unpublished report dated March 21, 2005.

Guidelines: Not applicable.

GLP: Not applicable.

Material and methods: The discussion paper expands on the discussion of the toxicological significance of the degradates of folpet.

Findings:

The degradates of folpet should not be included in the residue expression, as defined by the Guideline:

-Their basic toxicology

The physical and chemical properties of a chemical determine the nature and severity of effects in mammals. Folpet has an active moiety that is responsible both for its fungicidal properties and its toxicological effects in mammals. The degradates phthalimide, phthalamic acid and phthalic acid lack this active moiety and thus have a spectrum of effects distinct from their parent. These three degradates are not acutely toxic, are not developmental or reproductive toxins, are not mutagenic, are not carcinogenic, and do not exhibit any relevant systemic long term or sub-chronic toxicity.

The absence of significant toxicity is reflected in a Structure Activity Relationship (SAR) analysis for the three degradates of folpet (TOPKAT 2000). Where predictions could be made (i.e., where similar molecules/functional groups existed in the database with associated toxicity), low potential for mutagenicity and carcinogenicity were calculated. Interestingly, the SAR analysis did predict mutagenicity for folpet in the Ames Assay, providing some sense of validation for the analysis as folpet is an in vitro mutagen but not mutagenic in vivo (TOPKAT 2000).

-Their presence in significant amounts

The definition of "significant amounts" is not clear in the guideline; however, phthalimide is only present in the environment in a transient way, as it degrades further to phthalic acid via the intermediate phthalamic acid. Phthalamic acid has been shown not to be present in plants in "significant" amounts, based on laboratory studies.

Phthalic acid is present in the environment at relatively high levels, compared to the contribution expected with the agricultural use of folpet (Neyroud and Schnitzer 1977; Schnitzer 1977). This background level of phthalic acid is due to the industrial production of both phthalic acid and its anhydride (Slooff, Bont et al. 1994; Kleerebezem, Pol et al. 1999).

Inclusion of phthalic acid in the residue expression would confound the understanding of folpet residues present as the majority of phthalic acid found would be from sources other than folpet.

Conclusions:

The collective data on folpet degradates shows that the appropriate residue definition for folpet is the parent molecule, only, due to the lack of toxicity exhibited by these substances. This is in conformity with the conclusions of the JMPR and US EPA (FAO/WHO 1996; US-EPA 1999) This is in conformance with DG SANCO Guideline for Metabolism and Distribution in Plants (n° 7028/VI/95 rev.3, 22 July) note that:

Residues are expressed as parent compound if there are no metabolites or if the metabolites are known to be of no toxicological significance.

The metabolites present a significantly lower hazard to man than folpet, evidenced by the complete lack of systemic toxicity observed in the folpet long term and subchronic toxicity studies. In addition, direct comparisons of folpet and phthalimide and other metabolite aquatic toxicity further reinforces the differences due primarily to its

mode of action as a primary irritant. Key to resolving the differences in toxicity between folpet, phthalimide and other systemically circulating metabolites is the exceptionally rapid degradation of folpet in the presence of blood. As such, all systemic toxicity observed in folpet studies is attributed to the metabolites along with secondary effects of folpet's irritation of the GI tract.

The metabolites do not contribute to the overall toxicological burden.

RMS comments

The new evidences provided by the main data submitter seem to confirm that the metabolites of folpet phthalic acid, phthalamic acid and phthalimide are not of toxicological concern, in comparison to the parent compound folpet.
Conclusions are based on toxicological data, available for phthalic acid and phthalimide, and/or predictive models (TOPKAT) for phthalamic acid.

The residue definition in plants for risk assessment is therefore folpet alone (provisionally, waiting for results of the specific hydrolysis studies).

For animal commodities, as shown by table B.7.2.4 of the DAR, folpet is the only possible indicator, since other (possible) intermediate/s are rapidly transformed into natural compounds in muscle and milk. The need for a residue definition in animal commodities should be discussed during the next expert meeting.

- **Point IIA, 6.9 (open point 3.1): Acute dietary exposure**

Calculations of dietary exposure for assessing acute hazards posed by pesticide residues are based on consumption of a large portion of a single commodity containing residues assuming to be at the highest residue level detected (incorporating processing factors for processed commodities) .
An ARfD of 0.1 mg/kg bw has been proposed. Calculations of the acute dietary exposure (NESTI) for consumers were performed for adults and toddler by using UK models.

Acute intake estimates, termed National Estimates of Short-term Intake (NESTI), are calculated according to the recommendations of the PSD, on the basis of single day consumption data for adults and toddlers (UK registration handbook, 2001).

$$NESTI = \frac{\{U * HR\text{-}P * v\} + \{(F\text{-}U) * STMR\text{-}P\}}{\text{Mean body weight}}$$

Where:

U is the weight of the first commodity unit (kg)
F is the full portion consumption data (kg/person/day). Where F is less than or equal to U, then the second term of the equation drops out.
HR-P is the highest residue level detected (mg/kg), incorporating processing or edible portion factors.
v is the variability factor. It applies in case of commodities for which there may be a high variability of residue levels between the individual units within composite samples.
STMR-P is the supervised trials median residue in the edible portion, incorporating processing factors.

The NESTI values for folpet are presented in tables B.7.15.1.2.

Table B.7.15.1.1: Acute residue intake for folpet for adults (NESTI)

(ARfD =0.1 mg/kg bw)

Commodity	**U**	**HR-P**	**STMR-P**	**v**	**F**	**NESTI**	
	[kg]	[mg/kg]	[mg/kg]		[kg/person/day]	[mg/kg]	[%ARfD]
Adult (70.1 kg body weight)							
Grape	0.5	4.7	1.80	5	0.19	0.064	64.0
Tomatoes	0.085	2.0	0.83	7	0.157	0.018	17.8
Toddler 1½-4½ year-old (14.5 kg body weight)							
Grape	0.5	4.7	1.80	5	0.158	0.256	256.0
Tomatoes	0.085	2.0	0.83	7	0.093	0.082	82.2

Using the UK model for the determination of the acute intake, the ARfD for table grape is exceeded by the 807 % in toddler and by the 167% in adults.

Other values are 17.8% of the ARfD for tomatoes in adults and 82.2% of the ARfD for tomatoes in toddler. Contribution of wine (PF <0.1) and tomatoes processed (PF<0.1) and of wheat (HR = 0.02) were not assessed because considered not relevant.

EFSA Note: The calculations presented in table B.7.15.1.1 were conducted using the HR found in supervised trials. However the MRL is proposed to be fixed at higher level (5 for table grapes and 3 for tomatoes. At level of these MRLs, the short term exposures would be:

For Adults:

Grapes: 68% of the ARfD

Tomatoes: 29% of the ARfD

For Toddlers:

Grapes: 272% of the ARfD

Tomatoes: 124% of the ARfD

New references, by Annex point

Annex point / reference number	**Author(s)**	**Year**	**Title** **Source (where different from company)** **Company, Report No.** **GLP or GEP status (where relevant)** **Published or not**	**Data Protection Claimed** **Y/N**	**Owner**
IIA, 6.5.1./01	Goodyear, A.P.	2004	Folpet. Position paper on effects on the nature of the residue. TSGE report July 2004. Not GLP, Unpublished.	N	Makhteshim
IIA, 6.5.2./07	Pollmann, B.	2005	Determination of the residues of folpet in/on tomato and processed fractions after spraying of Folpan 80 WDG in Spain 2004. GAB, Report 20044043/S1-FPTO. GLP, Unpublished.	Y	Makhteshim
IIA, 5.8.1./01	Seilfried, H.E.	2000	Review: Toxicological risk characterisation of potential folpet metabolites. The toxicity profiles of phthalic and phthalamic acids and phthalimide – is there a significant risk from metabolite exposure. Consultants, report dated August 1, 2000 (Company file: R-12331). Not GLP, Unpublished.	Y	Makhteshim
IIA, 5.8.1./02	Gordon, E.	2005	Folpet. Toxicological significance of relevant degradates. Makhteshim, report dated March 21, 2005. Not GLP, Unpublished.	N	Makhteshim

Folpet

Dossier According to Directive 91/414/EEC

Summary Documentation

Tier II

Annex II and Annex III

Identity, Physical and chemical properties, Details of uses and further information, Methods of analysis

Addendum to dossier

April 2005

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Introduction

This document contains new information on identity, physical and chemical properties, details of uses and further information, and methods of analysis submitted by Makhteshim Chemical Works Ltd to the RMS.

New information is presented here in the order of the Evaluation table for folpet, cross-referencing the Open point numbers and Reporting table numbers. New information is summarised under the dossier numbering system.

Document D1: Critical Good Agricultural Practice

The GAP is presented in the table below.

Critical Good Agricultural Practice for folpet in the EU

Crop	Member state or country	Product name	F, G or I[a]	Pests or group of pests controlled	Formulation		Application			Application rate per treatment			PHI (days)	Remarks:
					Type	Conc. of a.s.	method kind	growth stage	number[b] (max.)	kg a.s./hL (max.)	water L/ha	kg a.s./ha (max.)		
Winter wheat	South EU	'Folpan' 80 WDG	F	*Septoria* Brown rust	WG	800 g/kg	Foliar spray; down-ward	Up to Z65	2	0.375	200	0.75	42	
Tomatoes	South EU	'Folpan' 80 WDG	F	Various [c]	WG	800 g/kg	Foliar spray; down-ward	From beginning of fruit set	4	0.125	1000	1.25	7	
	South EU	'Folpan' 80 WDG	G	Various [c]	WG	800 g/kg	Foliar spray; down-ward	From beginning of fruit set	3	0.16	1000 - 1300	1.6	7	
Grapes	North and south EU	'Folpan' 80 WDG	F	Various [d]	WG	800 g/kg	Airblast foliar spray; upwards/ sideways	Shoot emergence to veraison	10	0.75	200 - 400	1.5	28	

[a] F= field; G = greenhouse.
[b] Sprays on all crops are applied typically at intervals of 7 to 28 days.
[c] *Alternaria solanum, Cladospora, Colletotrichum, Septoria, Botrytis*
[d] Black rot, *Botrytis cinerea phomosis*. *Plasmopara viticola*.

New information on identity, physical and chemical properties, details of uses and further information, and methods of analysis

Evaluation table number	Reporting table number	Open Point number
-	1(1)	1.1
Conclusions of the EFSA Evaluation Meeting: *RMS to clarify whether [redacted] has to be regarded as a relevant impurity or not.*		

- **Point IIA, 1.10: Impurities**

CONFIDENTIAL BUSINESS INFORMATION:

available at RMS

Evaluation table number	Reporting table number	Open Point number
1.1	**1(6)**	**-**
Conclusions of the EFSA Evaluation Meeting: ***Notifier to provide data concerning the boiling point and temperature of decomposition, respectively.***		

- **Point IIA, 2.1.2 (Boiling point and temperature of composition)**

Test or study & Annex point	**Guideline and method**	**Test material and purity specification**	**Findings**	**Comments**	**GLP Y/N**	**Reference**
Boiling point (IIA 2.1.2)	Test substance decomposes below its boiling point – See Annex point IIA 2.1.3					
Decompo-sition or sublimation temperature (IIA 2.1.3)	EEC A.2 OECD 103	214-167-02 99.4%	The test substance decomposed above its melting point starting at 184°C.	-	Y	Turner, 2005

Evaluation table number	Reporting table number	Open Point number
1.2	**1(9)**	**1.5**

Conclusions of the EFSA Evaluation Meeting:
Notifier to submit the position paper: "Folpet. Position Paper on Residue Analytical Methods (May 2004)".

- **Point IIA, 4.2.1: Residues in and/or on plants, plant products, foodstuffs (of plant and animal origin), feedingstuffs**

4.2.1/08

Report: Burden, A.N. (2004). Folpet . Position paper on residue analytical methods. TSGE, unpublished report May 2004.

Guidelines: Not applicable.

GLP: No.

Material and methods: The DAR volume 1 concludes the following:
A number of crop residue methods were presented in the dossier. The methods have been validated for a range of crop types and the independent laboratory validation has confirmed the reproducibility of the methods between laboratories. Some modifications were made to the original procedures: these would not be expected to impact on the validity of the methods, but this aspect has not been fully investigated. Analytical methods are available for all of these crop groups, but confirmatory assays (based on the use of different detector systems, 8 different analytical columns or different elution conditions; Byast, 1996; Simek, 2002) have been provided only for wheat. No confirmatory assays for crops with high water, acid or oil content (including tomatoes and grapes, which are relevant crops included in the GAP). Independent laboratory validation has been performed for crops with high water content, high oil content and fruits with high acid content.

For animal tissues and milk, the method can be acceptable in principle, but requires independent laboratory validation and a confirmatory assay.

The position paper includes summaries of all the analytical methods, the validation data, a summary of the various chromatographic methods available for determination of folpet and the response to the data requirements/deficiencies.

Findings:

Confirmatory procedures for residues in plant products:
It is considered that residues may be confirmed using the many other chromatographic conditions presented for folpet residue determination (crops, soil, water, air). These methods are based on capillary GC with electron capture detection using a range of stationary phases of varying polarity and reverse-phase HPLC with either ultraviolet or diode array detection. The various conditions will be sufficient for use in confirmation of folpet residues. The guidance document SANCO/825/00 states that acceptable confirmatory techniques may be based on differences in the chromatographic principle (HPLC, GC), alternative detection, and different stationary and/or mobile phases. Therefore, it is considered unnecessary to conduct further work on confirmation when there are numerous existing chromatographic conditions available.

Determination of folpet residues in animal products:
It is considered that the analytical method described by Mende under Annex Point IIA, 4.2.1/06 has been adequately validated in all respects except that an independent laboratory validation has not been conducted. The comments above regarding confirmation for crop residue methods also apply to animal tissue methods - it is considered that residues may be confirmed using the many other chromatographic conditions presented for folpet residue determination (crops, soil, water, air). These methods are based on capillary GC with electron capture detection using a range of stationary phases of varying polarity and reverse-phase HPLC with either ultraviolet or diode array detection. The various conditions will be sufficient for use in confirmation of folpet residues. The guidance document SANCO/825/00 states that acceptable confirmatory techniques may be based on differences in the chromatographic principle (HPLC, GC), alternative detection, and different stationary and/or mobile phases. Therefore, it is considered unnecessary to conduct further work on confirmation when there are numerous existing chromatographic conditions available.

In any case, due to the absence of independent laboratory validation, it is considered appropriate to retract the original claim in the dossier that the method is suitable for monitoring purposes. However, further validation work is not required for the following reason.

The metabolism studies in goat demonstrated that residues of folpet in edible animal tissues following administration of a worst-case dietary concentration were below the limit of quantification. Therefore, feeding studies in ruminants are not required. Metabolism and feeding studies in poultry are not required as the dietary concentration of folpet is less than 0.1 mg/kg total diet as received. Consequently, MRLs for animal tissues, milk and eggs are not applicable. Therefore, an analytical method for monitoring purposes is not required under these circumstances (as defined by Commission Directive 96/46/EC) and the validity of the methods presented need not be evaluated. The method presented for determination of folpet in animal tissues, eggs and milk should be considered as supporting information for the methods dossier and any deficiencies in their validation are irrelevant.

Conclusions: No additional data are necessary to fulfil the Annex point requirement.

Evaluation table number	Reporting table number	Open Point number
-	**1(11)**	**1.6**

Conclusions of the EFSA Evaluation Meeting:
The need for further information regarding the flowability should be discussed in an expert meeting.

- **Point IIIA, 2.8.8.1: Flowability**

The results of the flowability test (granules agglomerated to an extent such that 15.2% was retained on a 5 mm sieve screen after 5 drops and 5.6% was retained after 20 drops) indicted that Folpan 80 WDG didn't remain fully flowable following storage under combined elevated temperatures and compression. The results do indicate that, to an extent, any agglomerates that formed were friable enough to be broken by simply dropping the sieve a distance of 1 cm.

The applicant contends that the flowability parameter has little practical importance in this case. When used, water dispersible granules are mixed with and dispersed in water. The important technical parameters for this procedure are suspensibility, dispersibility and wet sieve. The results of these tests were all acceptable according to the Draft SANCO document; 'Guidance document for the generation of data on the physical, chemical and technical properties of the plant protection products regulated under council directive 91/414/EEC'.

Evaluation table number	Reporting table number	Open Point number
-	**1(18)**	**1.9**
Conclusions of the EFSA Evaluation Meeting: ***The need for an analytical method for the determination of residues in surface water should be discussed in an expert meeting.*** ***Depending on the outcome of the fate and behaviour meeting, it could be that no analytical method for the determination of residues of folpet in surface water is required.***		

- **Point IIA, 4.2.3: Analytical method for determination of residues in water**

Discussion

Firstly, it is a reasonable assumption that the method presented, which is extremely sensitive for drinking water (LOQ = 0.02 μg/L) with a highly specific detection technique (UV photodiode array), will be directly applicable to surface water at relevant concentrations.

Of more relevance to the folpet dossier, it is concluded that the requirement of an analytical method for surface water may be waived, as confirmed by the reviewer from Germany "A method for residues in surface water is not required because of the low stability of Folpet (DT_{90} < 1 day)". The EU guidance document SANCO/825/00 states that analytical methods for residues in water are not necessary if the DT_{90} is less than three days.

It has been calculated from the hydrolysis data (presented originally in the dossier under IIA, 7.2.1.1) that the DT_{90} for folpet is in the range 51.5 seconds to 2.8 hours depending on pH. The DT_{90} values are newly calculated data that have not been previously submitted (see calculation details below). In addition, the results of the water/sediment study described under IIA, 7.2.1.3.2/01, demonstrated that folpet was not detectable in the surface water 24 hours after application.

Calculation of the hydrolysis DT_{90} for folpet

The hydrolysis degradation rate (DT_{90}) of folpet under sterile conditions in aqueous buffer at pH 5, 7 and 9 was calculated using data reported by Ruzo, L.O. and Ewing, A.D. (1988, Annex Point IIA, 7.2.1.1/01). These data are shown in the following table:

Sampling interval (hours)	Percent of applied radioactivity remaining as folpet	Sampling interval (hours)	Percent of applied radioactivity remaining as folpet	Sampling interval (seconds)	Percent of applied radioactivity remaining as folpet
pH 5		**pH 7**		**pH 9**	
0	89.7	0	90.6	15 – 30 [1]	59.5
1.0	76.7	0.5	60.1	70 – 71 [1]	47.2
3.0	49.3	1.0	50.5	131 – 147 [1]	27.2
5.0	28.5	2.0	26.8	191 – 196 [1]	16.0
9.5	9.7	3.0	24.6	366 – 371 [1]	4.4
24	0.5	4.0	17.3	611 – 613 [1]	0.3
-	-	8.0	3.0	-	-

[1] For the best fit determination the mean between the start time and the finish time was used.

The DT_{90} values were calculated using the Solver function in a Microsoft Excel spreadsheet to find the best fit between the experimental data and the following first order rate equation:

$C_T = C_0 \times \exp^{-KT}$

The line of best fit was determined by minimising the sum of the squares of the residuals between the actual data and the best fit line. This was achieved using the Solver function to change the values of C_0 and K and converge on a minimum value for the sum of the squares of the residuals. The rate constant, K, was then used to determine the DT_{90} value using the expression LN(10)/K.

The results obtained were as follows:

pH	DT_{90}	Co	K	R^2
5	2.8 hours	92.093	0.2210	0.996
7	1.1 hours	85.040	0.4945	0.971
9	51.5 seconds	72.137	0.0071	0.992

Conclusion

Therefore, it is concluded that, as degradation of folpet in water is extremely rapid, it would be practically impossible to monitor the active substance in the aquatic environment. Consequently, a monitoring method is not appropriate for folpet.

Evaluation table number	Reporting table number	Open Point number
1.3	**1(23)**	**-**
Conclusions of the EFSA Evaluation Meeting: ***Notifier to submit data regarding the purity and source (commercially available or not) of the starting material.***		

- **Point IIA, 1.8: Method of manufacture**

CONFIDENTIAL BUSINESS INFORMATION:

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Evaluation table number	Reporting table number	Open Point number
1.4	**1(24)**	**-**
Conclusions of the EFSA Evaluation Meeting: ***Notifier to justify the given specification for the impurities or submit a new one.***		

- **Point IIA, 1.11: Analytical profile of batches**

CONFIDENTIAL BUSINESS INFORMATION:

available at RMS

Evaluation table number	Reporting table number	Open Point number
1.5	**1(25)**	**-**

Conclusions of the EFSA Evaluation Meeting:
Data to confirm the identity of the impurities revealed by chemical analysis must be provided to address the requirement of the Directive on the specificity of the method(s).

- **Point IIA, 4.1.2: Methods for determination of impurities**

Specificity of the impurity methods has been adequately addressed in the dossier. Specificity was confirmed by comparison of chromatograms of certified analytical standards and blank solvent. Absence of interfering peaks is taken as confirmation of specificity.

Regarding identity of the impurities, this has been confirmed by the use of certified reference standards in the validation procedures. There is no sound scientific basis on which to reject this argument. Confirmation of the identity of the impurities is inherent in the proven specificity of the method. The Directive does not directly require any further confirmation of the identity of the impurities.

This conclusion is consistent with the opinions provided by the RMS.

New references, by Annex point

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company, Report No. GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N	Owner
IIA, 2.1.3/01	Turner, B.J.	2005	Folpet Boiling temperature. Huntingdon Life Sciences Ltd. Report number MAK 855/052248. GLP, Unpublished.	Y	Makhteshim
IIA, 4.2.1/08	Burden, A.N.	2004	Folpet . Position paper on residue analytical methods. TSGE report May 2004. Not GLP, Unpublished.	N	Makhteshim

Addendum
to the Draft Assessment Report

FOLPET

Volume 3
B.2 – B.5

May 2005

Rapporteur Member State: Italy

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New references, by Annex point 210

Introduction

This document contains new information on identity, physical and chemical properties, details of uses and further information, and methods of analysis submitted by Makhteshim Chemical Works Ltd to the RMS.

New information is presented here in the order of the Evaluation table for folpet, cross-referencing the Open point numbers and Reporting table numbers. New information is summarised under the dossier numbering system.

Document D1: Critical Good Agricultural Practice

The GAP is presented in the table below.

Critical Good Agricultural Practice for folpet in the EU

Crop	Member state or country	Product name	F, G or I[a]	Pests or group of pests controlled	Formulation		Application			Application rate per treatment			PHI (days)	Remarks:
					Type	Conc. of a.s.	method kind	growth stage	number[b] (max.)	kg a.s./hL (max.)	water L/ha	kg a.s./ha (max.)		
Winter wheat	South EU	'Folpan' 80 WDG	F	*Septoria* Brown rust	WG	800 g/kg	Foliar spray; down-ward	Up to Z65	2	0.375	200	0.75	42	
Tomatoes	South EU	'Folpan' 80 WDG	F	Various [c]	WG	800 g/kg	Foliar spray; down-ward	From beginning of fruit set	4	0.125	1000	1.25	7	
	South EU	'Folpan' 80 WDG	G	Various [c]	WG	800 g/kg	Foliar spray; down-ward	From beginning of fruit set	3	0.16	1000 - 1300	1.6	7	
Grapes	North and south EU	'Folpan' 80 WDG	F	Various [d]	WG	800 g/kg	Airblast foliar spray; upwards/ sideways	Shoot emergence to veraison	10	0.75	200 - 400	1.5	28	

[a] F= field; G = greenhouse.
[b] Sprays on all crops are applied typically at intervals of 7 to 28 days.
[c] *Alternaria solanum, Cladospora, Colletotrichum, Septoria, Botrytis*
[d] Black rot, *Botrytis cinerea phomosis*. *Plasmopara viticola*.

B.2 Physical and chemical properties

Evaluation table number	Reporting table number	Open Point number
1.1	**1(6)**	**-**

Conclusions of the EFSA Evaluation Meeting:
Notifier to provide data concerning the boiling point and temperature of decomposition, respectively.

- **Point IIA, 2.1.2 (Boiling point and temperature of composition)**

Test or study & Annex point	Guideline and method	Test material and purity specification	Findings	Comments	GLP Y/N	Reference
Boiling point (IIA 2.1.2)	Test substance decomposes below its boiling point – See Annex point IIA 2.1.3					
Decompo-sition or sublimation temperature (IIA 2.1.3)	EEC A.2 OECD 103	214-167-02 99.4%	The test substance decomposed above its melting point starting at 184°C.	-	Y	Turner, 2005

Conclusions: Study acceptable

- **Point IIIA, 2.8.8.1: Flowability**

Evaluation table number	Reporting table number	Open Point number
-	**1(11)**	**1.6**

Conclusions of the EFSA Evaluation Meeting:
The need for further information regarding the flowability should be discussed in an expert meeting.

The results of the flowability test (granules agglomerated to an extent such that 15.2% was retained on a 5 mm sieve screen after 5 drops and 5.6% was retained after 20 drops) indicted that Folpan 80 WDG didn't remain fully flowable following storage under combined elevated temperatures and compression. The results do indicate that, to an extent, any agglomerates that formed were friable enough to be broken by simply dropping the sieve a distance of 1 cm.
The applicant contends that the flowability parameter has little practical importance in this case. When used, water dispersible granules are mixed with and dispersed in water. The important technical parameters for this procedure are suspensibility, dispersibility and wet sieve. The results of these tests were all acceptable according to the Draft SANCO document; 'Guidance document for the generation of data on the physical, chemical and technical properties of the plant protection products regulated under council directive 91/414/EEC'.

Conclusions: acceptable.

B.5. Methods of Analysis

Evaluation table number	Reporting table number	Open Point number
1.2	**1(9)**	**1.5**

Conclusions of the EFSA Evaluation Meeting:
Notifier to submit the position paper: "Folpet. Position Paper on Residue Analytical Methods (May 2004)".

- **Point IIA, 4.2.1: Residues in and/or on plants, plant products, foodstuffs (of plant and animal origin), feedingstuffs**

4.2.1/08

Report: Burden, A.N. (2004). Folpet . Position paper on residue analytical methods. TSGE, unpublished report May 2004.

Guidelines: Not applicable.

GLP: No.

Material and methods: The DAR volume 1 concludes the following:
A number of crop residue methods were presented in the dossier. The methods have been validated for a range of crop types and the independent laboratory validation has confirmed the reproducibility of the methods between laboratories. Some modifications were made to the original procedures: these would not be expected to impact on the validity of the methods, but this aspect has not been fully investigated. Analytical methods are available for all of these crop groups, but confirmatory assays (based on the use of different detector systems, 8 different analytical columns or different elution conditions; Byast, 1996; Simek, 2002) have been provided only for wheat. No confirmatory assays for crops with high water, acid or oil content (including tomatoes and grapes, which are relevant crops included in the GAP). Independent laboratory validation has been performed for crops with high water content, high oil content and fruits with high acid content.

For animal tissues and milk, the method can be acceptable in principle, but requires independent laboratory validation and a confirmatory assay.

The position paper includes summaries of all the analytical methods, the validation data, a summary of the various chromatographic methods available for determination of folpet and the response to the data requirements/deficiencies.

Findings:

Confirmatory procedures for residues in plant products:
It is considered that residues may be confirmed using the many other chromatographic conditions presented for folpet residue determination (crops, soil, water, air). These methods are based on capillary GC with electron capture detection using a range of stationary phases of varying polarity and reverse-phase HPLC with either ultraviolet or diode array detection. The various conditions will be sufficient for use in confirmation of folpet residues. The guidance document SANCO/825/00 states that acceptable confirmatory techniques may be based on differences in the chromatographic principle (HPLC, GC), alternative detection, and different

stationary and/or mobile phases. Therefore, it is considered unnecessary to conduct further work on confirmation when there are numerous existing chromatographic conditions available.

Determination of folpet residues in animal products:
It is considered that the analytical method described by Mende under Annex Point IIA, 4.2.1/06 has been adequately validated in all respects except that an independent laboratory validation has not been conducted. The comments above regarding confirmation for crop residue methods also apply to animal tissue methods - it is considered that residues may be confirmed using the many other chromatographic conditions presented for folpet residue determination (crops, soil, water, air). These methods are based on capillary GC with electron capture detection using a range of stationary phases of varying polarity and reverse-phase HPLC with either ultraviolet or diode array detection. The various conditions will be sufficient for use in confirmation of folpet residues. The guidance document SANCO/825/00 states that acceptable confirmatory techniques may be based on differences in the chromatographic principle (HPLC, GC), alternative detection, and different stationary and/or mobile phases. Therefore, it is considered unnecessary to conduct further work on confirmation when there are numerous existing chromatographic conditions available.

In any case, due to the absence of independent laboratory validation, it is considered appropriate to retract the original claim in the dossier that the method is suitable for monitoring purposes. However, further validation work is not required for the following reason.

The metabolism studies in goat demonstrated that residues of folpet in edible animal tissues following administration of a worst-case dietary concentration were below the limit of quantification. Therefore, feeding studies in ruminants are not required. Metabolism and feeding studies in poultry are not required as the dietary concentration of folpet is less than 0.1 mg/kg total diet as received. Consequently, MRLs for animal tissues, milk and eggs are not applicable. Therefore, an analytical method for monitoring purposes is not required under these circumstances (as defined by Commission Directive 96/46/EC) and the validity of the methods presented need not be evaluated. The method presented for determination of folpet in animal tissues, eggs and milk should be considered as supporting information for the methods dossier and any deficiencies in their validation are irrelevant.

Conclusions: The notifier concludes that no additional data are necessary to fulfil the Annex point requirement. For what concerns the Methods of Analysis for residues in plants and Plant products, we disagree, because specificity, using a confirmatory method, must be provided for each method and representative matrices. The many chromatographic methods, based on GC with ECD using a range of stationary phases (and HPLC) have been applied to soil, water or air, but among crops, only to wheat, and not to crops with high water contents (tomatoes and grapes). In fact, in Appendix I of the Position Paper, it is clearly evident that a confirmatory assay, using 2 different GC capillary columns, has been applied only to cereals (Simek, 2002), and not to other representative matrices, which are very different in composition from cereals. For this reason specificity should be provided for tomatoes and grapes.
Regarding the Analytical methods for food of animal origin, conclusions are acceptable, since no MRLs are proposed.

- **Point IIA, 4.2.3: Analytical method for determination of residues in water**

Evaluation table number	Reporting table number	Open Point number
-	**1(18)**	**1.9**

Conclusions of the EFSA Evaluation Meeting:
The need for an analytical method for the determination of residues in surface water should be discussed in an expert meeting.
Depending on the outcome of the fate and behaviour meeting, it could be that no analytical method for the determination of residues of folpet in surface water is required.

Discussion

Firstly, it is a reasonable assumption that the method presented, based on a highly specific detection technique (UV photodiode array), is sufficiently sensitive for drinking water (LOQ = 0.02 μg/L). Anyway, it can not be directly applicable to surface water, because surface water is a more complex matrix than drinking water.

In addition, it has been concluded that the requirement of an analytical method for surface water may be reconsidered, as confirmed by the reviewer from Germany "A method for residues in surface water is not required because of the low stability of Folpet (DT_{90} < 1 day)". The EU guidance document SANCO/825/00 states that analytical methods for residues in water are not necessary if the DT_{90} is less than three days.

It has been calculated from the hydrolysis data (presented originally in the dossier under IIA, 7.2.1.1) that the DT_{90} for folpet is in the range 51.5 seconds to 2.8 hours depending on pH. The DT_{90} values have been newly calculated (the data have not been previously submitted, see calculation details below). In addition, the results of the water/sediment study described under IIA, 7.2.1.3.2/01, demonstrated that folpet was not detectable in the surface water 24 hours after application.

Calculation of the hydrolysis DT_{90} for folpet

The hydrolysis degradation rate (DT_{90}) of folpet under sterile conditions in aqueous buffer at pH 5, 7 and 9 was calculated using data reported by Ruzo, L.O. and Ewing, A.D. (1988, Annex Point IIA, 7.2.1.1/01). These data are shown in the following table:

Sampling interval (hours)	Percent of applied radioactivity remaining as folpet	Sampling interval (hours)	Percent of applied radioactivity remaining as folpet	Sampling interval (seconds)	Percent of applied radioactivity remaining as folpet
pH 5		**pH 7**		**pH 9**	
0	89.7	0	90.6	15 – 30 [1]	59.5
1.0	76.7	0.5	60.1	70 – 71 [1]	47.2
3.0	49.3	1.0	50.5	131 – 147 [1]	27.2
5.0	28.5	2.0	26.8	191 – 196 [1]	16.0
9.5	9.7	3.0	24.6	366 – 371 [1]	4.4
24	0.5	4.0	17.3	611 – 613 [1]	0.3
-	-	8.0	3.0	-	-

[1] For the best fit determination the mean between the start time and the finish time was used.

The DT_{90} values were calculated using the Solver function in a Microsoft Excel spreadsheet to find the best fit between the experimental data and the following first order rate equation:

$C_T = C_0 \times \exp^{-KT}$

The line of best fit was determined by minimising the sum of the squares of the residuals between the actual data and the best fit line. This was achieved using the Solver function to change the values of C_0 and K and converge on a minimum value for the sum of the squares of the residuals. The rate constant, K, was then used to determine the DT_{90} value using the expression LN(10)/K.

The results obtained were as follows:

pH	DT_{90}	Co	K	R^2
5	2.8 hours	92.093	0.2210	0.996
7	1.1 hours	85.040	0.4945	0.971
9	51.5 seconds	72.137	0.0071	0.992

Conclusions : **A**s degradation of folpet in water is extremely rapid, it would be practically impossible to monitor the active substance in the aquatic environment. Consequently, a monitoring method is not required for folpet.

New references, by Annex point

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company, Report No. GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N	Owner
IIA, 2.1.3/01	Turner, B.J.	2005	Folpet Boiling temperature. Huntingdon Life Sciences Ltd. Report number MAK 855/052248. GLP, Unpublished.	Y	Makhteshim
IIA, 4.2.1/08	Burden, A.N.	2004	Folpet . Position paper on residue analytical methods. TSGE report May 2004. Not GLP, Unpublished.	N	Makhteshim

Addendum
to the Draft Assessment Report

Folpet

Volume 4

Annex C

(Confidential Information)

May 2005

Rapporteur Member State: Italy

CONFIDENTIAL BUSINESS INFORMATION:

available at RMS

European Commission

Peer Review Programme

ECCO-Meetings

Folpet

Volume 3

Annex B
Addendum: definition of the residue

Rapporteur Member State: Italy

B.7.3 DEFINITION OF THE RESIDUE (ANNEX IIA 6.7; ANNEX IIIA 8.6).....211

B.7.15 REFERENCES RELIED ON 231

B.7.3 Definition of the residue (Annex IIA 6.7; Annex IIIA 8.6)

Folpet: The residue definition for the fungicide folpet should be folpet only as the metabolite phthalimide is neither of toxicological significance nor does it pose a significant dose to humans.

The collective data (toxicological data and residue data leading to estimated dose to humans) support the conclusion that the residue definition for folpet should be **folpet** only.

The DG SANCO Guideline notes (European Commission, 1997): Residue Definition – Of the three general considerations that are fundamental to the decision as to whether or not specific metabolites/degradation products should be included in the definition and expression of a residue, two are relevant to this discussion: (1) Their basic toxicology and (2) Their presence in significant amounts.

1) Phthalimide basic toxicology

Three lines of evidence show that the metabolite of folpet, phthalimide is not of toxicological significance:

a). Direct measurements of toxicity.

b). QSAR Analysis

c). Comparison of folpet and phthalimide in bioassays that are particularly sensitive to the toxicological properties of folpet.

a). Direct measurements of toxicity

Phthalimide is not acutely toxic. Its LD_{50} in mice is above 5 g/kg bw[10].

Phthalimide is not mutagenic. When tested in the multiple strains in the Ames Assay, it is negative (Riggin *et al.,* 1983).

Phthalimide is not a developmental toxin (Fabro *et al.,* 1964; Kennedy *et al.,* 1968).

b). QSAR Analysis

Phthalimide does not have structural alerts that indicate it poses a toxicological risk (Siegfried, 2000).

c). Comparison of folpet and its major metabolite in bioassays that are particularly sensitive to the toxicological properties of folpet.

The most sensitive bioassays for measuring toxicity of folpet are those involving aquatic organisms. This follows from the mode of action of folpet, which is irritation-based, due to its reaction with thiol groups.

In the case of rainbow trout, phthalimide is more than 3,000-fold less toxic than folpet (Bowman, 1988c), based on LC_{50} values, below. Bluegill sunfish are over 800-fold less sensitive to phthalimide than folpet (Bowman. 1989).

Test system	Folpet	Phthalimide	Ratio*
Trout, LC_{50}	0.015 mg/L	49 mg/L	3,266

[10] **(PAN Pesticides Database (2005). U.S. National Toxicology Program acute toxicity studies for Phthalimide (metabolite of folpet). http://www.pesticideinfo.org/List_NTPStudies.jsp?Rec_Id=PC40165.**

Bluegill, LC_{50}	0.047 mg/L	38 mg/L	809

*ratio of folpet toxicity to phthalimide toxicity: > 3,000 and > 800

The toxicity of folpet is entirely attributable to the reactive side chain of folpet which is not present in the phthalimide metabolite. The high reactivity of the side chain of folpet produces irritation to the tissues. Phthalimide has low activity and is not an irritant.

In conclusion, phthalimide poses no significant toxicological risk for adverse effects.

2) Their presence in significant amounts

The amount of phthalimide in milk and meat was determined in a goat metabolism study (Corden 1997a, 1997b). Goats were fed ^{14}C-folpet at 14 ppm labelled in the benzene ring for 6 days. Tissues were harvested and samples with 3% total radioactive residue or more were characterized. The majority of radioactivity was excreted in the urine and faeces. The following residue were analysed in meat and milk:

	Phthalimide[11]
Meat	<0.004 mg/kg
Milk	<0.001 mg/kg

The maximum possibly daily intake of phthalimide in milk and meat was calculated according to the worst scenarios for all consumer groups including toddlers and infants, which are the most sensitive consumer groups, and resulted with 0.0000 mg/kg bw/day (detailed calculations appear under point 2) c) below).

Considering the low toxicity of phthalimide and the zero exposure to human from animal products when calculated using conservative assumptions, there is no basis for rationally including phthalimide in the folpet residue expression.

In conclusion, the residue expression for folpet should be expressed as parent compound, folpet, only.

The references submitted in support of the above position are summarised below.

---

1) Phthalimide basic toxicology

a) Teratogenic activity of thalidomide and related compounds (Fabro, S., Schumacher, R. L., Smith, R. L. and Williams, R. T., 1964; IIA 7.3/01)

The paper tests a hypothesis that the teratogenic activity of thalidomide may be associated with the presence of a glutarimide ring in the molecule and interference in glutamic acid or glutamine metabolism. The significance of the glutarimide ring in the thalidomide molecule was investigated by testing other similar molecules, including the folpet metabolite phthalimide.

The study predated guidelines and was not to GLP. However, the study demonstrated that foetal malformations could be induced by a known positive control, and is considered valid.

Female rabbits of the New Zealand and Chinchilla strains were mated with males of the same strain and dosed orally by gavage with phthalimide at 150 mg/kg bw/day from day 7 to day 12 of pregnancy. Dams were killed on day 28 of pregnancy, and uterine parameters recorded. Foetuses were examined for external malformations only, especially those of the head and limbs. Other groups were dosed with other glutarimide-ring molecules, including thalidomide, at 150 mg/kg bw/day.

[11] **Other analytes measured included phthalamic acid, phthalic anhydride and phthalic acid (although the analytical method could not always separate these).**

There were 18 dams in the control group, 161 implantations, 13 resorptions and 148 externally normal foetuses (no malformations). Ten dams were given thalidomide, from which there were 78 implantations, 35 resorptions, 16 externally malformed and 27 externally normal foetuses. The malformations were typical of those induced by thalidomide. Of the three dams given phthalimide, there were 25 implantations, 3 resorptions and 22 externally normal foetuses (no external malformations).

The incidence of malformation was not increased in other molecules that contained the glutarimide ring. The results for phthalimide, control and other compounds are shown below (Table B.7.3.1).

Table B.7.3.1: Embryotoxic effects of phthalimide and other compounds in the rabbit

Compound	No. of animals (dams)	Implan-tations	Re-sorptions	Malformed foetuses	Normal foetuses
Control	**18**	**161**	**13**	**0**	**148**
Thalidomide	**10**	**78**	**35**	**16[a]**	**27**
3-Nitrothalidomide	4	40	9	1[b]	30
α-Aminoglutarimide	4	37	4	0	33
Hexahydrothalidomide	3	21	2	0	19
α-Succinimidoglutarimide	3	18	5	0	13
Phthalimide	**3**	**25**	**3**	**0**	**22**
1-Phthalimidobutane	5	49	6	0	43[e]
2-Phthalimidoacetamide	2	21	3	0	18
4-Phthalimidobutyramide	2	31	0	1[c]	30
α-Phthalimidoaspartimide	4	36	3	0	33
Phthalimidobenzene	7	65	4	2[d]	59
3-Phthalimidopyridine	4	40	4	0	36
2-Phthalimidoglutaric acid anhydride	6	64	12	0	52

[a] malformations of fore and hind limbs and cranioschisis typical for thalidomide
[b] cranioschisis.
[c] malformation of fore-limb- hook-like protrusion.
[d] malformation of fore-limb in one foetus, cyclopia in second foetus.
[e] one foetus with massive subcutaneous cranial haemorrhage, second foetus with large haemorrhage on left limb.

Conclusion: Maternal administration of phthalimide was not associated with increased incidence of resorptions or malformed foetuses when administered to rabbits during pregnancy.

b) An investigation of the teratogenic potential of captan, folpet, and difolatan (Kennedy, G., Fancher, O. E., and Calandra, J. C., 1968; IIA 7.3/02).

Study of effects of captan, folpet, the captan metabolite tetrahydrophthalimide (THPI), and the folpet metabolite phthalimide (PI) on the pregnant rabbit. Technical grade captan and folpet, and pure samples of THPI and PI were used. The related fungicide difoltan and the structurally similar drug thalidomide were also tested. The latter may be considered a positive control.

The study predated guidelines and was not to GLP. However, the study demonstrated that foetal malformations could be induced by a known positive control, and is considered valid.

Test materials were administered in gelatine capsules to groups of mated female Dutch Belted rabbits from day 6 to day 16 of pregnancy. Animals were weighed at three day intervals and

killed on day 29, when uterine contents were examined, and foetuses examined. Live foetuses were placed in an incubator for 24 hours after which they were killed and dissected. The carcasses were cleared and the skeleton stained with alizarin and examined. PI was administered at 75 mg/kg bw/day to a group of 10 females. Thalidomide was administered at 75.0 mg/kg bw/day to both strains of rabbit.

Maternal weight gains were not adversely affected by PI at 75.0 mg/kg/day, and there were no deaths.

Incidence of foetal resorptions was not adversely affected by PI administration.

One control foetus (of 105, from 17 litters) showed shortening and flexure of the forelimb. There were no malformations in the 63 foetuses from 10 dams treated with PI. Post-natal survival, crown-rump length, foetal weight and incidence of visceral and skeletal anomalies were not adversely affected by maternal treatment with PI. Thalidomide induced typical 'clubbing' (phocomelia) in 38 of 100 foetuses from 17 litters, demonstrating that the test system was capable of detecting malformations. The folpet metabolite phthalimide (PI) showed no malformed foetuses, and therefore no adverse effects on the developing rabbit foetus.

The results are summarised below (Table B.7.3.2).

Table B.7.3.2: Summary of effects of folpet, phthalimide and controls in rabbits

Compound	Oral dose (mg/kg)	No. of preg-nant females	Rabbit strain	No. of implants	No of resorp-tions	No of normal foetuses	No. (%) mal-formed foetuses	Mean litter size
Control	-	7	DB	52	0	51	1 (1.9)	7.4
	-	10	NZW	66	2	64	0 (0)	6.4
Thalidomide	75.0	7	BD	55	15	26	14 (35.0)	5.7
		10	NZW	74	10	40	24 (37.5)	6.4
Folpet	75.0	9	DB	66	0	65	1 (1.5)	7.3
	18.75	5	NZW	37	1	36	0 (0)	7.2
	37.5	5	NZW	35	11	24	0 (0)	4.8
	75.0	7	NZW	52	32	20	0 (0)	2.9
Phthalimide	75.0	10	DB	66	3	63	0 (0)	6.3

Conclusion: Phthalimide (PI) showed no adverse effects on the developing rabbit foetus.

c) Study of the cytogenetic activity of certain metabolites of a number of pesticides representing several classes of chemical compounds (Pilinskaya, M. A., 1986; IIA 7.3/03).

Phthalimide was tested in a human lymphocyte chromosome aberration assay.

The paper does not give sufficient detail to judge if the method was similar to recognised guidelines, but did give a positive result with some compounds, apparently demonstrating that the assay worked. The study was not to GLP.

Phthalimide was tested at 10,000, 1.0 and 0.1 μg/ml in 100, 200 and 200 metaphases, respectively, and 400 control metaphases were also evaluated. The frequency of metaphases with aberrations was not increased. Metabolites of the pesticides ziram, and betanal, tetramethylthiourea (TMTU) and methyl-3-hydroxyphenyl-carbamate (MHPC) respectively were positive in the assay. The compound methyl-benzimidazole-2-yl-carbamate (BMC), stated to be a metabolite of benomyl-type pesticides, produced hyperspiralisation of chromosomes and accumulation of mitoses.

Table B.7.3.3: Results of cytogenetic study

Concentration of substance (µg/mL)	No. of investigated metaphases	Frequency of aberrations (%)	Concentration of substance (µg/mL)	No. of investigated metaphases	Frequency of aberrations (%)
TMTU			**BMC**		
10,000	200	3.5*	200.00	200	2.00+
1.00	200	4.5	100.00	300	2.33+
0.10	200	6.00*	10.0	200	1.50
0.01	200	2.00	Control	400	2.33
Control	400	2.50			
Phthalimide			**MHPC**		
10,000	100	2.00	200.00	200	11.00***
1.00	200	1.50	100.00	200	3.00***
0.10	200	2.00	10.0	200	1.00
Control	400	2.00	Control	400	1.25

* $p < 0.1$
*** $p<0.05$
\+ a colchicine-type effect noted.

Conclusion: Phthalimide was not mutagenic in the human lymphocyte chromosome aberration assay.

d) Characterization of impurities in commercial lots of sodium saccharin produced by the Sherwin-Williams process (Riggin, R. M., Margard, W. L., and Kinzer, G. W., 1983; IIA 7.3/04).

Impurities and contaminants present or suspected to be present in commercial lots of the artificial sweetener saccharine, including Phthalimide, were tested in the Ames test.

The study was not performed to current guidelines, although it followed the method of Ames. The study was not to GLP.

A number of conflicting long-term animal feeding studies had been performed on the artificial sweetener saccharine, at levels up to 7.5% w/w diet. At such levels, the amount of impurities consumed may be significant, and the study was designed to investigate impurities and contaminants found in commercial lots of saccharine. The compounds were extracted using solvents, and the extracts (of all impurities/contaminants) subjected to the Ames test.

The origin of the impurities or contaminants was not always stated: several were stated to have appeared to have been derived from the polythene (polyethylene) materials used in packaging the lots. Insufficient quantities of the impurities could be obtained directly by solvent extraction for individual testing of each compound, and so various known or suspected saccharine contaminants were obtained and tested in the Ames test, at dose levels of 2000 or 400 µg/plate, using *S. typhimurium* strain TA98 with S-9 activation only. The mutagenicity was expressed as relative to the DMSO control.

The S-9 activation system was derived by injecting male rats (200g strain not specified) i.p. with 200 mg/mL Arclor 1254 in corn oil at 0.5 mg/g bodyweight. Rats were killed after 5 days, and the liver removed, homogenised in KCl and centrifuged for 10 minutes at 9000 g. The (S-9) supernatant was decanted and frozen. Samples were defrosted before use. The microsomal mix was prepared according to Ames and contained (per mL): S-9 (0.15 mL), $MgCl_2$ (8 µmole), KCl (33 µmole), glucose-6-phosphate (5 µmole), NADP (4 µmole), and sodium phosphate pH 7.4 (100 µmole). Fresh S-9 was prepared daily.

For the assay, a 0.1 mL aliquot of bacterial culture was added to 2 mL molten top agar, which was then mixed with 0.1-0.3 mL of sample solvent extractdissolved in DMSO. A 0.5 mL

aliquot of the S-9 mix was added to the agar immediately prio to pouring onto the plate. The poured top agar was allowed to solidify and the plates were incubated for 48 hours, after which the number of colonies were counted. Positive control (10 µg benzo[a]pyrene and a solvent (DMSO) blank control were assayed in triplicate.

Mutagenicity data for the potential contaminants in saccharine, including phthalimide, are summarised below (Table B.7.3.4).

Table B.7.3.4: Mutagenicity data for the potential contaminants in saccharine

Impurity	**Concentration (µg/plate)**	**Relative mutagenicity***
α-Sulphamoylbenzoic acid	400	1.2
	2000	0.9
α-Sulphobenzoic acid	400	1.0
	2000	0.8
α-Chlorobenzoic acid	400	0.9
	2000	0.6
6-Methylsaccharin	400	1.0
	2000	1.2
N-methylsaccharin	400	1.1
	2000	1.3
α-Toluenesulphonamide	400	0.7
	2000	1.2
Phthalimide	**400**	**1.0**
	2000	**0.9**
Methyl anthranilate	400	1.1
	2000	0.9
5-Chlorosaccharin	40	1.0
	200	0.9
	1000	0.8
Trioctyl phosphate	2000	0.7
Di-*tert*-butyl-*p*-benzoquinone	2000	0.7
α-Chlorobenzamide	2000	1.4
1,2-Benzisothiazolin-3-one	10	1.1
	100	toxic
3-Aminobenzisothiazole-1,2-dioxide	200	0.7
	1000	0.6
1,2-Benzisothiazoline-1,1-dioxide	200	0.6
	1000	0.6
Trichlorobenzene	133	1.0
	667	0.8

*Relative to DMSO control

The study did not give any information as to how phthalimide may be either an impurity or contaminant of saccharine. Phthalimide was not mutagenic in the assay, with relative mutagenicity of 1.0 and 0.9 compared to controls (DMSO). None of the other impurities/contaminants were positive in the assay, although one was stated to be toxic to the bacteria. The study found that the solvent-extracted impurities/contaminants exhibited a low level of mutagenicity, despite also demonstrating that the individual compounds, tested separately, showed no mutagenic activity. The study also showed that acetone extraction did not show mutagenic activity, but that chloroform/methanol extracts showed low levels of mutagenicity. The study concentrates on assays of batches of saccharine and on analysis of various solvents to try and determine the origin of the initial mutagenic activity, after concluding that the impurities normally present in saccharine were not responsible for the mutagenic activity seen in the initial solvent extractions. These data are not relevant to phthalimide. The authors concluded that as large amounts of solvent were required to extract

the impurities/contaminants, that contamination of the solvents themselves may be responsible for the mutagenic activity seen.

Conclusion: Phthalimide was not mutagenic in the Ames test, when tested in strain TA98 with metabolic activation.

e) Review: Toxicological risk characterisation of potential folpet metabolites. The toxicity profiles of phthalic and phthalamic acids and phthalimide – is there a significant risk from metabolite exposure? (Siefried, H.E., 2000; IIA 7.3/05). [This report was previously submitted with the toxicology addendum in March 2005.]

The position paper includes summaries the toxicity findings of the folpet metabolites. Phthalamic acid, a major degradate when folpet undergoes hydrolysis, is the main metabolite following oral administration to rats. Phthalic acid is a minor metabolite. Phthalamic acid is the main metabolite in goats and phthalic acid is not seen in the urine but is present in the kidney. Phthalamic acid is hydrolyses to phthalic acid at acid pH. TOPKAT was used to predict that phthalamic acid would have an acute oral rat LD_{50} of ~ 700 mg/kg bw, and would be negative in the Ames test. As a metabolite in the rat, animals are considered to have been exposed during oral toxicity studies. It is not possible to establish a risk level due to the lack of toxicological data on the compound itself, but based on the low toxicity of phthalate and phthalimide, the level of toxicity of phthalamic acid is expected to be low.

Phthalimide is an intermediate metabolite, capable of being metabolised to phthalamic acid, phthalate and possibly methylphthalate. It is not mutagenic in the Ames test, in yeast, mouse lymphoma assay or in a cytogenetic assay in human lymphocytes. The weight of evidence suggests a low level of risk. TOPKAT was used to predict that phthalimide would have an acute oral rat LD_{50} of ~ 980 mg/kg bw, and would be negative in the Ames test.

Phthalic acid is not mutagenic in Ames or other bacterial assays, but does act synergistically with some but not all heterocyclic amine mutagens. It is not carcinogenic based on negative rodent bioassays with phthalic anhydride (which converts to phthalic acid). Phthalic acid does not accumulate in the body and is essentially cleared by 48 hours after oral administration. Phthalic acid is not teratogenic in rats. The reported activity on male and female reproductive systems in some less-than-robust studies is not well supported when all results are taken into consideration and the weight of evidence for all folpet metabolites is considered. TOPKAT was used to predict that phthalic acid would have an acute oral rat LD_{50} of ~ 2500 mg/kg bw, and would be negative in the Ames test.

