CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2

Chemical name:

folpet (ISO); *N*-(trichloromethylthio)phthalimide

EC Number: **205-088-6**

CAS Number: **133-07-3**

Index Number: **613-045-00-1**

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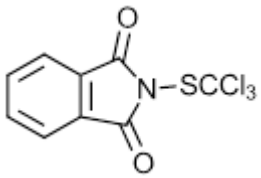
CONTENTS

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 1: Substance identity and information related to molecular and structural formula of the substance

Name(s) in the IUPAC nomenclature or other international chemical name(s)	N-(trichloromethylthio) phthalimide
Other names (usual name, trade name, abbreviation)	-
ISO common name (if available and appropriate)	folpet
EC number (if available and appropriate)	205-088-6
EC name (if available and appropriate)	-
CAS number (if available)	133-07-3
Other identity code (if available)	CIPAC: 75
Molecular formula	$C_9H_4Cl_3NO_2S$
Structural formula	
SMILES notation (if available)	-
Molecular weight or molecular weight range	296.6 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	-
Description of the manufacturing process and identity of the source (for UVCB substances only)	-
Degree of purity (%) (if relevant for the entry in Annex VI)	Min. 940 g/kg

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	**Concentration range (% w/w minimum and maximum in multi-constituent substances)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self-classification and labelling (CLP)**
none			

Table 3: Impurities (non-confidential information) if relevant for the classification of the substance

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling
PCMM 594-42-3	Max 3.5 g/kg	-	H301, H311, H314, H330,	No
Captan 133-06-2	Max 3 g/kg	H318, H331, H351, H317, H400	-	No
CCl4 56-23-5	Max 2 g/kg	H301, H311, H331, H351, H372, H412, H420	-	No

Table 4: Additives (non-confidential information) if relevant for the classification of the substance

Additive (Name and numerical identifier)	Function	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The additive contributes to the classification and labelling
none					

Table 5: Test substances (non-confidential information) (this table is optional)

Identification of test substance	Purity	Impurities and additives (identity, %, classification if available)	Other information	The study(ies) in which the test substance is used

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 6:

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATEs	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	613-045-00-1	folpet (ISO) N-(trichloromethylthio)phthalimide	205-088-6	133-07-3	Carc. 2 Acute Tox 4 Eye Irrit. 2 Skin Sens. 1 Aquatic Acute 1	H351 H332 H319 H317 H400	GHS09 GHS08 GHS07 Wng	H351 H332 H319 H317 H400		M=10	
Dossier submitters proposal	613-045-00-1	folpet (ISO); *N*-(trichloromethylthio)phthalimide	205-088-6	133-07-3	**Retain** Carc. 2 Aquatic Acute 1 **Add** STOT-RE 1 Skin Irrit. 2 Aquatic chronic 1 **Modify** Acute Tox 2 Eye Dam. 1 Skin Sens. 1A	**Retain** H351 H317 H400 **Add** H372 H315 H410 **Modify** H330 H318	**Retain** GHS08 GHS09 **Add** GHS05 GHS06 **Modify** Dgr **Remove** GHS07	**Retain** H351 H317 **Add** H372 H315 **Modify** H330 H318 H410		**Retain** M=10 **Add** inhalation: ATE = 0.39 mg/L (dusts and mists) Skin Sens.: SCL ≥ 0.001% M=1	
Resulting Annex VI entry if agreed by RAC and COM	613-045-00-1	folpet (ISO); *N*-(trichloromethylthio)phthalimide	205-088-6	133-07-3	Carc. 2 Acute Tox 2 STOT RE 1 Skin Irrit. 2 Eye Dam. 1 Skin Sens. 1A Aquatic Acute 1 Aquatic chronic 1	H351 H330 H372 H315 H318 H317 H400 H410	GHS05 GHS06 GHS08 GHS09 Dgr	H351 H330 H372 H315 H318 H317 H410		inhalation: ATE = 0.39 mg/L (dusts and mists) Skin Sens.: SCL ≥ 0.001% M=10 M=1	

Table 7: Reason for not proposing harmonised classification and status under public consultation

Hazard class	Reason for no classification	Within the scope of consultation
Explosives	hazard class not assessed in this dossier	No
Flammable gases (including chemically unstable gases)	hazard class not assessed in this dossier	No
Oxidising gases	hazard class not assessed in this dossier	No
Gases under pressure	hazard class not assessed in this dossier	No
Flammable liquids	hazard class not assessed in this dossier	No
Flammable solids	hazard class not assessed in this dossier	No
Self-reactive substances	hazard class not assessed in this dossier	No
Pyrophoric liquids	hazard class not assessed in this dossier	No
Pyrophoric solids	hazard class not assessed in this dossier	No
Self-heating substances	hazard class not assessed in this dossier	No
Substances which in contact with water emit flammable gases	hazard class not assessed in this dossier	No
Oxidising liquids	hazard class not assessed in this dossier	No
Oxidising solids	hazard class not assessed in this dossier	No
Organic peroxides	hazard class not assessed in this dossier	No
Corrosive to metals	hazard class not assessed in this dossier	No
Acute toxicity via oral route	data conclusive but not sufficient for classification	Yes
Acute toxicity via dermal route	data conclusive but not sufficient for classification	Yes
Acute toxicity via inhalation route	harmonised classification proposed	Yes
Skin corrosion/irritation	harmonised classification proposed	Yes
Serious eye damage/eye irritation	harmonised classification proposed	Yes
Respiratory sensitisation	data lacking	No
Skin sensitisation	harmonised classification proposed	Yes
Germ cell mutagenicity	data conclusive but not sufficient for classification	Yes
Carcinogenicity	harmonised classification proposed	Yes
Reproductive toxicity	data conclusive but not sufficient for classification	Yes
Specific target organ toxicity-single exposure	data conclusive but not sufficient for classification	Yes
Specific target organ toxicity-repeated exposure	harmonised classification proposed	Yes
Aspiration hazard	hazard class not assessed in this dossier	No
Hazardous to the aquatic environment	harmonised classification proposed	Yes
Hazardous to the ozone layer	Hazard class not assessed in this dossier.	No

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

The existing active substance folpet was first included in Annex I of Directive 91/414/EEC on 1st October 2007 (Commission Directive 2007/5/EC). The active substance was deemed to be approved under Regulation (EC) 1107/2009 via Implementing Regulation (EU) 540/2011 with an entry in Annex Part A of said regulation. With Commission Implementing Regulation (EU) No 2021/745, a new expiry date of the approval of folpet was set to 31st July 2022.

Folpet is approved as a biocide from 01/01/2016 until 31/12/2025.

In accordance with Commission Regulation (EU) 844/2012 of 18 September 2012, ADAMA Agriculture BV (formerly: Makhteshim Agan International) as the representative of ADAMA Makhteshim Ltd. (formerly: Makhteshim Chemical Works) and a Task Force (representing SAPEC Agro SA and ADAMA Agriculture BV (formerly: Makhteshim Agan International)) submitted separate dossiers to support the renewal of the approval of folpet. Austria acting as the Rapporteur Member State (RMS) evaluated all aspects of the renewal dossiers via a Draft Renewal Assessment Report (DRAR). The DRAR was the subject of a peer review by the Co-RMS Italy.

The RMS also paid attention to new criteria for classification and labelling according to Regulation (EC) 1272/2008. The following harmonised classification is available (Table 3.1 of Annex VI of Regulation (EC) No 1272/2008 as amended): Acute Tox. 4 – H332, Eye Irrit. 2 – H319, Skin Sens. 1 – H317, Carc. 2 – H351 and Aquatic Acute 1 – H400. Regarding human health the RMS proposes to add classification for chronic toxicity (STOT RE 1 – H372) and to update classification for skin sensitisation (Skin Sens. 1A – H317). In a newly submitted acute inhalation toxicity study, the LC50 for males was 0.39 mg/L. Therefore, the current classification as Acute Tox. Cat.4, H332 according to Regulation (EC) No 1272/2008 should be changed to Acute Tox. Cat. 2, H330. Moreover, classification for skin irritancy Cat. 2 might be warranted. Furthermore, newly submitted eye irritation studies show eye effects, which persist until the end of the recovery period. Therefore, classification as eye damage/eye irritation Cat. 1, H318 is more appropriate. Regarding ecotoxicity, new proposal for classification and labelling has been established (Aquatic acute, cat. 1 - H400 and Aquatic chronic, cat. 1 - H410 instead of current Aquatic chronic, cat. 2 - H411), based on the new studies with adverse endpoints included in the supplementary dossier for the renewal

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

[B.] Justification that action is needed at Community level is required.

Reason for a need for action at Community level:

no justification is needed (biocidal and PPP active substance)

5 IDENTIFIED USES

Folpet belongs to the class of phthalimide family.

Folpet is a broad spectrum fungicide with activity against many diseases. When applied before or at the onset of fungal attack, it prevents disease infection and establishment.

Folpet has a non-specific fungitoxic mode of action preventing disease infection and establishment. It inhibits many oxidative enzymes, carboxylases and enzymes involved with phosphate metabolism and citrate synthesis. Folpet reacts with the sulfhydryl groups of nuclear proteins, leading to an inhibition of the cell division.

In addition, it is a non-systemic fungicide and is not translocated in plants.

Protectant contact multi-site fungicides such as folpet prevent spores from germinating and infecting the plant if applied prior to spore release. Once infection has occurred and the fungus has penetrated the leaf, this type of fungicides will no longer control the disease. Folpet inhibits the germination of spores and the mycelium growth. Its fungicidal activity is based on multiple modes of action on different target sites.

Folpet enters treated conidia and reacts with many conidial constituents. The toxicity is attributed to the $SCCl_3$ group. Furthermore, it inhibits many oxidative enzymes, carboxylases and enzymes involved in the phosphate metabolism and citrate synthesis. The main mechanisms involved are the inhibition of a number of mitochondrial reactions, including oxidative phosphorylation and the oxidation of the reduced form of nicotinamide adenine dinucleotide ($NADH_2$), as well as reactions with vital cellular thiols. The effect of folpet on cell metabolism is summarized as follows:

• The reaction with endogenic thiol and the reaction of thiophosgen exhausts the endogenic thiols of the cells. As a result, enzymatic activity and metabolism cease.

• The reaction with sulphydryl groups of the nuclear proteins leads to inhibition of cell division.

• Respiration inhibition interferes with the electron transport.

• Toxic doses cause changes in carbohydrates, amino acids and the phosphate metabolism of fungi.

• Folpet deactivates Coenzyme A by oxidizing it to Coenzyme A trithiocarbonate.

• Folpet apparently prevents formation of high energy phosphate bonds by inhibiting incorporation of inorganic phosphate into organic molecules.

Folpet is not converted to a metabolite or degradation product in order to exert its intended effect.

Field of use envisaged:

Agriculture: Horticulture (vegetable production), viticulture, arable crops

Biocide: PT06 (Preservatives for products during storage), PT07 (Film preservatives) and PT09 (Fibre, leather, rubber and polymerised materials preservatives)

No REACH uses are known to the dossier submitter.

6 DATA SOURCES

DRAR – Draft renewal assessment report for folpet

Even if the CAR - Competent Authority Report was not used for this CLH report, the applicant confirmed that there is no data submitted in the biocide dossier that is not present in the pesticide dossier.

7 PHYSICOCHEMICAL PROPERTIES

Table 8: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	Solid	RAR (2019)	observed
Melting/freezing point	Melting point: 179°C	RAR (2019)	EC A.1
Boiling point	Decomposition above melting starting at 184 °C	RAR (2019)	EC A.2
Relative density	Not determined	-	-
Vapour pressure	7.6 to 17 x 10^{-6} Pa (20 °C)	RAR (2019)	EC A.4
Surface tension	Not determined, water solubility is below 1.0 mg/L	RAR (2019)	-
Water solubility	0.80 mg/L (max) at 25 °C 0.50 mg/L (mean) at 15 °C	RAR (2019)	EC A.6
Partition coefficient n-octanol/water	log P_{OW} = 3.107 (25°C)	RAR (2019)	EC A.8
Flash point	Not applicable	-	Melting point above 40°C
Flammability	Not classified as flammable	RAR (2019)	EC A.10
Explosive properties	Not explosive	RAR (2019)	EC A.14
Self-ignition temperature	Not self-igniting	RAR (2019)	EC A.16
Oxidising properties	Not oxidising	RAR (2019)	EC A.17
Granulometry	Not determined	-	-
Stability in organic solvents and identity of relevant degradation products	Not determined	-	-
Dissociation constant	Folpet does not dissociate at the pH ranges encountered in aqueous solution	RAR (2019)	Theoretical assessment based on structure
Viscosity	Not applicable	-	-

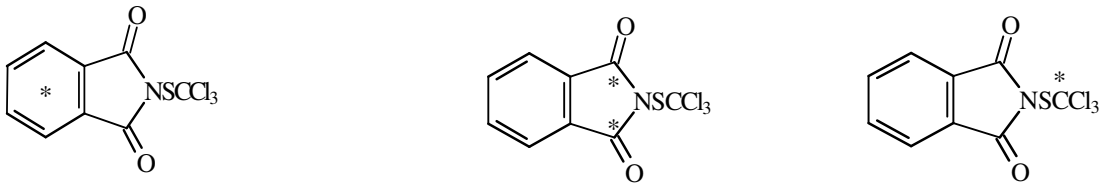
8 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Folpet is a contact fungicide, it rapidly reacts with thiols upon which it is degraded. The key degradation product is thiophosgene which is highly reactive and also reacts with thiols and other functional groups.