The related compounds phthalic anhydride (which converts to phthalic acid in aqueous media) and phthalamide have been tested for carcinogenicity in rats and mice under a US Government testing programme. Neither compound showed increased incidence of tumours.

Phthalic acid is ubiquitous in the environment from industrial sources (used as plasticizers and in the production of polyester) and can be formed from environmental phthalate esters via hydrolysis where they can be found widely distributed, generally at low levels in air, rain water, sediment, soil and biota, food samples, and human and animal tissues.

In conclusion, phthalimide together with other folpet metabolites metabolites, has a very low level of hazard to humans when exposed through the diet and to the environment compared to parent folpet. The appropriate residue expression for folpet is folpet per se.

2) Their presence in significant amounts

a) *^{14}C-folpet metabolism in the lactating goat (part A). ^{14}C- trichloromethyl folpet: material balance of dosed radioactivity. (Cordon, M.T. 1997a; Annex IIA, 6.2/01; IIA 7.3/06)*

NOTE: The summary below already appears in the DAR under B.7.2.a Metabolism, distribution and expression of residues in livestock (Annex IIA 6.2 and Annex IIIA 8.1).

[Trichloromethyl-^{14}C] folpet (radiochemical purity 99.3%) dissolved in dichloromethane was administered in gelatine capsules orally once daily for three consecutive days to a miniature lactating goat at a measured dietary concentration of 20 mg/kg diet. Milk was collected twice a day, from one day prior to dosing until sacrifice, urine and faeces were collected from one day prior to dosing until sacrifice and expired air was collected in potassium hydroxide traps. The goat was sacrificed 23 hours after the final dose. Radioactivity was determined in excreta, tissues, milk, gastrointestinal tract, cage wash and expired air by LSC and combustion/LSC.

The total recovery of radioactivity was 102%, of which 31.4% was recovered in air traps, 41.9% in faeces, 16.9% in the gastrointestinal tract and 10.2% in the urine. Very low levels of ^{14}C radioactivity were found in milk (1.0% of administered dose) and tissues (0.8% of administered dose). Significant residues were found in the liver (0.5% of administered dose, equivalent to 0.34 mg folpet equivalents/kg), kidney (0.1% of administered dose, equivalent to 0.26 mg folpet equivalents/kg), muscle (0.2% of administered dose, equivalent to 0.04 mg folpet equivalents/kg) and fat (< 0.1% of administered dose, equivalent to 0.01 mg folpet equivalents/kg).

The distribution of applied radioactivity is given in Table B.7.3.5.

Table B.7.3. 5: Distribution of ^{14}C following oral administration of [trichloromethyl-^{14}C] folpet to a lactating goat for three days

Matrix/tissue	% Applied dose	Residue (mg folpet equivalents/kg or L)
Tissues & milk		
subcutaneous fat	< 0.1	0.01
peritoneal fat	< 0.1	0.01
muscle (fore)	0.1	0.03
muscle (rump)	0.1	0.04
kidney	0.1	0.26
liver	0.5	0.34
milk 0-24 hr	0.2	0.23
milk 24-48 hr	0.4	0.38
milk 48-71 hr	0.4	0.34
total	1.8	-
Urine		
0-24 hr	2.1	-
24-48 hr	0.6	-
48-71 hr	6.4	-
bladder	1.1	-
total	10.2	
Faeces		
0-24 hr	8.7	-
24-48 hr	11.5	-
48-71 hr	21.7	-
total	41.9	-
Expired $^{14}CO_2$		
0-12 hr	6.8	-
12-24 hr	2.0	-
24-36 hr	7.9	-
36-48 hr	3.6	-
48-60 hr	8.9	-
60-71 hr	2.2	-
total	31.4	
Gastrointestinal tract		
intestine	10.8	-
rumen & reticulum	5.7	-
omasum & abomasum	0.4	-
total	16.9	
Bile	< 0.1	
Cage wash	0.2	-
Total	**102**	-

b) ^{14}C-folpet metabolism in the lactating goat (part B). (Cordon, M.T. 1997b; IIA, 6.2/02; IIA 7.3/07)

NOTE: The summary below already appears in the DAR under B.7.2.b Metabolism, distribution and expression of residues in livestock (Annex IIA 6.2 and Annex IIIA 8.1).

[Trichloromethyl-^{14}C] folpet (radiochemical purity 97%) and [U-phenyl -^{14}C] folpet (radiochemical purity 98%) dissolved in dichloromethane, were each administered to separate miniature lactating goats. Administration was in gelatine capsules orally once daily for six consecutive days at a measured dietary concentration of 24 mg/kg diet and 14 mg/kg diet for the [trichloromethyl-^{14}C] folpet and [U-phenyl -^{14}C] folpet, respectively. Milk was collected twice a day from one day prior to dosing until sacrifice. Urine and faeces were collected from

one day prior to dosing until sacrifice. The goat was sacrificed 23 hours after the final dose. Radioactivity was determined in excreta, tissues, milk, gastrointestinal tract and cage wash by LSC and combustion/LSC. Metabolites were characterised by TLC.

Following administration of [trichloromethyl-^{14}C] folpet, the majority of the administered radioactivity was excreted and recovered in the faeces and urine. The distribution results were comparable to those recorded in the distribution study (Cordon, M.T. 1997a). Significant residues were found in the kidney (0.16 mg folpet equivalents/kg), liver (0.25 mg folpet equivalents/kg), muscle (0.02 mg folpet equivalents/kg) and milk (up to 0.20 mg folpet equivalents/L). Residues in milk plateaued approximately 4 days after the start of administration. Residues in fat were less than 0.01 mg folpet equivalents/kg. The distribution of applied radioactivity is given in Table B.7.3.6.

Table B.7.3.6: Distribution of ^{14}C following oral administration of [trichloromethyl-^{14}C] folpet to a lactating goat for six days

Matrix/tissue	% Applied dose	Residue (mg folpet equivalents/kg or L)
Tissues & milk		
subcutaneous fat	< 0.1	< 0.01
peritoneal fat	< 0.1	< 0.01
muscle (fore)	< 0.1	0.02
muscle (rump)	< 0.1	0.03
liver	0.2	0.25
kidney	< 0.1	0.16
milk 0-24 hr	< 0.1	0.098
milk 24-48 hr	0.1	0.163
milk 48-72 hr	0.1	0.174
72-96 hr	0.1	0.177
96-120 hr	0.1	0.203
120-143 hr	0.1	0.192
total	0.7	-
Urine		
0-24 hr	0.5	-
24-48 hr	1.0	-
48-72 hr	0.5	-
72-96 hr	1.6	-
96-120 hr	0.7	-
120-143 hr	0.4	-
bladder	0.1	-
total	4.8	
Faeces		
0-24 hr	0.5	-
24-48 hr	5.3	-
48-72 hr	6.6	-
72-96 hr	12.7	-
96-120 hr	8.5	-
120-143 hr	1.3	-
total	34.9	-
Bile	< 0.1	
Cage wash	0.2	-
Total	**40.6**[a]	-

[a] Plus 31.4% present in expired air, 16.9% present in gastrointestinal tract (see Point 6.2/01).

Following administration of [U-phenyl -^{14}C] folpet, the majority of the administered radioactivity was recovered in the faeces (34.9%) and urine (58.3%), with small quantities in the cage wash (2.1%) and tissues plus milk (< 0.1%). The overall recovery was 95.3% of the administered dose. Significant residues were found in the kidney (0.05 mg folpet equivalents/kg) and liver (0.02 mg folpet equivalents/kg). Residues in muscle and fat were less than 0.01 mg folpet equivalents/kg; residues in milk were less than 0.01 mg folpet equivalents//L. The distribution of applied radioactivity is given in Table B.7.3.7.

Table B.7.3.7: Distribution of ^{14}C following oral administration of [U-phenyl -^{14}C] folpet to a lactating goat for six days

Matrix/tissue	% Applied dose	Residue (mg folpet equivalents/kg or L)
Tissues & milk		
subcutaneous fat	< 0.1	0.004
peritoneal fat	< 0.1	< 0.001
muscle (fore)	< 0.1	0.003
muscle (rump)	< 0.1	0.003
liver	< 0.1	0.022
kidney	< 0.1	0.052
milk 0-24 hr	< 0.1	0.004
milk 24-48 hr	< 0.1	0.006
milk 48-72 hr	< 0.1	0.005
72-96 hr	< 0.1	0.005
96-120 hr	< 0.1	0.005
120-143 hr	< 0.1	0.006
total	< 0.1	-
Urine		
0-24 hr	9.2	-
24-48 hr	12.1	-
48-72 hr	8.7	-
72-96 hr	6.4	-
96-120 hr	11.2	-
120-143 hr	10.7	-
total	58.3	
Faeces		
0-24 hr	1.4	-
24-48 hr	6.4	-
48-72 hr	7.7	-
72-96 hr	6.1	-
96-120 hr	6.3	-
120-143 hr	7.0	-
total	34.9	-
Bile	< 0.1	
Cage wash	2.1	-
Total	**95.3**	-

Following administration of [trichloromethyl-^{14}C] folpet, thiazolidine was found in the urine and faeces at 17.4% and 2.9%, respectively, of the radioactivity (equivalent to 0.8% and 1.0% of the administered radioactivity, respectively). Low levels of unmetabolised folpet were found only in the faeces (8.0% of the radioactivity, equivalent to 2.8% of the administered radioactivity). Folpet was extensively metabolised in tissues and the radiolabelled carbon was incorporated into naturally occurring compounds. These were amino acids (in the liver, kidney, milk, muscle), glucose and fats (in the liver), cholesterol (in the kidney) and lactose (in the milk).

Following administration of [U-phenyl -^{14}C] folpet, phthalamic acid was the major constituent of the urine (84.8% of the radioactivity, equivalent to 49.4% of the administered radioactivity). The faeces contained phthalimide (26.4% of the radioactivity, equivalent to 9.2% of the administered radioactivity) and a small amount of unmetabolised folpet (0.9% of the radioactivity, equivalent to 0.3% of the administered radioactivity). The majority of the radioactivity in the faeces was unextracted. The major metabolites in liver, kidney and milk were phthalimide and either phthalamic acid, phthalic anhydride or phthalic acid. No folpet was detected in tissues or milk.

The characterisation of radioactivity is summarised in Table B.7.3.8.

Table B.7.3.8: Characterisation of ^{14}C radioactivity in tissues, milk and excreta following administration of folpet to a lactating goat for six days

Identity of residue	% ^{14}C radioactivity (% of dosed radioactivity)										
	liver		kidney		urine		faeces		milk		muscle
	1	2	1	2	1	2	1	2	1	2	2
folpet	-	-	-	-	-	-	0.9 (0.3)	8.0 (2.8)	-	-	-
thiazolidine	-	-	-	-	-	17.4 (0.8)	-	2.9 (1.0)	-	-	-
phthalamic acid	27.8	-	69.1[c]	-	84.8 (49.4)	-	-	-	7.2[c]	-	
phthalimide	2.6	-	0.7	-	-	-	26.4 (9.2)	-	5.8	-	
natural compounds[a]	-	26.9	-	19.2					-	52.7	35.8
unknowns[b]	7.2 {12} [1.3]	10.8 {8} [3.5]	3.6 {5} [1.3]	20.5 {9} [8.7]	9.4 {4} [4.6]	33.3 {4} [13.3]	0.3	1.0 {1}	-	3.6 {3} [3.0]	10.9 {5} [6.0]
baseline	23.1	9.6	10.1	10.3	0.8	38.1	3.0	< 0.1	-	6.8	2.3
remainder	5.7	9.8	11.6	21.2	-	-		-	6.6	11.6	12.8
unextracted residue	-	10.0	4.4	-	-	-	68.0	87.0	4.1	15.9	31.8
other[d]	33.6	59.8	0.9	28.7	5.1	11.2	1.4	1.2	76.3	8.7	6.4

1 = [U-phenyl -^{14}C] folpet, 2 = [trichlormethyl-^{14}C] folpet.
[a] Amino acids, cholesterol, glucose, lactose, etc.
[b] Value in {} parenthesis = number of unknown components which make up the total radioactive residue; value in [ ] parenthesis = % of total radioactive residue represented by the major unknown component.
[c] Includes phthalic anhydride and phthalic acid.
[d] Unanalysed and losses during work-up.
Values in ( ) parenthesis are % of dosed radioactivity.

c) Dietary Risk assessment of Folpet Metabolite: Phthalimide

The amount of phthalimide in milk and meat was determined in a goat metabolism study (Corden 1997a, 1997b). Goats were fed ^{14}C-folpet at 14 ppm labelled in the benzene ring for 6 days. Tissues were harvested and samples with 3% total radioactive residue or more were characterized. The majority of radioactivity was excreted in the urine and faeces.

	Phthalimide
Meat	<0.004 mg/kg
Milk	<0.001 mg/kg

Estimation of the potential and actual exposure of phthalimide through animal products diet

Chronic exposure

Theoretical Maximum Daily Intake (TMDI)

The TMDI is calculated by multiplying the MRL or actual residues by the estimated average daily consumption for a given food commodity.

TMDI = ∑ MRL x F

where:
MRL = Maximum residue limit or actual residues for a given food commodity
F = Consumption of that food commodity.

This calculation is performed using:

1) An International diet (European Region) based on data from the World Health Organisation (WHO)[12].
2) The UK Dietary model (PSD, 1999[13])

<u>WHO European diet</u>

The TMDI calculation is presented in Table B.7.3.9.

[12] **WHO (1989). Guidelines for predicting dietary intake of pesticide residues. Prepared by the joint UNEP/FAO/WHO Food Contamination Monitoring Programme in collaboration with the Codex Committee on Pesticide Residues. World Health Organisation, Geneva.**

[13] **PSD (1999). Guidance on the estimation of dietary intakes of pesticides residues. The Registration Handbook. Pesticides Safety Directorate, Ministry of Agriculture, Fisheries and Food.**

Table B.7.3.9: TMDI calculation for Phthalimide based on WHO diet

Commodity	Phthalimide (mg/kg)	Consumption (kg/person/day)	TMDI (mg/person/day)
Total milk	< 0.001 (0.0005*)	0.3408	0.0002
Cattle meat	< 0.004 (0.002*)	0.0633	0.0001
Total			**0.0003**

*Since phthalimide residues were below the LOQ of the analytical method used, one half of the LOQ as worst case scenario was taken into consideration as appear in the brackets.

The total TMDI of Phthalimide is 0.0003 mg/person/day day or 0.0000 mg/kg bw/day for a 60 kg adult.

UK diet

UK consumption data for adults, children, toddlers and infants (mean consumers and high, i.e. 97.5th percentile, consumers) are presented in Table B.7.3.10

Table B.7.3.10: UK consumption data for adults, children, toddlers and infants

Commodity	5. Consumption data (kg/day)							
	Adults 6. (70.1 kg bw)		Children 7. (43.6 kg bw)		Toddlers 8. (14.5 kg bw)		Infants 9. (8.7 kg bw)	
	Mean	High [1]	Mean	High	Mean	High	Mean	High
Milk	0.2573	0.6659	0.0304	0.6745	0.3064	0.8017	0.33775	0.8719
Meat	0.0841	0.2050	0.0641	0.1339	0.0276	0.0869	0.1339	0.0121

The TMDI for Phthalimide was calculated for all consumer groups of milk and meat (high consumption intake).

Table B.7.3.11: consumption of Phthalimide by adults, children, toddlers and infants based on UK high consumption intakes

Commodity	Phthalimide (mg/kg)	10. TMDI (mg/kg bw/day)			
		Adults 11. (70.1 kg bw)	Children 12. (43.6 kg bw)	Toddlers 13. (14.5 kg bw)	Infants 14. (8.7 kg bw)
Milk	0.0005	0.0000	0.0000	0.0000	0.0000
Meat	0.002	0.0000	0.0000	0.00001	0.0000
Total exposure		**0.0000**	**0.0000**	**0.0000**	**0.0000**

The TMDIs of Phthalimide in all consumer groups including toddlers and infants, which are the most sensitive consumer groups, is 0.0000 mg/kg bw/day.

Comparison of TMDI of phthalimide with the ADI

The TMDI values for different consumer groups and diets are summarised in Table B.7.3.12.

Table B.7.3.12: TMDI values for different consumer groups and diets

Diet	15. Body weight (kg)	16. TMDI 17. (mg/kg bw/day)
WHO adult	60	0.0000
UK adult	70.1	0.0000
UK child	43.6	0.0000
UK toddler	14.5	0.0000
UK infant	8.7	0.0000

Based on the proposed ADI for folpet of 0.1 mg/kg bw/day, the TMDI for Phthalimide according to the worst case consumption scenarios represents 0 % of the ADI for all the different consumer groups and different dietary intakes of milk and meat.

The maximum daily intake of Phthalimide in animal products is zero for all consumer groups including the most sensitive consumer groups and compare to the ADI for folpet according to the worst case exposure.

d) Toxicity of phthalimide to aquatic organisms

Note: Summaries of all the relevant studies are presented below. These are already included in the DAR in Point B.9.2.1.

Fish

(i) Acute toxicity of phthalimide to rainbow trout (Salmo gairdneri). (Bowman, J.H. 1988c; IIA, 8.2.1/12; IIA 7.3/08)

The 96-hour acute toxicity of phthalimide (purity 98%) to the rainbow trout (*Salmo gairdneri* now known as *Oncorhynchus mykiss*) was determined in a static test system. Ten fish per glass vessel each containing 15 L (16 hour photoperiod, 12 °C) were exposed to nominal concentrations of phthalimide (dissolved in dimethylformamide) of 10, 18, 32, 56 and 100 mg/L in comparison with a dilution water control (hardness 40 to 46 mg/L $CaCO_3$) and a solvent control (0.1 mL/L). The fish were not fed for 48 to 96 hours prior to or during exposure. The test media were not renewed throughout the test. Samples of all test media for analysis of phthalimide by HPLC, were taken at the start and end of the exposure period. Measurements of pH, dissolved oxygen and temperature were taken at 0, 48 and 96 hours. Fish mortality and behaviour were recorded once every 24 hours.

The study met the essential criteria of EEC C1. However, standard lengths were measured whereas total lengths are stated in the EU guideline. No details were given of fish mortality during holding. It was conducted according to Good Laboratory Practice.

The mean measured concentrations of phthalimide were 9.4, 17, 26, 43 and 66 mg/L representing 94, 94, 81, 77 and 66% of nominal. There was little loss of phthalimide from 0 to 96 hours. At measured concentrations of 26, 43 and 66 mg/L there was a white precipitate on the surface and at the bottom of the test vessels at 0-hours. The amount of precipitate increased with nominal concentration, but became less visible with time. This suggests that in media at 26 mg/L and above, phthalimide was present in excess, possibly above the limit of water solubility and hence toxicity to rainbow trout at these concentrations may not be related to inherent toxicity but to excess test material in the test system. The water quality parameters were all within expected limits.

The cumulative mortality is presented in Table B.7.3.13. Sublethal effects at 26, 43 and 66 mg/L were surfacing, loss of equilibrium, fish on the bottom of the test vessels, quiescence and/or distended abdomen.

Table B.7.3.13: Mortality of rainbow trout exposed to phthalimide following 96-hours exposure in a static test system

Mean measured concentration of phthalimide (mg/L)	Cumulative mortality (%)			
	24 hr	48 hr	72 hr	96 hr
Water control	0	0	0	0
Solvent control	0	0	0	0
9.4	0	0	0	0
17	0	0	0	0
26	0	0	0	0
43	0	0	10	20
66	80	100	100	100

The 96-hour LC_{50} of phthalimide to rainbow trout under static test conditions was 49 mg/L (with 95% confidence limits of 26 to 66 mg/L) based on measured concentrations. The NOEC for mortality was 17 mg/L. The 24, 48 and 72-hour LC_{50} values were 58, 53 and 51 mg/L, respectively.]

(ii) Acute toxicity of phthalimide to bluegill sunfish (Lepomis macrochirus) in a static renewal system. (Bowman, J.H. 1989; IIA, 8.2.1/13; IIA 7.3/09)

The 96-hour acute toxicity of phthalimide (purity 98%) to the bluegill sunfish (*Lepomis macrochirus*) was determined in a semi-static test system with renewal of the test media after 48 hours. Ten fish per glass vessel each containing 15 L (16 hour photoperiod, 21 to 23 °C) were exposed to nominal concentrations of phthalimide (dissolved in dimethylformamide) of 10, 18, 32, 56 and 100 mg/L in comparison with a dilution water control (hardness 42 mg/L $CaCO_3$) and a solvent control (0.1 mL/L). The fish were not fed for 48 to 72 hours prior to or during exposure. Samples of all test media for analysis of phthalimide by HPLC, were taken at the start, after 48 hours and at the end of the exposure period. Measurements of pH, dissolved oxygen and temperature were taken at 0, 48 and 96 hours. Fish mortality and behaviour were recorded once every 24 hours. The 32 mg/L treatment was repeated with a concurrent solvent control treatment as three fish were lost during renewal of the test media in the first definitive test at this concentration.

The study met the essential criteria of EEC C1. However, standard lengths were measured whereas total lengths are stated in the EU guideline. No details were given of fish mortality during holding. Temperature, dissolved oxygen and pH should have been measured daily rather than at 0, 48 and 96 hours. It was conducted according to Good Laboratory Practice.

The mean measured concentrations of phthalimide were 6.8, 13, 22, 31 and 52 mg/L representing 68, 72, 69, 55 and 52% of nominal . At 22, 31 and 52 mg/L there was a white precipitate on the surface of the test media and at the bottom of the test vessels at 0-hours and 48-hours (freshly prepared media). The amount of precipitate increased with nominal concentration, but became less visible with time. The nominal 100 mg/L medium had a white precipitate at the bottom of the test vessel at both renewal time periods. This suggests that in media at 22 mg/L and above, phthalimide was present in excess, possibly above the limit of water solubility at the start of the renewal period but then may have fully dissolved on completion of the renewal period in all but the 100 mg/L medium. Therefore, the toxicity of phthalimide to bluegill sunfish at these concentrations may not be related to inherent toxicity but to excess test material in the test system. The water quality parameters were all within expected limits.

The cumulative mortality is presented in Table B.7.3.14. Sublethal effects at 31 and 52 mg/L were light discolouration, vertical orientation, quiescence and/or laboured respiration.

Table B.7.3.14: Mortality of bluegill sunfish exposed to phthalimide following 96-hours exposure in a semi-static test system

Mean measured concentration of phthalimide (mg/L)	Cumulative mortality (%)			
	24 hr	48 hr	72 hr	96 hr
Water control	0	0	0	0
Solvent control	0	0	0	0
6.8	0	0	0	0
13	0	0	0	0
22	0	0	0	0
31	10	10	10	10
52	90	90	100	100

The 96-hour LC_{50} of phthalimide to bluegill sunfish, under semi-static test conditions, was 38 mg/L (with 95% confidence limits of 31 to 52 mg/L) based on measured concentrations. The NOEC was 22 mg/L based on toxicological symptoms observed at 31 and 52 mg/L. The 24, 48 and 72-hour LC_{50} values were 40, 40 and 38 mg/L, respectively.

(iii) Acute flow-through toxicity of folpet technical to rainbow trout (Salmo gairdneri). (Bowman, J.H. 1988a; IIA, 8.2.1/01; IIA 7.3/10)

The 96-hour acute toxicity of folpet technical active substance (purity 90.3%) to the rainbow trout (*Salmo gairdneri* now known as *Oncorhynchus mykiss*) was determined in a flow-through test system. Two groups of 10 fish per replicate 15 L aquarium (16 hour photoperiod, 12 to 13 °C) were exposed to nominal concentrations of folpet (dissolved in DMF) of 0.0065, 0.013, 0.025, 0.05 and 0.10 mg/L in comparison with a dilution water control (hardness 40 to 46 mg/L $CaCO_3$) and a solvent control (0.1 mL/L). The fish were not fed for 48 hours prior to or during exposure. The test media were renewed 7.4 times each day. Samples of all test media for analysis of folpet, by high performance liquid chromatography (HPLC), were taken at the start and end of the exposure period. Measurements of pH, dissolved oxygen and temperature were taken at 0, 48 and 96 hours. Fish mortality and behaviour were recorded once every 24 hours.

The study met the essential criteria of EC method C1. However, fish lengths may be smaller, but standard lengths were measured whereas total lengths are stated in the EU guidelines. No details were given of fish mortality during holding. Temperature, dissolved oxygen and pH should have been measured daily rather than at 0, 48 and 96 hours. It was conducted according to Good Laboratory Practice.

The mean measured concentrations of folpet were 0.0022, 0.0056, 0.012, 0.026 and 0.13 mg/L representing 34, 43, 48, 52 and 130% of nominal. The 0 hour measurement for the 0.10 mg/L medium was 220% of nominal which was attributed to a precipitate in the splitter cell. For this reason and because the other measured adjacent concentrations were approximately 50% of each other, the 96 hour measured concentration at the highest nominal concentration was used in the LC_{50} calculation. The water quality parameters were all within expected limits.

The cumulative mortality is presented in Table B.7.3.15. Sublethal effects at 0.0056, 0.012 and 0.026 mg/L were loss of equilibrium, and fish on the bottom of the test vessels.

Table B.7.3.15: Mortality of rainbow trout following 96-hours exposure to folpet in a flow-through test system

Mean measured concentration of folpet (mg/L)	Cumulative mortality (%)			
	24 hr	48 hr	72 hr	96 hr
Water control	0	0	0	0
Solvent control	0	0	0	0
0.0022	0	0	0	0
0.0056	0	0	0	0
0.012	0	0	5	5
0.026	0	45	80	85
0.033*	100	100	100	100

*Based on the 96 h measured value.

The 96-hour LC_{50} of folpet to rainbow trout under flow-through conditions was 0.015 mg/L (with 95% confidence limits of 0.013 to 0.048 mg/L) based on measured concentrations. The NOEC was 0.0022 mg/L based on toxicological symptoms observed at 0.0056 mg/L and above. The 24, 48 and 72-hour LC_{50} values were 0.029, 0.026 and 0.015 mg/L, respectively.

(iv) Acute flow-through toxicity of folpet technical to bluegill sunfish (Lepomis macrochirus). (Bowman, J.H. 1988b, IIA, 8.2.1/02; IIA 7.3/11).

The 96-hour acute toxicity of folpet technical active substance (purity 90.3%) to the bluegill sunfish (*Lepomis macrochirus*) was determined in a flow-through test system. Two groups of 10 fish per replicate 15 L aquarium (16 hour photoperiod, 22 °C) were exposed to nominal concentrations of folpet (dissolved in DMF) of 0.065, 0.13, 0.25, 0.5 and 1.0 mg/L in comparison with a dilution water control (hardness 40 to 46 mg/L $CaCO_3$) and a solvent control (0.1 mL/L). It should be noted that the limit of water solubility of folpet at 25 °C is 0.8 mg/L. Therefore, at the highest nominal concentration folpet would be present in excess of its water solubility. The fish were not fed for 48 hours prior to or during exposure. The test media were renewed 7.4 times each day. Samples of all test media for analysis of folpet, by HPLC, were taken at the start and end of the exposure period. Measurements of pH, dissolved oxygen and temperature were taken at 0, 48 and 96 hours. Fish mortality and behaviour were recorded once every 24 hours.

The study met the essential criteria of EEC C1. However, standard lengths were measured whereas total lengths are stated in the EU guidelines. No details were given of fish mortality during holding.It was conducted according to Good Laboratory Practice.

The mean measured concentrations of folpet were 0.016, 0.033, 0.068, 0.20 and 0.25 mg/L representing 25, 25, 27, 40 and 25% of nominal (Table B.7.3.16). A white precipitate was observed in the diluter mixing cell and in the aquaria with the highest nominal concentration of folpet. This is consistent with the quantity of folpet added to water which was above the limit water solubility. The water quality parameters were all within expected limits.

Table B.7.3.16: Measured concentrations of folpet technical during a 96-hour flow-through toxicity test with bluegill sunfish

Folpet nominal concentration (mg/L)	Folpet measured concentration (mg/L)			Mean measured conc. as a % of nominal
	0-hr	96-hr	Mean	
Control	< 0.010	< 0.010	-	-
Solvent control	< 0.010	< 0.010	-	-
0.065	0.017	0.015	0.016	25
0.13	0.028	0.037	0.033	25
0.25	0.059	0.076	0.068	27
0.50	0.12	0.28	0.20	40
1.0	0.17	0.33	0.25	25
Stock solution (9500)	9900	9900	9900	104

[a] Precipitate present in vessel.
[b] 96-hour concentration used in LC_{50} calculation.

The cumulative mortality is presented in Table B.7.3.17. There were no sublethal effects recorded at 0.033 mg/L or below.

Table B.7.3.17: Mortality of bluegill sunfish following 96-hours exposure to folpet in a flow-through test system

Mean measured concentration of folpet (mg/L)	Cumulative mortality (%)			
	24 hr	48 hr	72 hr	96 hr
Water control	0	0	0	0
Solvent control	0	0	0	0
0.016	0	0	0	0
0.033	0	0	0	0
0.068	100	100	100	100
0.20	100	100	100	100
0.25	100	100	100	100

The 96-hour LC_{50} of folpet to bluegill sunfish under flow-through conditions was 0.047 mg/L (with 95% confidence limits of 0.033 to 0.068 mg/L) based on measured concentrations. The NOEC was 0.033 mg/L based on mortality at 0.068 mg/L. The 24, 48 and 72-hour LC_{50} values were 0.047 mg/L.

Table B.7.3.18: Summary of acute toxicity of folpet and PI

Compound	18. LC_{50} (mg/L) 19. Blue Gill sunfish	20. LC_{50} (mg/L) 21. Rainbow trout	22. References	
PI	38	49	*Bowman, J.H. 1989; IIA, 8.2.1/13; IIA 7.3/09*	*Bowman, J.H. 1988c; IIA, 8.2.1/12; IIA 7.3/08*
folpet	0.047	0.015	*Bowman, J.H. 1988b, IIA, 8.2.1/02; IIA 7.3/11*	*Bowman, J.H. 1988a; IIA, 8.2.1/01; IIA 7.3/10*
Ratio	809	3266		

B.7.17 References relied on

B.7.17.1 Active substance

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company, Report No. GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N	Owner
IIA 7.3/01	Fabro, S., Schumacher, R. L., Smith, R. L., Williams, R. T.	1964	Teratogenic activity of thalidomide and related compounds. *Life Sciences* **3**, 987-992. (Company file R-9963). Not GLP; Published.	N	-
IIA 7.3/02	Kennedy, G., Fancher, O. E., Calandra, J. C.	1968	An investigation of the teratogenic potential of captan, folpet, and difolatan. *Toxicol. Appl. Pharmacol.* **13**, 420-430. (Company file R-169). Not GLP; Published	N	-
IIA 7.3/03	Pilinskaya, M. A.	1986	Study of the cytogenetic activity of certain metabolites of a number of pesticides representing several classes of chemical compounds. *Tsitol. Genet.* **20**, 143-145. (Company file R-11352) Not GLP; Published.	N	-
IIA 7.3/04	Riggin, R. M., Margard, W. L., Kinzer, G. W.	1983	Characterization of impurities in commercial lots of sodium saccharin produced by the Sherwin-Williams process. II. Mutagenicity. *Fd Chem. Toxic.* **21**, 11-17. (Company file R-11350). Not GLP; Published.	N	-
IIA 7.3/05	Siefried, H.E.	2000	Review: Toxicological risk characterisation of potential folpet metabolites. The toxicity profiles of phthalic and phthalamic acids and phthalimide – is there a significant risk from metabolite exposure? Consultants, report dated August 1, 2000 (Company file: R-12331). Not GLP, Unpublished.	Y	Makhteshim
IIA 7.3/06	Cordon, M.T.	1997a	^{14}C-folpet metabolism in the lactating goat (part A). ^{14}C- trichloromethyl folpet: material balance of dosed radioactivity. [redacted] Report No. MBS72a/972856 (Company file: R-9137a). GLP, Unpublished.	Y	Makhteshim

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company, Report No. GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N	Owner
IIA 7.3/07	Cordon, M.T.	1997b	^{14}C-folpet metabolism in the lactating goat (part B). [redacted], Report No. MBS 72b/972856 (Company file: R-9137). GLP, Unpublished.	Y	Makhteshim
IIA 7.3/08	Bowman, J.H.	1988c	Acute toxicity of phthalimide to rainbow trout (*Salmo gairdneri*). [redacted] Report No. 36789 (Company file R-4956). GLP, Unpublished.	Y	Makhteshim
IIA 7.3/09	Bowman, J.H.	1989	Acute toxicity of phthalimide to bluegill sunfish (*Lepomis macrochirus*) in a static renewal system. [redacted], Report No. 36788 (Company file R-5255). GLP, Unpublished.	Y	Makhteshim
IIA 7.3/010	Bowman, J.H.	1988a	Acute flow-through toxicity of folpet technical to rainbow trout (*Salmo gairdneri*). [redacted] Report No. 36785 (Company file R-4954). GLP, Unpublished.	Y	Makhteshim
IIA 7.3/011	Bowman, J.H.	1988b	Acute flow-through toxicity of folpet technical to bluegill sunfish (*Lepomis macrochirus*). [redacted] Report No. 36784 (Company file R-4955). GLP, Unpublished.	Y	Makhteshim

Folpet

Addendum to Address Issues Raised at EPCO Meeting (11-14 April 2005)

Rapporteur Member State: Italy

EU review under Directive 91/414 EEC

Relating to Annex B (Volume 3) of the DAR

October 2005

Mammalian toxicology

Data on exposure

October 2005

Data on exposure

Operator exposure

Evaluation table number	Reporting table number	Open Point number
-	2(40)	2.14
Conclusions of EPCO 23 (10-15.5.2005): ***A new estimation of operator exposure has to be submitted for all uses (based on a dermal absorption value of 10%)***		

Amended calculations of operator exposure to Folpan 80WDG using the German BBA model are presented below in comparison with an AOEL of 0.1 mg/kg bw/day using a dermal absorption value of 10%. The AOEL value of 0.1 mg/kg bw/day has arisen following discussions between the RMS/EFSA/Member States on the DAR.

Estimation of operator exposure

For assessing operator exposure, the maximum rate, maximum concentration and the method of application for each crop type are given in Table 1.

Table 1 Summary of recommendations of 'Folpan' 80 WDG for operator exposure assessment

Type of crop	Method of application	Maximum application rate (kg a.s./ha)	Minimum spray volume (L/ha)	Maximum in-use spray concentration (mg a.s./mL)
Tomatoes (field)	tractor-mounted/tractor-drawn field crop sprayer or hand-held knapsack sprayers	1.25	1,000	1.25
Wheat	tractor-mounted/tractor-drawn field crop sprayer	0.75	200	3.75
Grapes	tractor-mounted/tractor drawn airblast	1.5	200	7.5
Tomatoes (glasshouse)	hand-held low-level knapsack	1.6	1,000	1.6

Estimation of operator exposure using the German BBA Model

The German BBA model[14] uses standard figures for different parameters. Models are available for the estimation of exposure for liquid and solid (WP and WG) pesticide formulations using a tractor-mounted sprayer for application to field crops and 'high' crops (i.e. those such as orchards or vineyards where the product is applied sideways and/or upwards) and hand-held equipment for application to 'high' crops.

[14] Westphal, D., Zels, J., Hoernicke, E. and Lundehn, J-R. (1993). Instructions for the protection of operators and other persons in the directions for use. Braunschweig Federal Research Centre, Department for Plant Protection Products and Application Techniques. Guidelines for the examination of plant protection products in the authorisation procedure, Part I 3-3, Third Edition March, 1993.

Percutaneous absorption through human skin is assumed to be 10%.

Based on the GAP uses, operator exposure estimates were calculated for worst-case uses, i.e. using the highest application rates and the highest spray concentrations which will lead to highest exposure of the spray operator as presented in Table 2. The estimate for hand-held equipment for application to 'high' crops has been used for application to tomatoes by a knapsack sprayer and represents a worst-case for a low-level crop such as tomatoes. The individual estimates are presented in Appendix I.

Assessment

Table 2. Estimation of operator exposure to folpet using German BBA Model and the proportion of the AOEL accounted for

Type of application/crop	Systemic operator exposure (mg/kg bw/day); proportion of AOEL	
	Without protective equipment	**With protective equipment[3]**
tractor-mounted/tractor-drawn field crop sprayer; tomatoes (1.25 kg a.s./ha)[1]	0.148 **148%**	0.077 **77%**
orchard tractor-mounted/tractor drawn airblast; grapes	0.236 **236%**	0.034 **34%**
hand held knapsack; tomatoes (1.6 kg a.s./ha)[2]	0.148 **148%**	0.076 **76%**

[1] The use on tomatoes represents the worst-case for applications made by tractor-mounted sprayer to field crops. Operator exposure following applications to wheat which is treated at a lower application rate is less than the values shown for tomatoes.
[2] The use on tomatoes at the application rate recommended for greenhouse grown crops represents the worst-case for applications made by hand-held sprayer. Operator exposure following applications to field grown tomatoes treated at a lower application rate is less than the values shown.
[3] For applications to tomato by tractor sprayer: gloves during mixing/loading. For applications to tomato by knapsack sprayer: gloves during mixing/loading and gloves during application.
For applications to grapes: gloves during mixing/loading; gloves and protective garment and sturdy footwear during application.

The results of the BBA Model calculations demonstrate that for the different spray application techniques and different crops, 148 to 236% of the AOEL is accounted for by exposure when spray operators wear no protective clothing. When protective equipment is worn (gloves during mixing/loading for applications to tomato using tractor-mounted sprayer; gloves during mixing/loading and application for applications to tomato by hand-held knapsack sprayer; gloves during mixing/loading and gloves and protective garment/sturdy footwear during application to grapes using tractor mounted airblast sprayer) estimated exposure accounts for 34 to 77% of the AOEL.

The BBA model does not specifically estimate exposure for applications made to greenhouse crops but the application rate for greenhouse tomatoes has been used for the hand-held estimate of exposure to represent the worst-case. In addition, even if inhalation exposure in greenhouses is higher than for outdoor crops (dermal exposure following applications to greenhouse crops and outdoor crops would be similar), inhalation exposure is small (0.0074 mg/kg bw/day – see Estimate for hand-held application in Appendix 1) compared to dermal exposure (0.6883 mg/kg bw/day) and so any increase would not significantly increase total systemic exposure. Furthermore, folpet has low vapour pressure (2.1 x 10^{-5} Pa at 25^{o}C).

There is, therefore, a wide margin of safety for spray operators in greenhouses wearing gloves during mixing/loading and gloves during application.

In addition, estimates are provided in the following for operator exposure in glass-houses, according to the IVA model (IVA, 1996)[15]. The results are shown in Table 3. Exposure is below the AOEL only when PPE (coverall) during application is worn.

The assessment is made with reference to:

1) "Operator exposure in greenhouses during practical use of plant protection products". Industrieverband Agrar e. V. (IVA - German Agrochemical Association).

2) The European Predictive Operator Exposure Model (EUROPOEM).

Dermal exposure for folpet is assumed to be 10% for the concentrate and diluted product in all calculations below.

1) Assessment of operator exposure using operator exposure study

The GAP for folpet in greenhouses is presented below and forms the basis of the operator exposure assessment presented.

Table 3: Summary of recommendations of 'Folpan' 80 WDG for operator exposure assessment in greenhouse crops

Crop	Method of application	Maximum application rate (kg folpet/ha)	Minimum spray volume (L/ha)	Maximum in-use spray concentration (mg folpet/mL)
Tomatoes	hand-held knapsack	1.6	1,000	1.6

The study was sponsored by Industrieverband Agrar e. V. (IVA, the German Agrochemical Association) and conducted by ECON Forschung GmbH in Germany according to GLP.

Mich, G. (1996). Operator exposure in greenhouses during practical use of plant protection products.
Econ Forschung GmbH, unpublished report no. EF 94-02-03.Bekanntmachung der Neufassung des Chemikaliengesetzes der Bundesrepublik Deutschland vom 25 Juli 1994 (BGBI. IS. 1703).Study meets the requirements of 'Guidance document for the conduct of studies of occupational exposure to pesticides during agricultural applications' OECD, 1997.
GLP: Yes
.
Materials and methods:
An operator exposure study was conducted in 1994 using three proprietary pesticides to assess the exposure of operators in greenhouses during application.

The three products used were 'Euparen' a WP formulation containing 51.1% of the fungicide dichlofluanid, 'Rody', an EC formulation containing 11.3% of the insecticide fenpropathrin and 'Saprol Neu', an EC formulation containing 17.7% of the fungicide triforine. Inhalation and dermal exposure was assessed for operators during the following operations wearing cotton overalls and impermeable coveralls, protective gloves, dust mask and, for mixing/loading, a peaked cap.

[15] IVA, 1996: Industrieverband Agrar e.V. (German Agrochemical Association). "Operator Exposure in Greenhouses during practical use of plant protection products" – Final Report/June 6, 1996

(1) Mixing and loading of the WP formulation into a knapsack sprayer prior to application. There were 12 trials and for each trial, an operator prepared 10 L of spray liquid (5 replicates per trial). The task included opening a 1 kg container, transferring the contents using a spoon to a weighing balance and then mixing the required weight in a bucket with the water, and then transferring the solution to a knapsack sprayer.

(2) Application of dilute spray to ornamentals, low level. There were 12 trials, four with each product, and applications were made to ornamental species (10 to 25 cm in height) grown in ventilated greenhouses on tables 1.15 m high using a spray gun with a flow rate of 8.4 L/minute at a pressure of 14 bar. Operators dragged the spray hose to the appropriate area of the greenhouse and applied 50 L dilute spray to the plants by moving backwards between the aisles of the plants.

(3) Application of dilute spray to roses, high level. There were 12 trials, four with each product, and applications were made to rose species (1.2 to 1.75 m in height) grown in ventilated greenhouses using a pressurised hose sprayer and cone nozzle delivering a coarse spray at a pressure of 15 bar. Operators dragged the spray hose to the appropriate area and applied 180 L dilute spray to the underside of the leaves by stepping backwards between the aisles of roses.

(Samples of air were also taken from the greenhouses 0 to 0.5, 1 to 2, 3 to 6 and 14 to 22 hours after application for the assessment of re-entry exposure. These results are not considered here.)

Inhalation exposure of operators was assessed using air samplers to collect air in tubes containing XAD-2 (for dichlofluanid or fenpropathrin) or Chromosorb 102 (for triforine) fixed on the shoulder of the operators at mouth height. Dermal exposure to hands was assessed by collecting rinsings in 1-propanol from the hands and from gloves. Dermal exposure to other parts of the body was assessed using cellulose patches inside a plastic cover (11.5 x 11.5 cm) with a 6.5 cm diameter opening attached to 13 locations on the outside of the protective clothing. After the operations, the exposed cellulose was cut from the plastic cover. The operator's underwear was cut into portions to measure exposure of the protected body.

Air samples in absorption tubes, cellulose patches, underwear and hand/glove rinsings were analysed for the respective active substances using gas chromatography with electron capture detection (GC-ECD) after extraction and clean-up appropriate to the active substance. Analytical methods were validated by spiking all matrices with standard solutions of each active substance. Samples of all matrices were also spiked in the greenhouses with dilutions of the formulated products. These field-fortified samples were exposed to the environment and were subject to the same packing, shipping and storage conditions as the study samples.

Inhalation exposure was calculated by the content of active substance in the sampling tube multiplied by a breathing rate of 1,740 L/hour. Dermal exposure was calculated by multiplying the active substance found by appropriate body surface areas according to Lundehn et al., 1992. Concentrations below the LOQ were assumed to be 50% of the LOQ for calculation purposes. The exposure for unprotected operators was assessed using the data for gloves plus hands (unprotected hands) and for patches on the outside of clothing (unprotected body and trunk). The exposure for protected operators wearing protective gloves, cotton overalls and a Gore-Tex suit (impermeable coveralls) was assessed using the data for hands (protected hands), for patches on the underwear under the outer clothing (protected body and trunk). The exposure of the head was calculated from the patches on the head, shoulders, chest, back and neck.

Findings:
Mean recovery of each active substance following laboratory fortification of the different matrices was within acceptable limits (70 to 110%) except for fenpropathrin/hand-washing solution where the overall mean recovery was 116%. Recovery from field fortified samples was more variable and this was thought to be related to uneven mixing of the formulations (particularly the WP) in water rather than degradation or decomposition during storage or

handling. The variable results of field spikings are not considered to affect the results of the study.

The results of the exposure measurements calculated as mg per kg a.s. handled are presented in Table 4. Inhalation exposure ranged from 0.1084 to 0.8946 mg/kg a.s. handled and was highest for operators involved with mixing/loading the WP formulation. The highest levels of dermal exposure occurred for unprotected operators during mixing/loading (47.3411 mg/kg a.s. handled) and application to high level plants (90.2047 mg/kg a.s. handled). Exposure was highest on the hands during mixing/loading and the body during application. Dermal exposure was considerably lower for protected compared to unprotected operators.

Table 4: Exposure of operators following mixing/loading and application of active substances in the greenhouse

Operation	Mean exposure[a] (mg/kg a.s. handled)					
	Inhala-tion[b]	Dermal				
		Hands	Head[c]	Body	Trunk	Total
Mixing and loading	0.8946	41.3121 (u) 0.0090 (p)	0.5552	2.8997 (u) 0.0650 (p)	2.5741 (u) 0.0806 (p)	47.3411 (u) 0.7098 (p)
Application (low level)	0.3985	0.7357 (u) 0.0089 (p)	0.4105	4.4649 (u) 0.1599 (p)	1.8551 (u) 0.0627 (p)	7.4662 (u) 0.6420 (p)
Application (high level)	0.1084	13.1884 (u) 0.0075 (p)	1.5412	70.0190 (u) 0.1922 (p)	12.4561 (u) 0.0357 (p)	97.2047 (u) 1.7766 (p)

(u) = unprotected
(p) = protected (protective gloves, cotton overalls and impermeable coveralls)
[a] Geometric mean of 12 operators.
[b] Operators wore dust masks but exposure was exposure was estimated in air tubes simulating unprotected exposure.
[c] Mean of the two methods used for calculating exposure.

The results of the operator exposure study with three active substances are presented as exposure in mg per kg of active substance handled during mixing/loading and application in greenhouses. The results with these surrogate active substances can be used to estimate exposure to folpet when mixing/loading and applying Folpan 80 WDG in greenhouses.

Folpan 80WDG is a non-dusty WG formulation and so the results of the measurements of operator exposure during mixing/loading of the dichlofluanid WP formulation are considered to be a worst-case for 'Folpan' 80 WDG. Once the dilute spray solution has been prepared, all formulations can be expected to lead to similar levels of operator exposure and so the results with dichlofluanid (WP formulation), fenpropathrin (EC formulation) and triforine (EC formulation) are considered to be applicable to 'Folpan' 80WDG. Tomatoes are treated in greenhouses with 'Folpan' 80 WDG using a hand-held knapsack applying the spray upwards and sideways. Therefore, the results from the studies applying the fungicides to ornamentals using a low-level spray gun and to roses using a high-level spray gun are considered to cover the worst-case for application to tomatoes.

Folpan 80 WDG is recommended for application to greenhouse grown tomatoes at a rate of 1.6 kg folpet/ha. It is assumed that the maximum work rate is 1 ha/day. Therefore, operators would handle 1.6 kg 'Folpan' 80 WDG per day. Thus, the exposure to folpet can be estimated for operators by multiplying the exposure values obtained in the operator exposure study by 1.6. Systemic exposure for folpet for a 70 kg body weight operator can be calculated assuming dermal absorption for folpet of 10%.

The results for folpet are presented in Table 5. Systemic exposure during mixing/loading plus application for unprotected operators ranged from 0.1549 to 0.3534 mg/kg bw/day. Systemic exposure for protected operators ranged from 0.0286 to 0.0327 mg/kg bw/day.

Table 5: Exposure of operators to folpet following mixing/loading and application of 'Folpan' 80 WDG in the greenhouse based on the operator exposure study

Operation	Protective clothing	Exposure to folpet		
		Inhalation[a] (mg/day)	Dermal[a] (mg/day)	Total systemic (mg/kg bw/day)[b]
Mixing and loading	unprotected	1.4314	75.7458	0.1287
	protected	1.4314	1.1357	0.0221
Application (low level)	unprotected	0.6376	11.9459	0.0262
	protected	0.6376	1.0272	0.0106
Application (high level)	unprotected	0.1734	155.5275	0.2247
	protected	0.1734	2.8426	0.0065
Total: mixing/loading plus application (low level)	unprotected			0.1549
	protected			0.0327
Total: mixing/loading plus application (high level)	unprotected			0.3534
	protected			0.0286

[a] Geometric mean exposure from operator exposure study x 1.6.
[b] Assumes 100% of inhalation and 10% of dermal absorption is absorbed, and operator body weight of 70 kg.

Conclusions
Based on surrogate operator exposure study in greenhouse (IVA, 1996), the estimated systemic exposure for mixing/loading plus application for unprotected operators ranged from 0.1549 to 0.3534 mg/kg bw/day. The estimated systemic exposure for mixing/loading plus application for protected operators wearing protective gloves, cotton overalls and impermeable coveralls ranged from 0.0286 to 0.0327 mg/kg bw/day, i.e. 29% to 33% of the AOEL for folpet of 0.1 mg/kg bw/day.

The estimated exposure of unprotected workers (measured on hands and the outside of clothing) is unrealistic since operators can be expected to wear at least cotton overalls during application.

2) Assessment of operator exposure using EUROPOEM

The European Predictive Operator Exposure Model (EUROPOEM) contains some exposure data relating to spraying protected crops using hand-held equipment.

The relevant EUROPOEM data are those derived from an operator monitoring study carried out in the Netherlands relating to the treatment of protected Chrysanthemums. In this study, 19 operators mixed, loaded and applied either a 200 g/L SC formulation (17 operators) or a 250 g/kg WP formulation (2 operators). A summary of the study application parameters is given in Table 6. Although the EUROPOEM database includes exposure measurements from other studies relating to the use of hand-held equipment to treat protected crops, these other studies involved treating small areas for brief periods and are not a suitable basis for estimating the level of exposure resulting from the supported use of 'Folpan 80 WDG' on protected tomatoes.

Table 6: EUROPOEM data relating to the treatment of protected chrysanthemums using hand-hand equipment

	Minimum	50th percentile	75th percentile	Maximum
Crop height	No data			
Application rate (kg a.s./ha)	0.1	0.18	0.22	0.5
Water volume (L/ha)	416	961	1000	1809
Area treated (ha)	0.3	0.72	0.85	1.35
Volume sprayed (L)	250	600	725	1900
Spray concentration (g a.s./L)	0.17	0.217	0.24	0.32
Monitoring time (hours)	0.75	1.15	1.84	2.73

In this EUROPOEM study, dermal exposure was determined using whole body dosimetry. Sample clothing worn throughout the entire mixing, loading and application operation consisted of a cotton coverall worn over a long sleeved T-shirt and long under-trousers, and cotton gloves. Protective gloves were not worn at any time. Exposure of the operators' heads and feet was not monitored. Inhalation exposure was measured for the whole mixing, loading and application operation using an IOM personal sampler with XAD filter cartridge positioned in each operator's breathing zone and connected to a personal air sampling pump. Acceptable field recoveries were achieved for all sampling media.
The dermal and inhalation exposure values from this study are summarised in Table 7. Although operators wore unprotected sampling gloves throughout the study, it is assumed that if protective gloves were worn these would reduce hand exposure by 90%.

Table 7: Summary of potential dermal exposure, actual dermal exposure and potential inhalation exposure values calculated from EUROPOEM data relating to the treatment of protected Chrysanthemums using hand-hand equipment.

Operator exposure mg a.s./kg a.s. mixed, loaded and applied					
	Potential dermal exposure *		Actual dermal exposure **		Potential inhalation exposure
	Body	Hands	Body	Hands	
Minimum	3.259	2.641	0.104	0.264	0.003
Arithmetic mean	27.619	128.349	2.524	12.835	0.115
Geometric mean	17.003	32.790	0.582	3.279	0.055
75th percentile	28.701	57.144	0.843	5.714	0.153
Maximum	168.245	1345.730	36.657	134.573	0.344

* potential body exposure from sum of a.s. measurements on inner and outer clothing
potential hand exposure from a.s. measurements on sampling gloves
** actual body exposure (under coveralls) from a.s. measurements on inner clothing
actual hand exposure (under protective gloves) assuming 10% penetration/transfer to hands

The levels of systemic operator exposure to folpet resulting from the supported use of 'Folpan 80 WDG' have been calculated using these data and assuming a dermal absorption value of 10% for the concentrate and the spray solution and an operator body weight of 70 kg. As the operators in the study used glasshouse sprayers with hose-fed hand lances, these data have been used in conjunction with the BBA dermal and inhalation exposure data for operators using knapsack sprayers (and the BBA assumption of 99% reduction in exposure when wearing protective gloves) to account for any additional exposure resulting from the need for a greater number of mixing and loading operations when using knapsack sprayers.