Folpet has been extensively studied in a series of guideline and non-guideline investigations. There are four *in vivo* studies in rat, of which one is conducted in both rat and mice. There are *in vitro* studies comparing test species and human metabolism and studies investigating the half time of folpet (and thiophosgene) in human blood. If not stated otherwise in the summary tables, the studies are considered as fully reliable.

Radiolabels have been incorporated in the aromatic ring, in the carbonyl ring, and in the trichloromethylthio side-chain (TCM). The aromatic ring was shown to be the most stable, and the trichloromethylthio side-chain the least stable part of the molecule.

Overall, the results are consistent between studies and in-line with folpet's fungicidal activity: there is no evidence for any relevant systemic exposure to the parent molecule. Radioactive recovery in the systemic compartment seems to be exclusively associated with its metabolites. Thus, the toxicokinetic studies are crucial for understanding and explaining folpet's hazard profile of acute local irritation, see Section 8.1-8.7, with subsequent adverse effects.



[U-phenyl -^{14}C] Folpet. [carbonyl-^{14}C] Folpet [trichloromethyl-^{14}C] Folpet

Figure 1: Different labelling positions in the folpet molecule

Radioactivity associated with folpet is rapidly absorbed, widely distributed and rapidly excreted after oral administration. Excretion patterns of males and females were essentially the same.

Irrespective of the position of the [14C]-radiolabel, the majority of radioactivity was recovered from the urine within 96 hours, with some in the faeces, when administered as low single doses, or low multiple doses (up to 14 days). Biliary excretion appears to play little or no part in elimination of folpet or its imide or TCM-based degradants.

A significant amount of radioactivity was excreted in expired air following administration of the [^{14}C]-TCM labelled form only. Tissue residues were negligible with the highest concentration being present 30 minutes after administered radioactivity (liver > muscle > blood > kidneys > fat). After 5 days radioactivity was essentially cleared from the body, with only very low levels in some organs and in the residual carcass.

Terminal half-life of radioactivity (^{14}C) in whole blood was no greater than approximately 12 hours. This may be compared with a half-life of the parent material in human blood at 37ºC of 4.9 seconds.

Excretion was also monitored over seven days following single high dose oral administration to rats. High dose levels were associated with slower rates of excretion in urine and faeces, and significantly greater proportions of radioactivity in the faeces, with unchanged folpet as the major radioactive amount.

The trichloromethylthio moiety is the reactive site of the folpet molecule. This side chain generates thiophosgene both via hydrolysis and its rapid reaction with thiols. Thiophosgene is both conjugated with the

cysteine moiety of glutathione (GSH) and excreted as thiazolidine and mineralised to CO_2, HCl and H_2S. Its half-life in human blood at 37°C is less than 1 second. In addition, disulphonic acid is detected in the urine. The changes in GSH levels after exposure to folpet reflect this relationship. Initial bolus administration of folpet results in a transient depletion of GSH that is rapidly followed by a rebound. With dietary administration, GSH levels are maintained at a high level showing a continued homeostatic response to the Folpet-triggered depletion.

Removal of the side-chain by hydrolysis or by detoxification mechanisms yields phthalimide, which is further metabolised to phthalamic acid, which may be converted to phthalic acid. Derivatives of phthalimide are excreted rapidly and extensively.

Folpet and its metabolites do not show any potential for accumulation.

Further, results of the *in vitro* metabolism study show that folpet degrades rapidly and extensively into its main degradation products. Phthalimide, phthalamic acid and phthalic acid were detected in both human and rat liver microsomal incubations performed with and without NADPH-regenerating system. Consequently, the results of these studies suggest that the degradation of folpet observed in human and rat liver microsomes was non-enzymatic. No relevant differences were observed between humans and rats.

There are also toxicokinetic publications in human available, which seem to indicate that absorption in human is substantially lower than in rats, however, they focused on phthalimide absorption and only in part on other subsequent metabolites, which may bias this assessment. Phthalimide and subsequent metabolites are also common metabolites of other pesticides and pharmaceuticals.

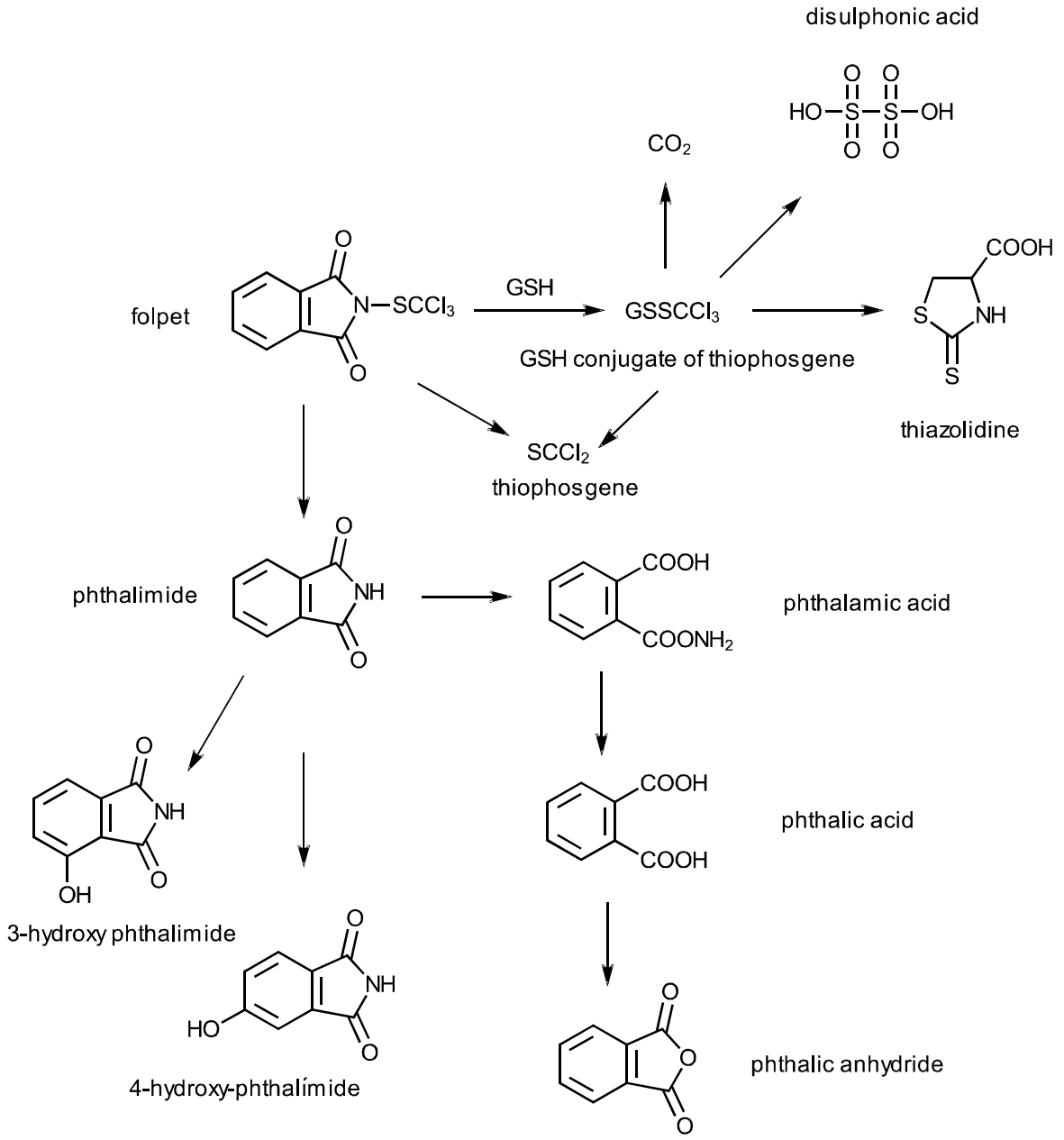


Figure 2: Generalised metabolic pathway for folpet in rodents following oral administration

Table 9: Summary table of toxicokinetic *in vivo* studies

Method; Deviations from OECD 417 (2010)	**Results**	**Reference (Company reference number)**
None stated, 5 SD rats/group/sex, treated with a single dose of 10 mg/kg bw or over 15 days or a single dose of 500 mg/kg bw [U-phenyl-^{14}C] folpet Deviations - Animals acclimatised for minimum of 2 days, compared to 5 days recommended in guideline - housing conditions were not reported	Single dose 10 mg/kg: Oral absorption >90%, predominantly excreted in the urine as phthalamic acid. At 5 days after dosing the dose was essentially cleared from the body. 15 days 10 mg/kg bw, similar values, no induction of metabolism or accumulation Single dose 500 mg/kg:	(R-5544) Study 1 (1991)

- a blood sample was solely taken at the end of the study period - tissue distribution was measured too late (i.e. due to the short half-life of folpet the substance might have been eliminated before study termination) - no bile cannulation or iv administration - toxicokinetic parameters were not calculated [e.g. Cmax, Tmax, half-life (t1/2), clearance, AUC] - a figure with the proposed metabolic pathway is not included in the study report	oral absorption ~60% and slower excretion. Residue in faeces was predominantly parent folpet (> 90%). Low levels of phthalimide and the initial ring degradant were also present (5-8%). Since no radioactivity was observed in a pilot study with one male and one female rat after a single dose of 10 mg/kg, air was not investigated in the main study.	
Not stated, four female SD rats treated with 15 mg [Carbonyl-^{14}C] folpet /kg bw and one control in metabolism cages. Deviations - only one dose level was administered - only one animal/ time point for tissue distribution - source of animals, acclimatisation period, feed and water supply housing conditions were not reported - expired air was not measured - a blood sample was solely taken at the end of the study period/ each animal - excretion in urine and faeces was combined - no bile cannulation or iv administration - toxicokinetic parameters were not calculated [e.g. Cmax, Tmax, half-life (t1/2), clearance, AUC] - recovery is not reported	Folpet was rapidly absorbed and metabolised. 24 hours after treatment, approximately 95% of the dose was excreted, mainly via urine. In the urine, approximately 80% was identified as phthalamic acid and 10% as phthalimide. Phthalamic acid and phthalimide were also the major metabolites found in faeces and tissues. Phthalic acid, 3-hydroxyphthalimide and 4-hydroxyphthalimide were minor metabolites.	(R-5441) Study 2 (1980)
None stated Adult Carworth CFY rats (SD-derived, 3/sex/group) Single 75 mg [carbonyl-^{14}C] folpet/kg bw Repeated 75 mg non-labelled folpet/kg/day for 7 days, 75 mg [carbonyl-^{14}C] folpet/kg on day 8, Groups of two rats per sex were sacrificed at 30 minutes, 1 day, 3 days and 8 days after radioactive dosing Supplementary experiment: Single 15 mg [^{14}C]-phthalimide /kg bw (2/sex) Deviations - (radiochemical) purity of folpet is not reported - only one dose group was administered - no information regarding the age or acclimatisation period of the animals - only 2 animals/ sex/ dose for the different time points of tissue distribution - only 3 animals/ sex/ dose for the different time points for blood sampling - tissue distribution was not measured in	Rapid, but not complete, absorption. Excretion was rapid and quantitative, most excreted within 24 hours in urine. Terminal $t_{1/2}$ < 12 hours in whole blood. Urine No parent recovered; major metabolite tentatively identified as an N-substituted phthalamic acid. Excretion pattern similar in animals given [^{14}C]-phthalimide. No accumulation, pre-treatment resulted in slightly different initial blood radioactivity concentration/time relationship, although the similarity of the terminal elimination phases indicates that repeated exposure does not influence folpet metabolism at the dosage given.	(R-5440) Study 3 (1974)

spleen - no bile cannulation or iv administration - toxicokinetic parameters were not calculated [e.g. Cmax, Tmax, clearance, AUC] - main metabolite was not clearly identified - individual values not reported for tissue distribution (except carcass) - no proposed metabolic pathway is included in the study report		
Not stated, extensive mode of action study to assess comparative metabolic fate and biochemical effects in male rats and mice Male SD rats and CD-1 mice received 50 and 5000 ppm folpet via diet for 21 days. In total 258 rats and 509 mice in various study cohorts. Deviations - tissue distribution was not measured in fat and spleen - expired air was not measured in the studies where bile was examined - a blood sample was solely taken at the end of the study period in rats - toxicokinetic parameters were not calculated [e.g. Cmax, Tmax, half-life (t1/2), clearance, AUC] - metabolites were not clearly identified	Glutathione and glutathione S-transferase are involved in the detoxification of the trichloromethylthio side-chain to thiazolidine and disulphonic acid, which are excreted in the urine. A proportion is metabolised fully to give CO_2, but the majority is conjugated and excreted in the urine. Biliary excretion appears to play only a minor part in elimination. Folpet administration was associated with generally increased glutathione and glutathione S-transferase activity, particularly in the duodenum and jejunum. In the mouse and to a lesser extend in the rat, pulse dose high levels of folpet were associated with short-term depletion of glutathione. Mice had a greater folpet intake than rats. Mice further relied more than rats on glutathione for detoxification of folpet, therefore glutathione supply in the mouse may be inadequate to deal with such high doses, which may explain the results observed for mice in the chronic/carcinogenicity studies, which are not observed in rats. A biochemical threshold in the defensive capability of glutathione and its associated glutathione S-transferase might exist which is exceeded in the target tissue in the overexposed mouse. Therefore, high concentrations of the reactive metabolites of Folpet cannot be detoxified and might cause local effects in target tissues of the mouse	(R-5232) Study 4 (1991)