These calculations are presented in Appendix 2 and summarised in Table 8 (hose-fed hand lance equipment) and Table 9 (knapsack sprayers).

Table 8: Operator exposure to folpet resulting from the use of 'Folpan 80 WDG' on protected tomato using hose-fed hand lance equipment (EUROPOEM 75th percentile data for the indoor use of hand-held equipment)

Dermal exposure mg/person/day	Inhalation exposure mg/person/day	Total systemic exposure *	
		mg/kg bw/day**	% of AOEL
No PPE			
13.735	0.245	0.200	200%
Gloves when handling the concentrate and during application			
5.506	0.245	0.082	82%
Gloves and coveralls when handling the concentrate and during application			
1.049	0.245	0.018	18%

* assuming a dermal absorption for folpet of 10% for the concentrate and the spray solution
** assuming a body weight of 70 kg
AOEL = 0.1 mg/kg bw/day

Table 9: Operator exposure to folpet resulting from the use of 'Folpan 80 WDG' on protected tomato using knapsack sprayers (EUROPOEM 75th percentile data for the indoor use of hand-held equipment with BBA mixing and loading values for knapsack sprayers)

Dermal exposure mg/person/day	Inhalation exposure mg/person/day	Total systemic exposure *	
		mg/kg bw/day**	% of AOEL
No PPE			
17.095	0.277	0.248	248%
Gloves when handling the concentrate and during application			
5.540	0.277	0.083	83%
Gloves and coveralls when handling the concentrate and during application			
1.083	0.277	0.019	19%

* assuming a dermal absorption for folpet of 10% for the concentrate and the spray solution
** assuming a body weight of 70 kg
AOEL = 0.1 mg/kg bw/day

The estimates based on EUROPOEM data summarised above indicate that the use of 'Folpan 80 WDG' on protected tomatoes through hose-fed hand lance equipment will result in an acceptable level of systemic exposure to folpet for a operator wearing protective gloves when handling the concentrate and during application (systemic exposure equivalent to 82% of the proposed systemic AOEL of 0.1 mg/kg bw/day).

Estimates using a combination of EUROPOEM data and the BBA data indicate that the use of 'Folpan 80 WDG' on protected tomatoes through knapsack sprayers will result in an acceptable level of systemic exposure to folpet for a operator wearing protective gloves when handling the concentrate and during application (systemic exposure equivalent to 83% of the proposed systemic AOEL of 0.1 mg/kg bw/day).

Overall conclusions for operator exposure to folpet in greenhouses

Two assessments of operator exposure are presented.

The first is based on a surrogate operator exposure study conducted in the greenhouse. Exposure was measured for operators without protective equipment and for operators wearing full protective equipment (gloves and a chemical proof garment over cotton overalls). For operators wearing no protective equipment, exposure exceeded the AOEL of 0.1 mg/kg bw/day. For operators wearing protective gloves, cotton overalls and impermeable (chemical proof) coveralls during mixing/loading and application, the estimated systemic exposure

during mixing/loading plus application ranged from 29% to 33% of the AOEL for folpet. The exposure study did not measure exposure for operators wearing protective gloves only.
An assessment is also presented using the EUROPOEM model for greenhouse applications (with and without BBA data to take account of any additional exposure that might occur when using knapsack sprayers). Exposure was estimated for operators without protective equipment, secondly for operators wearing protective gloves, and thirdly for operators wearing protective gloves plus a chemical proof garment during mixing/loading and application.
The worst-case estimates (i.e. based on EUROPOEM with BBA data) indicate that the use of 'Folpan 80 WDG' on protected tomatoes will result in an acceptable level of systemic exposure to folpet for operators wearing protective gloves when handling the concentrate and during application (systemic exposure was equivalent to 83% of the proposed systemic AOEL of 0.1 mg/kg bw/day).

Bystander exposure

Evaluation table number	Reporting table number	Open Point number
-	2(41)	2.15
Conclusions of EPCO 23 (10-15.5.2005): ***A calculation for bystander exposure taking into account the dermal absorption value of 10% has to be submitted.***		

Amended calculations of bystander exposure are presented below in comparison with an AOEL of 0.1 mg/kg bw/day using a dermal absorption value of 10%.

The vapour pressure of folpet is low 2.1 x 10^{-5} Pa at 25^{o}C and so the inhalation risk to bystanders is considered to be negligible.

Bystanders could be exposed to spray if they were walking next to a field which was being treated. At 10 m from the spray application, BBA data (BBA, 2000[16]) estimates that for grapes and tomatoes, the maximum drift estimate (90th percentile data; late application for grapes; vegetable crop greater than 50 cm in height for tomatoes) is 1.23%.

Based on the maximum application rate for folpet to grapes of 1.5 kg/ha, this gives a deposition concentration of 150 mg/m^2. Assuming a bystander is located 10 m from the field, they could receive 1.23% drift, i.e. the deposition could reach 1.85 mg folpet/m^2. Assuming that one-half the body surface (totalling approximately 1 m^2) is covered, the skin deposition could be 0.93 mg folpet.

Using 10% skin absorption, the absorbed dose of folpet would be 0.093 mg and assuming a 60 kg body weight, the systemic exposure would be 0.0016 mg/kg.

The exposure of bystanders can be compared with the AOEL for folpet of 0.1 mg/kg bw/day, and such a comparison shows that exposure of bystanders is approximately 1.6% of the AOEL.

Worker exposure

Evaluation table number	Reporting table number	Open Point number
-	-	2.16

[16] BBA, 2000: Bekanntmachung des Verzeichnisses risikomindernder Anwendungsbedingungen für Nichtzielorganismen. Bundesanzeiger Nr. 100, 9879-9880, May 26, 2000.

Conclusions of EPCO 23 (10-15.5.2005):
A calculation for worker exposure taking into account the dermal absorption value of 10% has to be submitted

Amended calculations of worker exposure are presented below in comparison with an AOEL of 0.1 mg/kg bw/day using a dermal absorption value of 10%.

Estimation of worker exposure

Grapes

An estimate of worker exposure using the EUROPOEM model[17] is presented.

'Folpan' 80 WDG is recommended on grapes at 1.5 kg folpet/ha with up to 10 applications from shoot emergence to ripening. The minimum interval between sprays is 7 days and the minimum PHI is 28 days.

Data on dislodgeable residues of folpet in leaves are not available. However, studies have been conducted with the closely related compound captan[18]. Dislodgeable residues of captan in leaves of grapes following 6 applications of captan as a 50% WP formulation at 3.36 kg a.s./ha (i.e. a total of 20.16 kg captan/ha), 28 days after the final application were 2.8 μg/cm^2. The half-life for captan (calculated by linear regression analysis) was 16 days, which is almost identical to the value of 15 days calculated for folpet based on residue studies in fruit. This similarity in the calculated half-life values further supports the relevance of the data obtained with captan to folpet. The individual application rate (3.36 kg a.s./ha) and the total application rate (20.16 kg a.s./ha) applied in the captan trial were higher than the individual application rate (1.5 kg a.s./ha) and the total rate of folpet recommended in grapes (15 kg a.s./ha). Therefore, the value of 2.8 μg/cm2 measured in the trial can be corrected according to the GAP for folpet in grapes. Based on the total rate applied per crop, dislodgeable residues of folpet 28 days after the final application are 2.08 μg/cm2 (2.8 x 15 ÷ 20.16). On the other side, DFR values reported above are calculated based on a 50% formulation, while Folpet 80 WDG is an 80% concentrated product. Thus, a further refinement is needed, leading to a final value of 3.34 μg/cm2

EUROPOEM assumes foliar dislodgeable residues of 3 μg/cm^2/kg a.s. However, based on data with captan, dislodgeable residues for folpet following applications according to the GAP for grapes (see above) are 3.34 μg/cm^2. Therefore, this value is substituted in the EUROPOEM model calculation below.

Estimate: Workers harvesting grapes treated with 'Folpan' 80 WDG 10 applications at 1.5 kg folpet/ha with a 7-day interval and 28 day PHI (EUROPOEM model).

Dermal exposure

D (without protective gloves) = FDR x TF x R
D (with protective gloves) = FDR x TF x R x P

where:
D = dermal exposure (mg/person/day)
FDR = foliar dislodgeable residues (3.34 μg/cm^2)
TF = transfer factor for harvesting berries (3,000 cm^2/person/hour)

[17] European Project Group (1996). The development, maintenance and dissemination of a European Predictive Operator Exposure Model (EUROPEM) database.
[18] Chetram, R.S. (1989). Captan 50-WP Dislodgeable residue study on California grapes. Pan-Agricultural Laboratories, Study PAL-EF-88-13. (R-5318).

R = working time (8 hours/day)

D (without protective gloves) = 3.34 x 3,000 x 8 ÷ 1000
= 80.16 mg/person/day

Systemic exposure

S = D ÷ bw x AF

where:
S = systemic exposure (mg/kg bw/day)
bw = worker body weight (60 kg)
AF = dermal absorption (10%)

S (without protective gloves) = 80.16 x 0.1 ÷ 60
= 0.133 mg/kg bw/day

The maximum exposure of workers to folpet following 10 applications to grapes at the maximum recommended rate in the absence of protective gloves is 0.133 mg/kg bw/day (based on the EUROPOEM model).

Tomatoes

'Folpan' 80 WDG is recommended on greenhouse tomatoes at a maximum rate of 1.6 kg folpet/ha with up to 3 applications from the beginning of fruit set. The minimum interval between sprays is 7 days and the minimum PHI is 7 days. (The recommended rate in field grown crops is lower at 1.25 kg folpet/ha, though there are 4 applications and so the total rate applied is the same as for greenhouse crops.)

Data on dislodgeable residues of folpet in plants are not available. However, the study with captan is directly relevant to folpet. Residues in leaves of grapes following 6 applications of captan as a 50% WP formulation at 3.36 kg a.s./ha (i.e. a total of 20.16 kg captan/ha), 7 days after the final application were 5.4 $\mu g/cm^2$. The individual application rate (3.36 kg a.s./ha) and the total application rate (20.16 kg a.s./ha) applied in the captan trial were higher than the individual application rate (1.6 kg a.s./ha) and the total rate of folpet recommended in tomatoes (4.8 kg a.s./ha). Therefore, the value of 5.4 $\mu g/cm^2$ measured in the trial can be corrected according to the GAP for folpet in tomatoes. Based on the total rate per crop, dislodgeable residues of folpet 7 days after the final application are 1.29 $\mu g/cm^2$ (5.4 x 4.8 ÷ 20.16). On the other side, DFR values reported above are calculated based on a 50% formulation, while Folpet 80 WDG is an 80% concentrated product: Thus, a further refinement is needed, leading to a final value of 2.06 $\mu g/cm^2$

EUROPOEM assumes foliar dislodgeable residues of 3 $\mu g/cm^2$/kg a.s. However, based on data with captan, dislodgeable residues for folpet following applications according to the GAP for tomato are 2.06 $\mu g/cm^2$. Therefore, this value is substituted in the EUROPOEM model calculation below.

Estimate: Workers harvesting tomatoes treated with 'Folpan' 80 WDG 3 applications at 1.6 kg folpet/ha with a 7-day interval and 7 day PHI (EUROPOEM model).

Dermal exposure

D (without protective gloves) = FDR x TF x R
D (with protective gloves) = FDR x TF x R x P

where:
D = dermal exposure (mg/person/day)

FDR = foliar dislodgeable residues (2.06 μg/cm^2)
TF = transfer factor for harvesting vegetables (2,500 cm^2/person/hour)
R = working time (8 hours/day)

D (without protective gloves) = 2.06 x 2,500 x 8 ÷ 1000
= 41.2 mg/person/day

Systemic exposure

S = D ÷ bw x AF

where:
S = systemic exposure (mg/kg bw/day)
bw = worker body weight (60 kg)
AF = dermal absorption (10%)

S (without protective gloves) = 41.2 x 0.1 ÷ 60
= 0.068 mg/kg bw/day

The maximum exposure of workers to folpet based on 3 applications to tomato at the maximum recommended rate in the absence of protective gloves is 0.068 mg/kg bw/day (based on the EUROPOEM model).

Overall assessment of worker exposure

Calculations of worker exposure show that exposure of workers harvesting grapes and tomatoes without protective gloves is 0.133 and 0.068 mg/kg bw/day, respectively, i.e. 133% and 68% of the AOEL.. Therefore, it is necessary for workers to wear protective gloves for harvesting operations in treated grapes. Worker exposure following applications of folpet to wheat can be expected to be lower than following applications to grapes or tomatoes.

Section 1. Appendix 1

BBA Model

Estimate Tractor-mounted application to tomatoes: 1.25 kg folpet/ha (2.25 kg product/ha).

Calculation of exposure during mixing/loading and application to field crops using tractor-mounted equipment according to the BBA Model

Task	Type of exposure [1]	Specific exposure (mg/person x kg a.s.)	Work rate (ha/day)	Application rate (kg a.s./ha)	Estimated exposure	
					(mg/person / day)	(mg/kg bw/day)
Mixing/loading	I_M	0.008	20	1.25	0.20	0.0029
	$D_{M(H)}$	2.0	20	1.25	50	0.7143
Application	I_A	0.001	20	1.25	0.025	0.0004
	$D_{A(H)}$	0.38	20	1.25	9.5	0.1357
	$D_{A(C)}$	0.06	20	1.25	1.5	0.0214
	$D_{A(B)}$	1.6	20	1.25	40	0.5714

[1] I_M Inhalation exposure during mixing/loading.
$D_{M(H)}$ Dermal hand exposure during mixing/loading.
I_A Inhalation exposure during application.
$D_{A(H)}$ Dermal hand exposure during application.
$D_{A(C}$ Dermal head (capita) exposure during application.
$D_{A(B)}$ Dermal body exposure during application.

Route of exposure	Exposure (mg/kg bw/day)		
	Without protective equipment	With protective equipment during mixing	
Inhalation			
Mixing/loading	0.0029	none	0.0029
Application	0.0004	none	0.0004
Total inhalation:	0.0033		0.0033
Dermal			
Mixing/loading			
- Hands	0.7143	gloves[1]	0.0071
Application			
- Hands	0.1357	none	0.1357
- Head	0.0214	none	0.0214
- Body	0.5714	none	0.5714
Total dermal:	1.4429		0.7356
Total systemic[2]	0.1476		0.0769

[1] 'Folpan' 80 WDG is classified as 'Irritating to eyes' and 'May cause sensitisation by skin contact' according to its acute toxicological properties by Council Directives 1999/45/EC (see Point IIIA 7.1). Therefore, the use of protective gloves during mixing/loading is obligatory.
[2] Assumes 10% dermal exposure and 100% inhalation exposure is absorbed.

Estimate Tractor-mounted application to grapes: 1.5 kg folpet/ha (1.875 kg product/ha).

Calculation of exposure during mixing/loading and application to 'high' crops using tractor-mounted equipment according to the BBA Model

Task	Type of exposure [1]	Specific exposure (mg/person x kg a.s.)	Work rate (ha/day)	Application rate (kg a.s./ha)	Estimated exposure	
					(mg/person / day)	(mg/kg bw/day)
Mixing/loading	I_M	0.008	8	1.5	0.096	0.0014
	$D_{M(H)}$	2.0	8	1.5	24	0.3429
Application	I_A	0.018	8	1.5	0.216	0.0031
	$D_{A(H)}$	0.7	8	1.5	8.4	0.1200
	$D_{A(C)}$	1.2	8	1.5	14.4	0.2057
	$D_{A(B)}$	9.6	8	1.5	115.2	1.6457

[1] I_M Inhalation exposure during mixing/loading.
$D_{M(H)}$ Dermal hand exposure during mixing/loading.
I_A Inhalation exposure during application.
$D_{A(H)}$ Dermal hand exposure during application.
$D_{A(C}$ Dermal head (capita) exposure during application.
$D_{A(B)}$ Dermal body exposure during application.

Route of exposure	Exposure (mg/kg bw/day)		
	Without protective equipment	With protective equipment during mixing	
Inhalation			
Mixing/loading	0.0014	none	0.0014
Application	0.0031	none	0.0031
Total inhalation:	0.0045		0.0045
Dermal			
Mixing/loading			
- Hands	0.3429	gloves[1]	0.0034
Application			
- Hands	0.1200	gloves	0.0012
- Head	0.2057	none	0.2057
- Body	1.6457	garment[3]	0.0823
Total dermal:	2.3143		0.2926
Total systemic[2]	0.2359		0.0338

[1] 'Folpan' 80 WDG is classified as 'Irritating to eyes' and 'May cause sensitisation by skin contact' according to its acute toxicological properties by Council Directives 1999/45/EC (see Point IIIA 7.1). Therefore, the use of protective gloves during mixing/loading is obligatory.
[2] Assumes 10% dermal exposure and 100% inhalation exposure is absorbed.
[3] Standard protective garment and sturdy footwear.

Estimate Hand-held application to tomatoes: 1.6 kg folpet/ha (2.0 kg product/ha).

Calculation of exposure during mixing/loading and application to 'high' crops using hand-held equipment according to the BBA Model

Task	Type of exposure [1]	Specific exposure (mg/person x kg a.s.)	Work rate (ha/day)	Application rate (kg a.s./ha)	Estimated exposure	
					(mg/person / day)	(mg/kg bw/day)
Mixing/loading	I_M	0.02	1	1.6	0.032	0.0005
	$D_{M(H)}$	21	1	1.6	33.6	0.4800
Application	I_A	0.3	1	1.6	0.48	0.0069
	$D_{A(H)}$	10.6	1	1.6	16.96	0.2423
	$D_{A(C)}$	4.8	1	1.6	7.68	0.1097
	$D_{A(B)}$	25	1	1.6	40	0.5714

[1] I_M Inhalation exposure during mixing/loading.
$D_{M(H)}$ Dermal hand exposure during mixing/loading.
I_A Inhalation exposure during application.
$D_{A(H)}$ Dermal hand exposure during application.
$D_{A(C}$ Dermal head (capita) exposure during application.
$D_{A(B)}$ Dermal body exposure during application.

Route of exposure	Exposure (mg/kg bw/day)		
	Without protective equipment	With protective equipment during mixing	
Inhalation			
Mixing/loading	0.0005	none	0.0005
Application	0.0069	none	0.0069
Total inhalation:	0.0074		0.0074
Dermal			
Mixing/loading			
- Hands	0.4800	gloves[1]	0.0048
Application			
- Hands	0.2423	gloves	0.0024
- Head	0.1097	none	0.1097
- Body	0.5714	none	0.5714
Total dermal:	1.4034		0.6883
Total systemic[2]	0.1477		0.0762

[1] 'Folpan' 80 WDG is classified as 'Irritating to eyes' and 'May cause sensitisation by skin contact' according to its acute toxicological properties by Council Directives 1999/45/EC (see Point IIIA 7.1). Therefore, the use of protective gloves during mixing/loading is obligatory.
[2] Assumes 10% dermal exposure and 100% inhalation exposure is absorbed.

Appendix 2

EUROPOEM (75th percentile values)

Hand-held sprayers (indoor use). Combined mixer/loader/applicator values.

Application rate (product)	2 kg/ha	Dermal absorption for the concentrate and spray solution	10 %
a.s. content	800 g/kg	AOEL	0.1 mg/kg bw/day
Work rate	1 ha/day		
Amount of a.s. handled/applied	1.6 kg/day		

	Component		kg a.s. handled		Exposure mg/kg a.s.	% absorption		mg/person/day	% of AOEL
No PPE	PIE	=	1.6	x	0.153	100	=	0.2448	3%
	PDE (b)	=	1.6	x	28.701	10	=	4.59216	66%
	PDE (h)	=	1.6	x	57.144	10	=	9.14304	131%
							Total	**13.98**	**200%**
Gloves only	PIE	=	1.6	x	0.153	100	=	0.2448	3%
	PDE (b)	=	1.6	x	28.701	10	=	4.59216	66%
	ADE (h)	=	1.6	x	5.714	10	=	0.91424	13%
							Total	**5.7512**	**82%**
Coveralls and gloves	PIE	=	1.6	x	0.153	100	=	0.2448	3%
	ADE (b)	=	1.6	x	0.843	10	=	0.13488	2%
	ADE (h)	=	1.6	x	5.714	10	=	0.91424	13%
							Total	**1.29392**	**18%**

PIE	Potential inhalation exposure (mix/load/apply)
PDE (b)	Potential body exposure less hands, feet and head (mix/load/apply) from sum of outer and inner body dosimeter measurements
PDE (h)	Potential hand exposure (mix/load/apply) from sampling glove measurements (no protective gloves worn)
ADE (b)	Actual body exposure less hands, feet and head (mix/load/apply) from inner body dosimeter measurements
ADE (h)	Actual hand exposure (mix/load/apply) assuming that protective gloves are worn providing 90% protection from penetration/transfer
% of AOEL	Assuming a body weight of 70 kg

EUROPOEM (75th percentile values) with additional mixing/loading data from the BBA model for knapsack sprayers

Hand-held sprayers (indoor use). Combined mixer/loader/applicator values.

Application rate (product)	2 kg/ha	Dermal absorption for the concentrate and spray solution	10 %
a.s. content	800 g/kg	AOEL	0.1 mg/kg bw/day
Work rate	1 ha/day		
Amount of a.s. handled/applied	1.6 kg/day		

	Component		kg a.s. handled		Exposure mg/kg a.s.	% absorption		mg/person/day	% of AOEL
No PPE	PIE	=	1.6	x	0.153	100	=	0.2448	3%
	PDE (b)	=	1.6	x	28.701	10	=	4.59216	66%
	PDE (h)	=	1.6	x	57.144	10	=	9.14304	131%
	PIE (hm)	=	1.6	x	0.02	100	=	0.032	0%
	PDE (hm)	=	1.6	x	21	10	=	3.36	48%
							Total	**17.372**	**248%**
	Component		kg a.s. handled		Exposure mg/kg a.s.	% absorption		mg/person/day	
Gloves only	PIE	=	1.6	x	0.153	100	=	0.2448	3%
	PDE (b)	=	1.6	x	28.701	10	=	4.59216	66%
	ADE (h)	=	1.6	x	5.714	10	=	0.91424	13%
	PIE (hm)	=	1.6	x	0.02	100	=	0.032	0%
	ADE (hm)	=	1.6	x	0.21	10		0.0336	0%
							Total	**5.8168**	**83%**
	Component		kg a.s. handled		Exposure mg/kg a.s.	% absorption		mg/person/day	
Coveralls and gloves	PIE	=	1.6	x	0.153	100	=	0.2448	3%
	ADE (b)	=	1.6	x	0.843	10	=	0.13488	2%
	ADE (h)	=	1.6	x	5.714	10	=	0.91424	13%
	PIE (hm)	=	1.6	x	0.02	100	=	0.032	0%
	ADE (hm)	=	1.6	x	0.21	10		0.0336	0%
							Total	**1.35952**	**19%**

PIE	Potential inhalation exposure (mix/load/apply)
PDE (b)	Potential body exposure less hands, feet and head (mix/load/apply) from sum of outer and inner body dosimeter measurements
PDE (h)	Potential hand exposure (mix/load/apply) from sampling glove measurements (no protective gloves worn)
ADE (b)	Actual body exposure less hands, feet and head (mix/load/apply) from inner body dosimeter measurements
ADE (h)	Actual hand exposure (mix/load/apply) assuming that protective gloves are worn providing 90% protection from penetration/transfer
P/ADE (hm)	BBA values for potential/actual hand exposure when mixing/loading knapsack sprayers
PIE (hm)	BBA values for potential inhalation exposure when mixing/loading knapsack sprayers
% of AOEL	Assuming a body weight of 70 kg

Environmental fate and behaviour

Relating to Annex B (Volume 3) of the DAR

October 2005

B.8 Environmental fate and behaviour

Introduction

This document is an Addendum to the Draft Assessment Report (DAR) for the EU review of **folpet** to address issues raised at the EPCO meeting held on 11-14 April 2005. The aim of this Addendum is to address 'Open points' and 'Data requirements' as raised in the official Evaluation Table (dated 12.08.05) in the area of **Environmental fate and behaviour**.
This Addendum includes summarisation and evaluation of new assessments submitted by Makhteshim Chemical Works Ltd.

Section numbering in this Addendum is in line with Annex B (Volume 3) of the DAR.

The Good Agricultural Practice (GAP) uses proposed by the Notifier for consideration under the review are specified in Table 1.

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Table 1: Critical Good Agricultural Practice for folpet in the EU

Crop	Member state or country	Product name	F, G or I[a]	Pests or group of pests controlled	Formulation		Application			Application rate per treatment			PHI (days)	Remarks:
					Type	Conc. of a.s.	method kind	growth stage	number[b] (max.)	kg a.s./hL (max.)	water L/ha	kg a.s./ha (max.)		
Winter wheat	South EU	'Folpan' 80 WDG	F	*Septoria* Brown rust	WG	800 g/kg	Foliar spray; down-ward	Up to Z65	2	0.375	200	0.75	42	
Tomatoes	South EU	'Folpan' 80 WDG	F	various[c]	WG	800 g/kg	Foliar spray; down-ward	From beginning of fruit set	4	0.125	1000	1.25	7	
	South EU	'Folpan' 80 WDG	G	various[c]	WG	800 g/kg	Foliar spray; down-ward	From beginning of fruit set	3	0.16	1000 - 1300	1.6	7	
Grapes	North and south EU	'Folpan' 80 WDG	F	various[d]	WG	800 g/kg	Airblast foliar spray; upwards / sideways	Shoot emergence to veraison	10	0.75	200 - 400	1.5	28	

[a] F= field; G = greenhouse.
[b] Sprays on all crops are applied typically at intervals of 7 to 28 days.
[c] *Alternaria solanum, Cladospora, Colletotrichum, Septoria, Botrytis*
[d] Black rot, *Botrytis cinerea phomosis*. *Plasmopara viticola*.

B.8.4 Fate and behaviour in water (Annex IIA 7.2.1; Annex IIIA 9.2.1, 9.2.3)

Surface Water

Data gap 4.5 Calculation of PEC sw with consideration of drainage needs to be done.

The Notifier has investigated the significance of drainage exposure routes to surface water following use of folpet, using the FOCUS SW methodology which includes assessment of exposure from drainage. All parameters selected have been previously evaluated and accepted (see Addendum to folpet DAR, January 2005) or were recommended by EPCO 21.

Report: *Terry, A. (2005) Folpet: Response to Environmental Fate and Behaviour data requirements arising from EPCO meeting 21. CEA, unpublished report September 2005.*

Surface water modelling for folpet and its soil metabolites phthalimide, phthalamic acid and phthalic acid has been undertaken to establish the relative significance of the drainage exposure route based on the proposed folpet EU GAPs. Substance parameters used in the modelling were as derived for previous PEC GW and SW calculations as evaluated and agreed in the Addendum to the folpet DAR (March, 2005) except for the following, which was to address a recommendation from EPCO 21 ('*The experts agreed to disregard Koc values from two LUFA but use Koc values from EUROSOILS. The experts agreed that Kfoc values should be used instead of the Koc values in this case*.'):

For phthalimide, adsorption and desorption was measured in five soils. However, due to the instability of phthalimide under neutral and alkaline conditions the soils selected all had pH values less than or equal to 6, to enable the study to be carried out.

In two of the soils tested there was evidence of a significant deviation from a linear sorption isotherm (1/n = 0.52, 1/n = 0.58). It is likely that this significant deviation from linearity is related to pH. The pH dependence of sorption, and the practical experimental difficulties that are associated with it also suggest that these values may be a less reliable basis for defining sorption. It was, therefore (following recommendation of EPCO 21), proposed that simulations be based upon a mean sorption coefficient (K_{fOC}) and coefficient (1/n) defining the Freundlich isotherm excluding the data for these two soils. The appropriate mean K_{fOC} value was, therefore, 208.7 cm^3/g. The appropriate mean coefficient (1/n) defining the Freundlich isotherm was 0.8706.

EPCO 21 also recommended: '*With respect to aerobic DT50: A new mean should be recalculated excluding DT50 value from the study conducted at 10 °C. Mean should be used in the risk assessment and therefore the median should be removed from the list of endpoints.'* Therefore, mean DT_{50} values have been re-calculated as summarised in Table B.8.6.11.

Table B.8.6.11: Summary of soil degradation rates of folpet and metabolites

First order DT_{50} (days)	Coefficient of fit (r^2)	Study (temperature of incubation)	DT_{50} normalised to pF 2.0 and 20°C (days)
Folpet			
0.2	0.999	Crowe, A. 2001 (20°C)	0.12
0.8	0.986	Crowe, A. 2001 (20°C)	0.49
3.8	0.995	Crowe, A. 2001 (20°C)	2.92
16.2	0.80	Daly, D. 1991 (25°C)	15.2
		Mean:	**4.68**
Phthalimide			
0.5	0.984	Crowe, A. 2001 (20°C)	0.29
1.7	0.992	Crowe, A. 2001 (20°C)	1.04
4.8	0.876	Crowe, A. 2001 (20°C)	3.69
28.2	0.83	Daly, D. 1991 (25°C)	26.5
		Mean:	**7.88**
Phthalic acid			
0.6	0.999	Crowe, A. 2001 (20°C)	0.35
1.0	0.954	Crowe, A. 2001 (20°C)	0.61
4.1	0.892	Crowe, A. 2001 (20°C)	3.15
		Mean:	**1.37**
Phthalamic acid			
0.4	0.999	Crowe, A. 2001 (20°C)	0.24
		Mean:	**NR**

NR: not relevant

On the basis of the information presented in Table B.8.6.11, the following DT_{50} values were selected:

- Folpet: 4.68 days (mean of four measurements in four soils)
- Phthalimide: 7.88 days (mean of four measurements in four soils)
- Phthalamic acid: 0.24 days (measurement in one soil)
- Phthalic acid: 3.15 days (worst-case of three measurements in three soils)

The substance parameters selected for the FOCUS SW investigations are summarised in Tables B.8.6.12 to B.8.6.15. All parameters selected have been previously evaluated and accepted (see Addendum to folpet DAR, March 2005) or were recommended by EPCO 21.

Table B.8.6.12: Summary of worst case sediment/water DT_{50} values for FOCUS modelling

Compound	$DT_{50,wat}$	$DT_{50,sed}$	$DT_{50,sys}$	Maximum % formed
Folpet (DT_{50} days)	0.1	0.1	0.1	N.A.
Phthalimide (DT_{50} days)	0.65	0.65	0.65	31.8
Phthalamic acid (DT_{50} days)	6.09	6.09	6.09	13.4
Phthalic acid (DT_{50} days)	6.45	6.45	6.45	41.3

N.A.: Not Applicable

Table B.8.6.13: Soil degradation parameters for FOCUS modelling

Compound	Normalised soil DT_{50} (days)	Maximum % formed
Folpet	4.68	N.A.
Phthalimide	7.88	64.9
Phthalamic acid	0.24	12.8
Phthalic acid	3.15	16.6

NR: not relevant; N.A.: not applicable; *pseudo-zero

Table B.8.6.14: Soil K_{OC} values used in FOCUS surface water assessment

Compound	K_{OC} (mL/g)	1/n
Folpet	304	0.90
Phthalimide	208.7	NR
Phthalamic acid	10	NR
Phthalic acid	73.06	NR

NR: not required at step 1 and 2; step 3 assessment not required for these compounds

Table B.8.6.15: Other parameter values employed for the FOCUS simulations

Parameter	Folpet	Phthalimide	Phthalamic acid	Phthalic acid
Vapour Pressure (Pa) at 20°C	2.1×10^{-5}	1.38×10^{-6}	1.53×10^{-4}	1.01×10^{-4}
Water Solubility (mg/L) at 20°C	0.8[a]	360	3.76×10^{4}	7010
Molecular Weight (g/mol)	296.59	147.13	166.1	181.2
Plant Uptake Factor	0	NR	NR	NR
Crop wash-off factor	0.015	NR	NR	NR

[a] measured at 25°C
NR: not required

FOCUS Step 1 inputs of runoff and erosion and/or drainage were evaluated as a single loading to the water body and worst case surface water concentrations were calculated. The crop types used were application to winter wheat (Southern Europe), application to tomatoes (Southern Europe), and early and late application to vines (Southern and Northern Europe).

The runoff/erosion/drainage loading to the water body is fixed at 10% of the application for all scenarios in the calculator at FOCUS Step 1. The runoff/erosion/drainage entry is distributed instantaneously between water and sediment at the time of loading according to the K_{OC} of the compound.

At Step 1, degradation in the water and sediment compartments is dependant on an overall dissipation rate, i.e. the total system DT_{50} (see Table B.8.6.12). Degradation is also assumed to follow 1st order kinetics. The Step 1 calculator reports instantaneous concentrations in surface water at intervals of time after application. The Initial maximum PEC_{SW} for folpet metabolites are summarised in Tables B.8.6.16 to B.8.6.18.

Table B.8.6.16: Calculated PEC_{SW} values for phthalimide (FOCUS Step 1)

Scenario		Max PEC in water (µg/L)
Winter wheat	SE	64.05
Tomatoes	SE	106.76
Vines (early)	SE	128.06
Vines (late)	SE	132.26
Vines (early)	NE	128.06
Vines (late)	NE	132.26

NE: Northern Europe, SE: Southern Europe

Table B.8.6.17: Calculated PEC_{SW} values for phthalamic acid (FOCUS Step 1)

Scenario		Max PEC in water (µg/L)
Winter wheat	SE	36.2
Tomatoes	SE	120.66
Vines (early)	SE	361.75
Vines (late)	SE	381.63
Vines (early)	NE	361.75
Vines (late)	NE	381.63

NE: Northern Europe, SE: Southern Europe

Table B.8.6.18: Calculated PEC_{SW} values for phthalic acid (FOCUS Step 1)

Scenario		Max PEC in water (µg/L)
Winter wheat	SE	45.56
Tomatoes	SE	151.85
Vines (early)	SE	454.86
Vines (late)	SE	516.5
Vines (early)	NE	454.86
Vines (late)	NE	516.5

NE: Northern Europe, SE: Southern Europe

The PEC_{SW} values obtained were compared to the worst-case runoff prediction made for folpet soil metabolites (577 µg/L) given in the official list of endpoints. The toxicity of phthalamide, phthalamic acid and phthalic acid is such that it was previously established that this PEC value was of no concern. In the case of the FOCUS step 1 calculations reported, the PEC_{SW} for these metabolites were below the worst-case PEC value. Therefore, no further assessment was required for them.

FOCUS Step 3 calculations were carried out to assess the movement and fate of folpet in surface waters. The relevant drainage scenarios are summarised in Table B.8.6.19.

Table B.8.6.19: Relevant drainage scenarios for FOCUS Step 3 investigation of folpet

Crop	Drainage Scenarios	
	Northern Europe	Southern Europe
Winter wheat	N.A.	D6
Tomatoes	N.A.	D6
Vines	*	D6

N.A. – not applicable; * - no relevant scenarios

Exposure following both multiple applications and single applications were investigated. The drift loadings to surface water expressed as percent areic mean are given in Table B.8.6.20. The late applications to vines are worst case. Percentage loadings are all higher for single applications.

Table B.8.6.20: Drift deposition into surface waters

Crop	Water body	Single Application	Multiple Applications
Winter wheat[a]	Ditch	1.9274	1.6838
	Stream	0.2191	0.1792
	Pond	1.7165	1.4844
Tomatoes[b]	Ditch	1.9274	1.2968
	Stream	0.2191	0.1449
	Pond	1.7165	1.1519
Vines[c]	Ditch	5.1730	3.9581
	Stream	0.6121	0.4589
	Pond	5.1516	3.9333

[a]: 2 applications used for multiple applications
[b]: 4 applications used for multiple applications
[c]: 8 applications used for multiple applications

The method of application of folpet to all crops is *via* foliar spray.

Multiple applications:

Application Rate: 2 x 750 g a.s./ha (Annual total = 1500 g a.s./ha)
Crop: **Winter wheat (Southern Europe)**

Application Rate: 4 x 1250 g a.s./ha (Annual total = 5000 g a.s./ha)
Crop: **Tomatoes (Southern Europe)**

Application Rate: 10 x 1500 g a.s./ha (Annual total = 15000 g a.s./ha)
Crop: **Vines (Southern Europe)**

FOCUS surface water models will only allow a maximum of eight applications. Therefore the GAP application rates detailed above for vines were altered to accommodate the model in the following way:

Vines (Southern Europe)
GAP: 10 x 1500 g a.s./ha
FOCUS model: (1500 x 10) / 8 = 1875 g a.s./ha x 8 applications

Application dates were generated using the scenario harvest date, pre-harvest interval (PHI) and the minimal interval between applications (7 days for all crops). The last date of the application window was calculated by subtracting the PHI from the scenario harvest date (Equation 1). The first date of the application window was calculated using Equation 2.

Harvest date – PHI = last date of application window **Equation (1)**

Equation (1) – (30 + ((number of applications – 1) x minimal interval between applications)) = first date of application window **Equation (2)**

Single applications:

The single application rates were a true representation of worst-case spray drift loadings. The appropriate maximum single application rate for each usage was used to simulate a worst-case single application according to the GAP.

PEC values generated for each scenario and crop type are reported in the following tables (Tables B.8.6.21 and B.8.6.22).

Table B.8.6.21: FOCUS Step 3 calculated PEC values for folpet; multiple applications

Crop	Scenario	Max PEC_{sw} (µg/L)
Winter Wheat	D6-ditch	4.189
Tomatoes	D6-ditch	5.326
Vines (SE)	D6-ditch	24.616

Table B.8.6.22 FOCUS Step 3 calculated PEC values for folpet; single applications

Crop	Scenario	Max PEC_{sw} (µg/L)
Winter Wheat	D6-ditch	4.796
Tomatoes	D6-ditch	7.911
Vines (SE)	D6-ditch	32.173

Given that in all cases the PEC values were worse for single applications compared to multiple applications, step 4 modelling was applied only to the single application scenarios.

The areic drift from 5 (winter wheat and tomatoes) or 10 m (vines) was calculated in the SWASH drift calculator and, for streams following adjustment for spray drift input from upstream (x 1.2), the corresponding parameter in the TOXSWA 'txw' file (*mldsd*) was manually altered as appropriate. In effect, this resulted in a reduction of the spray drift associated with the application. If the resulting PEC_{SW} value was significantly lower than the corresponding PEC_{SW} value calculated at step 3, then this would indicate that spray drift was the dominant exposure route for that scenario.
PEC_{SW} values generated for each scenario are reported in the following table (Table B.8.6.23). Included in Table B.8.6.23 are the corresponding PEC_{SW} values obtained at step 3 (for comparison).

Table B.8.6.23: FOCUS Step 4 calculated PEC_{SW} values for folpet (single applications)

Crop	Scenario	Maximum PEC_{SW} (µg/L)		No-spray buffer zone (m)
		Step 3	Step 4	
Winter Wheat	D6-ditch	4.796	1.300	5
Tomatoes	D6-ditch	7.911	2.144	5
Vines (SE)	D6-ditch	32.173	5.639	10

Clearly, the PEC_{SW} values were all reduced significantly at step 4. This indicates that the PEC_{SW} values were dominated by the spray drift exposure route and that drainage is not predicted to be a significant exposure route for folpet.

RMS comment: It is clear from this investigation that drainage is not a significant exposure route to surface water for folpet. PEC values for soil metabolites are less than the PEC previously calculated for run-off (which was already considered to present a low risk).

B.8.1 Route and rate of degradation in soil (Annex IIA 7.1.1; Annex IIIA 9.1.1)

B.8.1.1 Aerobic and anaerobic studies

Open point 4.21:
With respect to aerobic DT50:
A new mean should be recalculated excluding DT50 value from the study conducted at 10 ºC. Mean should be used in the risk assessment and therefore median should be removed from the list of end points.

The Notifier has recalculated a new mean value for the soil DT50's for folpet and its soil metabolites in order to carry out FOCUS GW calculations (*Terry, A. (2005) Predicted Environmental Concentrations of folpet and its degradation products in groundwater in the European Union using the FOCUS groundwater scenarios. CEA, unpublished report September 2005*). The recalculated mean values are summarised in Table B.8.1.1.12.

Table B.8.1.1.12: Summary of soil degradation rates of folpet and metabolites

Compound	Soil and incubation temperature (°C)	Soil pH	Observed DT_{50} (days)	Normalised DT_{50} (days)	Source
Folpet	Clay loam, 40% MWHC, 20°C	7.5	0.2	0.12	Crowe, 2001
	Silty loam, 40% MWHC, 20°C	6.2	0.8	0.49	Crowe, 2001
	Loamy sand, 40% MWHC, 20°C	4.8	3.8	2.92	Crowe, 2001
	Sandy loam, 75-80% Field Capacity, 25°C	5.4	16.2	15.2	Daly, 1991
			Mean	**4.68**	
Phthalimide	Clay loam, 40% MWHC, 20°C	7.5	0.5	0.29	Crowe, 2001
	Silty loam, 40% MWHC, 20°C	6.2	1.7	1.04	Crowe, 2001
	Loamy sand, 40% MWHC, 20°C	4.8	4.8	3.69	Crowe, 2001
	Sandy loam, 75-80% Field Capacity, 25°C	5.4	28.2	26.5	Daly, 1991
			Mean	**7.88**	
Phthalamic acid	Silty loam, 40% MWHC, 20°C	6.2	0.4	0.24	Crowe, 2001
Phthalic acid	Clay loam, 40% MWHC, 20°C	7.5	0.6	0.35	Crowe, 2001
	Silty loam, 40% MWHC, 20°C	6.2	1.0	0.61	Crowe, 2001
	Loamy sand, 40% MWHC, 20°C	4.8	4.1	3.15	Crowe, 2001
			Mean	**1.37**	

B.8.6 Predicted environmental concentrations in surface water and in ground water (PEC_{SW}, PEC_{GW}) (Annex IIIA 9.2.1, 9.2.3)

Groundwater

Data gap 4.6:
New FOCUS gw modelling is required with the mean values for DT 50 instead of median (Disregard DT50 values derived from the study conducted at 10°C for calculation of mean) and with Koc value for phthalimide metabolite derived from 3 EUROSOILS.

The Notifier has submitted a revised FOCUS gw assessment which uses the mean soil DT_{50} values for folpet and its metabolites appropriately calculated (i.e. values derived from the investigations conducted at 10°C have been excluded).
It also uses the mean phthalimide Koc value calculated from the data obtained with the 3 EUROSOILS only. All other parameters and methodology are as presented and evaluated in the addendum to the folpet DAR (March 2005).

Report: *Terry, A. (2005) Predicted Environmental Concentrations of folpet and its degradation products in groundwater in the European Union using the FOCUS groundwater scenarios. CEA, unpublished report September 2005.*

Groundwater modelling of folpet has been undertaken with the FOCUS groundwater scenarios using the PELMO model (FOCUS version 3.3.2). The modelling undertaken was based on the use of the 80 WDG formulation. Simulations were conducted with applications to vines based on an application rate of 1.5 kg a.s./ha. Simulations were also carried out for Southern Europe winter wheat usages at 0.75 kg a.s./ha. Simulations included the evaluation of three degradation products, phthalimide, phthalamic acid and phthalic acid. All substance parameters and methodology was as previously used and reported in XA1105 (Mackay, March 2002) except for the following.

For phthalimide, adsorption and desorption was measured in five soils. However, due to the instability of phthalimide under neutral and alkaline conditions the soils selected all had pH values less than or equal to 6, to enable the study to be carried out.

In two of the soils tested there was evidence of a significant deviation from a linear sorption isotherm (1/n = 0.52, 1/n = 0.58). It is likely that this significant deviation from linearity is related to pH. The pH dependence of sorption, and the practical experimental difficulties that are associated with it also suggest that these values may be a less reliable basis for defining sorption. It was, therefore (following recommendation of EPCO 21), proposed that simulations be based upon a mean sorption coefficient (K_{fOC}) and coefficient (1/n) defining the Freundlich isotherm excluding the data for these two soils. The appropriate mean K_{fOC} value was, therefore, 208.7 cm^3/g. The appropriate mean coefficient (1/n) defining the Freundlich isotherm was 0.8706.

The mean soil DT_{50} values for folpet and its soil metabolites have been re-calculated and used in the PEC GW determinations following recommendations from EPCO 21 that median values were not acceptable in this case and that the DT_{50} values derived at 10°C should be excluded from the calculations (see Table B.8.1.1.12, above).

The calculated PEC_{GW} values demonstrated that the predicted 80^{th} percentile concentrations for folpet, phthalimide, phthalamic acid and phthalic acid were all <0.001 μg/L at 1 m depth in all scenarios as simulated by FOCUS PELMO.

RMS comment: The revised FOCUSgw modelling is acceptable and shows a low risk to groundwater (PECgw <0.001 μg/L).

B.8.11 References relied on

B.8.11.1Active ingredient

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company, Report No. GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N	Owner
	Terry, A	2005	Folpet: Responses to Environmental Fate and Behaviour data requirements arising from EPCO meeting 21. CEA.103, unpublished report September 2005.	Y	Makhteshim

B.8.11.2 Formulation

Folpan 80 WDG

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company, Report No. GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N	Owner
	Terry, A.	2005	Predicted Environmental Concentrations of folpet and its degradation products in groundwater in the European Union using the FOCUS groundwater scenarios. CEA.093, unpublished report September 2005	Y	Makhteshim

Folpet

Addendum to Address Issues Raised at EPCO Meeting (11-15 April 2005):

Ecotoxicology

Relating to Annex B (Volume 3) of the DAR

October 2005

B.9 Ecotoxicology

Introduction

This document is an Addendum to the Draft Assessment Report (DAR) for the EU review of folpet to address issues raised at the EPCO meeting (no. 22) held on 11-15 April 2005. The aim of this Addendum is to address 'Open points' and 'Data requirements' as raised. in the official Evaluation Table (dated 12.08.05) in the area of Ecotoxicology.

This Addendum includes summarisation and evaluation of statements and risk assessments submitted by Makhteshim Chemical Works Ltd.

Section numbering in this Addendum is in line with Annex B (Volume 3) of the DAR.

The Good Agricultural Practice (GAP) uses proposed by the Notifier for consideration under the review are specified in Table 1.

Table 1: Critical Good Agricultural Practice for folpet in the EU

Crop	Member state or country	Product name	F, G or I[a]	Pests or group of pests controlled	Formulation		Application			Application rate per treatment			PHI (days)	Remarks:
					Type	Conc. of a.s.	method kind	growth stage	number[b] (max.)	kg a.s./hL (max.)	water L/ha	kg a.s./ha (max.)		
Winter wheat	South EU	'Folpan' 80 WDG	F	*Septoria* Brown rust	WG	800 g/kg	Foliar spray; down-ward	Up to Z65	2	0.375	200	0.75	42	
Tomatoes	South EU	'Folpan' 80 WDG	F	various [c]	WG	800 g/kg	Foliar spray; down-ward	From beginning of fruit set	4	0.125	1000	1.25	7	
	South EU	'Folpan' 80 WDG	G	various [c]	WG	800 g/kg	Foliar spray; down-ward	From beginning of fruit set	3	0.16	1000 - 1300	1.6	7	
Grapes	North and south EU	'Folpan' 80 WDG	F	various [d]	WG	800 g/kg	Airblast foliar spray; upwards/ sideways	Shoot emergence to veraison	10	0.75	200 - 400	1.5	28	

[a] F= field; G = greenhouse.
[b] Sprays on all crops are applied typically at intervals of 7 to 28 days.
[c] *Alternaria solanum, Cladospora, Colletotrichum, Septoria, Botrytis*
[d] Black rot, *Botrytis cinerea phomosis*. *Plasmopara viticola*.

New open point 5.13:
RMS to evaluate the risk to herbivorous birds and mammals in cereals. See open point 5.1. This open point was proposed at EPCO 22.

Folpet is not applied to early growth stages of winter wheat. It is only applied to late season winter wheat (in late spring/summer) for the treatment of *Septoria* and Brown rust. Hence, applications are made to wheat plants when the foliage would not be palatable for grazing birds or mammals. Therefore, there is a low risk.

New open point 5.14:
RMS to perform the long term risk assessment for birds with a NOEC of 78 mg a.s./kg bw. For the refinement of the long term risk assessment for birds a RUD value of 29 should be used. See open point 5.1. This open point was proposed at EPCO 22.

The Notifier has submitted the following risk assessment (Ref: Norman, 2005):

Following the recommendation of EPCO 22, long term toxicity exposure ratios (TER) for birds including a NOEC of 78.3 mg a.s./kg bw and an RUD of 29 for residues on 'small insects' are stated in Table 2 below.

Table 2. Folpet: Derivation of long term TER values for insectivorous birds.

Crop (scenario)	Indicator species	App. rate (kg/ha)	RUD[a]	FIR[b]	PT[c]	ETE mg/kg bw/d[d]	NOEL mg/kg bw/d[e]	TER
Winter wheat ('Cereals')	Insectivorous bird	0.75	29	1.04	0.61	13.8	78.3	5.67
Grapes ('Orchard/ vine/ hops')	Insectivorous bird	1.5	29	1.04	0.61	27.5	78.3	2.85
Tomatoes ('Leafy crops')	Insectivorous bird	1.25	29	1.04	0.61	23.0	78.3	3.4

a. Default RUD (Residue per Unit Dose) proposed by EPCO 22, from EU guidance document on risk assessment for birds and mammals (SANCO 4145/2000).
b. Default FIR (Food Intake Rate per unit bodyweight) for an insectivorous bird (SANCO 4145/2000).
c. PT (Proportion of diet obtained from Treated area) of 0.61. Understood to have been agreed by EPCO 22.
d. ETE: Estimated Theoretical Exposure (application rate x RUD x FIR x PT).
e. NOEL from reproduction study on mallard (highest treatment level).

TER for the use on winter wheat is greater than the Annex VI trigger of 5, indicating low risk. TER values for uses in grapes and tomato are below the trigger of 5, indicating that further assessment is required.

Long term risk assessment for birds including additional refinements:

Toxicity endpoint: It should be noted that there were no effects in the bird reproduction studies. Hence, the need for a refined assessment is not triggered by any adverse effect on reproduction. The TER values derived in Table 2 are simply a product of the highest concentration tested, i.e. 1000 ppm in the diet. Such a concentration is regarded as a reasonable upper limit for testing, and would have been high enough to justify low risk based on the previous commonly used risk assessment scheme (EPPO, 1992).

Residue per Unit Dose (RUD) of 29 for 'small insects': The default RUD value for 'small insects' quoted in the EU risk assessment guidance (SANCO/4145/2000) is extrapolated from residues on weed seeds (quoted from Hoerger and Kenaga, 1972). Hence, the values do not have a strong scientific basis. The EU guidance document states: *'The residue estimate for small insects appears unsatisfactory, and as soon as better information becomes available this surrogate should be replaced...'*. The available data on actual residues on insects suggests that the current default RUD values for small insects represent a significant overestimate. A research project (sponsored by ECPA) currently being undertaken by the contractor Rifcon is reviewing available insect residues trials. The project includes evaluation of modern insect residues trials conducted in the last few years. The project is well advanced, and the outcome is intended to be taken into account in a review of the existing default RUD values for insects. UK Central Science Laboratory is also undertaking research in this area. Following Annex I listing, risk assessments can be submitted at Member State level which include agreed *revised* RUD values for insects after these become available.

Refined risk assessment: As stated above the default RUD value for 'small insects' appears unsatisfactory (albeit precautionary). As there is currently no agreed alternative value, this value can be incorporated into a refined risk assessment for insectivorous birds. Under the EU guidance document on risk assessment for birds and mammals (SANCO/4145/2000) a refined assessment can be conducted by defining appropriate key (focal) species for the crop use scenario under discussion. This has now been conducted for the use of folpet in vineyards and tomatoes (Ref: Gerlach, 2005). A summary of this risk assessment is provided below. The refinements in this assessment are based on ecological information from the published literature on energy requirements, dietary composition and foraging behaviour of relevant insectivorous bird species:

Gerlach, J (2005): Folpet: Long term risk assessment for insectivorous birds. Rifcon GmbH report no. RC05-017. September 2005:

[The full text of this assessment is provided in Appendix 1 of this Addendum]

Species of concern:

Tomato: **Yellow wagtail** (*Motacilla flava*) is frequently observed in tomato fields and is known to nest within the crop. As a small insect-eating bird, is has been selected as a key species on which to base the refined risk assessment.

Vineyards (central EU): **Yellowhammer** has been reported as being regularly observed in vineyards in southern Germany, and is considered to be the most common bird species in vineyards in this region. Yellowhammer occurs throughout central and northern Europe. As a small insect-eating bird, is has been selected as a key species on which to base the refined risk assessment for central European vineyards.

Vineyards (south EU): South EU orchards are cultivated on bare soil. In south EU, the **cirl bunting** (*Emberiza cirlus*) occupies the same ecological niche as yellowhammer in central EU. The ranges of these species have some overlap (in France, Germany, Italy Greece). Literature suggests that cirl bunting is regularly found in vineyards. As a small insect-eating bird, is has been selected as a key species on which to base the refined risk assessment for south European vineyards.