Table 10: Summary table of toxicokinetic *in vitro* studies

Method	**Results**	**Reference (Company reference number)**
Comparative *in vitro* metabolism using human and rat liver microsomes, no OECD TG available. Incubations using 10 µM Phenyl-ring-U-[^{14}C]-folpet for 5 and 240 minutes with a microsomal protein concentration of 1.0 mg/mL	Folpet transformation predominantly by N-S cleavage, hydrolysis and protein reactivity. Biological metabolism negligible even at the longest incubation time compatible with functionality of the test system. Reactions in rat and human liver microsomes were similar and no unique human metabolite was observed.	(R-34967) Study 6 (2015)

Method	Results	Reference (Company reference number)
Comparative *in vitro* metabolism using human and rat liver microsomes, no OECD TG available. Incubations using 2 µM Phenyl-ring-U-[^{14}C]-folpet for 120 minutes with a microsomal protein concentration of 0.5 mg/mL	Folpet transformation predominantly non-enzymatic. However, degradation of folpet and production of one peak (#5) was enhanced by the presence of microsomes in the incubation system. No relevant differences were observed between humans and rats.	(000107203) Study 7 (2015)
[U-phenyl -^{14}C] Folpet stability in whole human blood, no OECD TG available.	Folpet degrades rapidly in whole human blood at 37ºC to phthalimide with a half-life of 4.9 seconds. No other short term degradants or intermediates were present, and there was no significant degradation in saline at 37ºC.	(R-11143) Study 8 (1999)
Thiophosgene stability in whole human blood, no OECD TG available.	Thiophosgene disappears rapidly when added in excess (100 µg/mL) to human whole blood *in vitro* at 37ºC. The half-life was calculated to be 0.6 seconds.	(R-17121) Study 9 (2004)

Table 11: Summary table of other toxicokinetic studies

Method	Results	Reference
No guideline cited Human volunteer study (n=5), 1 mg/kg bw oral	Folpet metabolites, phthalimide and phthalic acid, are rapidly and almost completely excreted over a 96 h period post-treatment. Phthalimide T_{max} on average 6 h post-ingestion in plasma $t_{1/2}$ in the order ~30 h. Negligible accumulation of phthalimide, only relatively small volumes of distribution (Vd), suggesting phthalimide remains mainly in the circulation. Furthermore, although the time courses of phthalimide and phthalic acid evolved in parallel, phthalimide represented only a small fraction of folpet dose and only 0.03% of the folpet dose was recovered in urine as phthalimide while 25% of the folpet dose was excreted in urine as phthalic acid over the 96 h period post-dosing. This is consistent with a rapid site-of-entry biotransformation of phthalimide into phthalamic acid and phthalic acid once formed, thus limiting the amounts of phthalimide available for absorption in blood. It also shows that the acids formed in the GI-tract following oral exposure are effectively absorbed. Folpet appears to be similarly metabolized in humans and rats.	Berthet et al. 2012a
No guideline cited Human volunteer study (n=4), 10 mg/kg bw dermal	Folpet metabolites, phthalimide and phthalic acid, are rapidly and almost completely excreted over a 96 h period post-treatment. Folpet ring metabolism T_{max} on average 10 h post-ingestion in plasma $t_{1/2}$ in the order ~30 h. The percentage of folpet dose recovered in urine as	Berthet et al. 2012b

Method	Results	Reference
	phthalimide and phthalic acid was 10- and 14-fold lower, respectively, following dermal exposure than after oral administration (on average 0.002 vs 0.02% for phthalimide and 1.8 vs 25% for phthalic acid), indicating a low dermal absorption fraction. NB using a 10x higher dose. Phthalimide and phthalic acid, exhibited similar time profiles, indicating that they were governed by the same essential biological processes. However, phthalic acid was found to be present in much higher amounts than phthalimide in urine.	
Male wistar rats were exposed with 10 mg folpet/kg bw formulated as Folpet 80 WG) by intraperitoneal injection or by intratracheal instillation Supplementary information (reliable with restrictions)	No folpet was detected in plasma either after intraperitoneal or intratracheal instillation. Phthalimide T_{max} = 0.25 h, $t_{1/2}$ = 2.2-2.6 h Phthalamic acid T_{max} = 0.25 h, $t_{1/2}$ = 4.7-4.97 h Phthalamic acid was the main degradation product for both administration routes. After intratracheal administration the C_{max} of phthalamic acid was 5.6 fold higher than for phthalimide and the AUC was 9.7 fold higher. At 24 h after administration phthalimide plasma concentrations were below 0.5 ng/mL (LOD) and phthalamic acid concentrations were close to the limit of quantification (25 ng/mL).	Canal-Raffin et al. 2008

Table 12: Summary table of toxicokinetic studies relating to dermal absorption

Method	Results	Reference
US EPA 85-2 Deviations - Age of the animals was not reported - Light/ dark cycle was extended for an extra 2.5 hours light on day one (for groups 1 and 2) and for extra 3 hours on day 4 (for groups 3 and 4) - Body weight variations exceeded 20% of the mean body weight - Recovery in carcass only 64.8% - Level in the carcass might be contaminated as the washing solution flowed from the test site over some of the untreated skin - High variation in the samples from the same group for liquid scintillation - Recovery was not reported (however, it was stated that all the values were corrected for 100%) Supplementary information (reliable with restrictions)	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. Therefore, the amount absorbed might be overestimated. Furthermore, no tape stripping was performed. 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.	Study 5 (1990) (R-5470)
No guideline stated, *in vivo* rat study Supplementary information	Percutaneous penetration of [trichloromethyl-^{14}C] folpet in the skin of rats showed a decreasing proportional absorption with increasing dose and there were no effects of age on skin penetration.	Shah et al. 1987

Method	Results	Reference
(reliable with restrictions)	On the one hand no washing or tape stripping was performed, which might overestimate dermal exposure. On the other hand, the occlusive dressing was glued, and some absorbed amount might be lost by removing the dressing.	
Position statement Supplementary information (only relevant in relationship to Study 5 and Shah et al. 1987)	Dermal absorption properties of folpet are reviewed with respect to the relevance of the dermal route to systemic exposure in occupational risk assessment. It is concluded that the dermal route is not relevant in occupational exposure scenarios. Please note that reliable *in vitro* studies in human skin were conducted for risk assessment of folpet products. The results were 0.1% for the concentrate and 9% for spry dilution of Folpan 80 WDG and 0.3% for the concentrate and 2% for spry dilution of Folpet 80 WG, respectively.	Study 10 (2005)

8.1 Short summary and overall relevance of the provided toxicokinetic information on the proposed classification(s)

The available studies consistently show that the systemic compartment is not exposed to folpet or its degradation product thiophosgene, but towards its metabolites phthalimide, phthalamic acid and to a lesser extent phthalic acid.

In vivo studies

There are four *in vivo* studies in rat and one of those together with mice available, none of which are fully compliant with OECD 417 (2010) as they predate the guideline. However, the results are consistent between studies and thus independently replicated. The results also complement each other, e.g. in Study 1 (1991) potentially exhaled radioactivity was only captured in a pre-test and not the main study, however, a dedicated cohort was used in Study 3 (1974). Study 4 (1991) is an extensive study to assess comparative metabolic fate and biochemical effects in male rats and mice. The results of Study 4 (1991) together with the acute local irritation effects observed in Sections 8.1-8.7, repeated exposure studies and dedicated mode-of action investigations explain why small intestinal tumours occur in mice but not in rats, see Section 8.9 (Carcinogenicity).

Study 1 (1991) investigated the metabolic fate of [U-phenyl-^{14}C] folpet in male and female CD (Sprague Dawley) rats (age: 6-8 weeks males, 8-10 weeks females) (weight: 200-250 g males, 175-225 g females). In a pilot study two rats (one male and one female) were given single oral dose of 10 mg/kg ^{14}C-folpet in 1% aqueous sodium carboxymethylcellulose and observed for up to 120 hours to determine if radioactivity was detectable in expired air, urine or faeces. Since no radioactivity was observed in air, it was not investigated in the main study. In the main study groups of 10 rats (5 male and 5 female) received [U-phenyl-^{14}C] folpet (radiopurity: 97-99%), adjusted with non-radiolabelled folpet to 29230 dpm/µg (for single and repeat dose studies at 10 mg/kg) and to 551.6 dpm/µg (for single dose study at 500 mg/kg. The nominal volumes were 5 mL/kg (10 mg/kg) or 10 mL/kg (500 mg/kg), as a fine suspension in aqueous 1 % (w/v) sodium carboxymethylcellulose, respectively. Orally administered [U-phenyl-^{14}C] folpet was well absorbed (> 90%) at doses of 10 mg/kg, and predominantly excreted in the urine as phthalamic acid. At 5 days after dosing the dose was essentially cleared from the body. Repeat dosing for 15 days at 10 mg/kg bw/day showed similar values to the single dose, indicating that induction of physiologic processes (e.g., enzymes) that affect metabolism of folpet is not occurring. Folpet or its imide ring degradants do not accumulate. There were no differences between the sexes. A single dose at 500 mg/kg was much less well absorbed (about 60%), and the rate of excretion in both urine and faeces was slower than in the low dose. In these high dose rats, the radioactivity present in the faeces was predominantly parent folpet (> 90%). Low levels of phthalimide, the initial ring degradant, was also present (5-8%).

Study 2 (1980) investigated [Carbonyl-^{14}C] folpet metabolism in four female Sprague Dawley rat (weight: 168-185 g) which were dosed orally with 2.7 mg [carbonyl-^{14}C] folpet (purity: 99.8%) in 0.5 ml acetone by a stomach tube, equivalent to approximately 15 mg/kg bw. One untreated female rat served as control. Folpet was rapidly absorbed and metabolised. 24 hours after treatment, approximately 95% of the dose was excreted, mainly all via urine. In the urine, approximately 80% was identified as phthalamic acid and 10% as phthalimide. Phthalamic acid and phthalimide were also the major metabolites found in faeces and tissues. Phthalic acid, 3-hydroxyphthalimide and 4-hydroxyphthalimide were minor metabolites.

Study 3 (1974) investigated [Carbonyl-^{14}C] folpet in adult Carworth CFY rats (SD-derived, bodyweight: 200 ± 10 g). In a repeat-dose phase, ten rats/sex were dosed orally by gavage with non-radiolabelled folpet at 75 mg/kg/day in 10 ml corn oil for seven days. On the eighth day, the rats were dosed by oral gavage with [carbonyl-^{14}C] folpet (11.2, Ci/mmol, purity >99%) at 75 mg/kg. After dosing with radiolabelled material, animals were housed individual in metabolism cages that allowed collection of expired air and excreta. Groups of two rats per sex were sacrificed at 30 minutes, 1 day, 3 days and 8 days after radioactive dosing. For assessment of whole-blood levels, three rats of each sex were given a single oral gavage dose of 75 mg/kg radiolabelled formulation. Blood samples were taken from the tail vein into heparinised tubes at 30 minutes, 45 minutes, 1, 2, 3, 4, 6 and 24 hours after dosing. A further three rats per sex were dosed orally by gavage with non-radiolabelled folpet at 75 mg/kg/day for seven days. On the eighth day, the rats were dosed with radiolabelled folpet at 75 mg/kg. Blood samples were taken from the tail vein as described above. For assessment of tissue distribution, six male rats were dosed orally by gavage with non-radiolabelled folpet at 75 mg/kg/day for seven days. On the eighth day, the rats were dosed with radiolabelled folpet at 75 mg/kg and sacrificed at 30 minutes, 1 day, 3 days and 8 days after radiolabelled dose. In a supplementary experiment two rats (m+f) were administered a single dose of 15 mg/kg [^{14}C]-phthalimide. Rats showed rapid, but not complete, absorption. Excretion was rapid and quantitative, with most of the radioactivity being excreted in the urine within 24 hours. Terminal half-life in whole blood was no greater than approximately 12 hours. No unchanged parent material was excreted in the urine. The major urinary metabolite was tentatively identified as an N-substituted phthalamic acid. Excretion was similar in animals given 15 mg/kg [^{14}C]-phthalimide. There were no indications that folpet or its metabolites would accumulate in tissues. Pre-treatment with non-radiolabelled folpet for seven days prior to administration of the radiolabelled dose resulted in slightly different initial blood radioactivity concentration-time relationships, although the similarity of the terminal elimination phases indicates that repeated exposure does not influence folpet metabolism at the dosage given.