Refinement of food intake rate (FIR):

Based on bodyweights, energetic requirement and energy value of relevant feed items, the food intake per day in terms of fresh weight can be estimated for a species. For **yellow wagtail** (approximate weight 17 g) this has been calculated to be 15 g of arthropods, giving as **FIR** of **0.88**. For **yellowhammer** (approximate weight 26.5 g) this has been calculated to be 20.5 g of arthropods, giving as **FIR** of **0.77** or 7.2 g of weed seeds, giving an **FIR** of **0.26**. For **cirl bunting** (approximate weight 23.6 g) this has been calculated to be 18.9 g of arthropods giving as **FIR** of **0.80** or 27.4 g of caterpillars giving an **FIR** of **1.16** or 6.6 g of weeds seeds giving an **FIR** of **0.27**.

Refinement of proportion of diet obtained from the treated area:

Yellow wagtail: For nesting, yellow wagtails require sufficient vegetation to cover the nest (which is why they have been observed to nest in tomato and potato fields). However, for feeding they prefer areas with no vegetation or short vegetation (short grazed pasture, mown meadow). Hence, they feed mainly outside the treated area. For a long term risk assessment, it is conservatively assumed that yellow wagtails will obtain at least half of their food from the treated area (i.e. **PT = 0.5**). This is supported by the EFSA PPR opinion (2004) on an avian risk assessment (on methamidophos) for yellow wagtails in potato fields (a similar crop to tomato in structure and taxonomy) which also used a PT of 0.5.

Yellowhammer / cirl bunting: No specific data available to refine PT. Hence, PT currently assumed to be 1. It is understood that EPCO 22 agreed, as a worst case, for the use of a PT of 0.61 for vineyards (extrapolated from orchards). This additional refinement will be used if necessary.

Refinement of portion of diet (PD):

Yellow wagtail: Information is available in the literature regarding size preference for insect food items for yellow wagtail. Based on this information it can be concluded that in terms of *number* of insects around 76.4% (**PD: 0.764**) are '**large insects'** and 23.6% (**PD: 0.236**) '**small insects'**. Data on mass of insects in relation to bodylength indicate that in terms of *mass* the PD for large insects would be higher than 0.764. Hence, the use of this value is conservative.

Yellowhammer: Based on a detailed survey of yellowhammer diet, realistic portions of the diet are estimated as 15% (**PD = 0.15**) **weeds seeds**, 75% (**PD=0.75**) **large insects** and 10% (**PD=0.1**) **small insects**.

Cirl bunting: A realistic diet during breeding season is considered to be 10% (**PD=0.1**) **small insects,** 20% (**PD = 0.2**) **weed seeds**, 20% (**PD=0.2**) **caterpillars** and 50% (**PD=0.5**) **large insects**.

Refinement of foraging strata:

Both yellowhammer and cirl bunting almost exclusively forage on the ground. Hence, foliar interception should be taken into account in the exposure assessment.

Exposure and risk assessment:
Yellow wagtail: The exposure assessment for yellow wagtails potentially foraging in **tomato** fields treated with folpet at a rate of 1.25 kg a.s./ha is depicted in Table 3.

Table 3 Exposure assessment for yellow wagtails in tomato fields

Diet proportions	large insects	small insects	Whole diet
Application rate [kg a.s./ha]	1.25	1.25	
RUD [mg/kg a.s./ha]	5.1	29	
Maximum initial concentration after last application [mg a.s./kg]	6.375	36.25	
Relative daily food intake (FIR/b.w.) [g fresh weight/g b.w./day]	0.88	0.88	
Portion of diet obtained in-crop (PT)	0.5	0.5	
Portion of diet (PD)	0.764	0.236	
Estimated theoretical exposure (ETE) [mg a.s./kg b.w./day]	2.14	3.76	**5.90**

Yellowhammer: The exposure assessment for yellowhammers foraging in **central European vineyards** treated with folpet at a rate of 1.5 kg a.s./ha is depicted in Table 4.

Table 4 Exposure assessment for yellowhammers potentially foraging in vineyards in Central Europe

Diet proportions	Arthropods large	Arthropods small	Weed seeds	
Application rate [kg a.s./ha]	1.5			
RUD [mg/kg a.s./ha]	5.1	29	40 [1)]	
Maximum initial concentration after last application [mg a.s./kg]	7.65	43.5	60	
Multiple application factor (MAF)	1	1	1	
Relative daily food intake (FIR/b.w.) [g fresh weight/g b.w./day]	0.77	0.77	0.26	
Portion of diet obtained in-crop (PT)	1	1	1	
Portion of diet (PD)	0.75	0.1	0.15	
Deposition factor	0.6	0.6	0.6	
Estimated theoretical exposure (ETE) [mg a.s./kg b.w./day]	2.65	2.01	1.40	**6.06**

1) Mean values of Fletcher et al. 1994 as derived from the Guidance Document (Anonymous 2002).

Cirl bunting: The exposure assessment for cirl buntings potentially foraging in **Southern European vineyards** treated with folpet at a rate of 1.5 kg a.s./ha is depicted in Table 5.

Table 5 Exposure assessment for cirl buntings potentially foraging in vineyards in Southern Europe

Diet proportions	Arthropods large	Arthropods small	Caterpillars	Weed seeds	
Application rate [kg a.s./ha]	1.5				
RUD [mg/kg a.s./ha]	5.1	29	5.1	40 [1)]	
Maximum initial concentration after last application [mg a.s./kg]	7.65	43.5	7.65	60	
Multiple application factor (MAF)	1	1	1	1	
Relative daily food intake (FIR/b.w.) [g fresh weight/g b.w./day]	0.8	0.8	1.16	0.27	
Portion of diet obtained in-crop (PT)	1	1	1	1	
Portion of diet (PD)	0.5	0.1	0.2	0.2	
Deposition factor	0.6	0.6	0.6	0.6	
Estimated theoretical exposure (ETE) [mg a.s./kg b.w./day]	1.84	2.09	1.06	1.94	**6.93**

1) Mean values of Fletcher et al. 1994 as derived from the Guidance Document (Anonymous 2002)

Toxicity exposure ratios have been calculated and are presented in Table 6.

Table 6 Refined long-term TER calculation for insectivorous birds

Species	**Scenario**	**Toxicity mg/kg b.w./day**	**ETE mg/ kg b.w.**	**TER_{lt}**
Yellow wagtail	Tomatoes	**78.3**	5.90	**13.27**
Yellowhammer	Grapes (Northern Europe)		6.06	**12.92**
Cirl bunting	Grapes (Southern Europe)		6.93	**11.30**

TER values are greater than the Annex VI trigger of 5, indicating low risk.

Comment from RMS on the above:

The RMS has considered the refined risk assessment report (Gerlach, 2005) and other argumentation provided above. The major point is that existing bird reproduction studies show no effects at the highest treatment level of 1000 ppm. Hence, there is no inherent concern over the potential for folpet to affect reproduction in birds. The refined risk assessment based on information from the published literature includes more realistic assumptions in terms of dietary composition (PD) and proportion of food taken from the treated area (PT). It is reasonable, especially over the long term, to assume that an individual insectivorous bird would consume a mixed diet including both small and large insects. Birds are obviously highly mobile, so modifications to PT over the long term are also justified (as agreed at EPCO 22). The choice of key species and refinement of parameters for these species in the higher tier assessment (Gerlach, 2005), are considered to be reasonable.

Considering the refined risk assessment, together with the fact that there were no effects in reproduction studies, a low risk can be concluded.

New open point 5.15:
RMS to revise the NOEL and if necessary revise the long-term risk assessment for mammals. See open point 5.1. This open point was proposed at EPCO 22.

The Notifier has submitted the following statement (ref: Norman, 2005):

In response to a recommendation from EPCO 22, the endpoint for the long term risk assessment for wild mammals has been reviewed:

In a 2-generation study on rat (Rubin, 1986), folpet was adminstered in the diet at 250, 1500 and 5000 ppm. At the highest treatment level food consumption was reduced compared with the control by less than or equal to 10% throughout the study. As a result adult bodyweights were lower than the controls to a similar extent. At this treatment level, the initial bodyweight of the F1 animals as weanlings was approximately 3% lower than the control. By end of lactation, the mean bodyweight of these weanlings was approximately 10% lower than the control, and was probably related to the reduced food intake of the mothers. The subsequent food consumption of the F1 generation was reduced to a similar extent as the F0 generation (i.e. ≤ 10%). Hyperkeratosis (thickening of the skin) on non-glandular gastric mucosa and oesophagus was observed at 5000 ppm (and to lesser extent at 1500 ppm). Folpet is an irritant, and this observation was related to the high dietary concentration irritating the mucal membranes. Overall, there was no effect on reproductive success (conception rate, fertility indices, litter size).

In another two generation study on rat conducted at 200, 800 and 3600 ppm (Richter, 1985). Similar results were observed compared with the other multi-generation study in terms of reduced food consumption, and resulting lower mean bodyweight compared with the control (≤10%). Reduced maternal feeding also resulted in a 17% (F1a and b litters) and 19% (F2 a and b litters) lower mean pup weight compared with the control at the highest treatment level. Overall, there were no effects on reproductive parameters.

The advice in the EU guidance document on risk assessment for birds and mammals is relevant on this issue. The key guidance is*: 'The usual approach is based on the consideration that effects on populations will not occur if the survival rate, reproduction rate and development of individuals are not affected. Therefore, in principle, only endpoints in toxicity tests which are related to these key factors of population dynamics are ecotoxicologically relevant.'* and also *'If not indicated otherwise by the overall toxicological data available, an endpoint relating to overall reproductive success should be selected to define the long term NOEC for birds and mammals*.'

In view of this guidance it is still considered that the 5000 ppm treatment level is an appropriate basis for the risk assessment, as there was no effect on reproductive success. Effect on bodyweight of adults and pups was likely to be the result of reduced food consumption due to reduced palatability of the treated feed. In the field, potential dietary exposure concentrations are much lower. Hence, the reduced feeding in the studies (and resulting lower bodyweights) is not relevant to the risk assessment. Hence, it is proposed that the endpoint of 5000 ppm, which is equivalent to daily dose of 548.6 mg/kg bw should be used in the risk assessment (as proposed in Norman and Wyness, 2003 and as stated in the DAR addendum of March 2005). Therefore, the risk assessment for wild mammals can remain unchanged and a low long term risk can be concluded.

Note on potential for exposure of herbivorous mammals: For the proposed use in tomato, there is unlikely to be exposure of herbivorous mammals because tomato foliage is unpalatable. For the proposed use in cereals, applications are only made to later growth stages, which are also not palatable. For use in grapevines in south EU, again there is minimal potential for exposure because there is usually no ground vegetation (removed

because of competition for water). Only the use in vineyards in central EU gives some potential for exposure. However, even in this case there is will be some management of ground vegetation which is likely to affect the attractiveness of the grass as a foraging habitat.

Comment from the RMS on the above statement:

The Notifier has provided an assessment of the endpoint similar to that previously submitted (Norman and Wyness, 2003). The endpoint has been reconsidered by the RMS in line with the Open Point. In the test group exposed to 5000 ppm (548.6 mg/kg bw/d) there was no effect on reproductive success. Following the EU guidance, this treatment level is considered to be an appropriate endpoint for the long term risk assessment (for which the focus is at the population level). Therefore, it is proposed that the risk assessment can remain as stated in the previous addendum to the DAR.

The comment from the Notifier regarding potential exposure is noted. For use in tomato, cereals and south EU vineyards, there is unlikely to be significant exposure of herbivorous mammals.

New open point 5.18:
RMS to conduct a long-term risk assessment for aquatic organisms based on NOEC values from chronic studies and the initial peak PECsw. See open point 5.9. This open point was proposed at EPCO 22.

The Notifier has submitted the following statement (ref: Norman, 2005):

This recommendation is regarding the potential use of the NOEC from the 28 day semi-static study on rainbow trout (as this study mimics repeated exposure) by comparing this with the initial peak PECsw.

To aid consideration of this issue, a summary of the 28 day semi-static study on rainbow trout (Jenkins, 1999) is included below:

The prolonged toxicity of Folpan 500 SC (containing 519 g folpet/L) to rainbow trout (*Oncorhynchus mykiss*) was determined under semi-static conditions. Groups of 10 fish were placed in 40 L test medium and were exposed to 0, 9.8, 19.5, 39, 78 and 156 µg a.s./L for 28 days. The test media were renewed at 48 or 72-hour intervals. Observations of abnormal behaviour and mortality were recorded after 15 minutes and daily thereafter. Individual fish wet weights and fork lengths were measured at the end of the test. Results are summarised in Table 7, 8 and 9.

Table 7: Cumulative mortality of rainbow trout under semi-static conditions

Day no.	Cumulative mortality (%)					
	Control	9.8 µg a.s./L	19.5 µg a.s./L	39 µg a.s./L	78 µg a.s./L	156 µg a.s./L
1	0	0	0	0	0	30
2	0	0	0	0	0	50
3	0	0	0	0	0	60
4	0	0	0	0	0	70
5	0	0	0	0	0	70
6	0	0	0	0	10	90
7	0	0	0	0	10	90
8	0	0	0	0	10	90
9	0	10	0	0	10	90
10 – 28	0	10	0	0	10	90

Table 8 Symptoms (% affected) of r. trout exposed under semi-static conditions

Day no.	symptoms and % affected [a]					
	control	9.8 µg a.s./L	19.5 µg a.s./L	39 µg a.s./L	78 µg a.s./L	156 µg a.s./L
1	-	-	E10	H20/L20	H40	H86/L29/O14
2	-	B10	B20	B30/H10	B10/H40	E20/H100
3	E20	E10	E10	E20/L10	B10/H30	E25/H100
4	E10	B10	E10	B20/H20	B10/H50/L10	B66/H100
5	-	B10	-	H30	H60	B33/H100
6	B10	B10	-	H50	H33	H100
7	-	B10	E10	B30/H30	H33	B100/H100
8	-	B10/H10	-	H30	B11/H33/C22	B100/H100
9	-	-	-	-	B11/H55	H100
10	-	-	-	H10	H33/S11	H100
11	-	-	-	H10	H33	H100
12	-	E11	-	E10/B10	B22/H33	H100
13	-	-	-	E10/B10	B11/H44/L11	H100
14	-	-	-	B10	B22/H33	H100
15	-	-	-	B10	E11/B11/H44	H100/L100
16	-	B11	-	B10	B22/H44	H100
17	-	-	-	B10	B22/H33	H100
18	-	B11	B10	-	B22/H33	B100/H100
19	-	-	B10	B10	E11/H22	E100
20	-	E11	H10	B10	E11/H33	E100
21	-	B11	B10	B10	B22/H33	E100/H100
22	-	-	B10	H10	B22/H22	E100
23	-	-	-	-	B11/H33	E100
24	-	-	-	B10	B11/H22/L11	E100
25	-	B22	-	B10	H22	-
26	-	-	-	-	B11/H11/L11	-
27	-	B11	B10	B10	B22/H11/L11	-
28	-	B11	B10	B10	B11/H11/L11	-

[a] Codes (below) shown with percentage of total surviving fish affected:
E: darkened pigmentation of the eye orbits
B: darkened pigmentation of the body
H: hyperventilation
L: lethargy
C: coughing
O: loss of co-ordination
S: erratic swimming.

Table 9: Wet weight and fork length of surviving rainbow trout exposed for 28 days under semi-static test conditions

Time	µg a.s./l	Growth parameter	
		Mean wet weight (g)	Mean fork length (mm)
Before test	-	1.68	56.2
End of test	Control	2.99	64.5
	9.5	2.98	63.4
	19.5	2.95	64.3
	39	2.67	62.7
	78	2.72	62.6
	156	1.30*	56.0

*Statistically significantly difference from control.

The 28-day LC_{50} was 110 µg a.s/L. At 9.8 to 78 µg a.s./L there was no significant difference in the final, mean wet weights and fork lengths compared to controls. The 28-day no observed lethality concentration (NOLC) was 39 µg a.s./L, based on 10% mortality at 78 µg a.s./L. The study author stated a NOEC of 19.5 µg a.s./L.

Implication of 28 day semi-static study on rainbow trout to the aquatic risk assessment:

There was no effect on growth (apart from for the one surviving fish at the highest treatment level, 156 µg a.s./L). Effects in the study were acute (e.g. hyperventilation) and were consistent with the mode of toxicity as an irritant to gill membranes. Sub-lethal effects were reversible. Hence, the No Observed Lethality Concentration (NOLC) is more relevant than the NOEC from this study.

There were 3 media exchanges per week during this 4 week study (total of 12 exchanges). There was no build up of mortality or extent of sub-lethal effects at 78 µg a.s./L (apart from 1 mortality after 6 days). The high level of mortality at 156 µg a.s./l (90% mortality) on day 6 was probably related to the short interval between the acute exposures (1st media exchange after 48 hours). This result at 156 µg a.s./L has limited relevance to the risk assessment because the proposed minimum spray interval in the GAP is **7** days. Overall, the study is sufficient to show that for a multiple spray program with a minimum spray interval of 7 days, there is unlikely to be a build up of effects compared with a single exposure.

It should also be noted that static acute toxicity studies on fish show reversibility of sub-lethal effects where lethality does not occur. In the acute study on brown trout, which was the most sensitive fish species tested (ref: Jenkins, 2002b. DAR ref: 8.2.1/04), 3 out of 7 fish showed hyperventilation at 66 µg/L after 2 hours, but 2 recovered by 48 hours, and the remaining 1 by 72 hours (no mortality at this treatment level). For rainbow trout (ref: Jenkins, 2002a. DAR ref: 8.2.1/03), at 117 µg/L 6 out of 7 fish showed hyperventilation after 24 hours, with 5 of these recovering at 72 h (i.e. 48 h later)(no mortality at this treatment level). These recovery periods are less than the proposed spray interval of 7 days in the GAP, so support the conclusion that there will be no build-up of effects.

It is also noted that in the field it is unlikely that the *same* individual fish would be exposed at a similar magnitude on several occasions as a result of the GAP.

Based on the above, it is proposed that the risk assessment should still be based on acute static toxicity studies on fish (lowest LD50 92 µg a.s./L for brown trout) where fish received a single exposure.

Comment from the RMS on the above:

The interval between exposures (media exchanges) of 2 or 3 days in the semi-static study is shorter than the minimum spray interval in the GAP (7days). Hence, the exposure regime in the study was more worst-case than could occur in the field. Therefore, it not considered relevant to use endpoints from this study for calculation of TERs. The study does in general show no build up of sub-lethal effects. Static acute toxicity studies on fish (as summarised in the DAR, Section B.9.2.1.1) generally show reversibility of sub-lethal effects where mortality has not occurred. This recovery is usually within 1 to 3 days. Overall, with a minimum spray interval of 7 days as in the GAP, it is unlikely that damage to fish would accumulate. It is proposed that the risk assessment should still be based on static acute toxicity studies on fish.

Data requirement 5.6: Notifier to repeat the 21 d Daphnia study under semi static conditions. The study should be conducted according to OECD guidelines. See open point 5.9. This data gap was identified at EPCO 22.

The following statement has been submitted by the Notifier (ref: Norman 2005):

The acute risk to fish should determine the outcome of the risk assessment. A 21 day semi-static study on *D. magna* is unlikely to affect this conclusion. However, to provide reassurance on this issue the Notifier agrees to conduct a 21-day semi-static study on *D. magna* for submission at Member State level in support of re-registration following Annex I listing.

Comment from RMS on the above:

RMS agrees with Notifier. A 21 day semi-static study on *D. magna* may be useful as supplementary information. However, it is unlikely to affect the risk assessment, which should be based on acute risk to fish. It is noted that the Notifier proposes to conduct this study, for submission at Member State level.

New references, by Annex point:

Annex point / reference number	Author(s)	Year	Title, Source (where different from company) Company, Report No. GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N	Owner
Annex IIIA **11.1**	Gerlach, J	2005	Folpet: Long-term risk assessment for insectivorous birds. September, 2005 Rifcon Report No. RC05-017 GLP not applicable, unpublished.	Y	Makhteshim
	Norman, S	2005	Folpet: Responses to questions raised at EPCO 22 on ecotoxicology. September 2005.	Y	Makhteshim

Appendix:1

Gerlach, 2005: Captan: Long term risk assessment for insectivorous birds (Rificon study report, September 2005):

1. Background information

Use pattern of Folpet

The use pattern of Folpet on which the refined risk assessment is based is shown in Table 1.

Table 1 Crops and application rates for Folpet

Crop	Northern Europe (NE)/ Southern Europe (SE)	Max. application rate kg a.s./ha	Max. no. of applications
Tomatoes	SE	1.25	4
Grapes	NE / SE	1.5	10

Effects on birds

The long-term toxicological endpoint for birds used in this refined risk assessment is presented in Table 2

Table 2 Toxicological endpoint for birds for Folpet

Organism	Duration	Test-substance	Ecotoxicological endpoint
Bobwhite quail	chronic, dietary	a.s.	NOEC = 1000 ppm equivalent to NOEC = 78.3 mg a.s./kg b.w./day

Refinement of factors

Species of concern

Tomatoes

According to SANCO/4145/2000 the risk for an insectivorous bird as a recommended indicator species has to be evaluated in the leafy crop category, i.e. in tomatoes.

The yellow wagtail (*Motacilla flava*) was frequently observed in tomato fields and was confirmed to be nesting inside the crop (Anonymous 2004).

Based on these data yellow wagtails are deemed to be a characteristic species to be encountered in tomato fields in Southern Europe. This species will be used in the risk assessment for Southern European tomato fields.

Grapes

Central Europe

A survey on the birds inhabiting vineyards has been conducted at four study sites in southwestern Germany from April to August 2003 by territory mapping (Pedall et al. 2003). The study sites differed in structure from richly structured small-scale vineyards to large scale monotonous vineyards. The bird contacts were assigned to certain habitat elements such as hedgerows, farm tracks, vines and open landscape to get some insight into the use of habitat element by certain bird species. As a result 44 species of birds were observed of which 24 were breeding within the study sites.

Among the birds typically foraging within the vineyards the linnet proved to be the representative species as it occurred at every study site. However, the linnets diet predominantly consists of small seeds (Frey 1989, Eybert and Constant 1998). Among the insectivorous guild, the yellowhammer and the blackbird were those species identified as characteristic, i.e. they are encountered regularly feeding in the vineyards by Pedall et al. 2003. The representative status of yellowhammer for Central European vineyards was corroborated by a comprehensive study in southern Germany on birds of vineyards (Seitz 1989). The yellowhammer was considered to be the most common bird species in vineyards in southern Germany (Braun 1985, Seiler 1986).

Southern Europe

Southern European vineyards are cultivated on bare soil. The main factor regulating different agricultural practices is precipitation and access to other water sources. For example, in Spain, vines are cultivated in regions of down to 250 mm precipitation per year (Hidalgo 2002). Only 7.5 percent of vineyards were supplied with artificial irrigation in 1997, while the remaining 92.5 percent were not irrigated.

In order to cope with the limited availability of water, vineyards in Spain are treated following the traditional “secano” technique, in which vineyards are ploughed three to four times a year in order to permit an optimal exploitation of the limited rainfall and to inhibit the growth of water consuming ground vegetation (Hidalgo 2002).

The yellowhammer occurs throughout Central and northern Europe. In southern Europe its ecological niche is occupied by a closely related species, the cirl bunting *Emberiza cirlus* whose range has some overlap with the yellowhammer in several parts of Europe, i.e. France, Germany, Italy and Greece (Glutz von Blotzheim and Bauer 1997).

The cirl bunting is considered to be the southern European representative of the yellowhammer. There are a number of hints in the scientific literature that the species is

regularly found in vineyards (Groh 1975, Glutz von Blotzheim and Bauer 1997, Hölzinger 1997, Sierro and Arlettaz 2003). In southwestern Germany cirl buntings were even observed nesting in vines (Groh 1975).

Refinement of the food intake rate (FIR)

Yellow wagtail

Yellow wagtails have a body weight of about 17 g (Dittberner & Dittberner 1984). The average daily food intake was estimated to amount 73.7 kJ/day according to (Crocker et al. 2002a) based on a body weight of 17.0 g (Dittberner and Dittberner 1984). Arthropods contain on the average 21.9 kJ/g dry weight and consist of 70.5% water. Therefore arthropods contain 6.5 kJ/g fresh weight. A yellow wagtail using 73.7 kJ/day will eat 11.4 g arthropods per day. Adjusting this figure for assimilation efficiency (76% for a passerine bird) this results in an average daily food intake of a yellow wagtail of 15 g arthropods per day. Related to the average body weight the FIR/bw will be **0.88**.

Yellowhammer

The body weight of a yellowhammer according to Crocker et al. 2002 is 26.5 g. The average daily food intake of a yellowhammer was estimated to amount 100.6 kJ/day according to (Crocker et al. 2002a) based on a body weight of 26.5 g.

Arthropods on the average contain 21.9 kJ/g dry weight and consist of 70.5% water. Therefore arthropods contain 6.5 kJ/g fresh weight. A yellowhammer using 100.9 kJ/day will eat 15.6 g arthropods per day. Adjusting this figure for assimilation efficiency (76% for a passerine bird) this results in an average daily food intake of a yellowhammer of 20.5 g arthropods per day. Related to the average body weight the FIR/bw of yellowhammer feeding on arthropods will be **0.77**.

Weed seeds on the average contain 21.0 kJ/g dry weight and consist of 11.9% water (Crocker et al. 2002a). Therefore weed seeds contain 18.5 kJ/g fresh weight. A yellowhammer using 100.9 kJ/day will have to eat 5.4 g weed seed per day. Adjusting this figure for assimilation efficiency (80% for a passerine bird) this results in an average daily food intake of a yellowhammer of 7.2 g weed seed per day. Related to the average body weight the FIR/bw of yellowhammers feeding on weed seed will be **0.26.**

Cirl bunting

The average body weight of German cirl buntings weighted between March and July was 23.9 g (n = 173) for males and 23.6 g (n = 16) for females (Groh 1975). The average daily food intake of a cirl bunting was estimated to amount 92.8 kJ/day according to (Crocker et al. 2002a) based on a body weight of 23.6 g.

Arthropods on the average contain 21.9 kJ/g dry weight and consist of 70.5% water. Therefore arthropods contain 6.5 kJ/g fresh weight. A cirl bunting using 92.8 kJ/day will eat 14.4 g arthropods per day. Adjusting this figure for assimilation efficiency (76% for a passerine bird) this results in an average daily food intake of a cirl bunting of 18.9 g arthropods per day. Related to the average body weight the FIR/bw of cirl bunting feeding on arthropods will be **0.80**.

Caterpillars on the average contain 21.7 kJ/g dry weight and consist of 79.5% water (Crocker et al. 2002a). Therefore caterpillars contain 4.4 kJ/g fresh weight. A cirl bunting using 92.8 kJ/day will have to eat 20.9 g caterpillars per day. Adjusting this figure for assimilation efficiency (76% for a passerine bird) this results in an average daily food intake of a cirl bunting of 27.4 g caterpillars per day. Related to the average body weight the FIR/bw of cirl buntings feeding on caterpillars will be **1.16**.

Weed seeds on the average contain 21.0 kJ/g dry weight and consist of 11.9% water

(Crocker et al. 2002a). Therefore weed seeds contain 18.5 kJ/g fresh weight. A cirl bunting using 92.8 kJ/day will have to eat 5.0 g weed seed per day. Adjusting this figure for assimilation efficiency (80% for a passerine bird) this results in an average daily food intake of a cirl bunting of 6.6 g weed seed per day. Related to the average body weight the FIR/bw of cirl buntings feeding on weed seed will be **0.27.**

Refinement of the proportion of diet obtained in the treated area (PT)

Yellow wagtail

The proportion of diet obtained within the tomato field is set at **0.5**. This is explained by the peculiar biology of the yellow wagtail since for the yellow wagtail a distinction between nesting habitat and foraging habitat is obvious and reported from several studies.

For nesting, yellow wagtails require sufficient vegetation to cover the nest. However, for foraging, the species prefers areas devoid of vegetation or characterized by short vegetation such as lawns, short-grazed pastures or mown meadows (Dittberner & Dittberner 1984). For example, optimum nesting habitats for yellow wagtails in Russia were characterized by a vegetation height of 40 – 60 cm and a coverage of 90% (Dittberner & Dittberner 1984). The preference for yellow wagtails to feed in areas of short vegetation (e.g. pasture) or bare soil is in accordance with the main foraging behaviour of the species, i.e. picking arthropods from the ground or fly-catching arthropods (Davies 1977, Dittberner and Dittberner 1984). Thus, a pronounced distinction between foraging habitat and nesting habitat is obvious. Commonly parent wagtails have to cover a distance of several hundreds of meters between nesting and foraging habitat (Dittberner & Dittberner 1984).

For a long-term risk assessment it is therefore assumed that yellow wagtails will obtain at least 50% of their diet outside the treated areas. This PT of 0.5 is still considered to be a conservative estimate.

This was corroborated by the EFSA PPR Panel that concluded, considering long-term exposure, that PT for yellow wagtails in potato fields would be less than 0.5 (Anonymous 2004). As observations of yellow wagtails in tomatoes showed similar numbers compared to potatoes (Anonymous 2004), the results are assumed to hold also true for tomato fields.

Refinement of the portion of diet (PD)

Yellow wagtail

In a study on the foraging behaviour of yellow wagtails in the UK the diet of solitary foraging yellow wagtails was examined on non-flooded areas of a meadow (Davies 1977). The predominant prey types of foraging yellow wagtails were flies around dung pats. The availability of the individual prey types was estimated by counting the number of prey individuals per 100 dung pats transect. The size distribution of available insects and ingested insects (from assessment of faecal material) was ascertained (Table 3). This research is valuable to the risk assessment. In effect, the yellow wagtails are presented with insects in a range of sizes (dung flies in a meadow in this case), from which their size preference is determined.

Table 3 The prey types eaten by solitary foraging yellow wagtails (adopted from Davies 1977)

Prey type	Body length	Availability	remains in droppings
	[mm]	[%]	[%]
Scatophagidae	5-10	77.1	35.1
Sphaeroceridae	1-2	6.9	2.3
Sphaeroceridae	3-4	10.1	41.3
Sepsidae	3-4	0.7	0.0
Coleoptera	2-3	5.1	6.4
others	--	0.1	14.9

Scatophagidae vary from 5 mm to 10 mm in body length with females being smaller. On the dung pats males outnumbered females by 3.7 to 1.0. Yellow wagtails preferred flies having about 7 mm in length. Prey up to this size is swallowed immediately in a very short period of time (< 1 sec). Larger prey, 10 mm in length, is bashed against a perch, sometimes dropped and took 5 – 10 sec to handle (Davies 1977).

From caloric specific values and the handling times for each size of prey, the energy intake per unit handling time was calculated and it could be seen that the size of the prey selected by wild wagtails corresponds to the optimum prey size they can handle. Thus small prey items (1-2 mm) were ignored because although quick to handle the ratio between energy used for foraging and energy gained from successful prey was too unfavorable for the bird. On the other end of the scale the largest Scatophagidae were rejected because although worth very much energy they took too long to handle (Davies 1977).

Based on the data presented by Davies (1977) which is the most comprehensive study on yellow wagtail diet available, the majority of prey items collected by yellow wagtails are 3 - 4 mm and greater.

The size definition of ‘small’ and ‘large’ insects is not stated in the EU guidance document on risk assessment for birds and mammals. The residue estimate for ‘small’ insects in the guidance document is derived from Kenaga (1973) on the basis of residues in weed seeds. Such seeds would typically be 1-2mm. The residues estimate for ‘large’ insects, which was previously quoted in the EPPO 1992 vertebrate scheme, came from the same published paper. This value was based on residues on wheat seeds. Wheat seeds are typically 4-5 mm in length. Hence, a working definition of ‘large’ (≥ 3 mm) and ‘small’ (<3mm) can be determined.

By summing the percentages for flies from the 3-4 mm and 5-10 mm categories (please see table 3), a total of 76.4% is derived (**PD** of **0.764**). PD for small insects is conservatively set at **0.236** (remaining groups).

These percentages are based on numbers of insects, *not* mass of insects. Clearly, the proportion of large insects based on *mass*, would be much more than 76.4%. In order to give an indication of the proportion according to mass, data are needed on the corresponding mass for a fly of known length. Makhteshim asked a contract laboratory (Huntingdon Life Sciences) to measure and weigh dung flies, and winged aphids (as surrogate for small dung flies). The data are presented below:

Table 4 Winged aphid wet weight and body length (weighed on 13 September 2004)[1]

Insect no.	*Rhopalosiphum padi* (Cereal aphid)		*Acyrthosiphon pisum* (Pea aphid)	
	Body length (mm)	Wet weight (mg)	Body length (mm)	Wet weight (mg)
1	1.41	N/A	2.98	2.1
2	1.66	N/A	2.83	1.8
3	1.72	N/A	3.41	2.1
4	1.28	N/A	3.07	2.1
5	1.74	N/A	3.00	1.8
6	1.38	N/A	3.14	1.9
7	1.68	N/A	2.53	1.4
8	1.66	N/A	2.77	1.7
9	-	-	2.69	1.4
10	-	-	2.44	1.1
11	-	-	2.58	1.8
12	-	-	2.94	1.5
13	-	-	2.46	1.4
14	-	-	2.59	1.5
15	-	-	3.12	1.9
16	-	-	3.33	1.7
17	-	-	2.96	1.2
18	-	-	3.08	1.5
19	-	-	2.67	1.2
20	-	-	2.50	1.0
21	-	-	3.01	1.7
Total	-	1.5	-	-
Mean	**1.5663**	**0.1875**	**2.8619**	**1.6095**

N/A = Not Applicable; insects were weighed together to arrive at mean body mass.
1: Alan Lawrence, HLS, personal communication, 13 Sept 2004.

Table 5 Winged aphid wet weight and body length (weighed on 13 September 2004)[1]

Fly number	*Scathophaga stercoraria*	
	Body length (mm)	Wet weight (mg)
1	7.62	25.6
2	5.99	19.3
3	6.89	24.9
4	7.04	26.3
5	7.62	31.2
6	7.19	22.0
7	7.70	30.3
8	7.12	23.6
9	5.87	13.8
10	6.15	17.7
11	8.58	45.9
12	7.72	23.2
13	9.56	41.2
Mean	**7.31**	**26.54**

1: Alan Lawrence, HLS, personal communication, 13 Sept 2004

Based on the above a fly of body length 7.3 mm, is around 17 times heavier than an aphid of 2.9 mm body length, which in turn is around 15 times heavier than an aphid of 1.6 mm bodyweight.

Hence, it can be determined that using a **PD** of **0.764** for large insects, based on information of number of insects, rather than mass, is particularly conservative.

Yellowhammer

The yellowhammer is known to feed on seeds, especially of grasses, while invertebrates are preyed in the breeding season and casually throughout remainder of the year (Perrins 1998).

A field study on the diet of the yellowhammer was conducted in an intensively managed richly structured agricultural area in Schleswig-Holstein, Germany between 6th June and 8th August 1987-90 (Lille 1996). The prey items of adult yellowhammers (12 pairs) were studied (1416 foraging flights of the adults) by means of photographical documentation (1691 photos) and direct observations. The prey items consisted of almost 84% animal and 16% vegetable items. Main components of the diet were 47% dipteran larvae (particularly Syrphid larvae), 16% cereal grains (especially oats), 12% lepidopteran larvae and further arachnids (8%), coleopterans (6%), dipteran imagines (4%), lepidopteran adults (2 %) and approximately 4% of the items could not be determined (Lille 1996).
This study also revealed data on the size of the prey items of yellowhammers. According to the results the prey size and prey length ranged from 3 mm and 5 mg in case of harvestmen (Opiliones) to 30 mm and 380 mg for craneflies (Tipulidae).

The majority of the nestlings diet of yellowhammers (42% of 4764 prey items) consists of small prey items with an average weight between 5 mg and 20 mg. This prey size class was dominated by small syrphid larvae (8 mm; 20 mg) (Lille 1996). The next prey size class included objects of 20-40 mg fresh weight (such as cereals grains) and of 40-60 mg fresh weight. 82% of the analysed food items had a fresh weight between 5 mg and 60 mg. 58% of

the prey weight was above 20 mg. The fresh weight per load delivered to the nestlings was found to range between 5 mg and 1,150 mg but 95 % of the loads had a weight below 580 mg (average weight 194 mg ± 187 mg, n = 1416) (Lille 1996).

Based on the results of the most comprehensive study on diet of yellowhammers (Lille 1996) it is obvious that the bulk of the food items of yellowhammer nestlings, which represent the worst case scenario regarding risk assessments of plant protection products intended to be used in vineyards, is equivalent or exceeds the size of cereal grain (5 mm). Thus as a realistic approach in a tier 2 assessment, the use of default residues of large insects (size of cereal grain or larger) would be justified for the bulk of arthropod prey species.

As a conclusion and synopsis of the results of the study presented above a realistic portion of diet of yellowhammer is estimated to comprise 15% weed seeds (cereal grain is expected to be non-available in vineyards), 75% large insects and 10% small insects. This is considered to be a worst case assumption as small amount of typical small insects such as aphids and collembolans was either reported to be rather small, e.g. few individuals of aphids (Bösenberg 1958), 1.2% of diet for aphids and collembolan respectively (Moreby and Stoate 2000), or not mentioned at all (Lille 1996). Thus the above suggested composition of diet is considered to be a realistic estimate of the diet of yellowhammers foraging in vineyards.

Cirl bunting

There is only limited data on the diet composition of adult cirl buntings. The diet consists mainly of seed. As in the yellowhammer invertebrates however predominate during the breeding season (Perrins 1998).

The nestlings of cirl buntings are given mostly invertebrates, but also seeds. In Devon, U.K., of 174 adult visits (where items identified) to 2 nests, 34% were with adult Lepidoptera, 20% Tettigoniidae, 18% larvae, 17% cereal grain, and 10% Acrididae; at a 3rd nest, 51% of 1108 visits were with barley and wheat grains, 25% invertebrates, and 24% unidentified; at 4th, of 41 invertebrates, 18 were Lepidoptera (16 caterpillars), 9 other larvae, 8 spiders, 3 Acrididae, 2 Diptera, and 1 Tettigoniidae. Of 7 faecal sacs from young, all 7 contained Diptera, 5 caterpillars, 5 Staphylinidae, 3 sawfly larvae, 3 Curculionidae; 6 contained seeds of barley, 5 nettle, and 4 Poa. At one nest, the male brought significantly more Lepidoptera, Diptera and spiders. More larval than adult Lepidoptera were brought to earlier nests, vice versa to later ones, perhaps reflecting abundance. Although, only invertebrates were recorded at early nests, grain is being taken as it ripens towards end of July, and adults collect grain even when invertebrates are easily obtainable, so cereal grains are apparently important components of the diet for later broods, especially in rain. Most food is gathered 20–100 m from the nest, though one pair flew 250 m to a barley field. (Sitters 1991 cited in Perrins 1998).

In south-west Germany cirl buntings have been observed to feed their young predominantly with larvae of green oak leafroller *Tortrix viridana*. White and grey Microlepidopterans and grasshoppers have been identified as additional major prey items of adult cirl buntings. Occassionally small to medium-sized beetles, cutworm moths (Noctuidae), mayflies, aphids and lumbricids are caught as food of nestlings (Groh 1975).

No data could be obtained in the scientific literature on the size of prey items of cirl buntings. Based on the predominant prey item in southwestern Germany, i.e. larvae of green oak leafroller (Groh 1975), it is assumed that the majority of prey items exceed the size of 5 mm. Based on the overall biology of the species and its close relationship the size of its prey items is assumed to be equivalent to the yellowhammer.

Based on these limited data, a realistic diet of cirl buntings during the breeding season is considered to be composed of 10% small insects, 20% weed seed (cereal grain is not assumed to be available in vineyards) 50% large insects and 20% caterpillars.

Refinement of foraging strata

Both key species, yellowhammer and cirl bunting, almost exclusively forage on the ground (Perrins 1998). The inclusion of a deposition factor is therefore deemed to be valid.

3. Exposure assessment

3.1 Yellow wagtail

The exposure assessment for yellow wagtails potentially foraging in tomato fields treated with Folpet at a rate of 1.25 kg a.s./ha is depicted in Table .

Table 6 Exposure assessment for yellow wagtails in tomato fields

Diet proportions	large insects	small insects	Whole diet
Application rate [kg a.s./ha]	1.25	1.25	
RUD [mg/kg a.s./ha]	5.1	29	
Maximum initial concentration after last application [mg a.s./kg]	6.375	36.25	
Relative daily food intake (FIR/b.w.) [g fresh weight/g b.w./day]	0.88	0.88	
Portion of diet obtained in-crop (PT)	0.5	0.5	
Portion of diet (PD)	0.764	0.236	
Estimated theoretical exposure (ETE) [mg a.s./kg b.w./day]	2.14	3.76	**5.90**

3.2 Yellowhammer

The exposure assessment for yellowhammers foraging in Northern European vineyards treated with Folpet at a rate of 1.5 kg a.s./ha is depicted in Table .

Table 7 Exposure assessment for yellowhammers potentially foraging in vineyards in Central Europe

Diet proportions	Arthropods large	Arthropods small	Weed seeds	
Application rate [kg a.s./ha]	1.5			
RUD [mg/kg a.s./ha]	5.1	29	40 [1)]	
Maximum initial concentration after last application [mg a.s./kg]	7.65	43.5	60	
Multiple application factor (MAF)	1	1	1	
Relative daily food intake (FIR/b.w.) [g fresh weight/g b.w./day]	0.77	0.77	0.26	
Portion of diet obtained in-crop (PT)	1	1	1	
Portion of diet (PD)	0.75	0.1	0.15	
Deposition factor	0.6	0.6	0.6	
Estimated theoretical exposure (ETE) [mg a.s./kg b.w./day]	2.65	2.01	1.40	**6.06**

1) Mean values of Fletcher et al. 1994 as derived from the Guidance Document (Anonymous 2002)

3.3 Cirl bunting

The exposure assessment for cirl buntings potentially foraging in Southern European vineyards treated with Folpet at a rate of 1.5 kg a.s./ha is depicted in Table .

Table 8 Exposure assessment for cirl buntings potentially foraging in vineyards in Southern Europe

Diet proportions	Arthropods large	Arthropods small	Caterpillars	Weed seeds	
Application rate [kg a.s./ha]	1.5				
RUD [mg/kg a.s./ha]	5.1	29	5.1	40 [1)]	
Maximum initial concentration after last application [mg a.s./kg]	7.65	43.5	7.65	60	
Multiple application factor (MAF)	1	1	1	1	
Relative daily food intake (FIR/b.w.) [g fresh weight/g b.w./day]	0.8	0.8	1.16	0.27	
Portion of diet obtained in-crop (PT)	1	1	1	1	
Portion of diet (PD)	0.5	0.1	0.2	0.2	
Deposition factor	0.6	0.6	0.6	0.6	
Estimated theoretical exposure (ETE) [mg a.s./kg b.w./day]	1.84	2.09	1.06	1.94	**6.93**

1) Mean values of Fletcher et al. 1994 as derived from the Guidance Document (Anonymous 2002)

4. Risk assessment

The risk assessment of Folpet for insectivorous birds in tomatoes and grapes is depicted in Table 6.

Table 9 Refined long-term TER calculation for insectivorous birds

Species	Scenario	Toxicity mg/kg b.w./day	ETE mg/ kg b.w.	TER_{lt}
Yellow wagtail	Tomatoes	**78.3**	5.90	**13.27**
Yellowhammer	Grapes (Northern Europe)		6.06	**12.92**
Cirl bunting	Grapes (Southern Europe)		6.93	**11.30**

A further refinement of the long-term risk assessment for insectivorous birds potentially foraging in tomatoes and grapes is not necessary. The TER-values derived from the application scenarios are above the Annex VI trigger of concern of 5 for long-term exposure, indicating a considerable margin of safety for wild birds from the use of Folpet in tomatoes and grapes under practical conditions.

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FOLPET

Addendum

Prepared by EFSA on 15. 11. 2005

Section Ecotoxicology

In the DAR no risk assessment was conducted for the risk to birds and mammals from the uptake of contaminated drinking water. It is not clear whether exposure to contaminated drinking water in puddles/leaf axils can be excluded for the representative uses of folpet. Therefore EFSA calculated the TER values according to the "Guidance Document on Risk Assessment for Birds and Mammals Under Council Directive 91/414/EEC" (Sanco/4145/2000 of 25. September 2002). The exposure concentration was calculated from the sprayed solution with a dilution factor of 5.

The endpoints from the list of endpoints of September 2005) were used for the TER calculations.

Table 1: Acute, short-term and long-term risk for birds and mammals from consumption of contaminated drinking water for the intended use in winter wheat for a sprayed solution of 3750 mg a.s./L.

Organisms	Risk	PEC* mg a.s./L	Body weigh t in kg	Total water ingestion . rate	Daily dose (mg a.s./kg bw/d)	LC50/NOE C (mg a.s./ kg bw/d	TER	AnnexV I trigger
Birds	Acute	750	0.01	0.002697	202.2615239	> 2510	> 12.4	10
	Short -term	750	0.01	0.002697	202.2615239	> 746	> 3.69	10
	Long-term	750	0.01	0.002697	202.2615239	78.3	0.39	5
Mammals	Acute	750	0.01	0.001569	117.6783195	> 2000	> 17	10
	Long-term	750	0.01	0.001569	117.6783195	548.6	4.66	5

* The PEC drinking water (= 20 % of the sprayed concentration) was calculated according to SANCO/4145/2000 (25. Sep. 2002).

Table 2: Acute, short-term and long-term risk for birds and mammals from consumption of contaminated drinking water for the intended use in tomatoes for a sprayed solution of 1250 mg a.s./L.

Organisms	Risk	PEC* mg a.s./L	Body weigh t in kg	Total water ingestion . rate	Daily dose (mg a.s./kg bw/d)	LC50/NOEC (mg a.s./ kg bw/d	TER	AnnexVI trigger
Birds	Acute	250	0.01	0.002697	67.42050797	> 2510	> 37.23	10
	Short -term	250	0.01	0.002697	67.42050797	> 746	> 11.06	10
	Long-term	250	0.01	0.002697	67.42050797	78.3	1.16	5
Mammals	Acute	250	0.01	0.001569	39.22610651	> 2000	> 50.99	10
	Long-term	250	0.01	0.001569	39.22610651	548.6	13.99	5

* The PEC drinking water (= 20 % of the sprayed concentration) was calculated according to SANCO/4145/2000 (25. Sep. 2002).

Table 3: Acute, short-term and long-term risk for birds and mammals from consumption of contaminated drinking water for the intended use in grapes for a sprayed solution of 7500 mg a.s./L.

Organisms	Risk	PEC* mg a.s./L	Body weigh t in kg	Total water ingestion . rate	Daily dose (mg a.s./kg bw/d)	LC50/NOEC (mg a.s./ kg bw/d	TER	AnnexVI trigger
Birds	Acute	1500	0.01	0.002697	404.5230478	> 2510	> 6.21	10
	Short -term	1500	0.01	0.002697	404.5230478	> 746	> 1.84	10
	Long-term	1500	0.01	0.002697	404.5230478	78.3	0.19	5
Mammals	Acute	1500	0.01	0.001569	235.3566391	> 2000	> 8.5	10
	Long-term	1500	0.01	0.001569	235.3566391	548.6	2.33	5

* The PEC drinking water (= 20 % of the sprayed concentration) was calculated according to SANCO/4145/2000 (25. Sep. 2002).

Table 4: Acute, short-term and long-term risk for birds and mammals from consumption of contaminated drinking water for the intended use in grapes for a sprayed solution of 3750 mg a.s./L.

Organisms	Risk	PEC* mg a.s./L	Body weigh t in kg	Total water ingestion . rate	Daily dose (mg a.s./kg bw/d)	LC50/NOE C (mg a.s./ kg bw/d	TER	AnnexV I trigger
Birds	Acute	750	0.01	0.002697	202.2615239	> 2510	> 12.41	10
	Short -term	750	0.01	0.002697	202.2615239	> 746	> 3.69	10
	Long- term	750	0.01	0.002697	202.2615239	78.3	0.39	5
Mammals	Acute	750	0.01	0.001569	117.6783195	> 2000	> 17	10
	Long- term	250	0.01	0.001569	39.22610651	548.6	4.66	5

* The PEC drinking water (= 20 % of the sprayed concentration) was calculated according to SANCO/4145/2000 (25. Sep. 2002).

The acute TER values for birds and mammals exceeded the relevant Annex VI trigger values except for the use in grapes if the solution is sprayed at the highest recommended concentration.

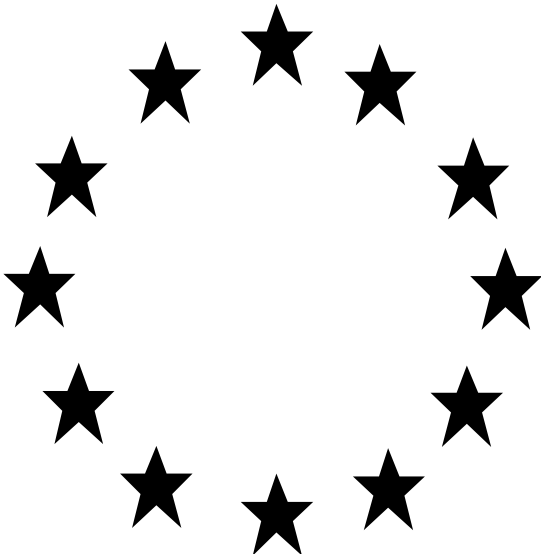
The short-term and long-term TER values for birds and the long-term TER values for mammals were below the Annex VI trigger values for the representative uses in winter wheat and grapes. For the representative use in tomatoes only a high long-term risk for birds was indicated.

A high acute risk for birds and mammals from uptake of contaminated drinking water is indicated for the representative use in grapes if the solution is prayed at the highest recommended concentration. The acute risk to birds and mammals is low if the solution is applied at the lowest recommended concentration.

The short-term and long-term risk to birds and the long-term risk to mammals is high for the representative uses in winter wheat and grapes. For the representative use in tomatoes a high long-term risk to birds was identified in the first tier risk assessment.

The long-term risk assessment for mammals presented is based on the endpoint which was suggested by the RMS. EFSA is of the opinion that the use of this endpoint is not fully scientifically justified (see EFSA conclusion on folpet, point 5.1). If the next lower endpoint (NOEC = 1500 ppm instead of 5000 ppm) is applied in the risk assessment the long-term TER values would be even more markedly below the Annex VI trigger of 5.

A refined risk assessment for the uptake of contaminated drinking water is required for the intended uses in winter wheat and grapes to address the short-term and long term risk to birds and the long-term risk to mammals. For the intended use in tomatoes a refined risk assessment is required to address the long-term risk to birds. A refined risk assessment or risk mitigation measures (e.g. restriction to the lowest recommended concentration) are required to address the high acute risk to birds and mammals from the use in grapes at the highest recommended concentration of folpet in the sprayed solution.



Final addendum to the

Draft Assessment Report (DAR)

- public version -

Initial risk assessment provided by the rapporteur Member State Italy for the existing active substance

FOLPET

of the second stage of the review programme referred to in Article 8(2) of Council Directive 91/414/EEC

January 2009

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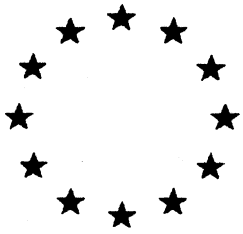

Folpet

Volume 3

Annex B
Addendum: Position paper relating to non-setting of Acute Reference Dose (ARfD)

Rapporteur Member State: Italy

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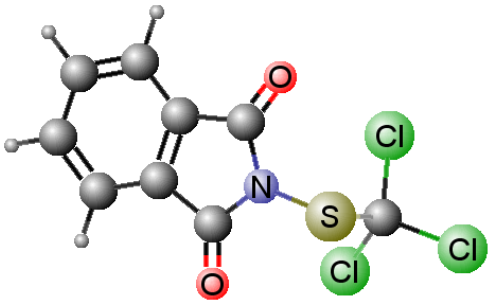
B.6.10.2 Acute Reference Dose (Annex IIA 5.10)



Folpet

Folpet: The JMPR (2004) set an ARfD of 0.2 mg/kg bw for women of childbearing age for Folpet based on foetal data from a rabbit developmental toxicity study, stating that it was not appropriate for the general population. In the EU an ARfD of 0.1 mg/kg bw has been proposed.

Both ARfDs are derived largely from the same toxicology database using rabbit prenatal development studies in their assessments. The foundation for the JMPR and the EU evaluation in selecting the prenatal development studies was based essentially on JMPR criteria used for assessing toxicological alerts when considering whether setting an ARfD is appropriate. Overall the database suggested that there was little concern for setting an ARfD and that an ARfD based largely on data from a prenatal development studies would provide a conservative ARfD for use in calculating an acute dietary risk assessment in the general population. The evidence for Folpet suggested that there were: no toxicological effects as a consequence of acute exposure, no teratogenic effects and no significant embryofoetal effects at the intermediate dosages used.

Folpet has a robust toxicological database and been evaluated by several international regulatory authorities. There have been a number of JMPR Monographs and WHO Evaluations for Folpet (e.g. JMPR 1995, 2004) and it has also been evaluated by the US EPA (1999). In the EU Folpet has been assessed under the EC Directive 91/414/EEC and has been included in Annex 1 of that directive for continued use in the EU.

For the JMPR and EU (and EU member states) evaluations of plant protection products the setting an appropriate acute reference dose (ARfD) may be considered if the toxicological database (toxicological alerts) for a molecule is considered to warrant it. Recently the JMPR (2004) considered Folpet as part of their evaluation for dietary risk assessment. In their judgement:

"…the establishment of ARfDs for (Captan and) Folpet was considered necessary only for women of child-bearing age."

The basis for this was that in their judgement Folpet produced no toxicological effects that might be considered to be a consequence of acute exposure (other than developmental effects). In setting their ARfD they stated that an ARfD for the general population (including children aged 1-6 years) was unnecessary. Furthermore they suggested that:

"…it might be necessary to establish an ARfD to protect the embryo or foetus from possible effects in utero. Such an ARfD would apply to women of childbearing age."

It is clear that the database for this fungicide suggests that setting an ARfD of 0.2 mg/kg bw/day, as JMPR have done, or 0.1 mg/kg bw/day as the EU have proposed may be considered a conservative approach for dietary risk assessment. There is no need to set an ARfD for the general population as the toxicology results suggest that there is a negligible risk to human health risk from acute dietary exposure. However the 2004 JMPR concluded:

"... that the database was insufficient (in particular, with regard to the absence of studies on the developmental effects of phthalimide) to establish the mode of action by which the increased incidence of hydrocephalus, observed in rabbits at 60 mg/kg bw per day (NOAEL, 20 mg/kg bw per day) was induced, and as a consequence, their relevance for deriving an ARfD could not be dismissed."

Considering this view a new developmental toxicity study (Blee, 2006) with phthalimide in the rabbit was conducted and is reviewed in the position paper prepared by the Notifier and presented in this addendum. Additionally in order to shed some light on one possible cause of maternal toxicity, and foetal effects, after Folpet exposure, two new studies are presented that investigated the effects of Folpet and phthalimide on micro-organisms selected as representative of those found in the rabbit gut. The dependence of the rabbit on its gut micro-flora is of paramount importance in ensuring normal gut function and nutrition (the rabbit is coprophagous and depends on its caecotrophs and gut micro-flora for normal nutrition). A perturbation in the gut micro-flora may lead to nutritional deficits in the dam which may impact on the outcome of her pregnancy.

The Notifier's position paper briefly reviews the relevant endpoints used for the ARfD assessment of Folpet, a new developmental toxicity study with phthalimide, two new MIC assays (Folpet and phthalimide) and suggests that there an ARfD for Folpet is not required.

1) Review of prenatal developmental toxicity studies for Folpet

The database contains five established studies, three in the rat and two in the rabbit. The references submitted in support of the above position are summarised below.

a) Prenatal development toxicity studies in the rat.

b) Prenatal development toxicity studies in the rabbit.

a). Prenatal development toxicity studies in the rat.

a) Folpan: teratology study in the rat. (Rubin, Y. 1985a; IIA, 5.6.2/01)

The study was a US EPA 83-3 guideline compliant study. The test substance: Folpet (purity 91.1%) was administered in the vehicle [redacted] by oral gavage to groups of 22 mated female CD rats at dose levels of 150, 550 and 2,000 mg/kg/day on Days 6 to 15 of gestation, inclusive. A similar group of rats was given the vehicle alone at the same dose volume. The dams were observed for mortality and clinical signs. Body weight and food consumption data were recorded. Dams were sacrificed on day 20 of gestation and a full *in utero* macroscopic examination performed followed by a detailed examination of the foetal pathology.

One high dose dam that died on Day 16 of gestation had multiple haemorrhagic ulcerations of the gastric mucosa that was considered to be related to treatment. There were no other deaths. Treatment-related clinical signs observed in the high dose animals included soft faeces, staining of the body fur and peri-anal staining.

Body weight was reduced in the high dose group from Day 8 of gestation through to Day 20 when compared with controls. The intermediate dose group body weights were slightly reduced from Day 8 but did not show significance until Day 17 of gestation. Gravid uterus weights were reduced in the intermediate and high dose groups which caused a reduction in the adjusted terminal body weight for the high dose group only. There was a marked depression in food consumption in the high dose group over the whole period of dosing but only over the first few days of dosing in the intermediate group. After cessation of dosing the food consumption by the high dose animals recovered and exceeded the control value.

There were no treatment-related changes in implantation numbers and numbers of foetuses or on pre- and post-implantation losses. Foetal weight was reduced in the intermediate and high dose groups, characterised by an increase in the number of small foetuses in both the intermediate and high dose groups and a reduction in the mean foetal weight in the high dose group.

Two foetuses in the high dose group had malformations; one with unilateral microphthalmia and one with multiple malformations. A number of skeletal variations characteristic of transient developmental retardation were observed in the intermediate and high dose groups. These included reduced ossification of cranial bones, sternebrae, pubes, metacarpals and metatarsals. Foetuses in the low dose group were not significantly affected except for the interparietal bone when analysed on a per litter basis. Angulated ribs occurred in all treated groups which showed significance only in the high dose group on a per foetus basis but significance for all treated groups on a per litter basis (Table B.6.10.2.1).

Table B.6.10.2.1: Selected foetal skeletal findings

	Dose level (mg/kg/day)			
	0	**150**	**550**	**2000**
Number of foetuses examined	171 **22**	160 **22**	138 **22**	134 **20**
Anterior fontanelle large	18 (10.5) **10**	19 (11.9) **9**	35 (25.4)** **14*** **	54 (40.3)*** **15*** **
Reduced ossification supraoccipital bone	25 (14.6) **10**	29 (18.1) **13**	28 (20.3) **10** **	40 (29.9)** **16*** **
Reduced ossification interparietal bone	23 (13.5) **12**	29 (18.1) **13***	22 (15.9) **12***	49 (36.6)*** **19*** **
Reduced ossification parietal bone	5 (2.9) **3**	10 (6.3) **5**	6 (4.4) **4** **	15 (11.2)** **11*** **
Reduced ossification squamosal bone	4 (2.3) **4**	5 (3.1) **4**	11 (8.0)* **6**	11 (8.2)* **9*** **
Angulated ribs	0 (0.0) **0**	5 (3.1) **3** **	4 (2.9) **3*** **	6 (4.5)* **5*** **
One or more sternebrae unossified 1-4	1 (0.6) **1**	3 (1.9) **2**	5 (3.6) **5*** **	14 (10.4)*** **9*** **
Reduced ossification pubic bone(s)	12 (7.0) **8**	11 (6.9) **7**	11 (8.0) **10***	22 (16.4)* **11*** **

Student's t-test following Freeman-Tukey transformation * (P<0.05) ** (P<0.01). *** (P<0.001)

Figures in bold = number of litters; ( ) = % of total number of foetuses.

Conclusion: The NOAEL for maternal toxicity was 150 mg/kg/day and the foetal NOAEL is less than 150 mg/kg/day.

b) Teratology study in rats with folpet technical. (Hoberman, A.M. 1983; IIA, 5.6.2/02*)*

The study essentially met the Directive 87/302/EEC Part B guideline requirements. Folpet (purity 89.5%), admixed with the vehicle [redacted] was administered by oral gavage to groups of 25 mated female Crl:COBS CD (SD)BR rats at dose levels of 10, 60 and 360 mg/kg/day on Days 6 to 19 of gestation, inclusive. A similar group of rats was given the vehicle alone at the same dose volume. The dams were observed for mortality and clinical signs. Body weight and food consumption data were recorded. Dams were sacrificed on day 20 of gestation and a full in utero macroscopic examination performed followed by a detailed examination of the foetal pathology.

Three high dose dams died during the study and although two of the animals had shown treatment related clinical signs e.g. excessive salivation prior to death their deaths were caused by tracheal intubation; the other animal showed no clinical signs or necropsy lesions.

Treatment related clinical signs observed in the high dose group included rales, excess salivation, chromorhinorrhoea, decreased motor activity, soft or liquid faeces, dyspnoea, urine stained abdominal fur and chromodacryorrhoea. Rales was observed in the intermediate dose group, on one day for each of three animals. No other treatment related signs were apparent.

There were no treatment related necropsy findings for the dams.

Body weight in the high dose group was lower than the controls from the start of treatment. Overall body weight change showed a significant reduction in the intermediate dose group when the body weight at Day 20 had been corrected for the gravid uterus weight. Other body weight changes were minimal and not significantly different from the controls. Food consumption was lower in the high dose group over the treatment period when compared with the controls. Other treated groups were similar to the controls.

There were no treatment related effects on the pregnancy rate, implantation or foetal viability. Mean foetal weight and foetal sex ratios were unaffected by treatment. There were no treatment related increases in foetal external, visceral or skeletal malformations or variations.

Conclusion: The maternal NOEL was 10 mg/kg/day and the embryo foetal NOEL was greater than 360 mg/kg/day, in contrast to Rubin (1985a) study.

c) Folpet study of effects on embryo-fetal development in CD rats treated by oral gavage administration.. (Myers, D.P. 2002; IIA, 5.6.2/04*).*

The study essentially met the Directive 87/302/EEC Part B guideline requirements. Folpet (purity 93.7%), admixed with the vehicle [redacted], was administered by oral gavage to groups of 22 mated female CD rats at dose levels of 20, 100 or 800 mg/kg/day on Days 6 to 19 of gestation, inclusive. A similar group of rats was given the vehicle alone at the same dose volume. The dams were observed for mortality and clinical signs and were given a physical examination at intervals through gestation. Body weight (Day 0, 3 and 6 to 20) and food consumption (over successive three day periods throughout gestation) data were recorded. Dams were sacrificed on day 20 of gestation and a full in utero macroscopic examination performed followed by a detailed examination of the foetal pathology.

There were no mortalities in any of the treated groups. There were generally no clinical signs of an adverse effect on the animals general condition observed in any of the treated groups. The only observation was the increased incidence of salivation in the high dose group – 15 of the 22 rats showed salivation after dosing on one or more occasions between days 13 and 19 of gestation, compared with a single animal with the sign prior to dosing. No such change was evident among rats dosed at 100 or 20 mg/kg bw/day.

Body weight gain was reduced in the high dose group for the day following initial dose administration but gains were similar to controls from Day 7 of gestation through to Day 15. A marginal reduction in weight gain was noted from Day 15 to Day 20 and the overall mean gain for the 800 mg/kg bw/day animals from Day 6 to 20 was approximately 9% lower than controls. When mean gains were adjusted for gravid uterus weights the high dose group was lower than controls (21% reduction), indicating the effect was affecting the dam rather than attributable to varying uterine weights. There was no effect of treatment on overall bodyweight or body weight gains for animals dosed at 100 or 20 mg/kg bw/day compared with controls (Tables B.6.10.2.3 and B.6.10.2.4). There was a reduction in food consumption in the high dose group for the first few days of dosing (Day 6 to 8) and then towards the end of gestation (Day 15 to 17). No treatment-related effects on food consumption were noted for rats dosed at 100 or 20 mg/kg bw/day.

Table B.6.10.2.3: Bodyweight and bodyweight gains

Dose level (mg/kg/day)	Absolute bodyweights								
	0	**6**	**7**	**9**	**12**	**15**	**16**	**17**	**20**
0	231	269	273	283	303	322	333	348	397
20	233	270	275	286	304	325	337	352	400
100	228	265	269	280	298	318	329	343	389
800	230	268	269	281	298	318	326	340	385
	Bodyweight gains								
	0-6	**6-7**	**6-8**	**6-12**	**6-15**	**6-16**	**6-17**	**6-19**	**6-20**
0	38	4	9	34	53	65	79	109	128
20	38	4	9	34	54	67	81	113	130
100	37	4	9	33	53	64	78	107	124
800	38	1**	7*	30*	50	58*	72*	100*	117*

* (P<0.05) ** (P<0.01)

Table B.6.10.2.4: Gravid uterine weight and adjusted bodyweight

Dose level (mg/kg/day)						
	Day 6	**Day 20**	**Day 6-20 bodyweight change**	**Gravid uterine weight**	**Adjusted bodyweight Day 20**	**Adjusted bodyweight change Day 6-20**
0	269	397	128	86.7	310	42
20	270	400	130	87.2	313	43
100	265	389	124	87.1	302	37
800	268	385	117*	84.2	302	33**

*(P<0.05) ** (P<0.01)

There were no relevant necropsy findings in any of the treated groups that were considered attributable to administration of Folpet at 20, 100 or 800 mg/kg bw/day.

All of the dams were pregnant with live young on Day 20. There were no treatment-related effects on embryo-foetal growth or survival as measured by implantation numbers, numbers of live young, embryo-foetal resorptions or pre- and post-implantation losses. Foetal weight, litter weight, placental weight and the sex distribution per litter also showed no effect of treatment with Folpet (Table B.6.10.2.5).

Table B.6.10.2.5: Group mean litter data

	Dose level (mg/kg/day)			
	0	**20**	**100**	**800**
Number of corpora lutea	**16.0**	**16.7**	**17.6**	**16.5**
Number of implantations	**15.2**	**15.5**	**15.7**	**15.6**
Resorptions				
early	0.6	0.8	0.8	0.6
late	0.0	0.0	0.0	0.0
total	0.6	0.8	0.8	0.6
Live young				
male	7.2	7.8	7.9	7.5
female	7.4	6.9	7.0	7.6
total	14.6	14.7	14.9	15.0
Sex ratio (% males)	49.3	52.6	53.2	49.2
Pre-implantation loss (%)	5.1	6.9	9.8	5.6
Post-implantation loss (%)	4.2	4.9	5.2	3.7
Placental weight	0.54	0.56	0.54	0.51
Litter weight	55.33	56.90	57.33	55.49
Foetal weight males	3.90	4.00	3.94	3.79
Foetal weight females	3.72	3.77	3.73	3.60
Foetal weight combined	3.80	3.89	3.85	3.69

The incidence of foetuses with major abnormalities, minor visceral abnormalities or minor skeletal variations showed no dose relationship or treatment effect. (Table B.6.10.2.6).

Table B.6.10.2.6: Foetal examinations – major abnormalities

	Dose level (mg/kg/day)							
	Foetuses				**Litters**			
	0	**20**	**100**	**800**	**0**	**20**	**100**	**800**
Number examined	321	323	328	331	22	22	22	22
Number affected	3	4	--	1	3	2	--	1
Exencephaly: anophthalmia: fused nasals, premaxillae, mandibles, upper and lower incisor sockets: cleft palate: misshapen basisphenoid, basioccipital:ventral cervical schisis: accentuated curvature clavicles: diaphragmatic hernia	1	--	--	--	1	--	--	--
Retroesophageal aortic arch	1	--	--	--	1	--	--	--
Duplicated inferior vena cava	--	1	--	--	--	1	--	--
Medially thickened/kinked ribs, marked: irregularly ossified ribs	--	3	--	--	--	1	--	--
Umbilical hernia	1	--	--	--	1	--	--	--
Termination of vertebral column lumbar region: imperforate anus: malrotated hindlimbs: threadlike tail	--	--	--	1	--	--	--	1

Conclusion: The NOEL for maternal toxicity was 100 mg/kg/day and the foetal NOEL was greater than 800 mg/kg/day.

b). Prenatal development toxicity studies in the rabbit.

a) Folpan: teratology study in the rabbit (Rubin, Y. 1985b; IIA, 5.6.2/03*)*

The study met the essential criteria of Directive 87/302/EEC Part B. Folpet (purity 91.1%), admixed with the vehicle [redacted] was administered by oral gavage to groups of 14 mated female HY/CR New Zealand White rabbits at dose levels of 10, 40 and 160 mg/kg/day on Days 7 to 19 of gestation, inclusive. A similar group of rabbits was given the vehicle alone at the same dose volume. The dams were observed for mortality and clinical signs. Body weight and food consumption data were recorded. The dams were killed on Day 29 of gestation and a full in utero macroscopic examination performed followed by a detailed examination of the foetal pathology.

There were no deaths. Treatment-related clinical signs observed in the high dose animals included soft faeces, yellow/orange discoloration of the urine and the production of few or no faeces at various times.

Body weight decreased for the high dose animals during the first four days of dosing and for the first two days of dosing for the intermediate group. There was reduced body weight gain in the high dose animals compared with controls from the commencement of treatment on Day 7 until termination, but the weight on day 29 net of gravid uterus was similar to the controls. During the period of dosing food consumption by the high dose group was reduced by more than half compared with the controls. A compensatory increase occurred in these animals after the cessation of treatment on Day 19 of gestation.

There were no effects of treatment on pregnancy rate. Post-implantation loss occurred more frequently among high dose animals than among controls. The proportion of foetuses defined as small (<30.0 g) was higher in the high dose group when compared with controls and the mean foetal weight was slightly lower but did not gain significance. Gravid uterine weight was reduced in the high and intermediate dose groups.

There were no treatment-related visceral or skeletal malformations or visceral variations. Evidence of slight developmental retardation (e.g. fewer than 16 caudal vertebrae centra ossified, reduced ossification of long bone epiphyses and sternebrae 1-4) was present in the high dose group and to a lesser extent in the intermediate dose group (Table B.6.10.2.7). There was a dose-related increase in the presence of a 13th (lumbar) pair of ribs and 13 thoracic vertebrae with 13 thoracic ribs occurred more frequently in the high dose group but present also in the intermediate dose group. Such axial skeletal variants may be related to the general developmental delay and could be related to a possible specific maternotoxic effect at the level of gastro-intestinal mucosae, and consequently to an imbalance in nutrients reaching the developing embryo. Low dose foetuses were not affected.

Table B.6.10.2.7: Foetal skeletal defects

	Dose level (mg/kg/day)			
	0	**10**	**40**	**160**
Number of foetuses examined	123 **14**	120 **14**	114 **14**	94 **12**
Fewer than 16 caudal vertebral centra ossified	0 (0.0) **0**	1 (0.9) **1**	2 (1.8) **2***	5 (5.7)* **5****
13th lumbar rib present bilaterally	64 (52.0) **13**	57 (47.5) **12**	67 (58.8) **14****	79 (84.0)**** **12****
13 thoracic vertebrae and 13 pairs of thoracic ribs	1 (0.8) **1**	0 (0.0) **0**	3 (2.6) **2***	5 (5.3) **3****
Reduced/irregular ossification among sternebrae 1-4	1 (0.8) **1**	1 (0.8) **1**	8 (7.0)* **5****	9 (9.6)** **5****
Reduced ossification of long bone epiphyses	26 (21.1) **10**	19 (15.8) **7**	28 (24.6) **10**	40 (42.6)** **10****

Student's t-test following Freeman-Tukey transformation * (P<0.05) ** (P<0.01) *** (P<0.001)
( ) = % of total number of foetuses Figures in **bold** = number of litters

Conclusions: The maternal NOAEL was 40 mg/kg/day; the foetal NOAEL was 40 mg/kg/day based on post-implantation loss.

b) Phaltan: Teratology study in rabbits with folpet technical. (Feussner, E.L. 1984. IIA, 5.6.2/07*)*

The study essentially agrees with OECD 414 and OPPTS 870.3700 guidelines. Folpet technical (purity 89.5%) was administered in the vehicle [redacted] to mated groups of 20 New Zealand White rabbits at 0 (control), 10, 20 or 60 mg/kg bw/day. The rabbits were treated over the period gestation days (GD) 6 to 28. Dosing was by oral gavage. Clinical observation were made at least once daily, body weights recorded on GD 0 and daily GD 6 to 28 and food intake was measured GD 5 to 28. On GD 29 the rabbits were killed and a full in utero macroscopic examination performed followed by a detailed examination of the foetal pathology.

One rabbit treated with 60 mg/kg bw/day was found dead on GD 27; the female had seven normal foetuses and one foetus with hydrocephaly and a meningocele. At 10 mg/kg bw/day one rabbit at was found dead on GD 17 due to a dosing error. No other unscheduled deaths occurred that were deemed to be treatment related. Two abortions were reported (one in the control and one at 60 mg/kg bw/day) that were not considered related to treatment with Folpet. Two females naturally delivered their young, one control and one at 60 mg/kg bw/day on GD 28 and 29 respectively.

There were no consistent clinical signs that were unequivocally treatment related. At 60 mg/kg bw/day there was an increase in hair loss, at 20 mg/kg bw/day an increase in anorexia and at 10 mg/kg bw/day an increase in excessive lacrimation, but these signs showed no clear treatment or dosage relationship.

Body weight performance is illustrated in the Table B.6.10.2.8 below.

Table B.6.10.2.8: Mean maternal body weight change (Kg)

	Dosage group (mg/kg bw/day)			
	0	10	20	60
No. pregnant	**19**	**16**	**16**	**14**
GD	Body weight change (kg)			
0-6	**0.09**	**0.08**	**0.09**	**0.07**
6-9	**0.02**	**0.01**	**-0.02***	**-0.07****
9-12	**0.00**	**0.16**	**0.28**	**0.02**
12-18	**0.08**	**0.07**	**0.02***	**-0.05****
18-24	**0.06**	**0.04**	**0.02**	**0.02**
24-29	**0.02**	**0.07**	**-0.06**	**0.08**
0-29	**0.28**	**0.26**	**0.07***	**0.11***
6-29	**0.19**	**0.19**	**-0.02***	**0.04**
6-29[1]	**0.22**	**0.15**	**-0.44***	**-0.31**

[1] Corrected body weight change (GD 29 body weight minus gravid uterine weight)

GD = Gestation Days. Significantly different from control; * $p<0.05$, ** p, 0.01

Body weight was unaffected at 10 mg/kg bw/day but there were some inconsistent changes at 20 or 60 mg/kg bw/day.

Food intake was reduced at 20 or 60 mg/kg bw/day. At 60 mg/kg bw/day the effect was more noticeable with a significant decrease ($p< 0.01$) recorded at GD 6 to 22. At 20 mg/kg bw/day significant decreases ($p<0.05$) were recorded at GD 12, 13, 22, 27 and 28. Food intake was unaffected at 10 mg/kg bw/day.

The litter data from uterine examinations on GD 29 are illustrated below (Table B.6.10.2.9). There were no significant changes in the numbers of corpora lutea, numbers of implantations, number of live foetuses or in implantation losses. Foetal body weights overall were not significantly affected, however for female foetuses only there was a slight decrease in foetal weight at 20 mg/kg bw/day. The sex ratio of the foetuses was unaffected by treatment.

Table B.6.10.2.9: Litter data (uterine examination data) on GD 29

	Dosage group (mg/kg bw/day)			
	0	10	20	60
No. Pregnant	**19**	**16**	**16**	**14**
No. Died	**0**	**1**	**0**	**1**
No. Aborted	**0**	**1**	**0**	**1**
No. Delivered	**1**	**0**	**0**	**1**
No. at uterine examination on GD 29	**18**	**14**	**16**	**11**
Mean number of:				
Corpora lutea	**9.7**	**10.8**	**11.8**	**11.5**
Implantations	**6.8**	**5.8**	**7.8**	**6.9**
Litter size	**5.3**	**5.2**	**7.2**	**5.8**
Resorptions	**1.5**	**0.6**	**0.6**	**1.1**
Foetal body weights g/litter	**46.82**	**47.98**	**41.58**	**44.64**

At the detailed foetal visceral and skeletal examinations there were no significant increases in foetal variations, either on a foetal or litter incidence basis. However there were four foetuses (three live and one dead) from three litters with hydrocephalus at 60 mg/kg bw/day, they also had skull, gastric and lung abnormalities. The finding of hydrocephalus was also generally associated with domed skull, dilated lateral ventricles and enlarged irregularly shaped fontanelle. The hydrocephalus and other findings at 60 mg/kg bw/day were not significant on a litter basis. In the top dosage group the litter incidence of dilatation of the lateral ventricles

was unchanged but showed an increase on a foetal basis. One foetus at 20 mg/kg bw/day was also found to be hydrocephalic and had a cleft palate. However, these findings did not achieve statistical significance.

When the incidence of hydrocephaly reported in this study is compared with available historical control data (see below) for the New Zealand White (NZW) rabbit it does not suggest unequivocal evidence for a treatment related effect, although the background incidence in the laboratory that conducted the study apparently had a low incidence of hydrocephaly:

Background data: Argus Lab., (USA): 285 litters (2,160 foetuses, including 23 dead foetuses and 24 late resorptions) hydrocephaly noted in 3 litters (1%); one foetus in each litter (0.1% of total foetuses examined).

The historical data were from a limited period (three years prior to study) and may not be truly representative of this particular foetal abnormality.

Hydrocephaly, which may be regarded as an abnormality/malformation, and dilated lateral ventricles which is considered a minor variant, may be reported separately or combined depending on the reporting convention used by the testing laboratory, and may have a variable background incidence in the NZW rabbit. Such findings have been suggested to occur spontaneously (Christian, 1985) i.e. in non-dose-related clusters. Moreover spontaneous incidences of hydrocephaly have been reported in inbred rabbit colonies at incidences of up to 13% (Robertson, 1965). This suggests that when low frequency incidences of such a finding occurs interpretation of these as a major effect on embryofoetal development should be tempered by such knowledge.

It is also interesting to note that the maximum incidences of hydrocephaly, reported in the US by the Mid-Atlantic Regional Teratology Association for teratology studies in the NZW rabbit (1989-1992), were 3% and 17% for foetal and litter incidence (Hood, 1996). The findings from the 1984 Feussner study are clearly within this range and further suggest that the incidence of hydrocephaly in that study should be prudently interpreted.

Conclusions: The study showed that embryofoetal toxicity did not occur in the absence of maternal toxicity and that Folpet was not teratogenic The NOEL for maternal toxicity was considered to be 10 mg/kg bw/day and the NOAEL for embryofoetal toxicity was 20 mg/kg bw/day.

c). Review of a prenatal development toxicity study for phthalimide

a) Phthalimide: Prenatal toxicity study in the rabbit by oral gavage administration (Blee, M.A.B. 2006).

Technical grade phthalimide, purity 100%, was used. The study was GLP compliant and run to current international regulatory guidelines: OECD 414, US EPA OPPTS 870.3700 and Japanese Ministry of Agriculture, Forestry and Fisheries 12 Nohsan No. 8147.

Twenty-five female rabbits, of the New Zealand White strain, per dosage group were mated with males of the same strain and source and were dosed orally by gavage with phthalimide at 0, 5, 15 or 30 mg/kg/day from Gestation Day (GD) 6 to GD 28. Dams were killed on GD 29 and a full in utero macroscopic examination performed followed by a detailed examination of the foetal pathology. Microscopic examination of the maternal duodenum was conducted on the control and top dose groups.

There were no deaths and no clinical signs that were attributed to treatment. Bodyweight (Table B.6.10.2.10) and food consumption (Table B.6.10.2.11) were unaffected by treatment.

Table B.6.10.2.10 : Bodyweight - group mean values (kg) for females during gestation (GD)

Group and phthalimide dose mg/kg bw/day		GD						
		0	6	7	14	21	28	29
Control 0	Mean	3.86	3.94	3.96	4.01	4.04	4.10	4.12
	SD	0.32	0.34	0.33	0.34	0.37	0.36	0.36
Gp 2 5	Mean	3.84	3.92	3.93	3.99	4.04	4.15	4.17
	SD	0.22	0.21	0.21	0.23	0.26	0.29	0.29
Gp 3 15	Mean	3.95	4.03	4.03	4.11	4.18	4.25	4.26
	SD	0.28	0.32	0.32	0.33	0.33	0.31	0.30
Gp 4 30	Mean	3.83	3.91	3.93	4.00	4.05	4.19	4.21
	SD	0.32	0.33	0.33	0.35	0.37	0.37	0.36

Table B.6.10.2.11 : Food consumption - group mean values (g/animal/day) for females during gestation (GD)

Group and phthalimide dose mg/kg bw/day		GD					
		1	6	7	14	21	28
Control 0	Mean	154	160	161	84	103	95
	SD	26	23	29	51	46	45
Gp 2 5	Mean	148	148	149	96	126	102
	SD	31	26	28	51	48	33
Gp 3 15	Mean	175	164	163	128	124	99
	SD	29	39	34	34	46	37
Gp 4 30	Mean	156	161	159	130	131	117
	SD	32	28	27	47	34	32

Macroscopic examination at necropsy of the dams did not reveal any treatment-related observations and microscopic examination of sections of the duodenum from animals in the Control and 30 mg/kg/day groups did not reveal any treatment-related findings.

Treatment did not adversely affect pregnancy outcome, embryo-foetal survival post-implantation, and foetal and placental weights were considered to be unaffected by treatment with phthalimide (Tables B.6.10.2.12 and B.6.10.2.13). The in utero progress and development of the foetuses up to GD 29 was similarly also unaffected by treatment.

Table B.6.10.2.12 : Litter data - group mean values on GD 29

Group and phthalimide dose mg/kg bw/day		**Corpora Lutea**	**Implantations**	**Resorptions**		**Live young**		**% implantation loss**	
				Early	**Late**	**Male**	**Female**	**Pre-**	**Post-**
Control 0	Mean	11.6	10.0	0.4	0.1	5.1	4.4	13.4	5.9
	SD	2.9	2.3			1.9	2.1		
Gp 2 5	Mean	11.8	9.6	0.6	0.1	4.5	4.4	16.9	7.1
	SD	2.0	1.5			1.8	1.6		
Gp 3 15	Mean	11.7	9.5	0.5	0.2	4.1	4.6	19.5	7.3
	SD	1.9	2.8			2.0	1.9		
Gp 4 30	Mean	11.2	8.3	0.4	0.4	3.5	4.0	25.7	10.3
	SD	2.1	2.5			2.0	2.3		

Table B.6.10.2.13 : Placental and foetal weights - group mean values (g) on GD 29

Group and phthalimide dose mg/kg bw/day		Placental weight	Foetal weight		
			Males	Females	Overall
Control 0	Mean	5.5	38.8	38.4	38.8
	SD	0.8	7.5	5.8	6.6
Gp 2 5	Mean	5.6	40.7	40.1	40.3
	SD	0.7	4.7	6.1	5.0
Gp 3 15	Mean	5.4	41.0	38.8	39.8
	SD	1.2	5.4	6.0	5.5
Gp 4 30	Mean	5.8	43.5	42.1	42.9
	SD	1.0	4.3	6.9	5.0

Foetal pathology examinations did not reveal any major skeletal/visceral malformations or abnormalities (Table B.6.10.2.14) or changes in minor skeletal abnormalities/variants (Table B.6.10.2.15) that were outside concurrent or the laboratories historical control data ranges (see report for full details). Thus foetal development was unaffected by maternal treatment with phthalimide.

Table B.6.10.2.14: Foetal examinations - major abnormalities - group incidences

	Foetuses				Litters			
Group	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
Number examined	**199**	**178**	**192**	**187**	**21**	**20**	**22**	**25**
Number affected	**1**	**2**	**2**	**3**	**1**	**2**	**2**	**3**
Single central naris: absent upper incisors: single nasal cavity: narrow nasal septum, misshapen nasal turbinates and conchae: absent olfactory lobes, fused frontal lobes: hydrocephaly	-	-	-	1	-	-	-	1
Distorted ribcage: termination of vertebral column lumbar region: lumbar spina bifida and scoliosis: abnormal orientation of pelvis: fused/misshapen pubis and ischium: unilateral amelia: one central rudimentary hindpaw consisting of calcaneum, metatarsal, phalanx, 2nd phalanx and claw: narrow pulmonary trunk, marked: gastroschisis: absent kidney, ureter, adrenal and ovary: small and misshapen kidney: fleshy tail	-	-	-	1	-	-	-	1
Bent humerus, scapula: dilated ascending aorta/aortic arch: diaphragmatic hernia	-	1	-	-	-	1	-	-
Umbilical hernia	1	-	-	-	1	-	-	-
Lumbar/sacral spina bifida	-	-	1	-	-	-	1	-
Lumbar scoliosis	-	-	1	-	-	-	1	-
Absent kidney and ureter	-	-	-	1	-	-	-	1
Hindlimb syndactyly: hindpaw brachydactyly: hindpaw hyperextension	-	1	-	-	-	1	-	-

Table B.6.10.2.15: Foetal examinations – minor skeletal abnormalities/variants - group incidences

		Foetuses				Litters			
Group		**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
Number examined		198	176	190	184	21	20	22	25
Number intact		100	92	96	92	21	20	22	25
Skeletal abnormalities									
Cranial	sutural bone	-	1	1	-	-	1	1	-
	extra sutures	-	-	-	1	-	-	-	1
	misaligned sutures	1	-	-	-	1	-	-	-
	partially fused maxilla to jugal	-	-	1	-	-	-	1	-
	unossified area	-	-	1	-	-	-	1	-
Vertebral elements abnormality									
	thoracic	-	1	1	-	-	1	1	-
	caudal	-	-	-	1	-	-	-	1
Ribs	medially thickened	1	-	-	-	1	-	-	-
	partially fused	1	-	-	-	1	-	-	-
	interrupted 13th	2	-	2	1	2	-	2	1
	malpositioned	1	-	-	-	1	-	-	-
Sternebrae	additional centre(s)	1	-	2	-	1	-	1	-
	bridge of ossification/partially fused/fused	-	-	2	2	-	-	1	2
	offset alignment	-	-	1	-	-	-	1	-
	wide	2	-	-	-	2	-	-	-
	elongated	-	1	1	1	-	1	1	1
Costal cartilage	offset alignment	1	-	-	-	1	-	-	-
	partially fused	1	-	1	-	1	-	1	-
	additional	-	-	1	-	-	-	1	-
	7th not connected to sternum	3	11	3	10	2	6	2	7
	8th connected to sternum	1	-	-	-	1	-	-	-
Appendicular	elongated acromion process	-	2	-	-	-	1	-	-
	bent spine of scapula	-	-	1	-	-	-	1	-
Total affected by one or more of the above		11	14	16	14	6	7	10	9

Table B.6.10.2.15: Foetal examinations – minor skeletal abnormalities/variants - group incidences (continued)

		Foetuses				Litters			
Group		**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
Number examined		198	176	190	184	21	20	22	25
Number intact		100	92	96	92	21	20	22	25
Rib and vertebral configuration									
Cervical rib		1	2	6	3	1	2	4	2
Number with 12/13 or 13/13 ribs		133	107	125	108	21	19	22	24
18 thoracolumbar vertebrae		-	-	-	1	-	-	-	1
20 thoracolumbar vertebrae		61	45	55	37	15	13	15	14
Offset alignment pelvic girdle		9	1	5	12	8	1	4	9
Incomplete ossification/unossified									
Enlarged posterior fontanelle		5	-	1	-	1	-	1	-
Cranial centres		-	-	-	1	-	-	-	1
Hyoid		1	-	-	-	1	-	-	-
Vertebral element	cervical	1	2	-	1	1	2	-	1
	thoracic	-	1	-	1	-	1	-	1
	caudal	-	1	-	-	-	1	-	-
Sternebrae	5^{th}	25	22	19	23	12	12	8	13
	other	7	5	4	4	3	3	3	3
	total	30	26	22	26	13	12	9	14
Epiphyses		20	3	4	6	8	2	2	5
Astragalus		3	1	2	-	3	1	2	-
Metacarpals/phalanges		22	23	13	14	9	8	8	8
Precocious ossification									
Small anterior fontanelle		1	-	2	1	1	-	2	1
Ossified olecranon processes		-	1	2	-	-	1	2	-

Conclusion: Maternal administration of phthalimide did not induce demonstrable maternal toxicity and did not affect the outcome of the pregnancies. The development of the foetus was unperturbed and foetal pathology was considered to be normal.

d) Studies on the anti-microbial potential of Folpet or Phthalimide

The position paper includes summaries of the toxicity findings of the folpet metabolites. Phthalamic acid, a major degradate when folpet undergoes hydrolysis, is the main metabolite following oral administration to rats. Phthalic acid is a minor metabolite. Phthalamic acid is the main metabolite in goats and phthalic acid is not seen in the urine but is present in the kidney. Phthalamic acid is hydrolyses to phthalic acid at acid pH. TOPKAT was used to predict that phthalamic acid would have an acute oral rat LD_{50} of ~ 700 mg/kg bw, and would be negative in the Ames test. As a metabolite in the rat, animals are considered to have been exposed during oral toxicity studies. It is not possible to establish a risk level due to the lack of toxicological data on the compound itself, but based on the low toxicity of phthalate and phthalimide, the level of toxicity of phthalamic acid is expected to be low.

a) Folpet: Determination of minimum inhibitory concentrations against selected micro-organisms representative of the rabbit gut micro-flora (Akhurst, L.C. 2005a).

Folpet can have bacteriostatic or bactericidal activity (e.g. see Guan et al, 2005). It may, therefore, be postulated that Folpet could affect the rabbit gut micro-flora and that an imbalance in the micro-flora may have consequences for the pregnant rabbit on both maternal and embryo-foetal nutrition. Such changes could, in theory, affect the developing foetus. The rabbit is a species particularly susceptible to gastrointestinal disturbances that may in part be mediated through changes in the gut micro-flora. To assess potential effects on micro-organisms representative of rabbit gut micro-flora the minimum inhibitory concentration (MIC) assay is an internationally accepted test for antimicrobial susceptibility testing and is commonly used to assess the effectiveness of intentional antimicrobial compounds. The test was adapted to assess selected micro-organisms representative of the rabbit gut micro-flora.

The study was conducted using an agar dilution procedure for determination of MIC values. The method was based on procedures described by the British Society for Antimicrobial Chemotherapy (Journal of Antimicrobial Chemotherapy 2001, 48, Supplement S1, 5-16). Ten species of the genus *Bacteroides*, one genus of *Enterococcus faecalis* and four isolates of *Candida albicans* were tested. Final concentrations of Folpet of 2000, 1000, 500, 200, 100, 50, 20, 10, 5 or 2 µg/ml were tested and solvent control and growth control plates were employed. Plates were incubated at 37°C for 48 hours. The lowest test substance concentration that completely inhibited growth of the test organism was recorded as the MIC.

Folpet demonstrated marked antimicrobial activity towards all microorganisms tested, although was more active against the yeast, *Candida albicans*, than the bacterial organisms. MIC values were in the range 20 –50 µg/ml for *Bacteroides* sp., 50 – 200 µg/ml for *Enterococcus faecalis* and 5 µg/ml for *Candida albicans*.

Conclusion: Folpet demonstrated significant antimicrobial activity against organisms selected as representatives of rabbit gut flora species tested in this study. The molecule has clear antimicrobial activity.

b) Phthalimide: Determination of minimum inhibitory concentrations against selected micro-organisms representative of the rabbit gut micro-flora (Akhurst, L.C. 2005b).

Phthalimide is not thought to have the same anti-microbial activity as the parent molecule Folpet, primarily because it is not capable of generating the highly reactive moiety thiophosgene, which in conjunction with Folpet is considered to be responsible for Folpet's mode of action on micro-organisms.

The test was essentially as that described above for Folpet.

The study was conducted using an agar dilution procedure for determination of MIC values. Ten species of the genus *Bacteroides*, one genus of *Enterococcus faecalis* and four isolates of *Candida albicans* were tested. Final concentrations of phthalimide of 1000, 500, 200, 100, 50, 20, 10, 5, 2 or 1 µg/ml were tested and solvent control and growth control plates were employed. Plates were incubated at 37°C for 48 hours. The lowest test substance concentration that completely inhibited growth of the test organism was recorded as the MIC.

Phthalimide demonstrated no antimicrobial activity towards *Bacteroides* sp. and *Enterococcus faecalis*, when tested up to a concentration of 1000 µg/ml, which was just above the limit of solubility in agar. It showed very weak activity against the yeast, *Candida albicans*, with very slight (qualitative) inhibition of growth only at 1000 µg/ml and in only one of the four strains tested.

Conclusion: Phthalimide demonstrated no significant antimicrobial activity against organisms selected as representatives of rabbit GI tract flora species tested in this study.

It is therefore unlikely that this molecule has the potential to affect the micro-flora of the rabbit GI tract.

2) Discussion and conclusions drawn from position paper

The rat study of Rubin (1985a) showed that Folpet was not a teratogen but that in the presence of maternal toxicity some effects on foetal development were noted at 150 mg/kg bw/day. In contrast the Hoberman study (1983) found that foetal development was unaffected at a dosage of 360 mg/kg bw/day. A finding that was vindicated by the more recent study of Myers (2002) where the foetal NOEL was greater than 800 mg/kg bw/day.

In the rabbit developmental toxicity study of Feussner (1984) maternal toxicity was seen at the high and intermediate dosages (20 or 60 mg/kg bw/day) as lower food consumption (a prime toxicity marker in rabbits) and lower body weight gain. One dam treated at 60 mg/kg bw/day died probably as a result of treatment and another aborted. At the highest dosage there were three live foetuses with hydrocephalus and one foetus with this at the intermediate dosage. The latter finding was not statistically different from the in-study control and fell within the range of the historical control range. No toxicity was seen at the lowest dosage tested in this study, 10 mg/kg bw/day. It was concluded that the NOAEL for embryo-foetal effects was 20 mg/kg bw/day. The rabbit developmental toxicity study of Rubin (1985) tested a much higher dosage of Folpet (160 mg/kg bw/day) and, interestingly, there were no treatment related incidences of hydrocephalus. The results from this study suggest that the New Zealand White rabbit, which is extensively used in these studies, may naturally have a number of such abnormalities that can occur spontaneously. At the high (160 mg/kg bw/day) and intermediate dosages (40 mg/kg bw/day) gravid uterine weight was low compared to control; only at the high dose was there any effect on implantation loss, but there were no significant effects on the number of live foetuses or foetal weights at either dosage. The data suggested that the effect on gravid uterine weight at 40 mg/kg bw/day was of doubtful toxicological significance. In the study no treatment related malformations were seen; there was some evidence of a slight development delay at the high dosage and to a lesser extent at the intermediate dosage. However, there was clear maternal toxicity at 160 mg/kg bw/day seen as markedly reduced food intake from the beginning of oral dosing. At 40 mg/kg bw/day, in the same early period of treatment, an effect on food intake was evident although did not reach statistical significance. The data suggest that bolus dosing with Folpet (i.e. local high concentrations may be reached on the gastric mucosa) may serve to precipitate a period of inappetance in rabbits, an effect that may be reversible on withdrawal of treatment. The cause may be gastric irritation, as Folpet has known irritancy properties. It is probable that such effects may have been present at 40 mg/kg bw/day as seen by a transient dip in food intake after dosing commenced. The slight delays in foetal development seen at 40 or 160 mg/kg bw/day may therefore be as a consequence of maternal toxicity. From this study it can be concluded that the NOAEL for embryo-foetal effects was 40 mg/kg bw/day.

A developmental toxicity study conducted with the Folpet metabolite phthalimide did not show any evidence of maternal or foetal toxicity in the rabbit (Blee, 2006). The data are interesting in that phthalimide cannot produce the reactive moiety thiophosgene that in conjunction with Folpet is considered to be responsible for the toxicological effects seen after treatment with this molecule. Thus the maternal effects seen for Folpet, low food consumption with an associated lower body weight gain, were not present for phthalimide. This suggests that the known irritancy effects of Folpet (particularly on the GI-tract) were mostly responsible for the maternal effects seen with this molecule. Such effects may have been exacerbated by the gavage dosing technique that would have resulted in local high concentrations in the stomach. Furthermore, the lack of foetal toxicity with phthalimide shows that maternal effects may be relevant to the slight delays in foetal development seen with Folpet. Such effects could be related to maternal well being, and nutrition, and not in this case to intrinsic toxicity. Indeed in a recent publication Cappon et al (2005) concluded that feed restriction in the rabbit may lead to developmental effects such as abortion, reduced foetal weight and changes in ossification – effects associated with reduced maternal weight performance. The data for Folpet would also suggest that this may be the case for this molecule.

It is also seems that the rabbit may be more sensitive to GI-tract perturbation than the rat – as demonstrated by the effects on food consumption and concomitant body weight gain. The effect is probably related to GI-tract irritancy following degradation of Folpet to form the reactive and short-lived moiety thiophosgene. Such effects would appear to be due to local GI-tract irritancy rather than systemic toxicity considering the very rapid reaction of Folpet with thiol groups and rapid hydrolysis in more alkaline conditions to produce thiophosgene. In such circumstances it is questionable whether the rabbit is the most appropriate model when considering the known irritancy of Folpet. What is more, the rabbits dependency on its gut micro-flora to maintain a healthy nutritional status means that compounds known to possess significant antimicrobial activity may have an affect on the animals well being (e.g. oral antibiotic use in rabbits may have adverse consequences because of undesirable effects on beneficial micro-organisms, e.g. see Hawk and Leary, 2004 and Morris, 1995). The bacteriostatic/bactericidal activity of Folpet may affect the production of caecotrophs that are critical for overall nutrition of the rabbit. The caecotrophs are generally produced 4 – 6 hours after eating by fermentation of ingested food in the caecum and are composed primarily of bacteria that contain fatty acids, amino acids, vitamins and minerals that were derived from the food but would otherwise not be available for absorption. The rabbit ingests these soft green pellets directly from the anus and digests the bacteria with their internal load of nutrients. The rabbit's dependency on ingestion of caecotrophs for nutrition may be adversely affected by the bacteriostatic/bactericidal action of Folpet. The MIC study with Folpet (Akhurst, 2005a) proved that this molecule has significant antimicrobial activity that may affect the production of caecotrophs whereas its stable metabolite, phthalimide (Akhurst, 2005b), did not. These data suggest that the effects on maternal nutrition and foetal development in the rabbit may also have a nutritional deficit component. Overall the findings indicate that the rabbit is not the most appropriate model for assessing embryo-foetal development when compounds that are irritants or bacteriostatic/bactericidal are administered by bolus dosing.

The 2004 JMPR, without the benefit of the new phthalimide prenatal development and MIC studies, concluded that 20 mg/kg bw/day was a NOAEL for embryo-foetal effects in the rabbit. Furthermore, they stated that:

"The maternal toxicity and the associated reductions in fetal body weight, delayed ossification and increased incidences in skeletal variations observed in studies of developmental toxicity in rabbits are likely to be caused by high local concentrations of folpet and are not considered to be relevant to dietary exposure. However, the increased incidence of hydrocephalus observed could not be attributed with confidence to maternal toxicity."

The 2004 JMPR proposal is considered to be a conservative approach. Their overall assessment of developmental toxicity data however is supported by the publication of Solecki at al (2005) on ARfD setting. In that publication, it is clearly stated:

"…that ARfDs based on reductions in foetal body weight gain arising from multiple dose studies are generally thought to be conservative."

The review of Solecki at al (2005) also makes it clear that it is *"…important to distinguish between a developmental effect from a secondary response"*, i.e. one that is as a consequence of maternal toxicity. The paper also advocates that maternal toxicity in pre-natal developmental studies may not be appropriate for ARfD setting by stating that:

"Maternal toxicity following repeated dosing in developmental toxicity studies may not be appropriate for setting an ARfD unless clinical observations or other toxicity in the dams are observed after a single dose of the test substance."

Considering the new data presented in the current position paper, in particular the developmental toxicity study in the rabbit with phthalimide that the JMPR thought critical, and the criteria for setting an ARfD (EC, 2001; JMPR, 2004 and Solecki et al, 2005) it is proposed that an ARfD for Folpet is not warranted. The reasons for this are:

a) There were no toxicological effects that might be considered to be as a consequence of acute exposure (i.e. manifest in a period of 24 h or less).
b) No teratogenic effects were obvious.
c) Embryo-foetal effects were associated with evidence of maternal toxicity.
d) No statistically significant embryo-foetal effects were seen at the intermediate dosages in the rabbit studies cited.
e) Reduced gravid uterine weight at 40 mg/kg bw/day (Ruben, 1985b) was not correlated with the number of live foetuses or foetal weights, the latter parameters being unaffected by treatment.
f) The slight embryo-foetal effects observed may be secondary to maternal toxicity and caused by high local concentrations of Folpet produced by administration by gavage and are not considered to be relevant to dietary exposure.
g) Data from a prenatal developmental study with phthalimide showed that this metabolite caused no maternal or foetal toxicity, suggesting that the maternal effects seen with Folpet (based on local irritant effects) were mostly responsible for the foetal effects seen with the parent molecule.
h) The rabbit may be considered to be a less appropriate experimental animal, as it appears to be very sensitive to local GI-tract irritancy and possible perturbation of its gut micro-flora. Such effects may not reflect systemic exposure.

It is the conclusion of the position paper that the weight of evidence for Folpet suggests that setting an acute reference dose for Folpet is unnecessary.

B.6.10.2 References relied on

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company, Report No. GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N	Owner
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##	Akhurst, L.C.	2005b	Phthalimide: Determination of minimum inhibitory concentrations against selected microorganisms representative of the rabbit gut micro-flora. Huntingdon Life Sciences, UK, unpublished report No. 889/053251 (company file: R-18734).	Y	Makhteshim
##	Blee, M.A.B.	2006	Phthalimide: Prenatal toxicity study in the rabbit by oral gavage. Report MAK 863/055231 administration (Company file: R-18201). GLP, Unpublished.	Y	Makhteshim
##	Cappon, G.D., Fleeman, T.L., Chapin, R.E. and Hurtt, M.E..	2005	Effects of feed restriction during organogenesis on embryo-fetal development in rabbit. Birth Defects Research Part B: Developmental and Reproductive Toxicology Volume 74, Issue 5, Pages 424–430 Not GLP; Published.	N	-
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##	Hood, R.D. (ed)	1996	Handbook of Developmental Toxicity. CRC Press, Inc. Published.	N	-
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##	JMPR, FAO/WHO.	2004	Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group on Pesticide Residues Rome, Italy, 20–29 September 2004. Published.	N	-
##	Morris, T. H.	1995	Antibiotic Therapeutics in Laboratory Animals. Laboratory Animals 29: 16-36, also see http://www.medirabbit.com/Unsafe_medication/dangerous_antibiotics.htm GLP, Unpublished.	N	-
IIA, 5.6.2/04	Myers, D.P.	2002	Folpet study of effects on embryo-fetal development in CD rats treated by oral gavage administration. [redacted] Study Number MAK 767/023430 (company file: R-14259). GLP, Unpublished.	Y	Makhteshim
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Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company, Report No. GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N	Owner
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##	US EPA	1999	Reregistration Eligibility Decision (RED) FOLPET: Available on the US EPA website: http://www.epa.gov/oppsrrd1/REDs/0630red.pdf	N	-

information presented in Addendum, not referenced in original submission

European Commission

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ECCO-Meetings

Folpet

Volume 3

Annex B
Addendum: definition of the residue

Rapporteur Member State: Italy

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B.7.3 Definition of the residue (Annex IIA 6.7; Annex IIIA 8.6)

Folpet: The residue definition for the fungicide folpet should be folpet only as the metabolite phthalimide is neither of toxicological significance nor does it pose a significant dose to humans.

The collective data (toxicological data and residue data leading to estimated dose to humans) support the conclusion that the residue definition for folpet should be **folpet** only.

The DG SANCO Guideline notes (European Commission, 1997): Residue Definition – Of the three general considerations that are fundamental to the decision as to whether or not specific metabolites/degradation products should be included in the definition and expression of a residue, two are relevant to this discussion: (1) Their basic toxicology and (2) Their presence in significant amounts.

1) Phthalimide basic toxicology

Four lines of evidence show that the metabolite of folpet, phthalimide is not of toxicological significance:

a) Direct measurements of toxicity.

b) QSAR Analysis

c) Measurement of minimal inhibitory concentration (MIC)

d) Comparison of folpet and phthalimide in bioassays that are particularly sensitive to the toxicological properties of folpet.

a). Direct measurements of toxicity

Phthalimide is not acutely toxic. Its LD_{50} in mice is above 5 g/kg bw[1].

Phthalimide is not mutagenic. When tested in the multiple strains in the Ames Assay, it is negative (Riggin *et al.,* 1983).

Phthalimide is not a developmental toxin (Fabro *et al.,* 1964; Kennedy *et al.,* 1968; Blee, 2006).

[1] (PAN Pesticides Database (2005). U.S. National Toxicology Program acute toxicity studies for Phthalimide (metabolite of folpet). http://www.pesticideinfo.org/List_NTPStudies.jsp?Rec_Id=PC40165.

b) QSAR Analysis

Phthalimide does not have structural alerts that indicate it poses a toxicological risk (Siegfried, 2000).

c) Measurement of minimal inhibitory concentration (MIC)

The MIC assay is designed to assess antimicrobial activity and efficacy *in vitro*. The study was deigned to assess the effects of phthalimide on micro-flora representative of that in the rabbit GI-tract. Ten species of *Bacteroides* and four isolates of *Candida albicans* were incubated in the presence of phthalimide at biologically significant concentrations. phthalimide had no antimicrobial activity (Akhurst, 2005).

d) Comparison of folpet and its major metabolite in bioassays that are particularly sensitive to the toxicological properties of folpet.