Study 4 (1991) needs to be understood as being part of an extensive mode of action study set to explore the aetiology of small intestinal tumours in mice, see Section 8.9. In particular, it aims to elucidate why such tumours occur in mice but not in rats; the hypothesis was potential differences in the metabolic fate and biochemical effects. For this, 258 SD-rats and 509 CD-1 mice (male) were allocated in different study cohorts and treated with 0, 50 and 5000 ppm folpet via diet for 21 days, i.e. a non-toxic effect level and a level associated with intestinal tumour formation in the mouse. At about the same time (+ 30 minutes) on the morning of Day 21, groups of animals were killed and gastro-intestinal and liver samples taken for measurements of certain biochemical and physiological parameters. Other animals received a pulse dose, by gavage, of [TCM-^{14}C] folpet (10 - 20% of the dietary dose) and were used in certain absorption, distribution, metabolism and excretion studies. Dietary doses of folpet were well tolerated and no treatment-related clinical signs or mortalities were detected. Mice consumed more diet than rats; consequently the dose level of folpet to mice was about 7 and 700 mg/kg respectively (50 and 5000 ppm) whereas that to rats was about 3 and 300 mg/kg respectively (50 and 5000 ppm). There was a marginal lowering of liver weight in animals receiving 5000 ppm, but a notable increase in gastro-intestinal mucosal tissue weight, as much as about 150% of control values. This latter increase is consistent with, and presumably reflects, the increase in upper intestinal tissue protein content that also occurred. *NB this weight increase could also reflect inflammation, which is seen in repeated dose studies, but was not investigated in detail here; no histopathology was performed in Study 4 (1991).* Together, GSH is involved at least in part with the degradation (bioactivation, toxication owing to thiophosgene formation) and detoxication (GSH conjugation, CO_2 formation) of folpet in the rat and mouse. Dietary folpet caused an increase in GSH levels and GSH S-transferase activity in the gastro-intestinal tract, particularly at sites of tumour formation (in the mouse). These increases were greater in the mouse than in the rat. As indicated by the lack of effect of folpet on liver GSH or GSH S-transferase activity, folpet or its reactive metabolite(s) did not reach the hepatoportal system to any extent. There was some evidence that GSH supply was insufficient to deal with high doses of folpet, as indicated by increased “covalent” binding in the gastro-

intestinal tract. The significance of this binding is unclear at this time. These studies suggest that reasons why folpet is tumorigenic in mouse upper gastro-intestinal tract but not in that of the rat could be due (i) to a much greater intake of folpet in the mouse, and consequent greater local target tissue exposure to reactive metabolite(s) of folpet that the mouse cannot detoxify, which passes the threshold required for tumorigenicity, (ii) to greater local effects on mouse target tissue in the upper gastro-intestinal tract as evidenced by the greater response in the measured biochemical parameters most notably to the higher dose level of folpet in mice and (iii) to a greater reliance by the mouse than the rat on GSH for the detoxification of folpet as evidenced by differences in the biochemical responses to folpet and in the metabolism of high doses of folpet. Support for this view is provided by the greater depletion of GSH in the mouse than in the rat, especially from the duodenum, when given single oral gavage doses of folpet. GSH supply in the mouse may be inadequate to deal with such high doses: i.e. a "GSH threshold" may exist which has been exceeded in the mouse. Furthermore, the excess local concentrations of GSH produced may upset cellular redox balance leading to tumour formation in the mouse by an epigenetic mechanism.

In vitro studies

There are four *in vitro* studies available. The comparative *in vitro* metabolism studies using rat and human liver microsomes show similar metabolite spectra between species. There is currently no internationally-accepted OECD test guideline available for such studies, however, the two studies, which used different experimental parameters, essentially show the same results. The *in vitro*/*ex situ* studies using human blood indicate that even if there is systemic exposure of folpet or thiophosgene, their systemic irritative properties would be very rapidly sequestered.

Peer-reviewed literature

The available human volunteer studies (Berthet et al. 2012a, 2012b) show that the human metabolism is essentially similar to that in rat, with only folpet metabolites, phthalimide and phthalic acid recovered in urine and no indication of accumulation. The studies predict also that the achieved systemic exposure is substantially less via dermal than oral route, i.e. <10% with a ten-fold higher dose of 10 mg/kg bw as compared to 1 mg/kg bw oral, which is relevant as this represents the typical exposure scenario for this chemical. The used single dermal dose was also higher than the NOAEL of 1 mg/kg bw/day for local dermal effects in rat upon repeated 28-day exposure, see Section 8.12.

Canal-Raffin et al. (2008) show that the systemic metabolites after intraperitoneal injection or intratracheal instillation are similar to those after oral exposure in rats.

Dermal absorption (supplementary information)

The available *in vitro* dermal absorption studies conducted with folpet show large fractions of [U-phenyl-^{14}C] folpet doses of 0.0064-4.8 mg/rat are absorbed, as radioactivity is recovered in the carcass. However, there was evidence that carcass levels were due to radioactivity seeping from the treated area during washing. Therefore, the amount absorbed might be overestimated. Furthermore, no tape stripping was performed. 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. Shah et al. 1987 demonstrate that very low fractions <<1% of radioactivity is recovered from the carcass in rats treated with [trichloromethyl-^{14}C] folpet. Study 10, 2005 reviewed these results and concluded that no intact folpet penetrates into the systemic compartment, hence the dermal route is not relevant for occupational exposure scenarios.

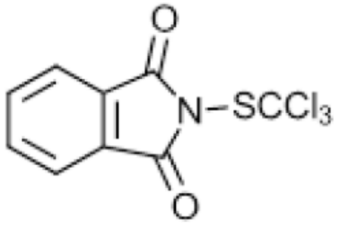
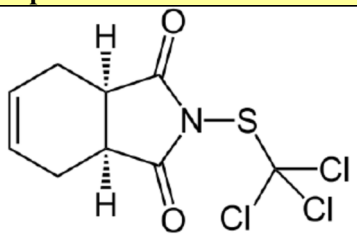
Overall conclusion with respect to toxicity

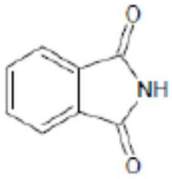
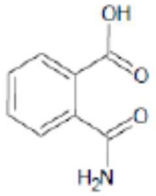
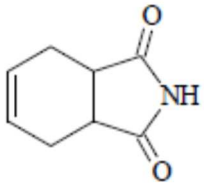
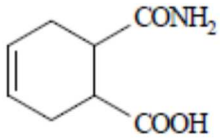
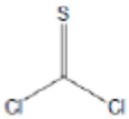
Together, folpet's metabolism is driven by rapid non-enzymatic reactions in rat and human. There is no evidence for systemic exposure towards the intact parent molecule, which incorporates the reactive toxicophore, i.e. the trichloromethylthio side-chain. Accordingly, irritative effects are expected to occur before entering the systemic compartment at the site of first exposure. Further, any observed systemic effects occur either subsequent to primary irritative effects, which may include effects associated with feeding if animals were exposed orally, are spurious or are facilitated by folpet's systemic metabolites. Intact folpet does not further appear to reach systemic circulation after dermal exposure.

8.2 Comparison folpet and captan

Folpet and captan belong to the group of phthalimide fungicides. They both share the same toxicophore (i.e. trichloromethylthio-side chain), which is responsible for the irritating properties. These irritating properties are claimed to be responsible for several hazard classes (i.e. acute Tox, Skin and Eye Irrit., Carc and STOT-RE). In the table below similarities are listed that support a read-across between these substances.

Table 13: Comparison folpet and captan

	Folpet	**Captan**
Chemical structure		
Proposed classification	Carc. 2 Acute Tox 2 (inhalation) STOT RE 1 Skin Irrit. 2 Eye Dam. 1 Skin Sens. 1A Aquatic Acute 1 Aquatic chronic 1	Carc. 2 Acute Tox. 2 (inhalation) STOT RE 1 Eye Dam. 1 Skin Sens. 1A Aquatic Acute 1 Aquatic Chronic 1
Absorption	> 80% (based on urinary excretion within 48 h)	> 80% (based on urinary excretion within 48 h)
Distribution	Widely distributed following initial absorption, but tissue residues negligible because of rapid excretion	Widely distributed following initial absorption, but tissue residues negligible because of rapid excretion
Metabolism	The trichloromethylthio (TCM) side chain generates thiophosgene via hydrolysis and its rapid reaction with thiols. Thiophosgene is conjugated with glutathione (GSH) and excreted as thiazolidine and mineralised to CO_2, HCl and H_2S. Removal of the side-chain by hydrolysis or by detoxification mechanisms yields phthalimide (10% in urine), which is further metabolised to phthalamic acid (80% in urine), which may be converted to phthalic acid.	Metabolic cleavage of nitrogen-sulphur bond resulting in thiophosgene and THPI (1,2,3,6-tetrahydrophthalimide; 11% in urine) occurs in GI tract prior absorption. Thiophosgene is conjugated with glutathione (GSH) and excreted as thiazolidine and mineralised to CO_2, HCl and H_2S. Hydroxylation of THPI resulting in 3-OH-THPI (42% in urine) or 5-OH-THPI (6% in urine), or metabolism of THPI to THPI-epoxide (5% in urine) (and further to the diol- 6% in urine) or THPAM (*cis/trans*-6-carbamoyl-3-cyclohexene-1 carboxylic acid) (7% in urine) (through opening of cyclohexene ring).
Excretion	Rapid and extensive (> 95 % within 48 h), mainly via urine (90 % within 24 h, 5 % via faeces within 48 h). No study measuring CO_2 traps is available with folpet.	Rapid and extensive (app. 95 % within 48 h); ring-labelled captan is excreted mainly via urine (75% within 24 h, 5% via faeces), trichloromethyl-labelled captan is also excreted via the pulmonary route as CO_2 (up to 25%; 40-50% via urine, up to 20% via faeces)

	Folpet	Captan
Similar metabolites	Phthalimide:  Phthalamic acid: 	THPI:  THPAM: 
Toxicophore	The trichloromethylthio (TCM) side chain which generates thiophosgene: 	

9 EVALUATION OF HEALTH HAZARDS

Acute toxicity

The predominant toxic effect of folpet in the acute studies is local irritation at the site of first contact, which is directly related to its fungicidal mode of action. The irritation appears to vary in severity based on the exposure route. It is not reported in the acute oral studies, however, as no mortalities occurred no extensive necropsy or histopathology investigations were conducted. Limited irritation is observed upon acute dermal exposure, however, irritation occurs when the *stratum corneum* is bypassed by intradermal injection, as observed in the skin sensitisation studies. The assays reported severe skin damage (eschar, necrosis) and prior to that dermal reactions immediately after topical induction, with a clear dose-response relationship and which decreased when the exposure stopped. Severe irritation is also observed in a 28-day repeated exposure dermal toxicity study using an organic solvent from the first observation time point on day 2. The skin effects were so severe that the high dose was first decreased, and then treatment stopped after 13 days.

Hence, irritation potency is driven not primarily by exposure route but by the duration of time the respective epithelia were in contact with folpet, which is plausible according to the rapid reaction and degradation observed in the kinetic studies. A bolus gavage dose shows relatively less irritation in the exposed epithelia than continuous 4 hour inhalation exposure. In the respiratory tract, rapidly degraded folpet is replenished by newly inhaled and deposited material, and this leads to the reported mortalities due to oedema. Irritative effects are also observed in the repeated dose and chronic studies with oral exposure, where the tissues' exposure time is also increased. The data consistently shows that underlying toxic effect has always the same aetiology – site of contact irritation.

If not stated otherwise in the summary tables, the studies are considered as fully reliable.

9.1 Acute toxicity - oral route

Two acute oral toxicity studies in rat are available for folpet. Both report an LD_{50} > 2000 mg/kg bw.