The most sensitive bioassays for measuring toxicity of folpet are those involving aquatic organisms. This follows from the mode of action of folpet, which is irritation-based, due to its reaction with thiol groups.

In the case of rainbow trout, phthalimide is more than 3,000-fold less toxic than folpet (Bowman, 1988c), based on LC_{50} values, below. Bluegill sunfish are over 800-fold less sensitive to phthalimide than folpet (Bowman. 1989).

Test system	Folpet	Phthalimide	Ratio*
Trout, LC_{50}	0.015 mg/L	49 mg/L	3,266
Bluegill, LC_{50}	0.047 mg/L	38 mg/L	809

*ratio of folpet toxicity to phthalimide toxicity: > 3,000 and > 800

The toxicity of folpet is entirely attributable to the reactive side chain of folpet which is not present in the phthalimide metabolite. The high reactivity of the side chain of folpet produces irritation to the tissues. Phthalimide has low activity and is not an irritant.

In conclusion, phthalimide poses no significant toxicological risk for adverse effects.

2) Their presence in significant amounts

The amount of phthalimide in milk and meat was determined in a goat metabolism study (Corden 1997a, 1997b). Goats were fed ^{14}C-folpet at 14 ppm labelled in the benzene ring for 6 days. Tissues were harvested and samples with 3% total radioactive residue or more were characterized. The majority of radioactivity was excreted in the urine and faeces. The following residue were analysed in meat and milk:

	Phthalimide[2]
Meat	<0.004 mg/kg
Milk	<0.001 mg/kg

The maximum possibly daily intake of phthalimide in milk and meat was calculated according to the worst scenarios for all consumer groups including toddlers and infants, which are the most sensitive consumer groups, and resulted with 0.0000 mg/kg bw/day (detailed calculations appear under point 2) c) below).

Considering the low toxicity of phthalimide and the zero exposure to human from animal products when calculated using conservative assumptions, there is no basis for rationally including phthalimide in the folpet residue expression.

[2] Other analytes measured included phthalamic acid, phthalic anhydride and phthalic acid (although the analytical method could not always separate these).

In conclusion, the residue expression for folpet should be expressed as parent compound, folpet, only.

The references submitted in support of the above position are summarised below.

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1) Phthalimide basic toxicology

a) Teratogenic activity of thalidomide and related compounds (Fabro, S., Schumacher, R. L., Smith, R. L. and Williams, R. T., 1964; IIA 7.3/01)

The paper tests a hypothesis that the teratogenic activity of thalidomide may be associated with the presence of a glutarimide ring in the molecule and interference in glutamic acid or glutamine metabolism. The significance of the glutarimide ring in the thalidomide molecule was investigated by testing other similar molecules, including the folpet metabolite phthalimide.

The study predated guidelines and was not to GLP. However, the study demonstrated that foetal malformations could be induced by a known positive control, and is considered valid.

Female rabbits of the New Zealand and Chinchilla strains were mated with males of the same strain and dosed orally by gavage with phthalimide at 150 mg/kg bw/day from day 7 to day 12 of pregnancy. Dams were killed on day 28 of pregnancy, and uterine parameters recorded. Foetuses were examined for external malformations only, especially those of the head and limbs. Other groups were dosed with other glutarimide-ring molecules, including thalidomide, at 150 mg/kg bw/day.

There were 18 dams in the control group, 161 implantations, 13 resorptions and 148 externally normal foetuses (no malformations). Ten dams were given thalidomide, from which there were 78 implantations, 35 resorptions, 16 externally malformed and 27 externally normal foetuses. The malformations were typical of those induced by thalidomide. Of the three dams given phthalimide, there were 25 implantations, 3 resorptions and 22 externally normal foetuses (no external malformations).

The incidence of malformation was not increased in other molecules that contained the glutarimide ring. The results for phthalimide, control and other compounds are shown below (Table B.7.3.1).

Table B.7.3.1: Embryotoxic effects of phthalimide and other compounds in the rabbit

Compound	No. of animals (dams)	Implan-tations	Re-sorptions	Malformed foetuses	Normal foetuses
Control	**18**	**161**	**13**	**0**	**148**
Thalidomide	**10**	**78**	**35**	**16**[a]	**27**
3-Nitrothalidomide	4	40	9	1[b]	30
α-Aminoglutarimide	4	37	4	0	33
Hexahydrothalidomide	3	21	2	0	19
α-Succinimidoglutarimide	3	18	5	0	13
Phthalimide	**3**	**25**	**3**	**0**	**22**
1-Phthalimidobutane	5	49	6	0	43[e]
2-Phthalimidoacetamide	2	21	3	0	18
4-Phthalimidobutyramide	2	31	0	1[c]	30
α-Phthalimidoaspartimide	4	36	3	0	33
Phthalimidobenzene	7	65	4	2[d]	59
3-Phthalimidopyridine	4	40	4	0	36
2-Phthalimidoglutaric acid anhydride	6	64	12	0	52

[a] malformations of fore and hind limbs and cranioschisis typical for thalidomide
[b] cranioschisis.
[c] malformation of fore-limb- hook-like protrusion.
[d] malformation of fore-limb in one foetus, cyclopia in second foetus.
[e] one foetus with massive subcutaneous cranial haemorrhage, second foetus with large haemorrhage on left limb.

Conclusion: Maternal administration of phthalimide was not associated with increased incidence of resorptions or malformed foetuses when administered to rabbits during pregnancy.

b) An investigation of the teratogenic potential of captan, folpet, and difolatan (Kennedy, G., Fancher, O. E., and Calandra, J. C., 1968; IIA 7.3/02).

Study of effects of captan, folpet, the captan metabolite tetrahydrophthalimide (THPI), and the folpet metabolite phthalimide (PI) on the pregnant rabbit. Technical grade captan and folpet, and pure samples of THPI and PI were used. The related fungicide difoltan and the structurally similar drug thalidomide were also tested. The latter may be considered a positive control.

The study predated guidelines and was not to GLP. However, the study demonstrated that foetal malformations could be induced by a known positive control, and is considered valid.

Test materials were administered in gelatine capsules to groups of mated female Dutch Belted rabbits from day 6 to day 16 of pregnancy. Animals were weighed at three day intervals and killed on day 29, when uterine contents were examined, and foetuses examined. Live foetuses were placed in an incubator for 24 hours after which they were killed and dissected. The carcasses were cleared and the skeleton stained with alizarin and examined. PI was administered at 75 mg/kg bw/day to a group of 10 females. Thalidomide was administered at 75.0 mg/kg bw/day to both strains of rabbit.

Maternal weight gains were not adversely affected by PI at 75.0 mg/kg/day, and there were no deaths.

Incidence of foetal resorptions was not adversely affected by PI administration.

One control foetus (of 105, from 17 litters) showed shortening and flexure of the forelimb. There were no malformations in the 63 foetuses from 10 dams treated with PI. Post-natal survival, crown-rump length, foetal weight and incidence of visceral and skeletal anomalies were not adversely affected by maternal treatment with PI. Thalidomide induced typical 'clubbing' (phocomelia) in 38 of 100 foetuses from 17 litters, demonstrating that the test system was capable of detecting malformations. The folpet metabolite phthalimide (PI) showed no malformed foetuses, and therefore no adverse effects on the developing rabbit foetus.

The results are summarised below (Table B.7.3.2).

Table B.7.3.2: Summary of effects of folpet, phthalimide and controls in rabbits

Compound	Oral dose (mg/kg)	No. of pregnant females	Rabbit strain	No. of implants	No of resorptions	No of normal foetuses	No. (%) malformed foetuses	Mean litter size
Control	-	7	DB	52	0	51	1 (1.9)	7.4
	-	10	NZW	66	2	64	0 (0)	6.4
Thalidomide	75.0	7	BD	55	15	26	14 (35.0)	5.7
		10	NZW	74	10	40	24 (37.5)	6.4
Folpet	75.0	9	DB	66	0	65	1 (1.5)	7.3
	18.75	5	NZW	37	1	36	0 (0)	7.2
	37.5	5	NZW	35	11	24	0 (0)	4.8
	75.0	7	NZW	52	32	20	0 (0)	2.9
Phthalimide	75.0	10	DB	66	3	63	0 (0)	6.3

Conclusion: Phthalimide (PI) showed no adverse effects on the developing rabbit foetus.

c) Phthalimide: Prenatal toxicity study in the rabbit by oral gavage administration (Blee, M.A.B., 2006 IIA 7.3/03)

A study of the effects of phthalimide on the pregnant rabbit was conducted. Technical grade phthalimide, purity 100%, was used. The study was GLP compliant and run to current international regulatory guidelines: OECD 414, US EPA OPPTS 870.3700 and Japanese Ministry of Agriculture, Forestry and Fisheries 12 Nohsan No. 8147.

Twenty-five female rabbits, of the New Zealand White strain, per dosage group were mated with males of the same strain and source and were dosed orally by gavage with phthalimide at 0, 5, 15 or 30 mg/kg/day from Gestation Day (GD) 6 to GD 28. Dams were killed on GD 29 of pregnancy, and uterine parameters recorded. Foetuses were examined macroscopically at necropsy and subsequently by detailed internal visceral examination of the head or at skeletal examination. Microscopic examination of the maternal duodenum was conducted on the control and top dose groups.

There were no deaths and no clinical signs that were attributed to treatment. Bodyweight (Table B.7.3.3) and food consumption (Table B.7.3.4) were unaffected by treatment.

Macroscopic examination at necropsy of the dams did not reveal any treatment-related observations and microscopic examination of sections of the duodenum from animals in the Control and 30 mg/kg/day groups did not reveal any treatment-related findings.

Treatment did not adversely affect pregnancy outcome, embryo-foetal survival post-implantation, and foetal and placental weights were considered to be unaffected by treatment with phthalimide (Table B.7.3.5 and Table B.7.3.6). The *in utero* progress and development of the fetuses up to GD 29 was similarly also unaffected by treatment.

Foetal pathology examinations did not reveal any major skeletal/visceral malformations or abnormalities or changes in minor skeletal abnormalities/variants that were outside concurrent or the laboratories historical control data ranges. Thus foetal development was considered to be unaffected by maternal treatment with phthalimide.

It may be concluded that maternal administration of phthalimide did not induce maternal toxicity and did not affect the outcome of the pregnancies. Foetal development was considered to be normal.

Table B.7.3.3: Bodyweight - group mean values (kg) for females during gestation (GD)

Group	:	1	2	3	4
Compound	:	Control	---------- Phthalimide ----------		
Dosage (mg/kg/day)	:	0	5	15	30

Group		GD						
		0	6	7	14	21	28	29
1	Mean	3.86	3.94	3.96	4.01	4.04	4.10	4.12
	SD	0.32	0.34	0.33	0.34	0.37	0.36	0.36
	n	21	21	21	21	21	21	21
2	Mean	3.84	3.92	3.93	3.99	4.04	4.15	4.17
	SD	0.22	0.21	0.21	0.23	0.26	0.29	0.29
	n	20	20	20	20	20	20	20
3	Mean	3.95	4.03	4.03	4.11	4.18	4.25	4.26
	SD	0.28	0.32	0.32	0.33	0.33	0.31	0.30
	n	22	22	22	22	22	22	22
4	Mean	3.83	3.91	3.93	4.00	4.05	4.19	4.21
	SD	0.32	0.33	0.33	0.35	0.37	0.37	0.36
	n	25	25	25	25	25	25	25

Table B.7.3.4: Food consumption - group mean values (g/animal/day) for females during gestation (GD)

Group	:	1	2	3	4
Compound	:	Control	---------- Phthalimide ----------		
Dosage (mg/kg/day)	:	0	5	15	30

Group		GD					
		1	6	7	14	21	28
1	Mean	154	160	161	84	103	95
	SD	26	23	29	51	46	45
	n	21	21	21	21	21	21
2	Mean	148	148	149	96	126	102
	SD	31	26	28	51	48	33
	n	20	20	20	20	20	20
3	Mean	175	164	163	128	124	99
	SD	29	39	34	34	46	37
	n	22	22	22	22	22	22
4	Mean	156	161	159	130	131	117
	SD	32	28	27	47	34	32
	n	25	25	25	25	25	25

Table B.7.3.5: Litter data - group mean values on GD 29

Group	:	1	2	3	4
Compound	:	Control	---------- Phthalimide ----------		
Dosage (mg/kg/day)	:	0	5	15	30

Group		Corpora Lutea	Implantations	Resorptions Early	Resorptions Late	Live young Male	Live young Female	% implantation loss Pre-	% implantation loss Post-
1	Mean	11.6	10.0	0.4	0.1	5.1	4.4	13.4	5.9
	SD	2.9	2.3			1.9	2.1		
	n	21	21	21	21	21	21	21	21
2	Mean	11.8	9.6	0.6	0.1	4.5	4.4	16.9	7.1
	SD	2.0	1.5			1.8	1.6		
	n	20	20	20	20	20	20	20	20
3	Mean	11.7	9.5	0.5	0.2	4.1	4.6	19.5	7.3
	SD	1.9	2.8			2.0	1.9		
	n	22	22	22	22	22	22	22	22
4	Mean	11.2	8.3	0.4	0.4	3.5	4.0	25.7	10.3
	SD	2.1	2.5			2.0	2.3		
	n	25	25	25	25	25	25	25	25

Table B.7.3.6: Placental and foetal weights - group mean values (g) on GD 29

Group	:	1	2	3	4
Compound	:	Control	---------- Phthalimide ----------		
Dosage (mg/kg/day)	:	0	5	15	30

Group		Placental weight	Foetal weight Males	Foetal weight Females	Foetal weight Overall
1	Mean	5.5	38.8	38.4	38.8
	SD	0.8	7.5	5.8	6.6
	n	21	21	21	21
2	Mean	5.6	40.7	40.1	40.3
	SD	0.7	4.7	6.1	5.0
	n	20	20	20	20
3	Mean	5.4	41.0	38.8	39.8
	SD	1.2	5.4	6.0	5.5
	n	22	22	22	22
4	Mean	5.8	43.5	42.1	42.9
	SD	1.0	4.3	6.9	5.0
	n	25	23	23	25

d) Study of the cytogenetic activity of certain metabolites of a number of pesticides representing several classes of chemical compounds (Pilinskaya, M. A., 1986; IIA 7.3/04).

Phthalimide was tested in a human lymphocyte chromosome aberration assay.

The paper does not give sufficient detail to judge if the method was similar to recognised guidelines, but did give a positive result with some compounds, apparently demonstrating that the assay worked. The study was not to GLP.

Phthalimide was tested at 10,000, 1.0 and 0.1 µg/ml in 100, 200 and 200 metaphases, respectively, and 400 control metaphases were also evaluated. The frequency of metaphases with aberrations was not increased. Metabolites of the pesticides ziram, and betanal, tetramethylthiourea (TMTU) and methyl-3-hydroxyphenyl-carbamate (MHPC) respectively were positive in the assay. The compound methyl-benzimidazole-2-yl-carbamate (BMC), stated to be a metabolite of benomyl-type pesticides, produced hyperspiralisation of chromosomes and accumulation of mitoses.

Table B.7.3.7: Results of cytogenetic study

Concentration of substance (µg/mL)	No. of investigated metaphases	Frequency of aberrations (%)	Concentration of substance (µg/mL)	No. of investigated metaphases	Frequency of aberrations (%)
TMTU			**BMC**		
10,000	200	3.5*	200.00	200	2.00+
1.00	200	4.5	100.00	300	2.33+
0.10	200	6.00*	10.0	200	1.50
0.01	200	2.00	Control	400	2.33
Control	400	2.50			
Phthalimide			**MHPC**		
10,000	100	2.00	200.00	200	11.00***
1.00	200	1.50	100.00	200	3.00***
0.10	200	2.00	10.0	200	1.00
Control	400	2.00	Control	400	1.25

* $p < 0.1$
*** $p<0.05$
\+ a colchicine-type effect noted.

Conclusion: Phthalimide was not mutagenic in the human lymphocyte chromosome aberration assay.

e) Characterization of impurities in commercial lots of sodium saccharin produced by the Sherwin-Williams process (Riggin, R. M., Margard, W. L., and Kinzer, G. W., 1983; IIA 7.3/05).

Impurities and contaminants present or suspected to be present in commercial lots of the artificial sweetener saccharine, including Phthalimide, were tested in the Ames test.

The study was not performed to current guidelines, although it followed the method of Ames. The study was not to GLP.

A number of conflicting long-term animal feeding studies had been performed on the artificial sweetener saccharine, at levels up to 7.5% w/w diet. At such levels, the amount of impurities consumed may be significant, and the study was designed to investigate impurities and contaminants found in commercial lots of saccharine. The compounds were extracted using solvents, and the extracts (of all impurities/contaminants) subjected to the Ames test.

The origin of the impurities or contaminants was not always stated: several were stated to have appeared to have been derived from the polythene (polyethylene) materials used in

packaging the lots. Insufficient quantities of the impurities could be obtained directly by solvent extraction for individual testing of each compound, and so various known or suspected saccharine contaminants were obtained and tested in the Ames test, at dose levels of 2000 or 400 µg/plate, using *S. typhimurium* strain TA98 with S-9 activation only. The mutagenicity was expressed as relative to the DMSO control.

The S-9 activation system was derived by injecting male rats (200g strain not specified) i.p. with 200 mg/mL Arclor 1254 in corn oil at 0.5 mg/g bodyweight. Rats were killed after 5 days, and the liver removed, homogenised in KCl and centrifuged for 10 minutes at 9000 g. The (S-9) supernatant was decanted and frozen. Samples were defrosted before use. The microsomal mix was prepared according to Ames and contained (per mL): S-9 (0.15 mL), $MgCl_2$ (8 µmole), KCl (33 µmole), glucose-6-phosphate (5 µmole), NADP (4 µmole), and sodium phosphate pH 7.4 (100 µmole). Fresh S-9 was prepared daily.

For the assay, a 0.1 mL aliquot of bacterial culture was added to 2 mL molten top agar, which was then mixed with 0.1-0.3 mL of sample solvent extractdissolved in DMSO. A 0.5 mL aliquot of the S-9 mix was added to the agar immediately prio to pouring onto the plate. The poured top agar was allowed to solidify and the plates were incubated for 48 hours, after which the number of colonies were counted. Positive control (10 µg benzo[a]pyrene and a solvent (DMSO) blank control were assayed in triplicate.

Mutagenicity data for the potential contaminants in saccharine, including phthalimide, are summarised below (Table B.7.3.8).

Table B.7.3.8: Mutagenicity data for the potential contaminants in saccharine

Impurity	Concentration (µg/plate)	Relative mutagenicity*
α-Sulphamoylbenzoic acid	400	1.2
	2000	0.9
α-Sulphobenzoic acid	400	1.0
	2000	0.8
α-Chlorobenzoic acid	400	0.9
	2000	0.6
6-Methylsaccharin	400	1.0
	2000	1.2
N-methylsaccharin	400	1.1
	2000	1.3
α-Toluenesulphonamide	400	0.7
	2000	1.2
Phthalimide	**400**	**1.0**
	2000	**0.9**
Methyl anthranilate	400	1.1
	2000	0.9
5-Chlorosaccharin	40	1.0
	200	0.9
	1000	0.8
Trioctyl phosphate	2000	0.7
Di-*tert*-butyl-*p*-benzoquinone	2000	0.7
α-Chlorobenzamide	2000	1.4
1,2-Benzisothiazolin-3-one	10	1.1
	100	toxic
3-Aminobenzisothiazole-1,2-dioxide	200	0.7
	1000	0.6
1,2-Benzisothiazoline-1,1-dioxide	200	0.6
	1000	0.6
Trichlorobenzene	133	1.0
	667	0.8

*Relative to DMSO control

The study did not give any information as to how phthalimide may be either an impurity or contaminant of saccharine. Phthalimide was not mutagenic in the assay, with relative mutagenicity of 1.0 and 0.9 compared to controls (DMSO). None of the other impurities/contaminants were positive in the assay, although one was stated to be toxic to the bacteria. The study found that the solvent-extracted impurities/contaminants exhibited a low level of mutagenicity, despite also demonstrating that the individual compounds, tested separately, showed no mutagenic activity. The study also showed that acetone extraction did not show mutagenic activity, but that chloroform/methanol extracts showed low levels of mutagenicity. The study concentrates on assays of batches of saccharine and on analysis of various solvents to try and determine the origin of the initial mutagenic activity, after concluding that the impurities normally present in saccharine were not responsible for the mutagenic activity seen in the initial solvent extractions. These data are not relevant to phthalimide. The authors concluded that as large amounts of solvent were required to extract the impurities/contaminants, that contamination of the solvents themselves may be responsible for the mutagenic activity seen.

Conclusion: Phthalimide was not mutagenic in the Ames test, when tested in strain TA98 with metabolic activation.

f) Review: Toxicological risk characterisation of potential folpet metabolites. The toxicity profiles of phthalic and phthalamic acids and phthalimide – is there a significant risk from metabolite exposure? (Siefried, H.E., 2000; IIA 7.3/06). [This report was previously submitted with the toxicology addendum in March 2005.]

The position paper includes summaries the toxicity findings of the folpet metabolites. Phthalamic acid, a major degradate when folpet undergoes hydrolysis, is the main metabolite following oral administration to rats. Phthalic acid is a minor metabolite. Phthalamic acid is the main metabolite in goats and phthalic acid is not seen in the urine but is present in the kidney. Phthalamic acid is hydrolyses to phthalic acid at acid pH. TOPKAT was used to predict that phthalamic acid would have an acute oral rat LD_{50} of ~ 700 mg/kg bw, and would be negative in the Ames test. As a metabolite in the rat, animals are considered to have been exposed during oral toxicity studies. It is not possible to establish a risk level due to the lack of toxicological data on the compound itself, but based on the low toxicity of phthalate and phthalimide, the level of toxicity of phthalamic acid is expected to be low.

Phthalimide is an intermediate metabolite, capable of being metabolised to phthalamic acid, phthalate and possibly methylphthalate. It is not mutagenic in the Ames test, in yeast, mouse lymphoma assay or in a cytogenetic assay in human lymphocytes. The weight of evidence suggests a low level of risk. TOPKAT was used to predict that phthalimide would have an acute oral rat LD_{50} of ~ 980 mg/kg bw, and would be negative in the Ames test.

Phthalic acid is not mutagenic in Ames or other bacterial assays, but does act synergistically with some but not all heterocyclic amine mutagens. It is not carcinogenic based on negative rodent bioassays with phthalic anhydride (which converts to phthalic acid). Phthalic acid does not accumulate in the body and is essentially cleared by 48 hours after oral administration. Phthalic acid is not teratogenic in rats. The reported activity on male and female reproductive systems in some less-than-robust studies is not well supported when all results are taken into consideration and the weight of evidence for all folpet metabolites is considered. TOPKAT was used to predict that phthalic acid would have an acute oral rat LD_{50} of ~ 2500 mg/kg bw, and would be negative in the Ames test.

The related compounds phthalic anhydride (which converts to phthalic acid in aqueous media) and phthalamide have been tested for carcinogenicity in rats and mice under a US Government testing programme. Neither compound showed increased incidence of tumours.

Phthalic acid is ubiquitous in the environment from industrial sources (used as plasticizers and in the production of polyester) and can be formed from environmental phthalate esters via hydrolysis where they can be found widely distributed, generally at low levels in air, rain water, sediment, soil and biota, food samples, and human and animal tissues.

In conclusion, phthalimide together with other folpet metabolites metabolites, has a very low level of hazard to humans when exposed through the diet and to the environment compared to parent folpet. The appropriate residue expression for folpet is folpet per se.

g) Phthalimide: Determination of minimal inhibitory concentrations against selected micro-organisms representative of the rabbit gut micro-flora (Akhurst, L.C., 2005 IIA 7.3/07).

It has been postulated that folpet may affect the rabbit GI tract micro-flora and that an imbalance in the micro-flora may have consequences for the pregnant rabbit on both maternal and embryo-fetal nutrition. Such changes could, in theory, affect the developing fetus. The rabbit is a species particularly susceptible to gastrointestinal disturbances which may in part be mediated through changes in the GI tract micro-flora. An *in vitro* approach to demonstrate changes in representative rabbit GI tract micro-flora was considered to be a simple and straightforward initial step to evaluate the potential effects of phthalimide on such micro-organisms.

Phthalimide is not thought to have the same anti-microbial activity as the parent molecule folpet partly because it is not capable of generating the highly reactive moiety thiophosgene.

The minimum inhibitory concentration (MIC) assay is an internationally accepted test for antimicrobial susceptibility testing and is commonly used to assess the effectiveness of intentional antimicrobial compounds. The test was adapted to assess selected micro-organisms representative of the rabbit gut micro-flora

The study was conducted using an agar dilution procedure for determination of MIC values. Ten species of the genus Bacteroides, one genus of Enterococcus faecalis and four isolates of Candida albicans were tested. Final concentrations of phthalimide of 1000, 500, 200, 100, 50, 20, 10, 5 , 2 or 1 µg/ml were tested and solvent control and growth control plates were employed. The lowest test substance concentration that completely inhibited growth of the test organism was recorded as the MIC.

Phthalimide demonstrated no antimicrobial activity towards Bacteroides sp. and Enterococcus faecalis, when tested up to a concentration of 1000 µg/ml, which was just above the limit of solubility in agar. It showed very weak activity against the yeast, Candida albicans, with very slight (qualitative) inhibition of growth only at 1000 µg/ml and in only one of the four strains tested.

It may be concluded that phthalimide demonstrated no significant antimicrobial activity against organisms selected as representatives of rabbit GI tract flora species tested in this study. It is unlikely that this molecule has the potential to affect the micro-flora of the rabbit GI tract.

2) Their presence in significant amounts

a) *^{14}C-folpet metabolism in the lactating goat (part A). ^{14}C- trichloromethyl folpet: material balance of dosed radioactivity. (Cordon, M.T. 1997a; Annex IIA, 6.2/01; IIA 7.3/08)*

NOTE: The summary below already appears in the DAR under B.7.2.a Metabolism, distribution and expression of residues in livestock (Annex IIA 6.2 and Annex IIIA 8.1).

[Trichloromethyl-^{14}C] folpet (radiochemical purity 99.3%) dissolved in [redacted] was administered in gelatine capsules orally once daily for three consecutive days to a miniature lactating goat at a measured dietary concentration of 20 mg/kg diet. Milk was collected twice a day, from one day prior to dosing until sacrifice, urine and faeces were collected from one day prior to dosing until sacrifice and expired air was collected in potassium hydroxide traps. The goat was sacrificed 23 hours after the final dose. Radioactivity was determined in excreta, tissues, milk, gastrointestinal tract, cage wash and expired air by LSC and combustion/LSC.

The total recovery of radioactivity was 102%, of which 31.4% was recovered in air traps, 41.9% in faeces, 16.9% in the gastrointestinal tract and 10.2% in the urine. Very low levels of ^{14}C radioactivity were found in milk (1.0% of administered dose) and tissues (0.8% of administered dose). Significant residues were found in the liver (0.5% of administered dose, equivalent to 0.34 mg folpet equivalents/kg), kidney (0.1% of administered dose, equivalent to 0.26 mg folpet equivalents/kg), muscle (0.2% of administered dose, equivalent to 0.04 mg folpet equivalents/kg) and fat (< 0.1% of administered dose, equivalent to 0.01 mg folpet equivalents/kg).

The distribution of applied radioactivity is given in Table B.7.3.9.

Table B.7.3. 9: Distribution of ^{14}C following oral administration of [trichloromethyl-^{14}C] folpet to a lactating goat for three days

Matrix/tissue	% Applied dose	Residue (mg folpet equivalents/kg or L)
Tissues & milk		
subcutaneous fat	< 0.1	0.01
peritoneal fat	< 0.1	0.01
muscle (fore)	0.1	0.03
muscle (rump)	0.1	0.04
kidney	0.1	0.26
liver	0.5	0.34
milk 0-24 hr	0.2	0.23
milk 24-48 hr	0.4	0.38
milk 48-71 hr	0.4	0.34
total	1.8	-
Urine		
0-24 hr	2.1	-
24-48 hr	0.6	-
48-71 hr	6.4	-
bladder	1.1	-
total	10.2	
Faeces		
0-24 hr	8.7	-
24-48 hr	11.5	-
48-71 hr	21.7	-
total	41.9	-
Expired $^{14}CO_2$		
0-12 hr	6.8	-
12-24 hr	2.0	-
24-36 hr	7.9	-
36-48 hr	3.6	-
48-60 hr	8.9	-
60-71 hr	2.2	-
total	31.4	
Gastrointestinal tract		
intestine	10.8	-
rumen & reticulum	5.7	-
omasum & abomasum	0.4	-
total	16.9	
Bile	< 0.1	
Cage wash	0.2	-
Total	**102**	-

b) ^{14}C-folpet metabolism in the lactating goat (part B). (Cordon, M.T. 1997b; IIA, 6.2/02; IIA 7.3/09)

NOTE: The summary below already appears in the DAR under B.7.2.b Metabolism, distribution and expression of residues in livestock (Annex IIA 6.2 and Annex IIIA 8.1).

[Trichloromethyl-^{14}C] folpet (radiochemical purity 97%) and [U-phenyl -^{14}C] folpet (radiochemical purity 98%) dissolved in [redacted] were each administered to separate miniature lactating goats. Administration was in gelatine capsules orally once daily for six consecutive days at a measured dietary concentration of 24 mg/kg diet and 14 mg/kg diet for the [trichloromethyl-^{14}C] folpet and [U-phenyl -^{14}C] folpet, respectively. Milk was collected twice a day from one day prior to dosing until sacrifice. Urine and faeces were collected from one day prior to dosing until sacrifice. The goat was sacrificed 23 hours after the final dose.

Radioactivity was determined in excreta, tissues, milk, gastrointestinal tract and cage wash by LSC and combustion/LSC. Metabolites were characterised by TLC.

Following administration of [trichloromethyl-^{14}C] folpet, the majority of the administered radioactivity was excreted and recovered in the faeces and urine. The distribution results were comparable to those recorded in the distribution study (Cordon, M.T. 1997a). Significant residues were found in the kidney (0.16 mg folpet equivalents/kg), liver (0.25 mg folpet equivalents/kg), muscle (0.02 mg folpet equivalents/kg) and milk (up to 0.20 mg folpet equivalents/L). Residues in milk plateaued approximately 4 days after the start of administration. Residues in fat were less than 0.01 mg folpet equivalents/kg. The distribution of applied radioactivity is given in Table B.7.3.10.

Table B.7.3.10: Distribution of ^{14}C following oral administration of [trichloromethyl-^{14}C] folpet to a lactating goat for six days

Matrix/tissue	% Applied dose	Residue (mg folpet equivalents/kg or L)
Tissues & milk		
subcutaneous fat	< 0.1	< 0.01
peritoneal fat	< 0.1	< 0.01
muscle (fore)	< 0.1	0.02
muscle (rump)	< 0.1	0.03
liver	0.2	0.25
kidney	< 0.1	0.16
milk 0-24 hr	< 0.1	0.098
milk 24-48 hr	0.1	0.163
milk 48-72 hr	0.1	0.174
72-96 hr	0.1	0.177
96-120 hr	0.1	0.203
120-143 hr	0.1	0.192
total	0.7	-
Urine		
0-24 hr	0.5	-
24-48 hr	1.0	-
48-72 hr	0.5	-
72-96 hr	1.6	-
96-120 hr	0.7	-
120-143 hr	0.4	-
bladder	0.1	-
total	4.8	
Faeces		
0-24 hr	0.5	-
24-48 hr	5.3	-
48-72 hr	6.6	-
72-96 hr	12.7	-
96-120 hr	8.5	-
120-143 hr	1.3	-
total	34.9	-
Bile	< 0.1	
Cage wash	0.2	-
Total	**40.6**[a]	-

[a] Plus 31.4% present in expired air, 16.9% present in gastrointestinal tract (see Point 6.2/01).

Following administration of [U-phenyl -^{14}C] folpet, the majority of the administered radioactivity was recovered in the faeces (34.9%) and urine (58.3%), with small quantities in the cage wash (2.1%) and tissues plus milk (< 0.1%). The overall recovery was 95.3% of the

administered dose. Significant residues were found in the kidney (0.05 mg folpet equivalents/kg) and liver (0.02 mg folpet equivalents/kg). Residues in muscle and fat were less than 0.01 mg folpet equivalents/kg; residues in milk were less than 0.01 mg folpet equivalents//L. The distribution of applied radioactivity is given in Table B.7.3.11.

Table B.7.3.11: Distribution of ^{14}C following oral administration of [U-phenyl -^{14}C] folpet to a lactating goat for six days

Matrix/tissue	% Applied dose	Residue (mg folpet equivalents/kg or L)
Tissues & milk		
subcutaneous fat	< 0.1	0.004
peritoneal fat	< 0.1	< 0.001
muscle (fore)	< 0.1	0.003
muscle (rump)	< 0.1	0.003
liver	< 0.1	0.022
kidney	< 0.1	0.052
milk 0-24 hr	< 0.1	0.004
milk 24-48 hr	< 0.1	0.006
milk 48-72 hr	< 0.1	0.005
72-96 hr	< 0.1	0.005
96-120 hr	< 0.1	0.005
120-143 hr	< 0.1	0.006
total	< 0.1	-
Urine		
0-24 hr	9.2	-
24-48 hr	12.1	-
48-72 hr	8.7	-
72-96 hr	6.4	-
96-120 hr	11.2	-
120-143 hr	10.7	-
total	58.3	
Faeces		
0-24 hr	1.4	-
24-48 hr	6.4	-
48-72 hr	7.7	-
72-96 hr	6.1	-
96-120 hr	6.3	-
120-143 hr	7.0	-
total	34.9	-
Bile	< 0.1	
Cage wash	2.1	-
Total	**95.3**	-

Following administration of [trichloromethyl-^{14}C] folpet, thiazolidine was found in the urine and faeces at 17.4% and 2.9%, respectively, of the radioactivity (equivalent to 0.8% and 1.0% of the administered radioactivity, respectively). Low levels of unmetabolised folpet were found only in the faeces (8.0% of the radioactivity, equivalent to 2.8% of the administered radioactivity). Folpet was extensively metabolised in tissues and the radiolabelled carbon was incorporated into naturally occurring compounds. These were amino acids (in the liver, kidney, milk, muscle), glucose and fats (in the liver), cholesterol (in the kidney) and lactose (in the milk).

Following administration of [U-phenyl -^{14}C] folpet, phthalamic acid was the major constituent of the urine (84.8% of the radioactivity, equivalent to 49.4% of the administered radioactivity). The faeces contained phthalimide (26.4% of the radioactivity, equivalent to 9.2% of the administered radioactivity) and a small amount of unmetabolised folpet (0.9% of the radioactivity, equivalent to 0.3% of the administered radioactivity). The majority of the

radioactivity in the faeces was unextracted. The major metabolites in liver, kidney and milk were phthalimide and either phthalamic acid, phthalic anhydride or phthalic acid. No folpet was detected in tissues or milk.

The characterisation of radioactivity is summarised in Table B.7.3.12.

Table B.7.3.12: Characterisation of ^{14}C radioactivity in tissues, milk and excreta following administration of folpet to a lactating goat for six days

Identity of residue	% ^{14}C radioactivity (% of dosed radioactivity)										
	liver		kidney		urine		faeces		milk		muscle
	1	2	1	2	1	2	1	2	1	2	2
folpet	-	-	-	-	-	-	0.9 (0.3)	8.0 (2.8)	-	-	-
thiazolidine	-	-	-	-	-	17.4 (0.8)	-	2.9 (1.0)	-	-	-
phthalamic acid	27.8	-	69.1[c]	-	84.8 (49.4)	-	-	-	7.2[c]	-	
phthalimide	2.6	-	0.7	-	-	-	26.4 (9.2)	-	5.8	-	
natural compounds[a]	-	26.9	-	19.2					-	52.7	35.8
unknowns[b]	7.2 {12} [1.3]	10.8 {8} [3.5]	3.6 {5} [1.3]	20.5 {9} [8.7]	9.4 {4} [4.6]	33.3 {4} [13.3]	0.3	1.0 {1}	-	3.6 {3} [3.0]	10.9 {5} [6.0]
baseline	23.1	9.6	10.1	10.3	0.8	38.1	3.0	< 0.1	-	6.8	2.3
remainder	5.7	9.8	11.6	21.2	-	-		-	6.6	11.6	12.8
unextracted residue	-	10.0	4.4	-	-	-	68.0	87.0	4.1	15.9	31.8
other[d]	33.6	59.8	0.9	28.7	5.1	11.2	1.4	1.2	76.3	8.7	6.4

1 = [U-phenyl -^{14}C] folpet, 2 = [trichlormethyl-^{14}C] folpet.
[a] Amino acids, cholesterol, glucose, lactose, etc.
[b] Value in {} parenthesis = number of unknown components which make up the total radioactive residue; value in [ ] parenthesis = % of total radioactive residue represented by the major unknown component.
[c] Includes phthalic anhydride and phthalic acid.
[d] Unanalysed and losses during work-up.
Values in ( ) parenthesis are % of dosed radioactivity.

c) Dietary Risk assessment of Folpet Metabolite: Phthalimide

The amount of phthalimide in milk and meat was determined in a goat metabolism study (Corden 1997a, 1997b). Goats were fed ^{14}C-folpet at 14 ppm labelled in the benzene ring for 6 days. Tissues were harvested and samples with 3% total radioactive residue or more were characterized. The majority of radioactivity was excreted in the urine and faeces.

	Phthalimide
Meat	<0.004 mg/kg
Milk	<0.001 mg/kg

Estimation of the potential and actual exposure of phthalimide through animal products diet

Chronic exposure

Theoretical Maximum Daily Intake (TMDI)

The TMDI is calculated by multiplying the MRL or actual residues by the estimated average daily consumption for a given food commodity.

$TMDI = \sum MRL \times F$

where:
MRL = Maximum residue limit or actual residues for a given food commodity
F = Consumption of that food commodity.

This calculation is performed using:

1) An International diet (European Region) based on data from the World Health Organisation (WHO)[3].
2) The UK Dietary model (PSD, 1999[4])

WHO European diet

The TMDI calculation is presented in Table B.7.3.13.

Table B.7.3.13: TMDI calculation for Phthalimide based on WHO diet

Commodity	Phthalimide (mg/kg)	Consumption (kg/person/day)	TMDI (mg/person/day)
Total milk	< 0.001 (0.0005*)	0.3408	0.0002
Cattle meat	< 0.004 (0.002*)	0.0633	0.0001
Total			**0.0003**

*Since phthalimide residues were below the LOQ of the analytical method used, one half of the LOQ as worst case scenario was taken into consideration as appear in the brackets.

[3] WHO (1989). Guidelines for predicting dietary intake of pesticide residues. Prepared by the joint UNEP/FAO/WHO Food Contamination Monitoring Programme in collaboration with the Codex Committee on Pesticide Residues. World Health Organisation, Geneva.

[4] PSD (1999). Guidance on the estimation of dietary intakes of pesticides residues. The Registration Handbook. Pesticides Safety Directorate, Ministry of Agriculture, Fisheries and Food.

The total TMDI of Phthalimide is 0.0003 mg/person/day day or 0.0000 mg/kg bw/day for a 60 kg adult.

UK diet

UK consumption data for adults, children, toddlers and infants (mean consumers and high, i.e. 97.5th percentile, consumers) are presented in Table B.7.3.14

Table B.7.3.14: UK consumption data for adults, children, toddlers and infants

Commodity	Consumption data (kg/day)							
	Adults (70.1 kg bw)		Children (43.6 kg bw)		Toddlers (14.5 kg bw)		Infants (8.7 kg bw)	
	Mean	High [1]	Mean	High	Mean	High	Mean	High
Milk	0.2573	0.6659	0.0304	0.6745	0.3064	0.8017	0.33775	0.8719
Meat	0.0841	0.2050	0.0641	0.1339	0.0276	0.0869	0.1339	0.0121

The TMDI for Phthalimide was calculated for all consumer groups of milk and meat (high consumption intake).

Table B.7.3.15: consumption of Phthalimide by adults, children, toddlers and infants based on UK high consumption intakes

Commodity	Phthalimide (mg/kg)	TMDI (mg/kg bw/day)			
		Adults (70.1 kg bw)	Children (43.6 kg bw)	Toddlers (14.5 kg bw)	Infants (8.7 kg bw)
Milk	0.0005	0.0000	0.0000	0.0000	0.0000
Meat	0.002	0.0000	0.0000	0.00001	0.0000
Total exposure		**0.0000**	**0.0000**	**0.0000**	**0.0000**

The TMDIs of Phthalimide in all consumer groups including toddlers and infants, which are the most sensitive consumer groups, is 0.0000 mg/kg bw/day.

Comparison of TMDI of phthalimide with the ADI

The TMDI values for different consumer groups and diets are summarised in Table B.7.3.16.

Table B.7.3.16: TMDI values for different consumer groups and diets

Diet	Body weight (kg)	TMDI (mg/kg bw/day)
WHO adult	60	0.0000
UK adult	70.1	0.0000
UK child	43.6	0.0000
UK toddler	14.5	0.0000
UK infant	8.7	0.0000

Based on the proposed ADI for folpet of 0.1 mg/kg bw/day, the TMDI for Phthalimide according to the worst case consumption scenarios represents 0 % of the ADI for all the different consumer groups and different dietary intakes of milk and meat.

The maximum daily intake of Phthalimide in animal products is zero for all consumer groups including the most sensitive consumer groups and compare to the ADI for folpet according to the worst case exposure.

d) Toxicity of phthalimide to aquatic organisms

Note: Summaries of all the relevant studies are presented below. These are already included in the DAR in Point B.9.2.1.

Fish

(i) Acute toxicity of phthalimide to rainbow trout (Salmo gairdneri). (Bowman, J.H. 1988c; IIA, 8.2.1/12; IIA 7.3/10)

The 96-hour acute toxicity of phthalimide (purity 98%) to the rainbow trout (*Salmo gairdneri* now known as *Oncorhynchus mykiss*) was determined in a static test system. Ten fish per glass vessel each containing 15 L (16 hour photoperiod, 12 °C) were exposed to nominal concentrations of phthalimide (dissolved in [redacted] of 10, 18, 32, 56 and 100 mg/L in comparison with a dilution water control (hardness 40 to 46 mg/L $CaCO_3$) and a solvent control (0.1 mL/L). The fish were not fed for 48 to 96 hours prior to or during exposure. The test media were not renewed throughout the test. Samples of all test media for analysis of phthalimide by HPLC, were taken at the start and end of the exposure period. Measurements of pH, dissolved oxygen and temperature were taken at 0, 48 and 96 hours. Fish mortality and behaviour were recorded once every 24 hours.

The study met the essential criteria of EEC C1. However, standard lengths were measured whereas total lengths are stated in the EU guideline. No details were given of fish mortality during holding. It was conducted according to Good Laboratory Practice.

The mean measured concentrations of phthalimide were 9.4, 17, 26, 43 and 66 mg/L representing 94, 94, 81, 77 and 66% of nominal. There was little loss of phthalimide from 0 to 96 hours. At measured concentrations of 26, 43 and 66 mg/L there was a white precipitate on the surface and at the bottom of the test vessels at 0-hours. The amount of precipitate increased with nominal concentration, but became less visible with time. This suggests that in media at 26 mg/L and above, phthalimide was present in excess, possibly above the limit of water solubility and hence toxicity to rainbow trout at these concentrations may not be related to inherent toxicity but to excess test material in the test system. The water quality parameters were all within expected limits.

The cumulative mortality is presented in Table B.7.3.17. Sublethal effects at 26, 43 and 66 mg/L were surfacing, loss of equilibrium, fish on the bottom of the test vessels, quiescence and/or distended abdomen.

Table B.7.3.17: Mortality of rainbow trout exposed to phthalimide following 96-hours exposure in a static test system

Mean measured concentration of phthalimide (mg/L)	Cumulative mortality (%)			
	24 hr	48 hr	72 hr	96 hr
Water control	0	0	0	0
Solvent control	0	0	0	0
9.4	0	0	0	0
17	0	0	0	0
26	0	0	0	0
43	0	0	10	20
66	80	100	100	100

The 96-hour LC_{50} of phthalimide to rainbow trout under static test conditions was 49 mg/L (with 95% confidence limits of 26 to 66 mg/L) based on measured concentrations. The

NOEC for mortality was 17 mg/L. The 24, 48 and 72-hour LC_{50} values were 58, 53 and 51 mg/L, respectively.]

(ii) Acute toxicity of phthalimide to bluegill sunfish (Lepomis macrochirus) in a static renewal system. (Bowman, J.H. 1989; IIA, 8.2.1/13; IIA 7.3/11)

The 96-hour acute toxicity of phthalimide (purity 98%) to the bluegill sunfish (*Lepomis macrochirus*) was determined in a semi-static test system with renewal of the test media after 48 hours. Ten fish per glass vessel each containing 15 L (16 hour photoperiod, 21 to 23 °C) were exposed to nominal concentrations of phthalimide (dissolved in [redacted] of 10, 18, 32, 56 and 100 mg/L in comparison with a dilution water control (hardness 42 mg/L $CaCO_3$) and a solvent control (0.1 mL/L). The fish were not fed for 48 to 72 hours prior to or during exposure. Samples of all test media for analysis of phthalimide by HPLC, were taken at the start, after 48 hours and at the end of the exposure period. Measurements of pH, dissolved oxygen and temperature were taken at 0, 48 and 96 hours. Fish mortality and behaviour were recorded once every 24 hours. The 32 mg/L treatment was repeated with a concurrent solvent control treatment as three fish were lost during renewal of the test media in the first definitive test at this concentration.

The study met the essential criteria of EEC C1. However, standard lengths were measured whereas total lengths are stated in the EU guideline. No details were given of fish mortality during holding. Temperature, dissolved oxygen and pH should have been measured daily rather than at 0, 48 and 96 hours. It was conducted according to Good Laboratory Practice.

The mean measured concentrations of phthalimide were 6.8, 13, 22, 31 and 52 mg/L representing 68, 72, 69, 55 and 52% of nominal . At 22, 31 and 52 mg/L there was a white precipitate on the surface of the test media and at the bottom of the test vessels at 0-hours and 48-hours (freshly prepared media). The amount of precipitate increased with nominal concentration, but became less visible with time. The nominal 100 mg/L medium had a white precipitate at the bottom of the test vessel at both renewal time periods. This suggests that in media at 22 mg/L and above, phthalimide was present in excess, possibly above the limit of water solubility at the start of the renewal period but then may have fully dissolved on completion of the renewal period in all but the 100 mg/L medium. Therefore, the toxicity of phthalimide to bluegill sunfish at these concentrations may not be related to inherent toxicity but to excess test material in the test system. The water quality parameters were all within expected limits.

The cumulative mortality is presented in Table B.7.3.18. Sublethal effects at 31 and 52 mg/L were light discolouration, vertical orientation, quiescence and/or laboured respiration.

Table B.7.3.18: Mortality of bluegill sunfish exposed to phthalimide following 96-hours exposure in a semi-static test system

Mean measured concentration of phthalimide (mg/L)	Cumulative mortality (%)			
	24 hr	48 hr	72 hr	96 hr
Water control	0	0	0	0
Solvent control	0	0	0	0
6.8	0	0	0	0
13	0	0	0	0
22	0	0	0	0
31	10	10	10	10
52	90	90	100	100

The 96-hour LC_{50} of phthalimide to bluegill sunfish, under semi-static test conditions, was 38 mg/L (with 95% confidence limits of 31 to 52 mg/L) based on measured concentrations. The NOEC was 22 mg/L based on toxicological symptoms observed at 31 and 52 mg/L. The 24, 48 and 72-hour LC_{50} values were 40, 40 and 38 mg/L, respectively.

(iii) Acute flow-through toxicity of folpet technical to rainbow trout (Salmo gairdneri). (Bowman, J.H. 1988a; IIA, 8.2.1/01; IIA 7.3/12)

The 96-hour acute toxicity of folpet technical active substance (purity 90.3%) to the rainbow trout (*Salmo gairdneri* now known as *Oncorhynchus mykiss*) was determined in a flow-through test system. Two groups of 10 fish per replicate 15 L aquarium (16 hour photoperiod, 12 to 13 °C) were exposed to nominal concentrations of folpet (dissolved in [redacted] of 0.0065, 0.013, 0.025, 0.05 and 0.10 mg/L in comparison with a dilution water control (hardness 40 to 46 mg/L $CaCO_3$) and a solvent control (0.1 mL/L). The fish were not fed for 48 hours prior to or during exposure. The test media were renewed 7.4 times each day. Samples of all test media for analysis of folpet, by high performance liquid chromatography (HPLC), were taken at the start and end of the exposure period. Measurements of pH, dissolved oxygen and temperature were taken at 0, 48 and 96 hours. Fish mortality and behaviour were recorded once every 24 hours.

The study met the essential criteria of EC method C1. However, fish lengths may be smaller, but standard lengths were measured whereas total lengths are stated in the EU guidelines. No details were given of fish mortality during holding. Temperature, dissolved oxygen and pH should have been measured daily rather than at 0, 48 and 96 hours. It was conducted according to Good Laboratory Practice.

The mean measured concentrations of folpet were 0.0022, 0.0056, 0.012, 0.026 and 0.13 mg/L representing 34, 43, 48, 52 and 130% of nominal. The 0 hour measurement for the 0.10 mg/L medium was 220% of nominal which was attributed to a precipitate in the splitter cell. For this reason and because the other measured adjacent concentrations were approximately 50% of each other, the 96 hour measured concentration at the highest nominal concentration was used in the LC_{50} calculation. The water quality parameters were all within expected limits.

The cumulative mortality is presented in Table B.7.3.19. Sublethal effects at 0.0056, 0.012 and 0.026 mg/L were loss of equilibrium, and fish on the bottom of the test vessels.

Table B.7.3.19: Mortality of rainbow trout following 96-hours exposure to folpet in a flow-through test system

Mean measured concentration of folpet (mg/L)	Cumulative mortality (%)			
	24 hr	48 hr	72 hr	96 hr
Water control	0	0	0	0
Solvent control	0	0	0	0
0.0022	0	0	0	0
0.0056	0	0	0	0
0.012	0	0	5	5
0.026	0	45	80	85
0.033*	100	100	100	100

*Based on the 96 h measured value.

The 96-hour LC_{50} of folpet to rainbow trout under flow-through conditions was 0.015 mg/L (with 95% confidence limits of 0.013 to 0.048 mg/L) based on measured concentrations. The NOEC was 0.0022 mg/L based on toxicological symptoms observed at 0.0056 mg/L and above. The 24, 48 and 72-hour LC_{50} values were 0.029, 0.026 and 0.015 mg/L, respectively.

(iv) Acute flow-through toxicity of folpet technical to bluegill sunfish (Lepomis macrochirus). (Bowman, J.H. 1988b, IIA, 8.2.1/02; IIA 7.3/13).

The 96-hour acute toxicity of folpet technical active substance (purity 90.3%) to the bluegill sunfish (*Lepomis macrochirus*) was determined in a flow-through test system. Two groups of 10 fish per replicate 15 L aquarium (16 hour photoperiod, 22 °C) were exposed to nominal concentrations of folpet (dissolved in [redacted] of 0.065, 0.13, 0.25, 0.5 and 1.0 mg/L in comparison with a dilution water control (hardness 40 to 46 mg/L $CaCO_3$) and a solvent control (0.1 mL/L). It should be noted that the limit of water solubility of folpet at 25 °C is

0.8 mg/L. Therefore, at the highest nominal concentration folpet would be present in excess of its water solubility. The fish were not fed for 48 hours prior to or during exposure. The test media were renewed 7.4 times each day. Samples of all test media for analysis of folpet, by HPLC, were taken at the start and end of the exposure period. Measurements of pH, dissolved oxygen and temperature were taken at 0, 48 and 96 hours. Fish mortality and behaviour were recorded once every 24 hours.

The study met the essential criteria of EEC C1. However, standard lengths were measured whereas total lengths are stated in the EU guidelines. No details were given of fish mortality during holding.It was conducted according to Good Laboratory Practice.

The mean measured concentrations of folpet were 0.016, 0.033, 0.068, 0.20 and 0.25 mg/L representing 25, 25, 27, 40 and 25% of nominal (Table B.7.3.20). A white precipitate was observed in the diluter mixing cell and in the aquaria with the highest nominal concentration of folpet. This is consistent with the quantity of folpet added to water which was above the limit water solubility. The water quality parameters were all within expected limits.

Table B.7.3.20: Measured concentrations of folpet technical during a 96-hour flow-through toxicity test with bluegill sunfish

Folpet nominal concentration (mg/L)	Folpet measured concentration (mg/L)			Mean measured conc. as a % of nominal
	0-hr	96-hr	Mean	
Control	< 0.010	< 0.010	-	-
Solvent control	< 0.010	< 0.010	-	-
0.065	0.017	0.015	0.016	25
0.13	0.028	0.037	0.033	25
0.25	0.059	0.076	0.068	27
0.50	0.12	0.28	0.20	40
1.0	0.17	0.33	0.25	25
Stock solution (9500)	9900	9900	9900	104

[a] Precipitate present in vessel.
[b] 96-hour concentration used in LC_{50} calculation.

The cumulative mortality is presented in Table B.7.3.21. There were no sublethal effects recorded at 0.033 mg/L or below.

Table B.7.3.21: Mortality of bluegill sunfish following 96-hours exposure to folpet in a flow-through test system

Mean measured concentration of folpet (mg/L)	Cumulative mortality (%)			
	24 hr	48 hr	72 hr	96 hr
Water control	0	0	0	0
Solvent control	0	0	0	0
0.016	0	0	0	0
0.033	0	0	0	0
0.068	100	100	100	100
0.20	100	100	100	100
0.25	100	100	100	100

The 96-hour LC_{50} of folpet to bluegill sunfish under flow-through conditions was 0.047 mg/L (with 95% confidence limits of 0.033 to 0.068 mg/L) based on measured concentrations. The NOEC was 0.033 mg/L based on mortality at 0.068 mg/L. The 24, 48 and 72-hour LC_{50} values were 0.047 mg/L.

Table B.7.3.22: Summary of acute toxicity of folpet and PI

Compound	LC_{50} (mg/L) Blue Gill sunfish	LC_{50} (mg/L) Rainbow trout	References	
PI	38	49	*Bowman, J.H. 1989; IIA, 8.2.1/13; IIA 7.3/09*	*Bowman, J.H. 1988c; IIA, 8.2.1/12; IIA 7.3/08*
folpet	0.047	0.015	*Bowman, J.H. 1988b, IIA, 8.2.1/02; IIA 7.3/11*	*Bowman, J.H. 1988a; IIA, 8.2.1/01; IIA 7.3/10*
Ratio	809	3266		

B.7.17 References relied on

B.7.17.1 Active substance

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company, Report No. GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N	Owner
IIA 7.3/01	Fabro, S., Schumacher, R. L., Smith, R. L., Williams, R. T.	1964	Teratogenic activity of thalidomide and related compounds. *Life Sciences* **3**, 987-992. (Company file R-9963). Not GLP; Published.	N	-
IIA 7.3/02	Kennedy, G., Fancher, O. E., Calandra, J. C.	1968	An investigation of the teratogenic potential of captan, folpet, and difolatan. *Toxicol. Appl. Pharmacol.* **13**, 420-430. (Company file R-169). Not GLP; Published	N	-
IIA 7.3/03	Blee, M.A.B.	2006	Phthalimide: Prenatal toxicity study in the rabbit by oral gavage. Report MAK 863/055231 administration (Company file: R-18201). GLP, Unpublished.	Y	Makhteshim
IIA 7.3/04	Pilinskaya, M. A.	1986	Study of the cytogenetic activity of certain metabolites of a number of pesticides representing several classes of chemical compounds. *Tsitol. Genet.* **20**, 143-145. (Company file R-11352) Not GLP; Published.	N	-
IIA 7.3/05	Riggin, R. M., Margard, W. L., Kinzer, G. W.	1983	Characterization of impurities in commercial lots of sodium saccharin produced by the Sherwin-Williams process. II. Mutagenicity. *Fd Chem. Toxic.* **21**, 11-17. (Company file R-11350). Not GLP; Published.	N	-
IIA 7.3/06	Siefried, H.E.	2000	Review: Toxicological risk characterisation of potential folpet metabolites. The toxicity profiles of phthalic and phthalamic acids and phthalimide – is there a significant risk from metabolite exposure? Consultants, report dated August 1, 2000 (Company file: R-12331). Not GLP, Unpublished.	Y	Makhteshim
IIA 7.3/07	Akhurst, L.C.	2005	Phthalimide: Determination of minimum inhibitory concentrations against selected micro-organisms representative of the rabbit gut micro-flora. Report MAK 889/053251 ((Company file:R-18734). GLP, Unpublished.	Y	Makhteshim

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company, Report No. GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N	Owner
IIA 7.3/08	Cordon, M.T.	1997a	^{14}C-folpet metabolism in the lactating goat (part A). ^{14}C- trichloromethyl folpet: material balance of dosed radioactivity. [redacted]Report No. MBS72a/972856 (Company file: R-9137a). GLP, Unpublished.	Y	Makhteshim
IIA 7.3/09	Cordon, M.T.	1997b	^{14}C-folpet metabolism in the lactating goat (part B). [redacted]Report No. MBS 72b/972856 (Company file: R-9137). GLP, Unpublished.	Y	Makhteshim
IIA 7.3/10	Bowman, J.H.	1988c	Acute toxicity of phthalimide to rainbow trout (*Salmo gairdneri*). [redacted]Report No. 36789 (Company file R-4956). GLP, Unpublished.	Y	Makhteshim
IIA 7.3/11	Bowman, J.H.	1989	Acute toxicity of phthalimide to bluegill sunfish (*Lepomis macrochirus*) in a static renewal system. [redacted]Report No. 36788 (Company file R-5255). GLP, Unpublished.	Y	Makhteshim
IIA 7.3/12	Bowman, J.H.	1988a	Acute flow-through toxicity of folpet technical to rainbow trout (*Salmo gairdneri*). [redacted]Report No. 36785 (Company file R-4954). GLP, Unpublished.	Y	Makhteshim
IIA 7.3/13	Bowman, J.H.	1988b	Acute flow-through toxicity of folpet technical to bluegill sunfish (*Lepomis macrochirus*). [redacted]Report No. 36784 (Company file R-4955). GLP, Unpublished.	Y	Makhteshim

European Commission

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ECCO-Meetings

Folpet

Volume 3

Annex B
Addendum: definition of the residue

Rapporteur Member State: Italy

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B.7.3 Definition of the residue (Annex IIA 6.7; Annex IIIA 8.6)

Folpet: The residue definition for the fungicide folpet should be folpet only as the metabolite phthalimide is neither of toxicological significance nor does it pose a significant dose to humans.

The collective data (toxicological data and residue data leading to estimated dose to humans) support the conclusion that the residue definition for folpet should be **folpet** only.

The DG SANCO Guideline notes (European Commission, 1997): Residue Definition – Of the three general considerations that are fundamental to the decision as to whether or not specific metabolites/degradation products should be included in the definition and expression of a residue, two are relevant to this discussion:
(1) Their basic toxicology and
(2) Their presence in significant amounts.

Introduction
Folpet was discussed at the December 10-13, 2007 EFSA meeting (PRAPeR 2007). At issue is the Definition of the Residue; specifically, whether the definition should include folpet only or folpet with its main degradate, phthalimide.

The decision on whether one or more degradates should be part of the residue definition rests on the toxicity of these compounds and whether their respective toxicities exceed the threshold level of concern that triggers inclusion.

Analysis of data related to this issue has been ongoing. It was earlier proposed that the residue definition should be folpet plus phthalimide:
"The metabolism of folpet in plants has been adequately elucidated. The main degradation products after release of the trichloromethylthio-side chain are phthalimide and phthalic acid. As it is not possible at this stage to fully characterize the toxicological properties of phthalimide, this metabolite needs to be included in the residue definition for plant products for monitoring and risk assessment purposes" (EFSA 2006).

The Rapporteur Member State proposed that the residue definition should be folpet, only:
Folpet: The residue definition for the fungicide folpet should be folpet only as the metabolite phthalimide is neither of toxicological significance nor does it pose a significant dose to humans.
The collective data (toxicological data and residue data leading to estimated dose to humans) support the conclusion that the residue definition for folpet should be folpet only (EC 2008).

The most recent discussion resulted in a request for additional data or argumentation regarding the toxicological significance of folpet's degradates as they impact the residue definition.

There were indications that the metabolite was not of higher concern than the parent compound; however, the submitted data package was likely incomplete. Furthermore, since the experts had not been able to fully access the relevant information provided by the RMS, it was decided to postpone the discussion on the metabolites of folpet/captan to the next meeting.

It was agreed that the RMS provides further information on the following endpoints on the metabolite phthalimide: Acute toxicity, genotoxicity, carcinogenicity, relevance of dog study and developmental effects in comparison to the parent compound (PRAPeR 2007).

This discussion document discusses folpet's degradate phthalimide and concludes that folpet's residue should be defined as parent only.

Definition of the Residue
There are competing needs when one sets out to define the definition of the residue (OECD

2006). On one hand there is the desire to consider the toxicity of the parent as well as the toxicity of all metabolites, degradates or other transformation products such that a sound risk assessment can be made and all relevant metabolites/degradates included. On the other hand there is the practical matter of defining the MRL such that it can, in fact, be monitored.

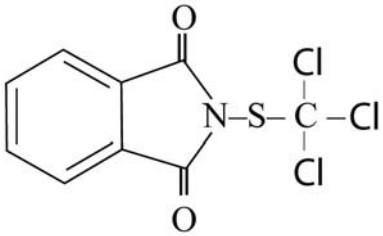
Guidance provided by OECD on the definition of the residue, as it relates to toxicity, includes the following (OECD 2006):
In order to assess metabolite/degradate toxicity and determine its potential effects, available information on the metabolite/degradate or similar compounds in databases or publications is evaluated. In most cases, however, toxicity data specific to the metabolite/degradate in question are not available or are limited to acute oral median lethal dose tests. In these instances weight of evidence evaluations are used to assess the toxic potential of the metabolite/degradate relative to that of the parent compound. The goal is to predict whether the metabolite/degradate is likely to be significantly less toxic than the parent, have comparable toxicity, be potentially significantly more toxic than the parent, or possess a different mechanism of toxicity. In many instances, it will not be clear as to whether a metabolite/degradate has the same mechanism of toxicity and/or how the level of toxicity would compare to that of the parent. The default position in such cases would be that the metabolite/degradate elicits the same effect as the parent and at comparable doses (i.e., equal toxicity). (OECD 2006, § 19)

Folpet Degradates
There are toxicological data for phthalimide on its mutagenicity, developmental toxicity and Minimum Inhibitory Concentration (*in vitro*) for select microorganisms. Additionally, since all mammalian studies in which folpet is dose orally, effectively test the systemic effects of phthalimide, there is adequate data to assess its toxicity with regard to the Definition of Residue. Finally, analysis of the chemical properties of phthalimide, compared to folpet, provide insight into their respective toxicities

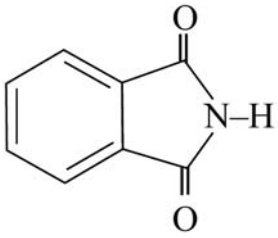
<u>The basis of folpet's toxicity</u>
Both acute and chronic toxicity endpoints are directly associated with the chemical structure of folpet and its interaction with biological materials. Specifically, it is the trichloromethylthio, TCMT, moiety that is responsible for both its pesticidal action and mammalian toxicity. The TCMT moiety reacts with thiol groups resulting in the denaturing of proteins and the degradation of folpet. The reactive product of this degradation, thiophosgene, continues the degradation of thiols as well as other functional groups. The relatively stable product of this degradation is phthalimide, the carrier moiety for TCMT.

The stable degradate phthalimide does not contain TCMT; does not react with thiol groups; does not generate thiophosgene; and, cannot, therefore, induce toxicity reactions that mimic the parent. This is not so evident from acute toxicity, since both parent and degradate are relatively non-toxic; but it is quite evident from mutagenicity studies, repeat dose studies, and developmental toxicity studies. We ask the Experts to consider the physical/chemical properties of folpet and phthalimide, when deriving the appropriate definition of residue.

<u>Comparative structures</u>
The structures of folpet and phthalimide are noted below.

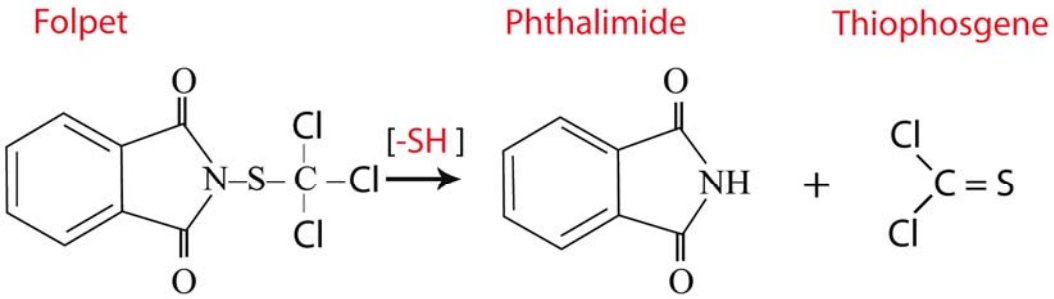


Folpet



Phthalimide

Note that phthalimide does not contain the trichloromethylthio moiety and therefore cannot replicate the chemical reaction that characterizes folpet's toxicity:



Folpet Metabolism

It is the process by which phthalimide is generated that is responsible for the irritative nature of folpet. Phthalimide does not react with thiols similarly to folpet.

Toxicity of phthalimide.

Acute toxicity
Both folpet and phthalimide have acute oral toxicity values greater than 5 g/kg bw.

Genotoxicity
There is a stark difference between folpet and phthalimide with regard to genotoxicity when assayed by *in vitro* test systems. Folpet is mutagenic *in vitro* while phthalimide is not. The mutagenicity has been associated with the generation of thiophosgene (Lukens 1966, Rideg 1982).

Phthalimide does not react with thiols, does not generate thiophosgene, and is not mutagenic.

It can be concluded that phthalimide is not genotoxic.

Carcinogenicity
Folpet induces gastrointestinal tumors in mice, primarily in the duodenum. Mechanistic data have been developed that elucidate the mode of action (MOA) responsible for these tumors. The salient features of the MOA are:

- Folpet is not mutagenic *in vivo.*
- Folpet is a local irritant to the villi of the duodenal lining.
- Villi are damaged and sloughed off into the duodenal lumen.
- The basal cells of the duodenal crypts respond with marked proliferation.
- Transformed cells, normally dormant, are resident in the crypt compartment.
- Increased proliferative pressure promotes these transformed cells to tumors.

Corollaries to this MOA include:

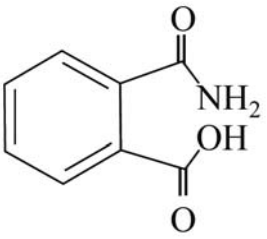
- Folpet's local irritation is due to the trichloromethylthio moiety (TCMT)
- The TCMT moiety is essential for the development of tumors.
- A threshold exists for TCMT-induced irritation and subsequent tumors.
- Phthalimide, lacking TCMT, cannot replicate captan's MOA for tumor induction.
- Phthalimide, therefore, cannot be carcinogenic in a similar way to folpet.

This MOA, as it applies to folpet's sister fungicide, captan, has been reviewed by outside Experts and by the US EPA. The supporting data were sufficiently robust as to have EPA revise their cancer classification of captan (US EPA 2004). Folpet is closely aligned chemically with captan and shares a common mechanism of toxicity (Bernard and Gordon 2000); thus, it is likely that the US EPA would arrive at a similar conclusion with regard to folpet's MOA.

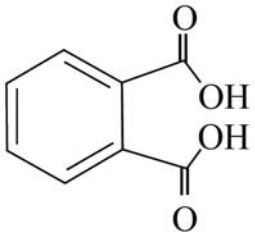
Oncogenicity studies with phthalimide have not been conducted (although phthalimide was tested by way of folpet's degradation in the bioassays). The fact that there were no systemic tumors, judged treatment-related, attests to the fact that folpet's degradates are not carcinogenic. In essence:

- Essentially 100% of folpet fed to rodents is degraded to phthalimide.
- Oral administration of folpet, therefore, results in systemic dosing of phthalimide.
- Phthalimide is metabolized in mammals to phthalamic acid and phthalic acid; thus,
- Rodent bioassays with folpet test for the systemic carcinogenicity of phthalimide, phthalamic acid and, to a lesser extent, phthalic acid.
- Rodent bioassays do not produce evidence of systemic tumors.

By way of completeness, the structures of phthalamic acid and phthalic acid are shown below.



Phthalamic Acid



Phthalic Acid

It can be concluded that neither phthalimide nor its metabolites are carcinogenic.

Relevance of dog study in comparison to the parent compound

The one-year dog study with folpet, conducted at 0, 10, 60 or 140 mg/kg bw/day administered by capsule, showed toxicity at 140 mg/kg bw/day requiring a dose adjustment to 120 mg/kg bw/day on Day 50. No significant treatment-related effects were seen at necropsy or during histopathological examination. There was a decrease in mean serum cholesterol, total protein, albumin and globulin levels in mid- and high-dose males. The high-dose females also showed reduced mean serum protein, albumin and cholesterol levels.

There was an initial weight loss in the high dose animals and those administered 60 mg/kg bw/day as well as decreased food intake. Based on these effects and the serum chemistries, the NOAEL was judged to be 10 mg/kg bw/day (Daly and Knezevich 1983).

Because phthalimide does not have the irritative properties of folpet, it is considered that the NOAEL in dogs would be higher than 10 mg/kg bw/day. It should be noted that apart from the local irritation effects due to folpet (leading to reduced food intake and likely associated with clinical chemistry changes), systemic effects reflected by microscopic changes (for which there were none) reflect phthalimide toxicity. This follows from the rapid degradation of folpet as soon as it is exposed to blood (Gordon et al. 2001).

Relevance of developmental effects in comparison to the parent compound
Folpet is not a frank teratogen, but has been associated with secondary developmental delays in fetuses associated with primary maternal toxicity. The maternal toxicity is due to the irritative nature of folpet on the gastrointestinal tract. This is particularly important in rabbits where folpet not only may induce irritation, but it also can adversely affect the intestinal bacterial flora that is critical for optimum nutrition, e.g., the MIC assays (Akhurst 2005).

Phthalimide has been tested extensively for developmental effects due to its association with the chemical thalidomide. The collective data show it is not teratogenic. It also does not induce maternal toxicity at equivalent folpet doses (Blee 2006). The question of dose selection for the phthalimide developmental study and its relevance to maternal toxicity is addressed below.

Dose selection for the phthalimide study.
Doses for the special developmental study with phthalimide (Blee 2006) were selected considering the degradation toxicokinetics of folpet and the toxicological question posed. In summary,

- The purpose of the study was to investigate whether phthalimide, *generated from the administered folpet*, contributed to the effects seen in rabbits.
- Folpet decomposes to phthalimide in a stochiometric manner (one molecule folpet results in one molecule phthalimide); thus, on a mg/kg bw basis the ratio of doses is approximately 2:1 (folpet molecular weight: 296.6; phthalimide molecular weight: 147.1).
- A bolus dose of folpet results in a slow increase of systemic phthalimide as folpet is degraded in the intestine and as it is absorbed into blood and degraded.
- In comparison, a bolus dose of phthalimide results in a marked increase in systemic phthalimide, since it is water soluble and readily absorbed; thus, bolus dosing of phthalimide at levels even below half that of the bolus folpet dose would be expected to produce peak blood levels far in excess of those produced by folpet alone.
- Since the developmental toxicity of phthalimide was investigated at folpet-relevant doses, the issue of maternal toxicity was moot.

Since systemic dosing of phthalimide results in the generation of phthalamic acid and, to a lesser extent, phthalic acid, all three degradation products were tested in the rabbit developmental study with THPI.

It can be concluded that phthalimide as well as its degradates are not developmental toxins.

Discussion
Rationale for not including folpet's metabolites in the residue definition is hereby set forth (EC 2008). These include its low toxicity, the lack of structural alerts upon QSAR analysis, comparative MIC data, and comparative toxicity in aquatic assays:
Folpet: The residue definition for the fungicide folpet should be folpet only as the metabolite phthalimide is neither of toxicological significance nor does it pose a significant dose to humans.
The collective data (toxicological data and residue data leading to estimated dose to humans) support the conclusion that the residue definition for folpet should be folpet only (EC 2008).

Analysis of the basic chemistry of folpet and phthalimide supports the RMS recommendations. Phthalimide, as well as its mammalian metabolites phthalamic acid and phthalic acid, do not have the reactive TCMT moiety that is responsible for folpet's fungicidal and mammalian toxicity.

Conclusion
Comparative toxicological studies show folpet, by reason of its trichloromethylthio moiety, elicits effects that are not seen with phthalimide, phthalamic acid, or phthalic acid, all of which lack this moiety. Studies include mutagenicity, developmental toxicity, carcinogenicity, Minimum Inhibitory Concentration (*in vitro*), and repeat dose studies in dogs.

The Residue Definition for folpet that best reflects its toxicity and the toxicity of its degradates is "folpet only."

1) Phthalimide basic toxicology

Four lines of evidence show that the metabolite of folpet, phthalimide is not of toxicological significance:

a) Direct measurements of toxicity.
b) QSAR Analysis
c) Measurement of minimal inhibitory concentration (MIC)
d) Comparison of folpet and phthalimide in bioassays that are particularly sensitive to the toxicological properties of folpet.

a). Direct measurements of toxicity
Phthalimide is not acutely toxic. Its LD_{50} in mice is above 5 g/kg bw[5].

Phthalimide is not mutagenic. When tested in the multiple strains in the Ames Assay, it is negative (Riggin *et al.,* 1983).

Phthalimide is not a developmental toxin (Fabro *et al.,* 1964; Kennedy *et al.,* 1968; Blee, 2006).

b) QSAR Analysis
Phthalimide does not have structural alerts that indicate it poses a toxicological risk (Siegfried, 2000).

c) Measurement of minimal inhibitory concentration (MIC)
The MIC assay is designed to assess antimicrobial activity and efficacy *in vitro*. The study was deigned to assess the effects of phthalimide on micro-flora representative of that in the rabbit GI-tract. Ten species of *Bacteroides* and four isolates of *Candida albicans* were incubated in the presence of phthalimide at biologically significant concentrations. phthalimide had no antimicrobial activity (Akhurst, 2005).

d) Comparison of folpet and its major metabolite in bioassays that are particularly sensitive to the toxicological properties of folpet.
The most sensitive bioassays for measuring toxicity of folpet are those involving aquatic organisms. This follows from the mode of action of folpet, which is irritation-based, due to its reaction with thiol groups.

In the case of rainbow trout, phthalimide is more than 3,000-fold less toxic than folpet (Bowman, 1988c), based on LC_{50} values, below. Bluegill sunfish are over 800-fold less sensitive to phthalimide than folpet (Bowman. 1989).

Test system	Folpet	Phthalimide	Ratio*
Trout, LC_{50}	0.015 mg/L	49 mg/L	3,266
Bluegill, LC_{50}	0.047 mg/L	38 mg/L	809

*ratio of folpet toxicity to phthalimide toxicity: > 3,000 and > 800

The toxicity of folpet is entirely attributable to the reactive side chain of folpet which is not present in the phthalimide metabolite. The high reactivity of the side chain of folpet produces irritation to the tissues. Phthalimide has low activity and is not an irritant.

In conclusion, phthalimide poses no significant toxicological risk for adverse effects.

[5] (PAN Pesticides Database (2005). U.S. National Toxicology Program acute toxicity studies for Phthalimide (metabolite of folpet). http://www.pesticideinfo.org/List_NTPStudies.jsp?Rec_Id=PC40165.

2) Their presence in significant amounts

Plants
The metabolism of folpet in plants proceeds according to the pathway shown below and each of the metabolites formed is also observed as a metabolite of folpet in mammalian studies. No plant unique metabolites are formed.

Folpet → Phthalimide → Phthalamic Acid → Phthalic Acid

Phthalimide was present at levels typically less than 10% of the total radioactive residue in radiolabelled studies conducted in avocados, potatoes, wheat and grapes. These residues are not considered significant in overall terms and in relation to the amount of folpet present.

A summary of the residues is shown in the following tables.

Summary of Folpet Related Radioactive Residues in Mature Avocados (Report No. 417W-2; Company file: R-7302)

Identity	^{14}C-Benzyl label	
	% TRR	ppm
Folpet	0.5	0.03
Phthalimide	3.9	0.22
Phthalic acid	81.9	4.49
Polar	7.3	0.40
Unknown A	0.4	0.02
Unknown B	2.8	0.15
Unknown C	1.6	0.09
Unknown D	0.9	0.05
Unknown E	0.5	0.03

Summary of Folpet Related Radioactive Residues in Mature Potato Tubers (Report No. MAK506/992098; Company file: R-10347)

Identity	^{14}C-Benzyl label	
	% TRR	ppm
Folpet	0.1	0.001
Phthalimide	0.5	0.005
Phthalamic acid	24.6	0.269
Phthalic acid	55.1	0.604
Acid conjugates	3.5	0.038
Unknown	0.3	0.003
Other	1.3	0.014
Residue	14.7	0.161

Summary of Folpet Related Radioactive Residues in Mature Wheat Straw and Grain (Report No. 95/MAK204/0049; Company file: R-7823)

Identity	^{14}C-Benzyl label			
	Straw		Grain	
	% TRR	ppm	% TRR	ppm
Folpet	27.2	4.09	35.8	8.56
Phthalimide	9.5	1.43	11.2	2.67
Phthalic acid	34.0	5.11	31.6	7.56

Summary of Folpet Related Radioactive Residues in Mature Grape Fruits (Report No. 14503B004; Company file: R-6403a)

Identity	^{14}C-Benzyl label	
	% TRR	ppm
Folpet	26.65	2.02
Phthalimide	10.60	0.81
Phthalic acid	5.82	0.44
Unknown 1	1.42	0.11
Unknown 2	41.39	3.14

Animals

The amount of phthalimide in milk and meat was determined in a goat metabolism study (Corden 1997a, 1997b). Goats were fed ^{14}C-folpet at 14 ppm labelled in the benzene ring for 6 days. Tissues were harvested and samples with 3% total radioactive residue or more were characterized. The majority of radioactivity was excreted in the urine and faeces. The following residues were analysed in meat and milk:

	Phthalimide[6]
Meat	<0.004 mg/kg
Milk	<0.001 mg/kg

The maximum possibly daily intake of phthalimide in milk and meat was calculated according to the worst scenarios for all consumer groups including toddlers and infants, which are the most sensitive consumer groups, and resulted with 0.00005 mg/kg bw/day (detailed calculations appear under point 2) h) below).

Considering the low toxicity of phthalimide, the low level of phthalimide residues in plants and the zero exposure to human from animal products when calculated using conservative assumptions, there is no basis for rationally including phthalimide in the folpet residue expression.

In conclusion, the residue expression for folpet should be expressed as parent compound, folpet, only.

The references submitted in support of the above position are summarised below.

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1) Phthalimide basic toxicology

a) Teratogenic activity of thalidomide and related compounds (Fabro, S., Schumacher, R. L., Smith, R. L. and Williams, R. T., 1964; IIA 7.3/01)

The paper tests a hypothesis that the teratogenic activity of thalidomide may be associated with the presence of a glutarimide ring in the molecule and interference in glutamic acid or glutamine metabolism. The significance of the glutarimide ring in the thalidomide molecule was investigated by testing other similar molecules, including the folpet metabolite phthalimide.

The study predated guidelines and was not to GLP. However, the study demonstrated that foetal malformations could be induced by a known positive control, and is considered valid.

Female rabbits of the New Zealand and Chinchilla strains were mated with males of the same strain and dosed orally by gavage with phthalimide at 150 mg/kg bw/day from day 7 to day 12 of pregnancy. Dams were killed on day 28 of pregnancy, and uterine parameters recorded. Foetuses were examined for external malformations only, especially those of the head and

[6] Other analytes measured included phthalamic acid, phthalic anhydride and phthalic acid (although the analytical method could not always separate these).

limbs. Other groups were dosed with other glutarimide-ring molecules, including thalidomide, at 150 mg/kg bw/day.

There were 18 dams in the control group, 161 implantations, 13 resorptions and 148 externally normal foetuses (no malformations). Ten dams were given thalidomide, from which there were 78 implantations, 35 resorptions, 16 externally malformed and 27 externally normal foetuses. The malformations were typical of those induced by thalidomide. Of the three dams given phthalimide, there were 25 implantations, 3 resorptions and 22 externally normal foetuses (no external malformations).

The incidence of malformation was not increased in other molecules that contained the glutarimide ring. The results for phthalimide, control and other compounds are shown below (Table B.7.3.1).

Table B.7.3.1: Embryotoxic effects of phthalimide and other compounds in the rabbit

Compound	No. of animals (dams)	Implan-tations	Re-sorptions	Malformed foetuses	Normal foetuses
Control	**18**	**161**	**13**	**0**	**148**
Thalidomide	**10**	**78**	**35**	**16**[a]	**27**
3-Nitrothalidomide	4	40	9	1[b]	30
α-Aminoglutarimide	4	37	4	0	33
Hexahydrothalidomide	3	21	2	0	19
α-Succinimidoglutarimide	3	18	5	0	13
Phthalimide	**3**	**25**	**3**	**0**	**22**
1-Phthalimidobutane	5	49	6	0	43[e]
2-Phthalimidoacetamide	2	21	3	0	18
4-Phthalimidobutyramide	2	31	0	1[c]	30
α-Phthalimidoaspartimide	4	36	3	0	33
Phthalimidobenzene	7	65	4	2[d]	59
3-Phthalimidopyridine	4	40	4	0	36
2-Phthalimidoglutaric acid anhydride	6	64	12	0	52

[a] malformations of fore and hind limbs and cranioschisis typical for thalidomide
[b] cranioschisis.
[c] malformation of fore-limb- hook-like protrusion.
[d] malformation of fore-limb in one foetus, cyclopia in second foetus.
[e] one foetus with massive subcutaneous cranial haemorrhage, second foetus with large haemorrhage on left limb.

Conclusion: Maternal administration of phthalimide was not associated with increased incidence of resorptions or malformed foetuses when administered to rabbits during pregnancy.

b) An investigation of the teratogenic potential of captan, folpet, and difolatan (Kennedy, G., Fancher, O. E., and Calandra, J. C., 1968; IIA 7.3/02).

Study of effects of captan, folpet, the captan metabolite tetrahydrophthalimide (THPI), and the folpet metabolite phthalimide (PI) on the pregnant rabbit. Technical grade captan and folpet, and pure samples of THPI and PI were used. The related fungicide difoltan and the structurally similar drug thalidomide were also tested. The latter may be considered a positive control.

The study predated guidelines and was not to GLP. However, the study demonstrated that foetal malformations could be induced by a known positive control, and is considered valid.

Test materials were administered in gelatine capsules to groups of mated female Dutch Belted rabbits from day 6 to day 16 of pregnancy. Animals were weighed at three day intervals and

killed on day 29, when uterine contents were examined, and foetuses examined. Live foetuses were placed in an incubator for 24 hours after which they were killed and dissected. The carcasses were cleared and the skeleton stained with alizarin and examined. PI was administered at 75 mg/kg bw/day to a group of 10 females. Thalidomide was administered at 75.0 mg/kg bw/day to both strains of rabbit.

Maternal weight gains were not adversely affected by PI at 75.0 mg/kg/day, and there were no deaths.

Incidence of foetal resorptions was not adversely affected by PI administration.

One control foetus (of 105, from 17 litters) showed shortening and flexure of the forelimb. There were no malformations in the 63 foetuses from 10 dams treated with PI. Post-natal survival, crown-rump length, foetal weight and incidence of visceral and skeletal anomalies were not adversely affected by maternal treatment with PI. Thalidomide induced typical 'clubbing' (phocomelia) in 38 of 100 foetuses from 17 litters, demonstrating that the test system was capable of detecting malformations. The folpet metabolite phthalimide (PI) showed no malformed foetuses, and therefore no adverse effects on the developing rabbit foetus.

The results are summarised below (Table B.7.3.2).

Table B.7.3.2: Summary of effects of folpet, phthalimide and controls in rabbits

Compound	Oral dose (mg/kg)	No. of pregnant females	Rabbit strain	No. of implants	No of resorptions	No of normal foetuses	No. (%) malformed foetuses	Mean litter size
Control	-	7	DB	52	0	51	1 (1.9)	7.4
	-	10	NZW	66	2	64	0 (0)	6.4
Thalidomide	75.0	7	BD	55	15	26	14 (35.0)	5.7
		10	NZW	74	10	40	24 (37.5)	6.4
Folpet	75.0	9	DB	66	0	65	1 (1.5)	7.3
	18.75	5	NZW	37	1	36	0 (0)	7.2
	37.5	5	NZW	35	11	24	0 (0)	4.8
	75.0	7	NZW	52	32	20	0 (0)	2.9
Phthalimide	75.0	10	DB	66	3	63	0 (0)	6.3

Conclusion: Phthalimide (PI) showed no adverse effects on the developing rabbit foetus.

c) Phthalimide: Prenatal toxicity study in the rabbit by oral gavage administration (Blee, M.A.B., 2006 IIA 7.3/03)

A study of the effects of phthalimide on the pregnant rabbit was conducted. Technical grade phthalimide, purity 100%, was used. The study was GLP compliant and run to current international regulatory guidelines: OECD 414, US EPA OPPTS 870.3700 and Japanese Ministry of Agriculture, Forestry and Fisheries 12 Nohsan No. 8147.

Twenty-five female rabbits, of the New Zealand White strain, per dosage group were mated with males of the same strain and source and were dosed orally by gavage with phthalimide at 0, 5, 15 or 30 mg/kg/day from Gestation Day (GD) 6 to GD 28. Dams were killed on GD 29 of pregnancy, and uterine parameters recorded. Foetuses were examined macroscopically at necropsy and subsequently by detailed internal visceral examination of the head or at skeletal examination. Microscopic examination of the maternal duodenum was conducted on the control and top dose groups.

There were no deaths and no clinical signs that were attributed to treatment. Bodyweight (Table B.7.3.3) and food consumption (Table B.7.3.4) were unaffected by treatment.

Macroscopic examination at necropsy of the dams did not reveal any treatment-related observations and microscopic examination of sections of the duodenum from animals in the Control and 30 mg/kg/day groups did not reveal any treatment-related findings.

Treatment did not adversely affect pregnancy outcome, embryo-foetal survival post-implantation, and foetal and placental weights were considered to be unaffected by treatment with phthalimide (Table B.7.3.5 and Table B.7.3.6). The *in utero* progress and development of the fetuses up to GD 29 was similarly also unaffected by treatment.

Foetal pathology examinations did not reveal any major skeletal/visceral malformations or abnormalities or changes in minor skeletal abnormalities/variants that were outside concurrent or the laboratories historical control data ranges. Thus foetal development was considered to be unaffected by maternal treatment with phthalimide.

It may be concluded that maternal administration of phthalimide did not induce maternal toxicity and did not affect the outcome of the pregnancies. Foetal development was considered to be normal.

Table B.7.3.3: Bodyweight - group mean values (kg) for females during gestation (GD)

Group	:	1	2	3	4
Compound	:	Control	---------- Phthalimide ----------		
Dosage (mg/kg/day)	:	0	5	15	30

Group			GD						
			0	6	7	14	21	28	29
1	Mean		3.86	3.94	3.96	4.01	4.04	4.10	4.12
	SD		0.32	0.34	0.33	0.34	0.37	0.36	0.36
	n		21	21	21	21	21	21	21
2	Mean		3.84	3.92	3.93	3.99	4.04	4.15	4.17
	SD		0.22	0.21	0.21	0.23	0.26	0.29	0.29
	n		20	20	20	20	20	20	20
3	Mean		3.95	4.03	4.03	4.11	4.18	4.25	4.26
	SD		0.28	0.32	0.32	0.33	0.33	0.31	0.30
	n		22	22	22	22	22	22	22
4	Mean		3.83	3.91	3.93	4.00	4.05	4.19	4.21
	SD		0.32	0.33	0.33	0.35	0.37	0.37	0.36
	n		25	25	25	25	25	25	25

Table B.7.3.4: Food consumption - group mean values (g/animal/day) for females during gestation (GD)

Group	:	1	2	3	4
Compound	:	Control	---------- Phthalimide ----------		
Dosage (mg/kg/day)	:	0	5	15	30

		GD					
Group		1	6	7	14	21	28
1	Mean	154	160	161	84	103	95
	SD	26	23	29	51	46	45
	n	21	21	21	21	21	21
2	Mean	148	148	149	96	126	102
	SD	31	26	28	51	48	33
	n	20	20	20	20	20	20
3	Mean	175	164	163	128	124	99
	SD	29	39	34	34	46	37
	n	22	22	22	22	22	22
4	Mean	156	161	159	130	131	117
	SD	32	28	27	47	34	32
	n	25	25	25	25	25	25

Table B.7.3.5: Litter data - group mean values on GD 29

Group	:	1	2	3	4
Compound	:	Control	---------- Phthalimide ----------		
Dosage (mg/kg/day)	:	0	5	15	30

Group		Corpora	Implantations	Resorptions		Live young		% implantation loss	
		Lutea		Early	Late	Male	Female	Pre-	Post-
1	Mean	11.6	10.0	0.4	0.1	5.1	4.4	13.4	5.9
	SD	2.9	2.3			1.9	2.1		
	n	21	21	21	21	21	21	21	21
2	Mean	11.8	9.6	0.6	0.1	4.5	4.4	16.9	7.1
	SD	2.0	1.5			1.8	1.6		
	n	20	20	20	20	20	20	20	20
3	Mean	11.7	9.5	0.5	0.2	4.1	4.6	19.5	7.3
	SD	1.9	2.8			2.0	1.9		
	n	22	22	22	22	22	22	22	22
4	Mean	11.2	8.3	0.4	0.4	3.5	4.0	25.7	10.3
	SD	2.1	2.5			2.0	2.3		
	n	25	25	25	25	25	25	25	25

Table B.7.3.6: Placental and foetal weights - group mean values (g) on GD 29

Group	:	1	2	3	4
Compound	:	Control	---------- Phthalimide ----------		
Dosage (mg/kg/day)	:	0	5	15	30

Group		Placental	Foetal weight		
		weight	Males	Females	Overall
1	Mean	5.5	38.8	38.4	38.8
	SD	0.8	7.5	5.8	6.6
	n	21	21	21	21
2	Mean	5.6	40.7	40.1	40.3
	SD	0.7	4.7	6.1	5.0
	n	20	20	20	20
3	Mean	5.4	41.0	38.8	39.8
	SD	1.2	5.4	6.0	5.5
	n	22	22	22	22
4	Mean	5.8	43.5	42.1	42.9
	SD	1.0	4.3	6.9	5.0
	n	25	23	23	25

d) Study of the cytogenetic activity of certain metabolites of a number of pesticides representing several classes of chemical compounds (Pilinskaya, M. A., 1986; IIA 7.3/04).

Phthalimide was tested in a human lymphocyte chromosome aberration assay.

The paper does not give sufficient detail to judge if the method was similar to recognised guidelines, but did give a positive result with some compounds, apparently demonstrating that the assay worked. The study was not to GLP.

Phthalimide was tested at 10,000, 1.0 and 0.1 µg/ml in 100, 200 and 200 metaphases, respectively, and 400 control metaphases were also evaluated. The frequency of metaphases with aberrations was not increased. Metabolites of the pesticides ziram, and betanal, tetramethylthiourea (TMTU) and methyl-3-hydroxyphenyl-carbamate (MHPC) respectively were positive in the assay. The compound methyl-benzimidazole-2-yl-carbamate (BMC), stated to be a metabolite of benomyl-type pesticides, produced hyperspiralisation of chromosomes and accumulation of mitoses.

Table B.7.3.7: Results of cytogenetic study

Concentration of substance (µg/mL)	No. of investigated metaphases	Frequency of aberrations (%)	Concentration of substance (µg/mL)	No. of investigated metaphases	Frequency of aberrations (%)
TMTU			**BMC**		
10,000	200	3.5*	200.00	200	2.00+
1.00	200	4.5	100.00	300	2.33+
0.10	200	6.00*	10.0	200	1.50
0.01	200	2.00	Control	400	2.33
Control	400	2.50			
Phthalimide			**MHPC**		
10,000	100	2.00	200.00	200	11.00***
1.00	200	1.50	100.00	200	3.00***
0.10	200	2.00	10.0	200	1.00
Control	400	2.00	Control	400	1.25

* $p < 0.1$
*** $p<0.05$
\+ a colchicine-type effect noted.

Conclusion: Phthalimide was not mutagenic in the human lymphocyte chromosome aberration assay.

e) Characterization of impurities in commercial lots of sodium saccharin produced by the Sherwin-Williams process (Riggin, R. M., Margard, W. L., and Kinzer, G. W., 1983; IIA 7.3/05).

Impurities and contaminants present or suspected to be present in commercial lots of the artificial sweetener saccharine, including Phthalimide, were tested in the Ames test.

The study was not performed to current guidelines, although it followed the method of Ames. The study was not to GLP.

A number of conflicting long-term animal feeding studies had been performed on the artificial sweetener saccharine, at levels up to 7.5% w/w diet. At such levels, the amount of impurities consumed may be significant, and the study was designed to investigate impurities and contaminants found in commercial lots of saccharine. The compounds were extracted using solvents, and the extracts (of all impurities/contaminants) subjected to the Ames test.

The origin of the impurities or contaminants was not always stated: several were stated to have appeared to have been derived from the polythene (polyethylene) materials used in

packaging the lots. Insufficient quantities of the impurities could be obtained directly by solvent extraction for individual testing of each compound, and so various known or suspected saccharine contaminants were obtained and tested in the Ames test, at dose levels of 2000 or 400 µg/plate, using *S. typhimurium* strain TA98 with S-9 activation only. The mutagenicity was expressed as relative to the DMSO control.

The S-9 activation system was derived by injecting male rats (200g strain not specified) i.p. with 200 mg/mL Arclor 1254 in corn oil at 0.5 mg/g bodyweight. Rats were killed after 5 days, and the liver removed, homogenised in KCl and centrifuged for 10 minutes at 9000 g. The (S-9) supernatant was decanted and frozen. Samples were defrosted before use. The microsomal mix was prepared according to Ames and contained (per mL): S-9 (0.15 mL), $MgCl_2$ (8 µmole), KCl (33 µmole), glucose-6-phosphate (5 µmole), NADP (4 µmole), and sodium phosphate pH 7.4 (100 µmole). Fresh S-9 was prepared daily.

For the assay, a 0.1 mL aliquot of bacterial culture was added to 2 mL molten top agar, which was then mixed with 0.1-0.3 mL of sample solvent extractdissolved in DMSO. A 0.5 mL aliquot of the S-9 mix was added to the agar immediately prio to pouring onto the plate. The poured top agar was allowed to solidify and the plates were incubated for 48 hours, after which the number of colonies were counted. Positive control (10 µg benzo[a]pyrene and a solvent (DMSO) blank control were assayed in triplicate.

Mutagenicity data for the potential contaminants in saccharine, including phthalimide, are summarised below (Table B.7.3.8).

Table B.7.3.8: Mutagenicity data for the potential contaminants in saccharine

Impurity	Concentration (µg/plate)	Relative mutagenicity*
α-Sulphamoylbenzoic acid	400	1.2
	2000	0.9
α-Sulphobenzoic acid	400	1.0
	2000	0.8
α-Chlorobenzoic acid	400	0.9
	2000	0.6
6-Methylsaccharin	400	1.0
	2000	1.2
N-methylsaccharin	400	1.1
	2000	1.3
α-Toluenesulphonamide	400	0.7
	2000	1.2
Phthalimide	**400**	**1.0**
	2000	**0.9**
Methyl anthranilate	400	1.1
	2000	0.9
5-Chlorosaccharin	40	1.0
	200	0.9
	1000	0.8
Trioctyl phosphate	2000	0.7
Di-*tert*-butyl-*p*-benzoquinone	2000	0.7
α-Chlorobenzamide	2000	1.4
1,2-Benzisothiazolin-3-one	10	1.1
	100	toxic
3-Aminobenzisothiazole-1,2-dioxide	200	0.7
	1000	0.6
1,2-Benzisothiazoline-1,1-dioxide	200	0.6
	1000	0.6
Trichlorobenzene	133	1.0
	667	0.8

*Relative to DMSO control

The study did not give any information as to how phthalimide may be either an impurity or contaminant of saccharine. Phthalimide was not mutagenic in the assay, with relative mutagenicity of 1.0 and 0.9 compared to controls (DMSO). None of the other impurities/contaminants were positive in the assay, although one was stated to be toxic to the bacteria. The study found that the solvent-extracted impurities/contaminants exhibited a low level of mutagenicity, despite also demonstrating that the individual compounds, tested separately, showed no mutagenic activity. The study also showed that acetone extraction did not show mutagenic activity, but that chloroform/methanol extracts showed low levels of mutagenicity. The study concentrates on assays of batches of saccharine and on analysis of various solvents to try and determine the origin of the initial mutagenic activity, after concluding that the impurities normally present in saccharine were not responsible for the mutagenic activity seen in the initial solvent extractions. These data are not relevant to phthalimide. The authors concluded that as large amounts of solvent were required to extract the impurities/contaminants, that contamination of the solvents themselves may be responsible for the mutagenic activity seen.

Conclusion: Phthalimide was not mutagenic in the Ames test, when tested in strain TA98 with metabolic activation.

f) Review: Toxicological risk characterisation of potential folpet metabolites. The toxicity profiles of phthalic and phthalamic acids and phthalimide – is there a significant risk from metabolite exposure? (Siefried, H.E., 2000; IIA 7.3/06). [This report was previously submitted with the toxicology addendum in March 2005.]

The position paper includes summaries the toxicity findings of the folpet metabolites. Phthalamic acid, a major degradate when folpet undergoes hydrolysis, is the main metabolite following oral administration to rats. Phthalic acid is a minor metabolite. Phthalamic acid is the main metabolite in goats and phthalic acid is not seen in the urine but is present in the kidney. Phthalamic acid is hydrolyses to phthalic acid at acid pH. TOPKAT was used to predict that phthalamic acid would have an acute oral rat LD_{50} of ~ 700 mg/kg bw, and would be negative in the Ames test. As a metabolite in the rat, animals are considered to have been exposed during oral toxicity studies. It is not possible to establish a risk level due to the lack of toxicological data on the compound itself, but based on the low toxicity of phthalate and phthalimide, the level of toxicity of phthalamic acid is expected to be low.

Phthalimide is an intermediate metabolite, capable of being metabolised to phthalamic acid, phthalate and possibly methylphthalate. It is not mutagenic in the Ames test, in yeast, mouse lymphoma assay or in a cytogenetic assay in human lymphocytes. The weight of evidence suggests a low level of risk. TOPKAT was used to predict that phthalimide would have an acute oral rat LD_{50} of ~ 980 mg/kg bw, and would be negative in the Ames test.

Phthalic acid is not mutagenic in Ames or other bacterial assays, but does act synergistically with some but not all heterocyclic amine mutagens. It is not carcinogenic based on negative rodent bioassays with phthalic anhydride (which converts to phthalic acid). Phthalic acid does not accumulate in the body and is essentially cleared by 48 hours after oral administration. Phthalic acid is not teratogenic in rats. The reported activity on male and female reproductive systems in some less-than-robust studies is not well supported when all results are taken into consideration and the weight of evidence for all folpet metabolites is considered. TOPKAT was used to predict that phthalic acid would have an acute oral rat LD_{50} of ~ 2500 mg/kg bw, and would be negative in the Ames test.

The related compounds phthalic anhydride (which converts to phthalic acid in aqueous media) and phthalamide have been tested for carcinogenicity in rats and mice under a US Government testing programme. Neither compound showed increased incidence of tumours.

Phthalic acid is ubiquitous in the environment from industrial sources (used as plasticizers and in the production of polyester) and can be formed from environmental phthalate esters via hydrolysis where they can be found widely distributed, generally at low levels in air, rain water, sediment, soil and biota, food samples, and human and animal tissues.

In conclusion, phthalimide together with other folpet metabolites, has a very low level of hazard to humans when exposed through the diet and to the environment compared to parent folpet. The appropriate residue expression for folpet is folpet per se.

g) Phthalimide: Determination of minimal inhibitory concentrations against selected micro-organisms representative of the rabbit gut micro-flora (Akhurst, L.C., 2005 IIA 7.3/07).

It has been postulated that folpet may affect the rabbit GI tract micro-flora and that an imbalance in the micro-flora may have consequences for the pregnant rabbit on both maternal and embryo-fetal nutrition. Such changes could, in theory, affect the developing fetus. The rabbit is a species particularly susceptible to gastrointestinal disturbances which may in part be mediated through changes in the GI tract micro-flora. An *in vitro* approach to demonstrate changes in representative rabbit GI tract micro-flora was considered to be a simple and straightforward initial step to evaluate the potential effects of phthalimide on such micro-organisms.

Phthalimide is not thought to have the same anti-microbial activity as the parent molecule folpet partly because it is not capable of generating the highly reactive moiety thiophosgene.

The minimum inhibitory concentration (MIC) assay is an internationally accepted test for antimicrobial susceptibility testing and is commonly used to assess the effectiveness of intentional antimicrobial compounds. The test was adapted to assess selected micro-organisms representative of the rabbit gut micro-flora

The study was conducted using an agar dilution procedure for determination of MIC values. Ten species of the genus Bacteroides, one genus of Enterococcus faecalis and four isolates of Candida albicans were tested. Final concentrations of phthalimide of 1000, 500, 200, 100, 50, 20, 10, 5 , 2 or 1 µg/ml were tested and solvent control and growth control plates were employed. The lowest test substance concentration that completely inhibited growth of the test organism was recorded as the MIC.

Phthalimide demonstrated no antimicrobial activity towards Bacteroides sp. and Enterococcus faecalis, when tested up to a concentration of 1000 µg/ml, which was just above the limit of solubility in agar. It showed very weak activity against the yeast, Candida albicans, with very slight (qualitative) inhibition of growth only at 1000 µg/ml and in only one of the four strains tested.

It may be concluded that phthalimide demonstrated no significant antimicrobial activity against organisms selected as representatives of rabbit GI tract flora species tested in this study. It is unlikely that this molecule has the potential to affect the micro-flora of the rabbit GI tract.

2) Their presence in significant amounts

a) *Folpet: distribution and metabolism in winter wheat. (Crowe, A. 1995; Annex IIA, 6.1/01; IIA 7.3/12)*

NOTE: The summary below already appears in the DAR under B.7.1 Metabolism, distribution and expression of residues in plants (Annex IIA 6.1 and Annex IIIA 8.1).

[U-phenyl -^{14}C] folpet (radiochemical purity > 98%) was dissolved in [redacted] (for the first application) or [redacted] for the second application) and formulated as a 80% water dispersible granule (WG) formulation with formic acid to pH 4 to prevent hydrolytic degradation. Sprays were applied to the foliage of wheat seedlings (variety Mercia) grown in pots at the first awns visible growth stage (Zadoks scale 49) and again at the end of flowering (Zadoks scale 69) at a nominal rate equivalent to 1.6 kg a.s./ha. The first sprays were applied on the 20 May 1994 and the second sprays on the 13 June 1994. The treated pots were covered with polythene for 24 hours then maintained outdoors in the UK. Whole plant samples were taken one day after each application, and at the early dough stage (Zadoks scale 83) and at normal harvest (Zadoks scale 92). At each sampling, the plants were separated into straw, roots and grain. Samples were combusted and total radioactive residues were determined by liquid scintillation counting (LSC). Samples were extracted with various solvents and the metabolites were characterised by high-performance liquid chromatography (HPLC) and thin-layer chromatography (TLC).

The measured radiochemical purities of the [^{14}C] folpet formulations prior to the first and second application were 97.7% and 98.6%, respectively. Measured application rates were 1.4 to 1.6 kg a.s./ha (first application) and 1.0 to 1.2 kg a.s./ha (second application).

^{14}C radioactivity was found mainly in the straw and grain. Radioactive residues increased at later samplings, reaching a maximum of 15.05 mg folpet equivalents/kg in straw and 23.92 mg folpet equivalents/kg in straw in samples taken at normal harvest. Residues in roots were less than 1 mg folpet equivalents/kg at all times. The distribution of total radioactive residue (TRR) is given in Table B.7.3.9.

Table B.7.3.9: Distribution of TRR and residue levels in wheat following applications of [^{14}C] folpet

Sampling time	Crop part	% TRR	Residue (mg folpet equivalents/kg)
Zadocks 49 + 1 day	roots	0.2	0.03
	straw	90.9	4.50
	grain	8.9	3.18
Zadocks 69 + 1 day	roots	0.5	0.23
	straw	84.6	9.42
	grain	14.9	7.50
Zadocks 83	roots	1.1	0.63
	straw	67.4	13.31
	grain	31.5	10.26
Zadocks 92 (normal harvest)	roots	0.9	0.74
	straw	44.6	15.05
	grain	54.5	23.92

The majority of the residue in straw and grain was extractable with solvents. At harvest (Zadocks 92), the unextractable residues in straw and grain were less than 10% TRR, and were equivalent to 1.4 and 1.9 mg folpet equivalents/kg.

The extractable residue in the samples taken one day after application was identified as folpet and phthalimide. In the samples taken at Zadoks 83, the majority of the extractable residue was folpet, with less than 10% TRR identified as phthalic acid, phthalimide, polar material and unknowns. In samples taken at normal harvest (Zadoks 92), extractable residues were identified as folpet, phthalic acid and phthalimide. Residues of these three compounds were equivalent to 8.56, 7.56 and 2.67 mg folpet equivalents/kg, respectively, in grain and 4.09, 5.11 and 1.43 mg folpet equivalents/kg, respectively, in straw. The distribution of the characterised metabolites in straw and grain is given in Table B.7.3.10.