Table 14: Summary table of animal studies on acute oral toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels, duration of exposure	Value LD_{50}	Reference
Not reported (protocol no. 1.51). Method	Rat, Sprague Dawley, 5/group, 10/group in	Folpet Technical, Purity not	5000, 6500, 8500, 11200, 14800, 20000 or 26300	M: 19500 mg/kg F: 43800 mg/kg	(R-7902) Study 1 (1983)

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels, duration of exposure	Value LD_{50}	Reference
was similar to OECD 401 (1981). Deviations from OECD 401 (1987): - Dose volume exceeded 2 ml/ 100g - More than 3 doses examined - 10 animals (5/sex) per dose	control	reported	mg/kg		
OECD 401 (1987), no deviations	Rat, Sprague Dawley, 5/group	Folpet tech., Batch No 930375, Purity: no data	2000 mg/kg bw	>2000 mg/kg bw	(R-6510) Study 2 (1992)

9.1.1 Short summary and overall relevance of the provided information on acute oral toxicity

Two acute oral toxicity studies are available for folpet, which report similar toxicity.

In Study 1 (1983), groups of five, fasted, 8-13 week old, Sprague Dawley rats per sex were given a single oral dose, by gavage, of folpet Technical in 1% carboxymethyl cellulose in distilled water at doses of 5000, 6500, 8500, 11200, 14800, 20000 or 26300 mg/kg bodyweight. A group of ten rats per sex were given a single oral dose, by gavage, of 1% carboxymethyl cellulose in distilled water, and served as the controls.

There were no mortalities in the control group or at 5000 mg/kg. There were 0/5, 0/5, 1/5, 0/5, 3/5 and 1/5, and 1/5, 0/5, 2/5, 3/5, 3/5 and 2/5 mortalities in males/females at doses of 6500, 8500, 11200, 14800, 20000 and 26300 mg/kg, respectively. Signs of toxicity observed during the study that were attributed to treatment with the test material were: decreased motor activity, reduced food intake, weakness, ocular discharge, nasal discharge, dyspnoea, scruffiness, discoloured fur, chewed feet and toes, collapse, and death. The mean body weight of the males dosed at 6500 mg/kg was significantly less ($p<0.01$) than that of controls at 7 and 14 days after dosing. There were no other significant differences in mean body weights between groups. At necropsy, slightly grainy livers and kidneys were observed in some animals. Histopathological examination of these tissues revealed no microscopic changes that could be attributed to treatment with the test material.

The acute oral median LD_{50} of folpet technical was 19500 mg/kg bw in females and 43800 mg/kg bw in males.

In Study 2 (1992), five male and five female rats were dosed orally, by gavage, at a level of 2000 mg/kg bw as a suspension in distilled water (dose volume: 10 ml/kg). Signs of toxicity and body weights were recorded up to 14 days after dosing. Gross pathological examinations were performed on all main study animals. No deaths and no clinical observations were recorded during the study. All animals showed expected gain in bodyweight throughout the study. No abnormalities were detected in any animal at necropsy. The acute oral median lethal dose (LD_{50}) of folpet technical was greater than 2000 mg/kg body weight.

9.1.2 Comparison with the CLP criteria

According to the criteria shown in the Table 3.1.1 of Annex I, Part 3 of CLP, substances can be allocated to one of four toxicity categories based on acute toxicity by the oral route. In general, classification is based on

the lowest ATE value available i.e. the lowest ATE in the most sensitive appropriate species tested. Acute toxicity values are expressed as approximate LD_{50} values (oral) or as acute toxicity estimates (ATE):

Acute oral toxicity - Category 4: 300 < ATE ≤ 2 000 mg/kg bw.

Since the LD_{50} values of all studies exceed 2000 mg/kg bw, folpet does not have to be classified for acute oral toxicity.

9.1.3 Conclusion on classification and labelling for acute oral toxicity

Folpet is proposed to be not classified for acute oral toxicity according to the CLP classification criteria.

9.2 Acute toxicity - dermal route

There are two acute dermal toxicity studies available for folpet, conducted with rabbits and rats. No study reported any mortalities. In the rabbit but not in rat, which was treated with a lower dose, treatment was associated with some local skin effects.

Table 15: Summary table of animal studies on acute dermal toxicity

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance,**	**Dose levels duration of exposure**	**Value LD_{50}**	**Reference**
Not given in the study but similar to OECD 402 (1981), No deviations but no LD_{50} value was determined in the study	New-Zealand White rabbits, 5/group/sex	Folpet tech, no purity data	5000 mg/kg bw	> 5000 mg/kg bw	(R-6139) Study 1 (1982)
OECD 402 (1981), no deviations	Sprague-Dawley rats, 5/group/sex	Folpet tech, no purity data	2000 mg/kg bw	> 2000 mg/kg bw	(R-6509) Study 2 (1991)

9.2.1 Short summary and overall relevance of the provided information on acute dermal toxicity

In Study 1 (1982), five male and five female rabbits with clipped fur and abraded skin were given a single dermal application of 5.0 g/kg bw of test material formulated with physiological saline (1:1 w/v) for 24 hours. Control animals (2 males, 2 females) were treated similarly with physiological saline. Application site was bandaged during the 24 hour application period and a collar was fitted for six days from the start of treatment. Signs of toxicity and body weights were recorded up to 14 days after dosing.

No deaths or clinical signs were observed during the study. There were no significant differences between body weights of treated and control groups. No gross pathological findings attributable to the test material were observed. Histopathological evaluation revealed mild hyperkeratosis (5 females, 1 male), mild non-suppurative dermatitis (4 females), mild acanthosis (1 female), diffuse hepatocellular vacuolisation (2 females) and diffuse chronic cholangitis (1 female). Diffuse hepatocellular vacuolisation (2 females) and diffuse hyperkeratosis (2 females, 1 male) was also seen in control animals.

The acute dermal median lethal dose (LD_{50}) of folpet technical in the rabbit was greater than 2000 mg/kg body weight.

In Study 2 (1991), undiluted test substance (moistened with distilled water) was applied to the shorn skin of five male and five female rats at a level of 2000 mg/kg body weight for a period of 24 hours. Application site was covered with a piece of surgical gauze and a semi-occlusive dressing during the 24 hour application period. Deaths, signs of toxicity and adverse dermal reactions were recorded for up to 14 days. Body weights were

recorded at intervals up to 14 days. All animals were given a macroscopic pathological examination at the end of the study.

No deaths, clinical signs or skin irritation were observed during the study. There were no significant differences between body weights of treated and control groups. No gross pathological findings attributable to the test material were observed.

The acute dermal median lethal dose (LD_{50}) of folpet technical in the rat was greater than 2000 mg/kg bw.

9.2.2 Comparison with the CLP criteria

According to the criteria shown in the Table 3.1.1 of Annex I, Part 3 of CLP, substances can be allocated to one of four toxicity categories based on acute toxicity by the dermal route. In general, classification is based on the lowest ATE value available i.e. the lowest ATE in the most sensitive appropriate species tested. Acute toxicity values are expressed as approximate LD_{50} values (dermal) or as acute toxicity estimates (ATE):

Acute dermal toxicity - Category 4: $1000 < ATE \leq 2\,000$ mg/kg bw

Since the LD_{50} values of all studies exceed 2000 mg/kg bw, Folpet does not have to be classified for acute dermal toxicity.

9.2.3 Conclusion on classification and labelling for acute dermal toxicity

Folpet is proposed to be not classified for acute dermal toxicity according to the CLP classification criteria.

9.3 Acute toxicity - inhalation route

Six acute inhalation toxicity studies are available for folpet, covering both whole-body and nose-only exposure. The studies report differences in observed toxicity, which seems to be associated with differences in achieved particle sizes, due to the use of different source materials. One study investigated the toxicity of non-micronized material. The studies report effects that are associated with exposure towards irritant particles, such as changes of the respiratory rate, laboured breathing, swollen lungs or increased lung weight and oedema.

Table 16: Summary table of animal studies on acute inhalation toxicity

Method, guideline, deviations from OECD 403 (2009) if any	**Species, strain, sex, no/group**	**Test substance, form and particle size (MMAD)**	**Dose levels, duration of exposure**	**Value** **LC_{50}**	**Reference**
GLP, Acute Inhalation – Rat US EPA OPP 81.3. Deviations from OECD 403 (2009): No justification of whole-body exposure, MMAD partly above recommended 1-4 µm	Rat, Sprague-Dawley, 5/sex/group	Folpet technical, 89.2%; MMAD 2.5-6.4 µm	0, 0.21, 0.53, 0.95, 1.49 mg/L for 4 hours Converted dose levels to 100% purity: 0, 0.19, 0.47, 0.85, 1.33 mg/L for 4 hours	M: 0.34 mg/L F: 1 mg/L Converted LC_{50} levels to 100% purity: M: 0.3 mg/L F: 0.89 mg/L	(000092041) Study 1 (1988)
AEPA, Proposed Guidelines for Registering Pesticides in the U.S., Part II, August 22, 1978	Rat, Sprague-Dawley-	Folpet technical, purity not stated MMAD (GSD) 2.4	0.64, 0.65, 0.67, 2.68, 3.61 mg/L for	M: 1.38 mg/L	(000039795) Study 2

Method, guideline, deviations from OECD 403 (2009) if any	Species, strain, sex, no/group	Test substance, form and particle size (MMAD)	Dose levels, duration of exposure	Value LC_{50}	Reference
Deviations from OECD 403 (2009): - No justification for whole body exposure - One exposure (No. 4) was run only for one hour and it was found that the concentration of test material in the chamber could not be controlled within the limits desired - MMAD: exceeded the recommended range at two low concentrations and exceeded the recommended geometric standard deviation (GSD), which indicates a multimodal distribution of particles Supplementary information (reliable with restrictions)	derived, 5/sex/group	(12), 2.3 (0.2), 5.3 (1.3), 2.0 (0.8), 3.0 (0.3) µm	4 hours	F: 1.30 mg/L	(1979)
GLP, OECD 403 (1981) Deviations from OECD 403 (2009): Room temperature was maintained at 16-21°C	Rat, Sprague-Dawley derived, 5/sex/group	Folpet technical, purity not stated MMAD: 1.7, 1.6, 1.8, 2.8 µm	0.14, 0.36, 1.06, 4.35 mg/L for 4 hours	M: 0.39 mg/L F: 0.43 mg/L	(000040833) Study 3 (1991)
GLP, EPA Guideline No. 83-1 (equivalent to OECD 403 (1981)) Deviations from OECD 403 (2009): Room temperature was maintained at 18-25°C	Rat, CD strain (Sprague-Dawley derived),	Folpet technical, 95.6% MMAD: 2.7-4.0 µm	M: 1.84, 2.14, 3.57, 4.35 mg/L F: 0.79, 1.11, 1.84, 2.14 mg/L for 4 hours	M: >4.35 mg/L F: 1.08 mg/L	(000041394) Study 4 (1993)
GLP, EPA Guideline No. 83-1 (equivalent to OECD 403 (1981)) Deviations from OECD 403 (2009): - Room temperature was maintained at 18-25°C - Only one concentration tested - MMAD 14.3 µm Supplementary information (reliable with restrictions)	Rat, CD strain (Sprague-Dawley derived), 5/sex	Folpet Technical (non-micronized), 98.99% MMAD 14.3 µm	2.14 mg/L for 4 hours	M: >2.14 mg/L F: >2.14 mg/L	(000041392) Study 5 (1993)
GLP, OECD 403 (1981) Deviations from OECD 403	Rat, CD strain	Folpet Technical, 98.99%	0, 0.8, 1.6, 1.99 mg/L for	M: 1.54 mg/L	(000009988) Study 6

Method, guideline, deviations from OECD 403 (2009) if any	Species, strain, sex, no/group	Test substance, form and particle size (MMAD)	Dose levels, duration of exposure	Value LC_{50}	Reference
(2009): - Particle size distribution was only measured once during the exposure period (instead of twice) - MMAD from 4.6-5.2 µm (recommended from 1-4 µm)	(Sprague-Dawley derived), 5/sex/group	MMAD: 4.6, 4.9, 5.2 µm	4 hours	F: 2.89 mg/L	(1993)

9.3.1 Short summary and overall relevance of the provided information on acute inhalation toxicity

Six acute inhalation toxicity studies are available for folpet, of which three are relevant for the acute inhalation toxicity classification; namely those with nose-only exposure and that are in the required test guideline particle size range, i.e. studies 3, 4 and to some extend 6, which had a slightly higher MMAD.

An overview of the observed mortalities is given in the following Figure. It shows both group mortalities, Mass Median Aerodynamic Diameter (MMAD) and probit fits per study, stratified by sex.