Table B.7.3.10: Characterisation of ^{14}C radioactivity in wheat straw and grain following applications of [^{14}C] folpet

Sampling time	Crop part	Identity of residue: % TRR (mg folpet equivalents/kg)				
		Folpet	Phthalic acid	Phthalimide	Polar material	Unknown
Zadocks 49	straw	76.9 (3.46)	ND	9.2 (0.41)	ND	ND
+ 1 day[a]	grain	57.1 (1.82)	ND	25.0 (0.80)	ND	ND
Zadocks 69	straw	50.2 (4.73)	ND	10.4 (0.98)	ND	ND
+ 1 day[a]	grain	63.4 (4.76)	ND	15.6 (1.17)	ND	ND
Zadocks 83[a]	straw	51.9 (6.91)	4.5 (0.60)	5.7 (0.76)	3.2 (0.43)	ND
	grain	46.2 (4.74)	5.6 (0.57)	9.5 (0.98)	4.8 (0.49)	2.9 (0.29)
Zadocks 92[b]	**straw**	**27.2 (4.09)**	**34.0 (5.11)**	**9.5 (1.43)**	-	-
	grain	**35.8 (8.56)**	**31.6 (7.56)**	**11.2 (2.67)**	-	-

ND = not detected. [a] Determined by HPLC analysis. [b] Mean of HPLC and TLC analysis.

b) *Folpet: nature of residue on grapes. (O'Connor, J. 1994; Annex IIA, 6.1/02; IIA 7.3/13)*

NOTE: The summary below already appears in the DAR under B.7.1 Metabolism, distribution and expression of residues in plants (Annex IIA 6.1 and Annex IIIA 8.1).

[U-phenyl -^{14}C] folpet (radiochemical purity 99.6%), was dissolved in [redacted] and formulated as a 50% wettable powder. Three sprays were applied to the foliage of field grown grapes (variety Thompson Seedless) in California, USA. Treatments were applied on the 28 May 1992 (berry set stage), 29 June 1992 (mid-berry stage) and 27 July 1992 (late-berry stage) to give a nominal rate at each application of 1.5 kg a.s./ha. Samples of fruit and leaves

were taken at normal harvest on 19/21 August 1992. Samples were washed and total radioactive residues were determined in extracts from the washed fruit and leaves, and in the rinsate, by combustion and LSC. Samples were extracted with various solvents and the metabolites were characterised by HPLC and liquid chromatography/mass spectrometry (LC-MS). The measured radiochemical purity of the [^{14}C] folpet was 99.6%. Measured application rates were 1.35 to 1.58 kg a.s./ha.

Rinsate accounted for 26% of TRR found on the fruit and 88% of TRR found on the leaves. The majority of radioactivity remaining in the washed fruit and leaves was extracted. Unextractable residues accounted for less than 10% of TRR, and were equivalent to 0.11 and 3.21 mg folpet equivalents/kg in fruit and leaves, respectively. The distribution of TRR is given in Table B.7.3.11.

Table B.7.3.11: Distribution of TRR and residue levels in grape samples following three applications of [^{14}C] folpet

Crop part	Sample	% TRR	Residue (mg folpet equivalents/kg)
Fruit	rinsate	25.71	1.95
	plant	74.29	
	organo-soluble	18.77	1.43
	water-soluble	54.03[a]	4.10
	unextractable	1.49	0.11
	total	**100**	**7.59**
Leaf	rinsate	87.83	257.94
	plant	12.16	
	organo-soluble	6.47	19.01
	water-soluble	4.60	13.51
	unextractable	1.09	3.21
	total	**100**	**293.67**

[a] 77% extracted by methanol wash from solid phase extraction.

The characterisation of radioactive residues in grapes is given in Table B.7.3.12. Folpet accounted for the majority of the residue in fruit and leaves (2.02 and 266.02 mg folpet equivalents/kg, respectively). Phthalic acid, phthalimide and a phthalic acid conjugate were also identified

Table B.7.3.12: Characterisation of ^{14}C radioactivity in grape samples following three applications of [^{14}C] folpet

Crop part	Sample	Identity of residue: % TRR (mg folpet equivalents/kg)						
		Folpet	**Phthalic acid**	**Phtha-limide**	**Unknown 1**	**Phthalic acid conjugate**	**Unknown 3**	**Unknown 4**
Fruit	rinsate	13.88 (1.05)	2.10 (0.16)	9.74 (0.74)	-	-	-	-
	plant	12.77 (0.97)	3.72 (0.28)	0.86 (0.07)	1.42 (0.11)	41.39 (3.14)	-	-
	total	**26.65 (2.02)**	**5.82 (0.44)**	**10.60 (0.81)**	**1.42 (0.11)**	**41.39 (3.14)**	**-**	**-**
Leaf	rinsate	85.39 (250.77)	-	2.44 (7.17)	-	-	-	-
	plant	5.19 (15.25)	2.38 (6.99)	0.55 (1.61)	-	-	2.27 (6.65)	0.69 (2.01)
	total	**90.58 (266.02)**	**2.38 (6.99)**	**2.99 (8.78)**	**-**	**-**	**2.27 (6.65)**	**0.69 (2.01)**

Samples of fruit and leaves were also analysed for folpet and metabolites after freezer storage for an additional seven to eight months. The level of folpet, phthalimide and other (unknown) metabolites as a % TRR was unaffected by storage. Results for the leaf rinsate showed that the rinsate portions were not homogenous.

c) *Nature of residue (^{14}C)-folpet (LX1145-05) in avocados applied under field conditions. (Toia, R.F., Collins, E.H. 1994; Annex IIA, 6.1/03; IIA 7.3/14)*

NOTE: The summary below already appears in the DAR under B.7.1 Metabolism, distribution and expression of residues in plants (Annex IIA 6.1 and Annex IIIA 8.1).

[U-phenyl -^{14}C] folpet (radiochemical purity > 98%), was dissolved in [redacted] and formulated as a 50% wettable powder. Three sprays were applied to an avocado tree during fruit development at a nominal rate at each application of 3.36 kg a.s./ha in California in the USA. Samples of immature fruit and leaves were taken 21 days after the final application and samples of mature fruit and leaves were taken 97 days after the final application. Samples were rinsed and total radioactive residues were determined in the rinsate. The rinsed mature fruit samples were peeled and pitted, and peel and pulp were extracted and analysed separately. Leaves and immature fruit samples were extracted and analysed in their entirety. Radioactive residues were determined by combustion and LSC. Samples were extracted with various solvents and the metabolites were characterised by HPLC and TLC.
The measured radiochemical purities of the [^{14}C] folpet prior to application were 99.00 to 99.84%. Measured rates at each application were 3.40 to 3.44 kg a.s./ha.
Rinsate accounted for a small proportion of the radioactivity found on the fruit but a significant proportion of the residue found on the leaves. The distribution of the residue is given in Table B.7.3.13.

Table B.7.3.13: Distribution of residue levels in avocado samples following three applications of [^{14}C] folpet

Crop part	Sample	Residue (mg folpet equivalents/kg)
Immature leaf	rinsate	47.51
	plant	88.12
Mature leaf	rinsate	20.91
	plant	52.72
Immature fruit	rinsate	0.70
	plant	10.15
Mature fruit	rinsate	0.01
	pulp	8.20
	peel	16.91
	whole fruit	8.95[a]

[a] Calculated from relative weight of pulp (356.4 g) and peel (33.66 g).

More than 93% of the radioactivity in the mature whole fruit was identified. Folpet was a minor component of the TRR (0.5%). Phthalic acid was the major component at 81.9% of the radioactivity. Phthalimide and polar materials were 3.9% and 7.3%, respectively. Five metabolites were also present in the mature fruit at 0.4% (0.02 mg/kg), 2.8% (0.15 mg/kg), 1.6% (0.09 mg/kg), 0.9% (0.05 mg/kg) and 0.5% (0.03 mg/kg), respectively. All were derivatives of phthalic acid.

The characterisation of radioactive residues in avocados is given in Table B.7.3.14.

Table B.7.3.14: Characterisation of ^{14}C radioactivity in avocado samples following three applications of [^{14}C] folpet

Crop Part	Sample	Identity of residue: % TRR (mg folpet equivalents/kg)									
		Folpet	**Phthalic acid**	**Phtha-limide**	**Polar[a]**	**Unknown A**	**Unknown B**	**Unknown C**	**Unknown D**	**Unknown E**	**Total unknowns**
Immature Fruit	rinsate	46.9 (0.29)	12.6 (0.08)	33.0 (0.20)	3.0 (0.02)	-	-	-	-	-	-
	plant[b]										
	1	3.1 (0.23)	83.8 (6.42)	5.5 (0.42)	1.0 (0.08)	-	-	-	-	-	3.1 (0.51)
	2	-	54.9 (0.77)	8.0 (0.11)	31.2 (0.44)	-	-	-	-	-	6.0 (0.09)[d]
	3	11.7 (0.01)	34.5 (0.03)	13.7 (0.01)	-	-	-	-	-	-	-
Mature fruit	peel[b]										
	1	1.1 (0.01)	71.7 (0.53)	14.7 (0.11)	-	0.8 (0.01)	3.5 (0.03)	7.8 (0.06)	0.3 (0.002)	-	12.4 (0.09)
	2	5.7 (0.01)	50.6 (0.11)	17.6 (0.04)	8.1 (0.02)	3.0 (0.01)	5.0 (0.01)	0.7 (0.002)	-	7.0 (0.02)	15.7 (0.03)
	5	14.8 (0.001)	49.9 (0.002)	-	-	-	-	20.6 (0.001)	-	-	20.6 (0.001)
	pulp[b]										
	1	-	94.2 (3.85)	1.6 (0.07)	-	0.2 (0.01)	2.7 (0.11)	0.4 (0.02)	0.5 (0.02)	0.3 (0.01)	4.1 (0.17)
	2	-	-	-	91.9 (0.38)[c]	-	-	2.0 (0.03)	6.1 (0.03)	-	8.1 (0.03)
	5	17.2 (0.004)	9.7 (0.002)	3.9 (0.001)	9.7 (0.002)	3.3 (0.001)	15.9 (0.004)	9.3 (0.002)	5.0 (0.001)	-	33.5 (0.01)
	Total[e] (%)	**0.5 (0.03)**	**81.9 (4.49)**	**3.9 (0.22)**	**7.3 (0.40)**	**0.4 (0.02)**	**2.8 (0.15)**	**1.6 (0.09)**	**0.9 (0.05)**	**0.5 (0.03)**	**-**
Immature Leaf	rinsate	57.1 (23.61)	9.7 (4.01)	25.1 (10.36)	2.3 (0.94)	-	-	-	-	-	-
	plant[b]										
	1	83.4 (53.31)	0.5 (0.35)	1.7 (1.11)	13.3 (8.52)	-	-	-	-	-	1.0 (0.66)
	4	2.7 (0.32)	94.6 (11.11)	0.5 (0.06)	1.1 (0.12)	-	-	-	-	-	1.1 (0.13)

[a] Eluting within or near the void volume of the column; characterised as glucose conjugates.
[b] 1 = acetronitrile portion of ethyl acetate, 2 = acetonitrile/water/acetic acid extract, 3 = methanol/HCl extract, 4 = acetonitrile/phosphoric acid extract, 5 = 3 N HCl extract.
[c] TLC shows as phthalic acid.
[d] Tentatively identified as benzoic acid.
[e] % of total residue, sum of extracted mg/kg in fruit in parenthesis.

e) *Folpet: metabolism in potatoes. (Crowe, A. 1999; IIA, 6.1/05; IIA 7.3/15)*

NOTE: The summary below already appears in the DAR under B.7.1 Metabolism, distribution and expression of residues in plants (Annex IIA 6.1 and Annex IIIA 8.1).

[U-phenyl -^{14}C] folpet (radiochemical purity > 98%), was dissolved in [redacted] and formulated as a 80% water dispersible granule with formic acid to pH 4 to prevent hydrolytic degradation. Five sprays were applied to the foliage of potato plants (variety Maris Piper) grown in pots at a nominal rate equivalent to 2.0 kg a.s./ha between July and October 1998. The treated pots were covered with polythene for two to four hours then maintained outdoors in the UK. Plant samples were taken two to four hours after applications one, three and five (77, 37 and seven days, respectively, before harvest), three days after the final application and seven days after the final application (normal harvest). At each sampling, the plants were separated into foliage and tubers. Samples were washed, combusted and total radioactive residues were determined by LSC. Samples were extracted with various solvents and the metabolites were characterised by HPLC and LC-MS.
The measured radiochemical purities of the [^{14}C] folpet formulations prior to each application ranged from 98.23 to 99.39%. Measured application rates were 1.86 to 1.95 kg a.s./ha.

^{14}C radioactivity in the foliage ranged from 57 to 111 mg folpet equivalents/kg. Residues in tubers increased from 0.6 after the third application to 1.2 mg folpet equivalents/kg in samples taken at normal harvest. The majority (85 to 98%) of the residue in the foliage was washed of the surface with acidified acetonitrile and the majority of the residue remaining was extractable (approximately equal proportions as organo-soluble and aqueous residue). The level of unextractable residues in the foliage was 0.2 to 1.2% TRR (0.21 to 1.06 mg folpet equivalents/kg). The majority (85.9 to 92.7%) of the radioactivity in the tubers was extractable, with most of this (79 to 88.5% TRR) remaining in the aqueous residues. The level of unextractable residues in the tubers was 14.7 to 22.2% TRR (0.10 to 0.16 mg folpet equivalents/kg). Radioactive residue levels are given in Table B.7.3.15.

Table B.7.3.15: Distribution of TRR and residue levels residue levels in potatoes following five applications of [^{14}C] folpet

Sampling time (days before normal harvest)	Crop part	% TRR (mg folpet equivalents/kg)			
		Surface wash	Extractable	Unextractable	Total
After first application (77)	foliage	98.3 (104.72)	1.2 (1.31)	0.2 (0.21)	99.8 (106.24)
	tubers	-	-	-	NA
After third application (37)	foliage	91.4 (58.83)	7.1 (4.58)	0.8 (0.55)	99.3 (63.95)
	tubers	-	87.1 (0.49)	17.2 (0.10)	104.3 (0.58)
After fifth application (7)	foliage	89.0 (91.35)	11.0 (11.31)	1.0 (1.06)	101.1 (103.71)
	tubers	-	92.7 (0.80)	16.6 (0.14)	109.3 (0.94)
Pre-harvest (3)	foliage	85.2 (48.58)	14.6 (8.30)	1.2 (0.67)	101.0 (57.55)
	tubers	-	85.9 (0.61)	22.2 (0.16)	108.1 (0.77)
Normal harvest (0)	**foliage**	**89.8 (99.05)**	**10.2 (11.22)**	**0.9 (1.00)**	**100.8 (111.27)**
	tubers	**-**	**92.6 (1.02)**	**14.7 (0.16)**	**107.3 (1.18)**

NA = not applicable.

The majority of the radioactivity in/on the foliage was identified as folpet (88 to 90% TRR). The proportion of phthalimide in the foliage increased after the sampling taken after the first application to reach 2 to 5% TRR (up to 3.14 mg folpet equivalents/kg); phthalamic acid and phthalic acid were present at up to 0.31 and 1.18 mg/kg folpet equivalents/kg, respectively. Unextractable residues in the foliage accounted for 0.9% TRR (1.00 mg folpet equivalents/kg) at harvest.

Several unknown organo-soluble metabolites were present in the tubers at low levels (up to 0.3% TRR, 0.003 mg/kg) and were not characterised further. Unextractable residues in the tubers accounted for 14.7% TRR (0.16 mg folpet equivalents/kg) at harvest and these were fully characterised as conjugated metabolites (8.4% TRR), membrane bound residues (2.1% TRR) and residues naturally incorporated into sugars and proteins (1.3% TRR). Bound residues in the tubers at harvest were 0.1% TRR (0.001 mg folpet equivalents/kg). Each of the characterised fractions in the unextractable residues was less than 10% TRR and not characterised further.

In the tubers, folpet was present at only 0.1% TRR (0.001 mg/kg) and the majority of the extractable residue was secondary polar metabolites, phthalamic acid, phthalic acid and their conjugates. The levels of phthalamic acid and phthalic acid at harvest were 24.6% and 55.1% TRR, respectively, (0.27 and 0.60 mg folpet equivalents/kg, respectively). Acid conjugates were present in the tubers up to 0.06 mg folpet equivalents/kg. The distribution of the characterised metabolites in the tubers is given in Table B.7.3.16.

Table B.7.3.16: Characterisation of ^{14}C radioactivity in potato tubers following five applications of [^{14}C] folpet

Sampling time (days before normal harvest)	% TRR (mg folpet equivalents/kg)								
	Folpet	**Phthal-imide**	**Phthal-imic acid**	**Phthalic acid**	**Acid con-jugates**	**Un-knowns**	**Other[a]**	**Bound residue**	**Total**
After third application (37)	ND	ND	25.6 (0.14)	50.5 (0.28)	ND	-	6.7 (0.04)	17.2 (0.10)	100.0 (0.56)
After fifth application (7)	0.1 (0.001)	0.6 (0.005)	32.4 (0.28)	43.3 (0.37)	6.8 (0.06)	0.3 (0.003)	ND	16.6 (0.14)	100.2 (0.86)
Pre-harvest (3)	0.1 (0.001)	0.4 (0.003)	28.7 (0.20)	46.7 (0.33)	ND	0.2 (0.002)	1.7 (0.01)	22.2 (0.16)	100.0 (0.71)
Normal harvest (0)	**0.1 (0.001)**	**0.5 (0.005)**	**24.6 (0.27)**	**55.1 (0.60)**	**3.5 (0.04)**	**0.3 (0.003)**	**1.3 (0.01)**	**14.7 (0.16)**	**100.0 (1.10)**

ND = not detected.

f) *^{14}C-folpet metabolism in the lactating goat (part A). ^{14}C- trichloromethyl folpet: material balance of dosed radioactivity. (Cordon, M.T. 1997a; Annex IIA, 6.2/01; IIA 7.3/16)*

NOTE: The summary below already appears in the DAR under B.7.2.a Metabolism, distribution and expression of residues in livestock (Annex IIA 6.2 and Annex IIIA 8.1).

[Trichloromethyl-^{14}C] folpet (radiochemical purity 99.3%) dissolved in [redacted] was administered in gelatine capsules orally once daily for three consecutive days to a miniature lactating goat at a measured dietary concentration of 20 mg/kg diet. Milk was collected twice a day, from one day prior to dosing until sacrifice, urine and faeces were collected from one day prior to dosing until sacrifice and expired air was collected in potassium hydroxide traps. The goat was sacrificed 23 hours after the final dose. Radioactivity was determined in excreta, tissues, milk, gastrointestinal tract, cage wash and expired air by LSC and combustion/LSC.

The total recovery of radioactivity was 102%, of which 31.4% was recovered in air traps, 41.9% in faeces, 16.9% in the gastrointestinal tract and 10.2% in the urine. Very low levels of ^{14}C radioactivity were found in milk (1.0% of administered dose) and tissues (0.8% of administered dose). Significant residues were found in the liver (0.5% of administered dose, equivalent to 0.34 mg folpet equivalents/kg), kidney (0.1% of administered dose, equivalent to 0.26 mg folpet equivalents/kg), muscle (0.2% of administered dose, equivalent to 0.04 mg folpet equivalents/kg) and fat (< 0.1% of administered dose, equivalent to 0.01 mg folpet equivalents/kg).

The distribution of applied radioactivity is given in Table B.7.3.17.

Table B.7.3.17: Distribution of ^{14}C following oral administration of [trichloromethyl-^{14}C] folpet to a lactating goat for three days

Matrix/tissue	% Applied dose	Residue (mg folpet equivalents/kg or L)
Tissues & milk		
subcutaneous fat	< 0.1	0.01
peritoneal fat	< 0.1	0.01
muscle (fore)	0.1	0.03
muscle (rump)	0.1	0.04
kidney	0.1	0.26
liver	0.5	0.34
milk 0-24 hr	0.2	0.23
milk 24-48 hr	0.4	0.38
milk 48-71 hr	0.4	0.34
total	1.8	-
Urine		
0-24 hr	2.1	-
24-48 hr	0.6	-
48-71 hr	6.4	-
bladder	1.1	-
total	10.2	
Faeces		
0-24 hr	8.7	-
24-48 hr	11.5	-
48-71 hr	21.7	-
total	41.9	-
Expired $^{14}CO_2$		
0-12 hr	6.8	-
12-24 hr	2.0	-
24-36 hr	7.9	-
36-48 hr	3.6	-
48-60 hr	8.9	-
60-71 hr	2.2	-
total	31.4	
Gastrointestinal tract		
intestine	10.8	-
rumen & reticulum	5.7	-
omasum & abomasum	0.4	-
total	16.9	
Bile	< 0.1	
Cage wash	0.2	-
Total	**102**	-

g) ^{14}C-folpet metabolism in the lactating goat (part B). (Cordon, M.T. 1997b; IIA, 6.2/02; IIA 7.3/17)

NOTE: The summary below already appears in the DAR under B.7.2.b Metabolism, distribution and expression of residues in livestock (Annex IIA 6.2 and Annex IIIA 8.1).

[Trichloromethyl-^{14}C] folpet (radiochemical purity 97%) and [U-phenyl -^{14}C] folpet (radiochemical purity 98%) dissolved in dichloromethane, were each administered to separate miniature lactating goats. Administration was in gelatine capsules orally once daily for six consecutive days at a measured dietary concentration of 24 mg/kg diet and 14 mg/kg diet for the [trichloromethyl-^{14}C] folpet and [U-phenyl -^{14}C] folpet, respectively. Milk was collected twice a day from one day prior to dosing until sacrifice. Urine and faeces were collected from one day prior to dosing until sacrifice. The goat was sacrificed 23 hours after the final dose.

Radioactivity was determined in excreta, tissues, milk, gastrointestinal tract and cage wash by LSC and combustion/LSC. Metabolites were characterised by TLC.

Following administration of [trichloromethyl-^{14}C] folpet, the majority of the administered radioactivity was excreted and recovered in the faeces and urine. The distribution results were comparable to those recorded in the distribution study (Cordon, M.T. 1997a). Significant residues were found in the kidney (0.16 mg folpet equivalents/kg), liver (0.25 mg folpet equivalents/kg), muscle (0.02 mg folpet equivalents/kg) and milk (up to 0.20 mg folpet equivalents/L). Residues in milk plateaued approximately 4 days after the start of administration. Residues in fat were less than 0.01 mg folpet equivalents/kg. The distribution of applied radioactivity is given in Table B.7.3.18.

Table B.7.3.18: Distribution of ^{14}C following oral administration of [trichloromethyl-^{14}C] folpet to a lactating goat for six days

Matrix/tissue	% Applied dose	Residue (mg folpet equivalents/kg or L)
Tissues & milk		
subcutaneous fat	< 0.1	< 0.01
peritoneal fat	< 0.1	< 0.01
muscle (fore)	< 0.1	0.02
muscle (rump)	< 0.1	0.03
liver	0.2	0.25
kidney	< 0.1	0.16
milk 0-24 hr	< 0.1	0.098
milk 24-48 hr	0.1	0.163
milk 48-72 hr	0.1	0.174
72-96 hr	0.1	0.177
96-120 hr	0.1	0.203
120-143 hr	0.1	0.192
total	0.7	-
Urine		
0-24 hr	0.5	-
24-48 hr	1.0	-
48-72 hr	0.5	-
72-96 hr	1.6	-
96-120 hr	0.7	-
120-143 hr	0.4	-
bladder	0.1	-
total	4.8	
Faeces		
0-24 hr	0.5	-
24-48 hr	5.3	-
48-72 hr	6.6	-
72-96 hr	12.7	-
96-120 hr	8.5	-
120-143 hr	1.3	-
total	34.9	-
Bile	< 0.1	
Cage wash	0.2	-
Total	**40.6**[a]	-

[a] Plus 31.4% present in expired air, 16.9% present in gastrointestinal tract (see Point 6.2/01).

Following administration of [U-phenyl -^{14}C] folpet, the majority of the administered radioactivity was recovered in the faeces (34.9%) and urine (58.3%), with small quantities in the cage wash (2.1%) and tissues plus milk (< 0.1%). The overall recovery was 95.3% of the

administered dose. Significant residues were found in the kidney (0.05 mg folpet equivalents/kg) and liver (0.02 mg folpet equivalents/kg). Residues in muscle and fat were less than 0.01 mg folpet equivalents/kg; residues in milk were less than 0.01 mg folpet equivalents//L. The distribution of applied radioactivity is given in Table B.7.3.19.

Table B.7.3.19: Distribution of ^{14}C following oral administration of [U-phenyl -^{14}C] folpet to a lactating goat for six days

Matrix/tissue	% Applied dose	Residue (mg folpet equivalents/kg or L)
Tissues & milk		
subcutaneous fat	< 0.1	0.004
peritoneal fat	< 0.1	< 0.001
muscle (fore)	< 0.1	0.003
muscle (rump)	< 0.1	0.003
liver	< 0.1	0.022
kidney	< 0.1	0.052
milk 0-24 hr	< 0.1	0.004
milk 24-48 hr	< 0.1	0.006
milk 48-72 hr	< 0.1	0.005
72-96 hr	< 0.1	0.005
96-120 hr	< 0.1	0.005
120-143 hr	< 0.1	0.006
total	< 0.1	-
Urine		
0-24 hr	9.2	-
24-48 hr	12.1	-
48-72 hr	8.7	-
72-96 hr	6.4	-
96-120 hr	11.2	-
120-143 hr	10.7	-
total	58.3	
Faeces		
0-24 hr	1.4	-
24-48 hr	6.4	-
48-72 hr	7.7	-
72-96 hr	6.1	-
96-120 hr	6.3	-
120-143 hr	7.0	-
total	34.9	-
Bile	< 0.1	
Cage wash	2.1	-
Total	**95.3**	-

Following administration of [trichloromethyl-^{14}C] folpet, thiazolidine was found in the urine and faeces at 17.4% and 2.9%, respectively, of the radioactivity (equivalent to 0.8% and 1.0% of the administered radioactivity, respectively). Low levels of unmetabolised folpet were found only in the faeces (8.0% of the radioactivity, equivalent to 2.8% of the administered radioactivity). Folpet was extensively metabolised in tissues and the radiolabelled carbon was incorporated into naturally occurring compounds. These were amino acids (in the liver, kidney, milk, muscle), glucose and fats (in the liver), cholesterol (in the kidney) and lactose (in the milk).

Following administration of [U-phenyl -^{14}C] folpet, phthalamic acid was the major constituent of the urine (84.8% of the radioactivity, equivalent to 49.4% of the administered radioactivity). The faeces contained phthalimide (26.4% of the radioactivity, equivalent to 9.2% of the administered radioactivity) and a small amount of unmetabolised folpet (0.9% of the radioactivity, equivalent to 0.3% of the administered radioactivity). The majority of the

radioactivity in the faeces was unextracted. The major metabolites in liver, kidney and milk were phthalimide and either phthalamic acid, phthalic anhydride or phthalic acid. No folpet was detected in tissues or milk.

The characterisation of radioactivity is summarised in Table B.7.3.20.

Table B.7.3.20: Characterisation of ^{14}C radioactivity in tissues, milk and excreta following administration of folpet to a lactating goat for six days

Identity of residue	% ^{14}C radioactivity (% of dosed radioactivity)										
	liver		kidney		urine		faeces		milk		muscle
	1	2	1	2	1	2	1	2	1	2	2
folpet	-	-	-	-	-	-	0.9 (0.3)	8.0 (2.8)	-	-	-
thiazolidine	-	-	-	-	-	17.4 (0.8)	-	2.9 (1.0)	-	-	-
phthalamic acid	27.8	-	69.1[c]	-	84.8 (49.4)	-	-	-	7.2[c]	-	
phthalimide	2.6	-	0.7	-	-	-	26.4 (9.2)	-	5.8	-	
natural compounds[a]	-	26.9	-	19.2					-	52.7	35.8
unknowns[b]	7.2 {12} [1.3]	10.8 {8} [3.5]	3.6 {5} [1.3]	20.5 {9} [8.7]	9.4 {4} [4.6]	33.3 {4} [13.3]	0.3	1.0 {1}	-	3.6 {3} [3.0]	10.9 {5} [6.0]
baseline	23.1	9.6	10.1	10.3	0.8	38.1	3.0	< 0.1	-	6.8	2.3
remainder	5.7	9.8	11.6	21.2	-	-		-	6.6	11.6	12.8
unextracted residue	-	10.0	4.4	-	-	-	68.0	87.0	4.1	15.9	31.8
other[d]	33.6	59.8	0.9	28.7	5.1	11.2	1.4	1.2	76.3	8.7	6.4

1 = [U-phenyl -^{14}C] folpet, 2 = [trichlormethyl-^{14}C] folpet.
[a] Amino acids, cholesterol, glucose, lactose, etc.
[b] Value in {} parenthesis = number of unknown components which make up the total radioactive residue; value in [ ] parenthesis = % of total radioactive residue represented by the major unknown component.
[c] Includes phthalic anhydride and phthalic acid.
[d] Unanalysed and losses during work-up.
Values in ( ) parenthesis are % of dosed radioactivity.

h) Dietary Risk assessment of Folpet Metabolite: Phthalimide

The amount of phthalimide in milk and meat was determined in a goat metabolism study (Corden 1997a, 1997b). Goats were fed ^{14}C-folpet at 14 ppm labelled in the benzene ring for 6 days. Tissues were harvested and samples with 3% total radioactive residue or more were characterized. The majority of radioactivity was excreted in the urine and faeces.

	Phthalimide
Meat	<0.004 mg/kg
Milk	<0.001 mg/kg

Estimation of the potential and actual exposure of phthalimide through animal products diet

Chronic exposure

Theoretical Maximum Daily Intake (TMDI)

The TMDI is calculated by multiplying the MRL or actual residues by the estimated average daily consumption for a given food commodity.

TMDI = ∑ MRL x F

where:
MRL = Maximum residue limit or actual residues for a given food commodity
F = Consumption of that food commodity.

This calculation is performed using:

3) An International diet (European Region) based on data from the World Health Organisation (WHO)[7].
4) The UK Dietary model (PSD, 1999[8])

<u>WHO European diet</u>

The TMDI calculation is presented in Table B.7.3.21.

Table B.7.3.21: TMDI calculation for Phthalimide based on WHO diet

Commodity	Phthalimide (mg/kg)	Consumption (kg/person/day)	TMDI (mg/person/day)
Total milk	< 0.001 (0.0005*)	0.3408	0.0002
Cattle meat	< 0.004 (0.002*)	0.0633	0.0001
Total			**0.0003**

*Since phthalimide residues were below the LOQ of the analytical method used, one half of the LOQ as worst case scenario was taken into consideration as appear in the brackets.

[7] WHO (1989). Guidelines for predicting dietary intake of pesticide residues. Prepared by the joint UNEP/FAO/WHO Food Contamination Monitoring Programme in collaboration with the Codex Committee on Pesticide Residues. World Health Organisation, Geneva.

[8] PSD (1999). Guidance on the estimation of dietary intakes of pesticides residues. The Registration Handbook. Pesticides Safety Directorate, Ministry of Agriculture, Fisheries and Food.

The total TMDI of Phthalimide is 0.0003 mg/person/day day or 0.000005 mg/kg bw/day for a 60 kg adult.

UK diet

UK consumption data for adults, children, toddlers and infants (mean consumers and high, i.e. 97.5th percentile, consumers) are presented in Table B.7.3.22

Table B.7.3.22: UK consumption data for adults, children, toddlers and infants

Commodity	Consumption data (kg/day)							
	Adults (70.1 kg bw)		Children (43.6 kg bw)		Toddlers (14.5 kg bw)		Infants (8.7 kg bw)	
	Mean	High [1]	Mean	High	Mean	High	Mean	High
Milk	0.2573	0.6659	0.0304	0.6745	0.3064	0.8017	0.33775	0.8719
Meat	0.0841	0.2050	0.0641	0.1339	0.0276	0.0869	0.1339	0.0121

The TMDI for Phthalimide was calculated for all consumer groups of milk and meat (high consumption intake).

Table B.7.3.23: consumption of Phthalimide by adults, children, toddlers and infants based on UK high consumption intakes

Commodity	Phthalimide (mg/kg)	TMDI (mg/kg bw/day)			
		Adults (70.1 kg bw)	Children (43.6 kg bw)	Toddlers (14.5 kg bw)	Infants (8.7 kg bw)
Milk	0.0005	0.0000047	0.00005	0.00005	0.00005
Meat	0.002	0.000001	0.00001	0.00001	0.00001
Total exposure		**0.000047**	**0.00005**	**0.00005**	**0.00005**

The TMDIs of Phthalimide in all consumer groups including toddlers and infants, which are the most sensitive consumer groups, is 0.00005 mg/kg bw/day.

Comparison of TMDI of phthalimide with the ADI

The TMDI values for different consumer groups and diets are summarised in Table B.7.3.24.

Table B.7.3.24: TMDI values for different consumer groups and diets

Diet	Body weight (kg)	TMDI (mg/kg bw/day)
WHO adult	60	0.000005
UK adult	70.1	0.0000047
UK child	43.6	0.000005
UK toddler	14.5	0.00005
UK infant	8.7	0.00005

Based on the proposed ADI for folpet of 0.1 mg/kg bw/day, the TMDI for Phthalimide according to the worst case consumption scenarios represents less than 0.00001 % of the ADI for all the different consumer groups and different dietary intakes of milk and meat.

The maximum daily intake of Phthalimide in animal products is zero for all consumer groups including the most sensitive consumer groups and compare to the ADI for folpet according to the worst case exposure.

i) Toxicity of phthalimide to aquatic organisms

Note: Summaries of all the relevant studies are presented below. These are already included in the DAR in Point B.9.2.1.

Fish

(1) Acute toxicity of phthalimide to rainbow trout (Salmo gairdneri). (Bowman, J.H. 1988c; IIA, 8.2.1/12; IIA 7.3/18)

The 96-hour acute toxicity of phthalimide (purity 98%) to the rainbow trout (*Salmo gairdneri* now known as *Oncorhynchus mykiss*) was determined in a static test system. Ten fish per glass vessel each containing 15 L (16 hour photoperiod, 12 °C) were exposed to nominal concentrations of phthalimide (dissolved in [redacted] of 10, 18, 32, 56 and 100 mg/L in comparison with a dilution water control (hardness 40 to 46 mg/L $CaCO_3$) and a solvent control (0.1 mL/L). The fish were not fed for 48 to 96 hours prior to or during exposure. The test media were not renewed throughout the test. Samples of all test media for analysis of phthalimide by HPLC, were taken at the start and end of the exposure period. Measurements of pH, dissolved oxygen and temperature were taken at 0, 48 and 96 hours. Fish mortality and behaviour were recorded once every 24 hours.

The study met the essential criteria of EEC C1. However, standard lengths were measured whereas total lengths are stated in the EU guideline. No details were given of fish mortality during holding. It was conducted according to Good Laboratory Practice.

The mean measured concentrations of phthalimide were 9.4, 17, 26, 43 and 66 mg/L representing 94, 94, 81, 77 and 66% of nominal. There was little loss of phthalimide from 0 to 96 hours. At measured concentrations of 26, 43 and 66 mg/L there was a white precipitate on the surface and at the bottom of the test vessels at 0-hours. The amount of precipitate increased with nominal concentration, but became less visible with time. This suggests that in media at 26 mg/L and above, phthalimide was present in excess, possibly above the limit of water solubility and hence toxicity to rainbow trout at these concentrations may not be related to inherent toxicity but to excess test material in the test system. The water quality parameters were all within expected limits.

The cumulative mortality is presented in Table B.7.3.25. Sublethal effects at 26, 43 and 66 mg/L were surfacing, loss of equilibrium, fish on the bottom of the test vessels, quiescence and/or distended abdomen.

Table B.7.3.25: Mortality of rainbow trout exposed to phthalimide following 96-hours exposure in a static test system

Mean measured concentration of phthalimide (mg/L)	Cumulative mortality (%)			
	24 hr	48 hr	72 hr	96 hr
Water control	0	0	0	0
Solvent control	0	0	0	0
9.4	0	0	0	0
17	0	0	0	0
26	0	0	0	0
43	0	0	10	20
66	80	100	100	100

The 96-hour LC_{50} of phthalimide to rainbow trout under static test conditions was 49 mg/L (with 95% confidence limits of 26 to 66 mg/L) based on measured concentrations. The NOEC for mortality was 17 mg/L. The 24, 48 and 72-hour LC_{50} values were 58, 53 and 51 mg/L, respectively.]

(2) Acute toxicity of phthalimide to bluegill sunfish (Lepomis macrochirus) in a static renewal system. (Bowman, J.H. 1989; IIA, 8.2.1/13; IIA 7.3/19)

The 96-hour acute toxicity of phthalimide (purity 98%) to the bluegill sunfish (*Lepomis macrochirus*) was determined in a semi-static test system with renewal of the test media after 48 hours. Ten fish per glass vessel each containing 15 L (16 hour photoperiod, 21 to 23 °C) were exposed to nominal concentrations of phthalimide (dissolved in [redacted] of 10, 18, 32, 56 and 100 mg/L in comparison with a dilution water control (hardness 42 mg/L $CaCO_3$) and a solvent control (0.1 mL/L). The fish were not fed for 48 to 72 hours prior to or during exposure. Samples of all test media for analysis of phthalimide by HPLC, were taken at the start, after 48 hours and at the end of the exposure period. Measurements of pH, dissolved oxygen and temperature were taken at 0, 48 and 96 hours. Fish mortality and behaviour were recorded once every 24 hours. The 32 mg/L treatment was repeated with a concurrent solvent control treatment as three fish were lost during renewal of the test media in the first definitive test at this concentration.

The study met the essential criteria of EEC C1. However, standard lengths were measured whereas total lengths are stated in the EU guideline. No details were given of fish mortality during holding. Temperature, dissolved oxygen and pH should have been measured daily rather than at 0, 48 and 96 hours. It was conducted according to Good Laboratory Practice.

The mean measured concentrations of phthalimide were 6.8, 13, 22, 31 and 52 mg/L representing 68, 72, 69, 55 and 52% of nominal . At 22, 31 and 52 mg/L there was a white precipitate on the surface of the test media and at the bottom of the test vessels at 0-hours and 48-hours (freshly prepared media). The amount of precipitate increased with nominal concentration, but became less visible with time. The nominal 100 mg/L medium had a white precipitate at the bottom of the test vessel at both renewal time periods. This suggests that in media at 22 mg/L and above, phthalimide was present in excess, possibly above the limit of water solubility at the start of the renewal period but then may have fully dissolved on completion of the renewal period in all but the 100 mg/L medium. Therefore, the toxicity of phthalimide to bluegill sunfish at these concentrations may not be related to inherent toxicity but to excess test material in the test system. The water quality parameters were all within expected limits.

The cumulative mortality is presented in Table B.7.3.26. Sublethal effects at 31 and 52 mg/L were light discolouration, vertical orientation, quiescence and/or laboured respiration.

Table B.7.3.26: Mortality of bluegill sunfish exposed to phthalimide following 96-hours exposure in a semi-static test system

Mean measured concentration of phthalimide (mg/L)	Cumulative mortality (%)			
	24 hr	48 hr	72 hr	96 hr
Water control	0	0	0	0
Solvent control	0	0	0	0
6.8	0	0	0	0
13	0	0	0	0
22	0	0	0	0
31	10	10	10	10
52	90	90	100	100

The 96-hour LC_{50} of phthalimide to bluegill sunfish, under semi-static test conditions, was 38 mg/L (with 95% confidence limits of 31 to 52 mg/L) based on measured concentrations. The NOEC was 22 mg/L based on toxicological symptoms observed at 31 and 52 mg/L. The 24, 48 and 72-hour LC_{50} values were 40, 40 and 38 mg/L, respectively.

(3) Acute flow-through toxicity of folpet technical to rainbow trout (Salmo gairdneri). (Bowman, J.H. 1988a; IIA, 8.2.1/01; IIA 7.3/20)

The 96-hour acute toxicity of folpet technical active substance (purity 90.3%) to the rainbow trout (*Salmo gairdneri* now known as *Oncorhynchus mykiss*) was determined in a flow-through test system. Two groups of 10 fish per replicate 15 L aquarium (16 hour photoperiod, 12 to 13 °C) were exposed to nominal concentrations of folpet (dissolved in [redacted] of 0.0065, 0.013, 0.025, 0.05 and 0.10 mg/L in comparison with a dilution water control (hardness 40 to 46 mg/L $CaCO_3$) and a solvent control (0.1 mL/L). The fish were not fed for 48 hours prior to or during exposure. The test media were renewed 7.4 times each day. Samples of all test media for analysis of folpet, by high performance liquid chromatography (HPLC), were taken at the start and end of the exposure period. Measurements of pH, dissolved oxygen and temperature were taken at 0, 48 and 96 hours. Fish mortality and behaviour were recorded once every 24 hours.

The study met the essential criteria of EC method C1. However, fish lengths may be smaller, but standard lengths were measured whereas total lengths are stated in the EU guidelines. No details were given of fish mortality during holding. Temperature, dissolved oxygen and pH should have been measured daily rather than at 0, 48 and 96 hours. It was conducted according to Good Laboratory Practice.

The mean measured concentrations of folpet were 0.0022, 0.0056, 0.012, 0.026 and 0.13 mg/L representing 34, 43, 48, 52 and 130% of nominal. The 0 hour measurement for the 0.10 mg/L medium was 220% of nominal which was attributed to a precipitate in the splitter cell. For this reason and because the other measured adjacent concentrations were approximately 50% of each other, the 96 hour measured concentration at the highest nominal concentration was used in the LC_{50} calculation. The water quality parameters were all within expected limits.

The cumulative mortality is presented in Table B.7.3.27. Sublethal effects at 0.0056, 0.012 and 0.026 mg/L were loss of equilibrium, and fish on the bottom of the test vessels.

Table B.7.3.27: Mortality of rainbow trout following 96-hours exposure to folpet in a flow-through test system

Mean measured concentration of folpet (mg/L)	Cumulative mortality (%)			
	24 hr	48 hr	72 hr	96 hr
Water control	0	0	0	0
Solvent control	0	0	0	0
0.0022	0	0	0	0
0.0056	0	0	0	0
0.012	0	0	5	5
0.026	0	45	80	85
0.033*	100	100	100	100

*Based on the 96 h measured value.

The 96-hour LC_{50} of folpet to rainbow trout under flow-through conditions was 0.015 mg/L (with 95% confidence limits of 0.013 to 0.048 mg/L) based on measured concentrations. The NOEC was 0.0022 mg/L based on toxicological symptoms observed at 0.0056 mg/L and above. The 24, 48 and 72-hour LC_{50} values were 0.029, 0.026 and 0.015 mg/L, respectively.

(4) Acute flow-through toxicity of folpet technical to bluegill sunfish (Lepomis macrochirus). (Bowman, J.H. 1988b, IIA, 8.2.1/02; IIA 7.3/21).

The 96-hour acute toxicity of folpet technical active substance (purity 90.3%) to the bluegill sunfish (*Lepomis macrochirus*) was determined in a flow-through test system. Two groups of 10 fish per replicate 15 L aquarium (16 hour photoperiod, 22 °C) were exposed to nominal concentrations of folpet (dissolved in [redacted] of 0.065, 0.13, 0.25, 0.5 and 1.0 mg/L in comparison with a dilution water control (hardness 40 to 46 mg/L $CaCO_3$) and a solvent control (0.1 mL/L). It should be noted that the limit of water solubility of folpet at 25 °C is

0.8 mg/L. Therefore, at the highest nominal concentration folpet would be present in excess of its water solubility. The fish were not fed for 48 hours prior to or during exposure. The test media were renewed 7.4 times each day. Samples of all test media for analysis of folpet, by HPLC, were taken at the start and end of the exposure period. Measurements of pH, dissolved oxygen and temperature were taken at 0, 48 and 96 hours. Fish mortality and behaviour were recorded once every 24 hours.

The study met the essential criteria of EEC C1. However, standard lengths were measured whereas total lengths are stated in the EU guidelines. No details were given of fish mortality during holding. It was conducted according to Good Laboratory Practice.

The mean measured concentrations of folpet were 0.016, 0.033, 0.068, 0.20 and 0.25 mg/L representing 25, 25, 27, 40 and 25% of nominal (Table B.7.3.28). A white precipitate was observed in the diluter mixing cell and in the aquaria with the highest nominal concentration of folpet. This is consistent with the quantity of folpet added to water which was above the limit water solubility. The water quality parameters were all within expected limits.

Table B.7.3.28: Measured concentrations of folpet technical during a 96-hour flow-through toxicity test with bluegill sunfish

Folpet nominal concentration (mg/L)	Folpet measured concentration (mg/L)			Mean measured conc. as a % of nominal
	0-hr	96-hr	Mean	
Control	< 0.010	< 0.010	-	-
Solvent control	< 0.010	< 0.010	-	-
0.065	0.017	0.015	0.016	25
0.13	0.028	0.037	0.033	25
0.25	0.059	0.076	0.068	27
0.50	0.12	0.28	0.20	40
1.0	0.17	0.33	0.25	25
Stock solution (9500)	9900	9900	9900	104

[a] Precipitate present in vessel.
[b] 96-hour concentration used in LC_{50} calculation.

The cumulative mortality is presented in Table B.7.3.29. There were no sublethal effects recorded at 0.033 mg/L or below.

Table B.7.3.29: Mortality of bluegill sunfish following 96-hours exposure to folpet in a flow-through test system

Mean measured concentration of folpet (mg/L)	Cumulative mortality (%)			
	24 hr	48 hr	72 hr	96 hr
Water control	0	0	0	0
Solvent control	0	0	0	0
0.016	0	0	0	0
0.033	0	0	0	0
0.068	100	100	100	100
0.20	100	100	100	100
0.25	100	100	100	100

The 96-hour LC_{50} of folpet to bluegill sunfish under flow-through conditions was 0.047 mg/L (with 95% confidence limits of 0.033 to 0.068 mg/L) based on measured concentrations. The NOEC was 0.033 mg/L based on mortality at 0.068 mg/L. The 24, 48 and 72-hour LC_{50} values were 0.047 mg/L.

Table B.7.3.30: Summary of acute toxicity of folpet and PI

Compound	LC_{50} (mg/L) Blue Gill sunfish	LC_{50} (mg/L) Rainbow trout	References	
PI	38	49	*Bowman, J.H. 1989; IIA, 8.2.1/13; IIA 7.3/09*	*Bowman, J.H. 1988c; IIA, 8.2.1/12; IIA 7.3/08*
folpet	0.047	0.015	*Bowman, J.H. 1988b, IIA, 8.2.1/02; IIA 7.3/11*	*Bowman, J.H. 1988a; IIA, 8.2.1/01; IIA 7.3/10*
Ratio	809	3266		

B.7.17 References relied on

B.7.17.1 Active substance

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company, Report No. GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N	Owner
7.3a	Lukens R.J.	1966	The fungitoxicity of compounds containing a trichloromethylthio-group. Journal of Agricultural Food Chemistry 14: 365-367.	N	Public domain
7.3b	Rideg K.	1982	Genetic toxicology of phthalimide-type fungicides. Mutation Research 97: 217.	N	Public domain
7.3c	US EPA	2004	Captan: Cancer Reclassification; Amendment of Reregistration Eligibility Decision; Notice of Availability. 69 Fed. Reg. 68357-68360 November 24, 2004.	N	Public domain
7.3d	Daly I.W.; Knezevich A.L.	1983	A one-year subchronic oral toxicity study in dogs with folpet technical. [redacted] Report no. No. 82-2677; R-6035. March 17, 1983.	N	--
7.3e	Gordon E.B. et al	2001	Measurement of the reaction between the fungicides captan or folpet and blood thiols. Toxicology Methods 11: 209-223.	N	Public domain
7.3f	Beranrd K.B.; Gordon E.B.	2000	An evaluation of the common mechanism approach to the Food Quality Protection Act: Captan and four related fungicides, a practical example. International Journal of Toxicology 19: 43-61.	N	Public domain
IIA 7.3/01	Fabro, S., Schumacher, R. L., Smith, R. L., Williams, R. T.	1964	Teratogenic activity of thalidomide and related compounds. *Life Sciences* **3**, 987-992. (Company file R-9963). Not GLP; Published.	N	-
IIA 7.3/02	Kennedy, G., Fancher, O. E., Calandra, J. C.	1968	An investigation of the teratogenic potential of captan, folpet, and difolatan. *Toxicol. Appl. Pharmacol.* **13**, 420-430. (Company file R-169). Not GLP; Published	N	-

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company, Report No. GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N	Owner
IIA 7.3/03	Blee, M.A.B.	2006	Phthalimide: Prenatal toxicity study in the rabbit by oral gavage. Report MAK 863/055231 administration (Company file: R-18201). GLP, Unpublished.	Y	Makhteshim
IIA 7.3/04	Pilinskaya, M. A.	1986	Study of the cytogenetic activity of certain metabolites of a number of pesticides representing several classes of chemical compounds. *Tsitol. Genet.* **20**, 143-145. (Company file R-11352) Not GLP; Published.	N	-
IIA 7.3/05	Riggin, R. M., Margard, W. L., Kinzer, G. W.	1983	Characterization of impurities in commercial lots of sodium saccharin produced by the Sherwin-Williams process. II. Mutagenicity. *Fd Chem. Toxic.* **21**, 11-17. (Company file R-11350). Not GLP; Published.	N	-
IIA 7.3/06	Siefried, H.E.	2000	Review: Toxicological risk characterisation of potential folpet metabolites. The toxicity profiles of phthalic and phthalamic acids and phthalimide – is there a significant risk from metabolite exposure? Consultants, report dated August 1, 2000 (Company file: R-12331). Not GLP, Unpublished.	Y	Makhteshim
IIA 7.3/07	Akhurst, L.C.	2005	Phthalimide: Determination of minimum inhibitory concentrations against selected micro-organisms representative of the rabbit gut micro-flora. Report MAK 889/053251 ((Company file:R-18734). GLP, Unpublished.	Y	Makhteshim
IIA 7.3/08	Rideg, K.	1992	Genetic toxicology of phthalimide-type fungicides. Mutation Research 97 (3): 217.	N	Public domain
IIA 7.3/12	Crowe, A.	1995	Folpet: distribution and metabolism in winter wheat. Pharmaco LSR Ltd., Report No. 95/MAK204/0049 (Company file: R-7823). GLP, Unpublished.	Y	Makhteshim
IIA 7.3/13	O'Connor, J.	1994	Folpet: nature of residue on grapes. Pharmaco LSR Ltd., Report No. 93/WLS019/0962. (Company file: R-6403a). GLP, Unpublished.	Y	Makhteshim

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company, Report No. GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N	Owner
IIA 7.3/14	Toia, R.F., Collins, E.H.	1994	Nature of residue (^{14}C)-folpet (LX1145-05) in avocados applied under field conditions. PTRL West Inc., Report No. 417W-2. (Company file: R-7302). GLP, Unpublished.	Y	Makhteshim
IIA 7.3/15	Crowe, A.	1999	Folpet: metabolism in potatoes. Huntingdon Life Sciences Ltd., Report No. MAK506/992098 (Company file: R-10347). GLP, Unpublished.	Y	Makhteshim
IIA 7.3/16	Cordon, M.T.	1997a	^{14}C-folpet metabolism in the lactating goat (part A). ^{14}C- trichloromethyl folpet: material balance of dosed radioactivity. [redacted]Report No. MBS72a/972856 (Company file: R-9137a). GLP, Unpublished.	Y	Makhteshim
IIA 7.3/17	Cordon, M.T.	1997b	^{14}C-folpet metabolism in the lactating goat (part B). [redacted]Report No. MBS 72b/972856 (Company file: R-9137). GLP, Unpublished.	Y	Makhteshim
IIA 7.3/18	Bowman, J.H.	1988c	Acute toxicity of phthalimide to rainbow trout (*Salmo gairdneri*). [redacted]Report No. 36789 (Company file R-4956). GLP, Unpublished.	Y	Makhteshim
IIA 7.3/19	Bowman, J.H.	1989	Acute toxicity of phthalimide to bluegill sunfish (*Lepomis macrochirus*) in a static renewal system. [redacted]Report No. 36788 (Company file R-5255). GLP, Unpublished.	Y	Makhteshim
IIA 7.3/20	Bowman, J.H.	1988a	Acute flow-through toxicity of folpet technical to rainbow trout (*Salmo gairdneri*). [redacted]Report No. 36785 (Company file R-4954). GLP, Unpublished.	Y	Makhteshim
IIA 7.3/21	Bowman, J.H.	1988b	Acute flow-through toxicity of folpet technical to bluegill sunfish (*Lepomis macrochirus*). [redacted]Report No. 36784 (Company file R-4955). GLP, Unpublished.	Y	Makhteshim