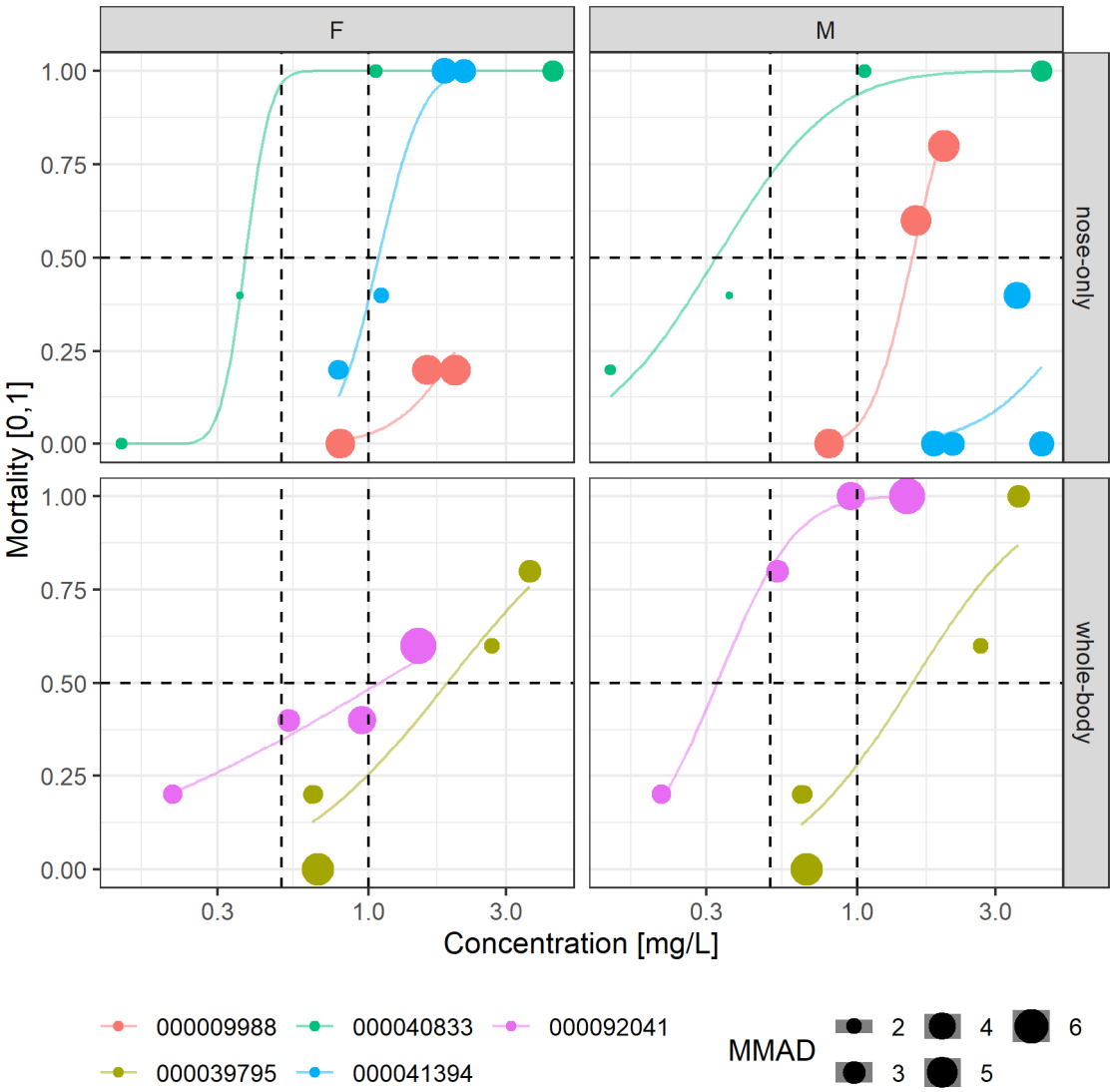


Figure 3: Overview of the observed mortalities, as a fraction between 0 (no mortality) and 1 (100% mortality in the group), in acute inhalation toxicity studies with folpet.

Figure 3 shows observed mortality in the acute inhalation toxicity studiers, MMAD and probit fits of nose-only (000009988 = Study 6 (1993); 000040833 = Study 3 (1991); 000041394 = Study 4 (1993) and whole-body (000039795 = Study 2(1979); 000092041 = Study 1(1988)) studies. Superimposed is 50% mortality and the classification concentration thresholds of 0.5 and 1 mg/L.

Studies with whole-body exposure

In Study 1 (1988), 5 rats/group/sex were exposed towards folpet concentrations of 0, 0.21, 0.53, 0.95, 1.49 mg/L for 4 hours in a whole-body exposure chamber, with a 14-day post exposure observation period. The average MMAD and GSD of the exposure dusts, based on gravimetric analysis, ranged from 2.5 to 6.4 µm and 2.3 to 2.6, respectively. The percent of dust smaller than 10 µm ranged from approximately 66 to 96 %. Possible compound-related macroscopic changes observed in the lung, trachea and liver were as follows: lung discolouration and/or discoloured foci (0.53, 0.95, and 1.49 mg/L); lung fluid (0.95 and 1.49 mg/L); tracheal fluid (0.95 mg/L males and 1.49 mg/L both sexes); and liver discolouration (0.53, 0.95, and 1.49 mg/L). There were compound-related lesions present in the lung and trachea of males and females at all exposure levels. Indirect compound-related lesions of the liver were present in some animals that died on study at all exposure levels. These lung lesions were typical of an acute response to an irritant at the highest exposure levels and a more chronic response to an irritant among the animals surviving longer and thus at the lower exposure levels.

The acute responses of the lung included fibrinous exudate in the alveoli, alveolar oedema, alveolar macrophages, interstitial oedema and alveolar haemorrhage. Chronic interstitial pneumonia was the most common response in those animals surviving longer. These lesions were not seen in all animals but there was a general dose-related trend in incidence and/or severity. Death of the exposed animals influenced the severity and interrupted the progression of the lesions from acute to chronic. A few other lesions were considered to be secondary to, or a progression of, the compound-related lesions described above and thus they were also compound-related even though their incidence was low. These included suppurative bronchopneumonia (one male at 0.53 mg/L), fibrosis (one female at 1.49 mg/L), and acute peribronchiolitis (one male and one female at 1.49 mg/L, and one male at 0.53 mg/L). Agonal congestion of the lung was present in most animals that died on study. Alveolar haemorrhage considered to be related to sacrifice procedures was present in one control female. Compound-related tracheal lesions were a typical acute response to an irritant and consisted of acute tracheitis in some animals of all exposure groups. A single animal had a trace lesion of chronic tracheitis. This was a typical spontaneous lesion for the rat and it was not considered to be compound-related. Lesions of the liver were considered to be spontaneous or agonal. The agonal lesions were an indirect compound-related effect. They included vacuolar change and necrosis, both centrilobular. The vacuolar change was typical hydropic change and was considered to be a result of anoxia associated with congestion and vascular stasis. The necrosis observed was an extension of the anoxic lesion. These lesions of the liver were more severe than those usually considered to be agonal, suggesting a prolonged vascular stasis and anoxia. The LC_{50} for males was 0.34 mg/L and 1 mg/L for females.

In Study 2 (1979), 5 rats/group/sex were exposed towards folpet concentrations of 0.64, 0.65, 0.67, 2.68 and 3.61 mg/L for 4 hours in a whole-body exposure chamber, with a 14-day post exposure observation period. The average MMAD ranged from 2.3 to 5.3 µm, but was about 3 µm in the highest exposure groups. All of the animals which died during these exposures did so within 4-5 days after the exposure especially at the lower concentrations of test substance. Signs of toxicity most often noted during the exposures included gasping, lacrimation, nasal discharge (in some instances these were bloody), dyspnoea and salivation. These signs continued, for the most part, in some animals during the first six hours post-exposure and for several days thereafter. Also often noted during the post-exposure period were short rapid respiration, piloerection and decreased locomotor activity. Gross pathology showed most of the animals which died had either gelatinous material or gas in the stomach, small and large intestines, caecum and colon. Many of the animals exposed to the higher concentrations of test substance (2.68 and 3.61 mg/L) also showed that the trachea and bronchi were filled with fluid and the lungs of most of the animals exposed at 3.61 mg/L were haemorrhagic and the thoracic cavities were filled with fluid. Also, the nasal passages of many of these animals (exposed at 3.61 mg/L) had white material which was apparently test material. The LC_{50} for males was 1.38 mg/L and 1.30 mg/L for females.

Studies with nose-only exposure

In Study 3 (1991), 5 rats/group/sex were exposed towards folpet concentrations of 0.14, 0.,36, 1.06 and 4.35 mg/L for 4 hours, with a 14-day post exposure observation period. The MMAD ranged from 1.7 to 2.8 µm. Animals exposed at 1.06 and 4.35 mg/L showed wet fur and decreased respiratory rate during the exposure period. On removal from the exposure chamber surviving animals in these two groups showed hunched posture, lethargy, piloerection, ptosis, ataxia, pallor of the extremities and decreased or laboured respiration. Two males exposed to 1.06 mg/L showed gasping respiration and one male showed red/brown staining around the snout. Surviving animals continued to show similar signs until death. Several animals treated with 0.36 mg/L showed wet fur during exposure and two females showed decreased respiratory rate at three hours. On removal from the chamber wet fur was common and all animals showed hunched posture, lethargy, piloerection and ptosis, ataxia was also observed in several animals. One hour after completion of exposure ataxia had subsided, with the exception of one female but decreased respiratory rate had become apparent with one female showing gasping respiration and one female showing laboured and noisy respiration. On day one following exposure to 0.36 mg/L hunched posture and pilot-erection were still evident in all animals, many were still lethargic with one female showing extreme lethargy. Several animals showed signs of respiratory distress and some showed ptosis. Isolated incidents of pallor of the extremities, dehydration, increased lacrimation and hypothermia were noted, and red/brown staining of the snout was seen in several animals. On day two surviving animals generally showed an improvement in condition although one male developed noisy respiration and the condition of two animals had worsened to include signs of gasping and noisy respiration,

pallor of the extremities, hypothermia, dehydration, increased activity, ptosis, ataxia and red/brown staining of the snout and mouth. By day three the surviving animals, apart from one female, had recovered and by day five all animals in this group appeared normal. Animals exposed to 0.14 mg/L showed wet fur throughout the exposure period. On removal from the chamber hunched posture, lethargy, piloerection and ptosis was shown in all animals, several showed ataxia. One hour after completion of exposure two animals appeared normal but respiratory rate had decreased in many animals and one male showed laboured respiration. A slight improvement was noted on day one following exposure, with four animals appearing normal, but red/brown staining of the snout was seen in two animals. Signs of toxicity including hunched posture, lethargy, piloerection, decreased respiratory rate and ptosis persisted in the other animals and two of these showed red/brown staining around the snout. Generally surviving animals in this group showed further improvement by day two, although one female showed noisy respiration as well as decreased respiratory rate and dehydration at this time. All surviving animals appeared normal on day three and for the rest of the study.

Surviving animals from the 0.36 mg/L and the 0.14 mg/L groups showed reduced bodyweight gain or bodyweight loss during the first week of the observation period. Weight gains generally recovered during the second week but remained slightly lower than would normally be expected in rats of this strain and age.

Animals exposed to 4.35 mg/L all showed haemorrhagic and swollen lungs. Several animals showed haemorrhage of the small intestine with isolated signs of congestion and general reddening and one showed haemorrhage of the large intestine. One female which was killed in extremis also showed patchy pallor of the liver and kidneys and test material was present in the stomach. Haemorrhagic lungs were seen in animals exposed to 1.06 mg/L, several lungs were also swollen. In two animals, congestion of the small intestine was noted and one showed haemorrhage of the small intestine. Animals treated with 0.14 mg/L showed various lung changes including haemorrhage, abnormal redness, pale and dark areas, dark foci and swelling or reduced size. The one male that died in this group also showed haemorrhagic and congested small intestine. Two females showed no abnormalities. Abnormalities of the lungs were also seen in animals exposed to 0.36 mg/L, most commonly dark patches were noted but abnormal redness, pallor, greyish discolouration and swelling were also apparent. Three animals that died in this group showed congestion and haemorrhage in the intestinal tract and patchy pallor of the liver.

The LC_{50} for males was 0.39 mg/L and 0.43 mg/L for females.

In Study 4 (1993), 5 males/group were exposed to 1.84, 2.14, 3.57, 4.35 mg/L and 5 females/group were exposed to 0.79, 1.11, 1.84, 2.14 mg/L for four hours, with a 14 day post exposure observation period. In Group 5 (2.14 mg/L) two females died during the first two hours immediately following exposure and the remaining three females were found dead during Day 1 of the observation period. In Group 6 (1.84 mg/L) three females were found dead during Day 1 of the observation period and the remaining two females were killed in extremis on Day 2 following exposure.

In Group 7 (3.57 mg/L) one male was found dead on the day following exposure and a further male was found dead on Day 2 of the observation period. In Group 8 (0.79 mg/L) one female was killed in extremis on Day 2 following exposure. In Group 10 (1.11 mg/L) two females were found dead on Day 2 of the observation period. Changes in respiratory rate and pattern comprising increased respiratory rate, shallow respiration and irregular respiration were observed during the treatment of males exposed to 3.57 or 4.35 mg/L and females exposed to 1.11 mg/L. These signs, together with observations of struggling in the restraint tube and wet fur recorded for animals exposed to 1.11, 2.14 or 3.57 mg/L, were considered to be a non-specific response to the inhalation of a particulate atmosphere.

Signs evident during the two hours immediately following the exposures were seen predominantly among females exposed to 1.84 or 2.14 mg/L. They included changes in respiratory rate and pattern, rales, gasping, underactivity, hunched posture, staggering gait, closed eyes, hypothermia and piloerection. A similar range of signs, but at a lower incidence, was observed for females exposed to 1.11 mg/L and males exposed to 3.57 mg/L. There were also isolated incidences of many of these signs in other exposure groups at this time. Wet fur was evident during the first two hours following exposure for most animals. Signs that persisted for several days, or that developed on the first day of the observation period, comprised: slow respiration, fast respiration, irregular respiration, deep respiration, shallow respiration, rales, gasping, underactivity, overactivity, hunched posture, staggering gait, thin body appearance, piloerection, hypothermia, pigmented staining on the snout and ungroomed appearance. These signs were observed most commonly among females. Males exposed to 1.84 or

4.35 mg/L were normal in appearance and behaviour from the day following exposure. Observed changes for males exposed to 2.14 mg/L were largely confined to the first three days following exposure, however, rales were evident for one animal (No. 121) until Day 5. The three males which survived the effects of exposure to a chamber concentration of 3.57 mg/L and females that survived exposure to 0.79 or 1.11 mg/L had fully recovered from all signs by Day 3 of the observation period. Animals that died during the observation period lost weight before death. Males exposed to 1.84, 2.14 or 4.35 mg/L lost weight on the day following treatment and gained weight at a reduced rate on the second day of the observation period; weight gain for one of these animals (No. 121 – exposed to 2.14 mg/L) remained low until Day 7. Males that survived exposure 3.57 mg/L lost weight or gained weight at a reduced rate for up to two days following exposure. The weight gains of these males from Day 3 and throughout the remainder of the observation period were considered to be unaffected by previous treatment. Reduced weight or low weight gain was seen for up to two days following exposure for females that survived treatment at 0.79 or 1.11 mg/L; thereafter, weight gains for these animals were similar to those seen before exposure.

Necropsy examination of animals that died during the observation period confirmed the signs fur staining recorded at despatch. Among internal findings for decedents, macroscopic observations attributed to treatment were confined to the respiratory system.

Incomplete collapse of the lungs was seen for one of the two males exposed to 3.57 mg/L which died, for two females in each of the groups exposed to 1.84 or 2.14 mg/L and for the female decedent exposed to 0.79 mg/L. In addition, observations of caseous material in the trachea and firm lungs for males exposed to 3.57 mg/L (Nos. 142 and 144, respectively) together with findings of dark lungs or dark areas on the lungs seen for many of the animals that died were probably associated with previous exposure to folpet Technical (micronized).

There was a range of other findings for animals that died as a result of exposure including single incidences of pale areas on the liver, dark thymus, large adrenals, distension of the stomach or jejunum with gas and pale kidneys. However, none of these observations was considered directly attributable to treatment.

All of the observations in animals that were killed after 14 days of observation following exposure were of the types normally encountered in control rats at these laboratories.

The lung weights of animals that died as a result of treatment were clearly high when compared with background data for animals of the same age and strain. Liver and kidney weights of these animals were unaffected.

Among animals that survived the effects of exposure to the test substance the bodyweight-relative lung weight for one male exposed to 2.14 mg/L (No. 121) was clearly high when compared with background control data; slightly high lung weights were also recorded for a male (No. 132) exposed to 1.84 mg/L, a female (No. 149) exposed to 0. 79 mg/L and the three female survivors (Nos. 166-168) exposed to 1.11 mg/L. Liver and kidney weights for animals which survived exposure to folpet Technical (micronized) were considered to be unaffected by treatment.

The LC_{50} for males was > 4.35 mg/L and 1.08 mg/L for females.

In Study 5 (1993), 5 animals/sex were exposed to non-micronized folpet at 2.14 mg/L. The MMAD was 14.3 µm. There were no mortalities, or clinical signs as observed for the other acute inhalation studies. The LC_{50} was >2.14 mg/L for males and females.

In Study 6 (1993), 5 animals/sex/group were exposed to folpet concentrations of 0, 0.8, 1.6 and 1.99 mg/L. The MMADs were 4.6, 4.9 and 5.2. The following mortalities occurred during or shortly following exposure: 0, 3, 4 for low-, mid-, and high-dosed males respectively and 0, 1, 1 for low-, mid-, and high-dosed females respectively. In the mid-dosed group one female was found dead after 10 minutes of exposure. No deaths occurred after Day 2. During exposure, irregular respiration and gasping were recorded, with the numbers of animals affected related to the atmosphere concentration. In the two hours following exposure, signs predominantly seen in animals exposed to 1.60 or 1.99 mg/L included changes in respiratory rates and pattern, gasping, rales, vocalisation, underactivity, hunched or prone posture, closed or partially closed eyes, pigmented staining of the snout, piloerection and wet fur. Similar signs were seen during the subsequent observation period with rales being the most persistent (until day 13). One female exposed to 0.8 mg/L also showed this sign.

Wet fur, underactivity and closed or partially closed eyes were also seen among animals exposed to 0.8 mg/L, in the two hours following exposure. In one male of the low dose group rales were recorded 2 days after treatment. Slow and/or deep respiration, rales, partially closed eyes, pigmented staining on the snout and hunched posture were recorded for one female of the low dose group. These effects lasted for up to 11 days post-exposure. Body weight loss was evident on the day following exposure for all animals that survived the initial effects of exposure. Further body weight loss or low weight gain in comparison with pre-exposure gains was also evident for some animals in all groups on the second day following treatment. Generally, weight gain was similar to that observed before exposure from Day 3 of the observation period.

Macroscopic changes attributed to treatment were seen only in decedents and comprised clear viscous fluid in the trachea, dark lungs, incomplete collapse of the lungs and occasional pale areas on the lungs. Skin and fur staining were also evident for a number of these animals. There were no treatment-related findings in surviving animals.

Treatment increased lung weights dose-dependently. The effect was most noticeable for the animals that died during the observation period were when compared with background data for animals of the same age and strain.

The LC_{50} for males was 1.54 mg/L and 2.89 mg/L for females.

9.3.2 Comparison with the CLP criteria

According to the criteria shown in the Table 3.1.1 of Annex I, Part 3 of CLP, substances can be allocated to one of four toxicity categories based on acute toxicity by the inhalation route. In general, classification is based on the lowest ATE value available i.e. the lowest ATE in the most sensitive appropriate species tested. Acute toxicity values are expressed as approximate LC_{50} values (inhalation) or as acute toxicity estimates (ATE):

Acute inhalation toxicity - Category 2: $0.05 < ATE \leq 0.5$ mg/L

Acute inhalation toxicity - Category 3: $0.5 < ATE \leq 1$ mg/L

Acute inhalation toxicity - Category 4: $1 < ATE \leq 5$ mg/L

While the majority of the responses for the nose-only exposure studies are exceeding the threshold of 0.5 mg/L, the study with the smallest achieved particle sizes is below the threshold and qualifies for a classification for Acute inhalation toxicity Category 2.

An ATE of 0.39 mg/L is proposed based on the lowest LC50 in males from a fully reliable study (i.e. Study 3).

9.3.3 Conclusion on classification and labelling for acute inhalation toxicity

Folpet is proposed to be classified for Acute inhalation toxicity Category 2 according to the CLP classification criteria. An ATE of 0.39 mg/L is proposed.

9.4 Skin corrosion/irritation

Contrary to the respiratory tract and the eye, there was only limited acute skin irritation in one of the two acute skin corrosion/irritation assays in rabbits. However, severe irritation was observed in a 28-day dermal toxicity study, already at the first observation time point, Day 2. The irritation potency increased with dose and exposure time to a level where the high dose treatment was first reduced and eventually stopped due to the severity of the skin effects. Topical induction in the sensitisation assays resulted in an immediate irritation dose-response and intra-dermal injection produced necrosis and eschar. Mice exposed in the chronic studies also showed skin lesions due to direct contact to high concentration folpet diet.

Overall, since the observed skin effects observed over the whole study package are of acute aetiology, a classification for acute skin irritation category 2 is considered appropriate to both describe and communicate the irritation hazard and to facilitate appropriate protection measures.

If not stated otherwise in the summary tables, the studies are considered as fully reliable.

Table 17: Summary table of acute skin corrosion/irritation animal studies

<table>
<tr><th>Method, guideline, deviations</th><th>Dose levels duration of exposure, Species, strain, sex, no/group</th><th>Results
-Observations and time point of onset
-Mean scores/animal
-Reversibility</th><th>Reference</th></tr>
<tr><td>GLP, EPA 81-5 870.2500 (1984)
Deviations from OECD 404 (2015):
6 animals instead of 3</td><td>0.5 g for 4 hours
Folpet, no purity reported
New Zealand Albino rabbits, 4 males, 2 females</td><td>Mild oedema in one rabbit, mean 0.3 (24-72 h), reversible within 24 hours.
Mild erythema at the one hour observation time point.
<table>
<tr><th colspan="2">Animal No.</th><th>Mean scores (24 - 72 h)</th></tr>
<tr><td rowspan="2">1</td><td>Erythema</td><td>0</td></tr>
<tr><td>Oedema</td><td>0</td></tr>
<tr><td rowspan="2">2</td><td>Erythema</td><td>0</td></tr>
<tr><td>Oedema</td><td>0</td></tr>
<tr><td rowspan="2">3</td><td>Erythema</td><td>0.3</td></tr>
<tr><td>Oedema</td><td>0</td></tr>
<tr><td rowspan="2">4</td><td>Erythema</td><td>0</td></tr>
<tr><td>Oedema</td><td>0</td></tr>
<tr><td rowspan="2">5</td><td>Erythema</td><td>0</td></tr>
<tr><td>Oedema</td><td>0</td></tr>
<tr><td rowspan="2">6</td><td>Erythema</td><td>0</td></tr>
<tr><td>Oedema</td><td>0</td></tr>
</table></td><td>(R-6508)
Study 1 (1991)</td></tr>
<tr><td>GLP, OECD 404 (1981)</td><td>0.5 g for 4 hours
Folpet, 95.6%
New Zealand White rabbits, 3 females</td><td>No dermal response (erythema or oedema)
<table>
<tr><th colspan="2">Animal No.</th><th>Mean scores (24 - 72 h)</th></tr>
<tr><td rowspan="2">1</td><td>Erythema</td><td>0</td></tr>
<tr><td>Oedema</td><td>0</td></tr>
<tr><td rowspan="2">2</td><td>Erythema</td><td>0</td></tr>
<tr><td>Oedema</td><td>0</td></tr>
<tr><td rowspan="2">3</td><td>Erythema</td><td>0.3</td></tr>
<tr><td>Oedema</td><td>0</td></tr>
</table></td><td>(R-7394)
Study 2 (1993)</td></tr>
</table>

Table 18: Other studies contributing evidence to skin corrosion/irritation

Method, guideline, deviations	Dose levels duration of exposure, Species, strain, sex, no/group	Results -Observations and time point of onset -Mean scores/animal -Reversibility	Reference
4-week rat	Folpet	Dose and exposure time dependent increase in skin irritation potency. Due to the severity of the skin effects one of the highest	(R-5452)

<table>
<tr><th>Method, guideline, deviations</th><th>Dose levels duration of exposure, Species, strain, sex, no/group</th><th>Results
-Observations and time point of onset
-Mean scores/animal
-Reversibility</th><th>Reference</th></tr>
<tr><td>No guideline stated. Similar to Directive 92/69/EEC B.9.

Deviations from OECD 410 (1981):

- Groups of six animals of each sex were used (instead of 10)

- Treatment for 5 days (not seven days) per week over 4 weeks</td><td>Purity: Not reported

0, 1, 10, 30, 30/20 mg/kg bw/day

Two 30 mg/kg bw/d groups

4-weeks

6/sex/group

SD rats</td><td>treatment levels was reduced from 30 to 20 mg/kg bw/day on day 6, while for the other dosing was discontinued after Day 13 and the animals were allowed to recover. However, for males the high dose treatment was stopped after day 6. The effects were less pronounced in females.

Day 2 erythema/oedema scores:
<table><tr><td>mg/kg bw/d</td><td>0</td><td>1</td><td>10</td><td>30</td><td>30/20</td></tr><tr><td>M</td><td>0.5/0</td><td>1.33/0.17</td><td>1.5/0.67</td><td>2.17/1</td><td>2.67/1.5</td></tr><tr><td>F</td><td>0.17/0</td><td>0.17/0</td><td>0.5/0.17</td><td>0.83/0.17</td><td>0.5/0.33</td></tr></table>
Day 9 erythema/oedema scores:
<table><tr><td>mg/kg bw/d</td><td>0</td><td>1</td><td>10</td><td>30</td><td>30/20</td></tr><tr><td>M</td><td>0.83/0.5</td><td>1.67/1.17a</td><td>3.67/2.33</td><td>3.83/2.5c</td><td>3.5/2.33c,d</td></tr><tr><td>F</td><td>0/0</td><td>0.33/0</td><td>1.67/0.67</td><td>3/1.67</td><td>2.67/1.5</td></tr></table>a: multiple pinpoint scabs; b: large scabs; c: sloughing; d: lacerations

bw gain ↓ (males) starting at 10 mg/kg bw/d, no differences in food consumption and body weight between the treated and control animals

Changes in haematological and clinical chemistry parameters, probably due to skin reactions at the highest dose (females)

Local effects: erythema, oedema, scabs, sloughing (from 10 mg/kg bw/d onwards) and lacerations (highest dose)

NOAEL: 1 mg/kg bw/d (males), 10 mg/kg bw/d (females)

For local effects LOAEL: 1 mg/kg bw/d</td><td>Study 3 (1988)</td></tr>
<tr><td>Acute toxicity dermal route</td><td colspan="2">Some irritation is observed in one acute dermal toxicity study with abraded skin [(i.e. suppurative dermatitis (4 females), mild acanthosis (1 female)].</td><td>Section 8.2 / Study 1 (1982)</td></tr>
<tr><td>Skin sensitisation: animal data</td><td colspan="2">After topical induction, there is an irritation dose-response, which decreased after exposure was ceased. Intradermal injection results in erythema, eschar and necrosis.</td><td>Section 8.7/Study 1 (1991))</td></tr>
<tr><td>Skin sensitisation: human data</td><td colspan="2">In some studies, with human volunteers the skin sensitisation potential of folpet was investigated. While erythema was observed the study designs do not allow to clearly distinguish skin sensitisation and skin irritation potential.</td><td>Section 10.7</td></tr>
<tr><td>Chronic toxicity: mouse</td><td colspan="2">Mice in all chronic toxicity studies showed skin effects in high dose group animals due to direct exposure to diet with high folpet concentrations. The skin lesions may be exacerbated by scratching since the exposed skin was not protected by covering.</td><td>Section 8.9/Study 1-3</td></tr>
</table>

Table 19: Summary table of acute skin corrosion/irritation *in vitro* studies

Method, guideline, deviations	**Dose levels duration of exposure, Species, strain, sex, no/group**	**Results** **-Observations and time point of onset** **-Mean scores/animal** **-Reversibility**	**Reference**
OECD TG 431 (Reconstructed Human Epidermis, corrosion)		Study is currently being conducted. Results will be amended during public commenting.	(000107034/ 20273724)
OECD TG 439 (Reconstructed Human Epidermis, irritation)		Study is currently being conducted. Results will be amended during public commenting.	(000107035/ 20273726)

9.4.1 Short summary and overall relevance of the provided information on skin corrosion/irritation

There are two skin corrosion/irritation tests available in rabbits, which showed only limited acute irritation potential that do not warrant classification. However, other studies provide evidence that make an acute skin irritation classification appropriate.

In Study 1 (1991), 6 rabbits (4 males and 2 females) were exposed towards 0.5 g folpet moistened with 0.6 mL distilled water under semi-occlusive dressing. Only one rabbit showed mild oedema, which was reversible within 24 hours, with a mean 0.3 (24-72 h). Erythema were noted in four animals, which were reversible within 24 hours. There were no signs of clinical toxicity mentioned in the study report.

In Study 2 (1993), 3 rabbits were exposed towards 0.5 g folpet for 4 hours, with a 3-day post exposure observation period. There were no dermal responses in any animal at any timepoint.

There is also only limited irritation is observed in an acute dermal toxicity study (Section 8.2/Study 1, 1982).

In a dermal toxicity study (Study 3, 1988), folpet was applied at a volume of 2 mL/kg bw in mineral oil to a clipped area of unabraded skin on the back of six male and six female Sprague-Dawley Crl:CDBR rats at dose levels of 0, 1, 10 and 30 (two groups) mg/kg bw/day daily for six hours for five days for four weeks, i.e. a total of 21 applications. The test material was applied to two alternating skin sites – once on the right side and the next day on the left. The dose level for the male animals in one of the high dose groups was reduced to 20 mg/kg/day on Day 6 but dosing was discontinued after Day 13 and the animals were allowed to recover. Dosing was terminated in the males in the other high dose group after Day 13 and the animals sacrificed on Day 15. Test formulations were applied to one of two sites, alternating on a daily basis.

The incidence and severity of skin irritation were greater in males than females and appeared to be dose-related. Due to the severity of skin irritation, the dose level of one of the high dose male groups was reduced to 20 mg/kg bw/day on Day 6 and in both the 20 mg/kg and 30 mg/kg males group dosing was discontinued on Day 13. The 30 mg/kg group was sacrificed (on day 15) and the 20 mg/kg group was carried as a recovery group. By Day 28 there was no or reduced irritation (3 animals showed no irritation, while 2 had large or multiple pinpoint scabs with slight oedema) indicating that the irritation associated with the test substance was reversible in males. Incidence and severity increased in both sexes with dose and duration of the study. Low dose males showed severe erythema with well-defined oedema and low dose females had well-defined erythema with slight oedema. Dry and flaky skin was observed in both sexes at all dose levels.

Table 20: Mean scores for erythema/ oedema from a 4-week dermal toxicity study with folpet (6 animals treated and scored per sex and group)

Day	Dose level (mg/kg bw/day)				
	0	1	10	30	30/20#
Males					
0	0/0	0/0	0/0	0/0	0/0
2	0.5/0	1.33/0.17	1.5/0.67	2.17/1	2.67/1.5
9	0.83/0.5	1.67/1.17a	3.67/2.33 a,b	3.83/2.5 c	3.5/2.33 c,d
14+	-	-	-	3.67/2.33	3.67/2.17 a,b,c
16	0.67/0	2.67/1.5a,b	3.83/2.33 a,b,c	-	3.67/1.83 b,c
23	0.17/0	2.33/1.5 a,b	3.5/2.5 a,b	-	1.5/0.5 a,b
28	0.17/0	1.33/0.67	3.5/2.33 a,b	-	1.33/0.33 a,b
Females					
0	0/0	0/0	0/0	0/0	0.17/0
2	0.17/0	0.17/0	0.5/0.17	0.83/0.17	0.5/0.33
9	0/0	0.33/0	1.67/0.67	3/1.67	2.67/1.5
16	0/0	0.33/0	2.17/1.17	3.5/2 a,c	3/1.5 c
23	0/0	0.67/0.17	2.5/1.67 c	3.83/2 b,c	3.33/1.67 c
28	0/0	0.5/0.17	2/1.17	3.5/2.33 b,c	3/2 b,c

dose level reduced Day 6; treatment ceased Day 13 in males only, in females: 30 mg/kg bw/day
+ only high dose males scored.
a: multiple pinpoint scabs
b: large scabs
c: sloughing
d: lacerations

The difference in irritation potential between the acute irritation studies and the acute dermal toxicity studies is probably related to differences in the application method and the test system. The the acute irritation studies tested either moistened material (Study 1) or dry material on moistened skin (Study 2) for four hours.

In Study 3 folpet was applied in a mineral oil vehicle for 6 hours and the formulation was replaced daily. The maximum possible application in the repeated exposure study was about 20% of that of the acute irritation studies; i.e. 30 mg/kg bw/day × 21 days × 1.5 hour exposure-ratio to acute studies × 0.25 kg assumed bw = 236.25 / 2 application areas = ~120 mg/application area vs 500 mg/application area. If the actual size difference of application areas is additionally considered, 6.25 cm^2 for rabbits and about 10% in dermal rat studies which is[1] 2.5 cm^2 according to OECD TG, the cumulative application per square centimetre is actually higher in the rat, i.e. 500/6.25 = 80 mg/cm^2 in rabbit and 236.25/2.5 = 94.5 mg/cm^2 in rat. As folpet reacts and degrades rapidly, the daily application and unhindered diffusion towards the target skin, due to lower daily concentrations (94.5/21= 4.5 mg/cm^2/day) and alternating sites, presumably maximised the amount available for skin reactions in the repeated exposure study, which explains the severity of the effect in rat when compared to the rabbit skin irritation study.

The data demonstrates that both dose and exposure time increase folpet's irritation potential, which supports the rapid and local acute irritation mode of action and allows to apply Haber's rule to pro-rata adjust doses when comparing studies.

Furthermore, folpet hydrolyses fast in water, while its solubility in organic solvents is limited (i.e. 0.45 g/L in heptane). It should further be noted that, captan (the sibling of folpet) did not induce such effects when applied up to 1000 mg/kg bw/d in water in a repeated dose dermal toxicity study.

There is also substantial acute irritation observed after topical application in a skin sensitisation study (Section 8.7/Study 1, 1991). Moderate and diffuse redness was noted in 18 of 19 animals as well as scattered mild redness in 1/19 animals one hour after patch removal. After 24 hours, 9/19 animals showed scattered mild

[1] FDA, 2005 "Guidance for Industry Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers" as well as ECHA, 2017 "Guidance on the Application of the CLP Criteria"

redness. Erythema could not be evaluated because of other adverse skin reactions, i.e. bleeding open wounds, small superficial scabs, dried blood, hardened light brown-coloured scabs and residual test material. The skin sensitisation study used again a non-aqueous vehicle, arachis oil, which presumably decreased degradation.

The human studies summarized in Section 8.7, reported only limited erythema upon folpet exposure. It is not clear from the study design whether potential sensitisation effects could be clearly distinguished from potential irritation effects.

Together, there is substantial evidence of skin irritation in the data package, which might not have been observed in the skin irritation studies in rabbits due to rapid degradation of folpet in water.

9.4.2 Comparison with the CLP criteria

According to the criteria shown in the Table 3.2.2 of Annex I, Part 3 of CLP, substances can be allocated to one of two skin corrosion/irritation categories based mean dermal responses.

The lowest category 2 (irritant) applies if the following is observed in the available data package:

> (1) Mean value of ≥ 2,3 - ≤ 4,0 for erythema/eschar or for oedema in at least 2 of 3 tested animals from gradings at 24, 48 and 72 hours after patch removal or, if reactions are delayed, from grades on 3 consecutive days after the onset of skin reactions; or
>
> (2) Inflammation that persists to the end of the observation period normally 14 days in at least 2 animals, particularly taking into account alopecia (limited area), hyperkeratosis, hyperplasia, and scaling; or
>
> (3) In some cases where there is pronounced variability of response among animals, with very definite positive effects related to chemical exposure in a single animal but less than the criteria above.

According to the acute skin corrosion/irritation studies, there is (1) only a mean value of 0.3 (24-72 h) in 1 of 6 animals in study 1 and a mean value of 0 in 3 of 3 animals in study 2 and (2) the observed effect is reservable within 24 hours and (3) there is no pronounced variability, hence, no classification for skin corrosion/irritation is applicable for folpet based on the skin irritation studies alone.

However, the repeated dose dermal toxicity study, show mean erythema and oedema scores of 2.67 and 1.5, respectively, after 2 days of 6-hour treatments of 6 male rats with 30 mg/kg bw/day. While a similar effect would correspond to about 5 g folpet distributed equally over hands for four hours, a classification for skin irritation is considered to appropriately reflect folpet's hazard profile. The irritation clearly increases with dose and exposure time and similar reactions are observed in other animal studies.

9.4.3 Conclusion on classification and labelling for skin corrosion/irritation

Folpet is proposed to be classified for skin corrosion/irritation Category 2 according to the CLP classification criteria.

9.5 Serious eye damage/eye irritation

Four studies are available, which were performed according OECD TG 405 or similar. All studies show irritation potential for folpet, however, indicate different potencies. A decontamination procedure 20-30 s after instillation greatly reduced or prevented irritation, which is however not relevant for classification and is omitted from the assessment.

If not stated otherwise in the summary tables, the studies are considered as fully reliable.

Table 21: Summary table of animal studies on serious eye damage/eye irritation

Method, guideline, deviations from OECD 405 (2012) if any	**Species, strain, sex, no/group**	**Test substance, purity**	**Dose levels duration of exposure**	**Results** **- Observations and time point of onset** **- Mean scores/animal** **- Reversibility**	**Reference**
GLP, OECD 405 Room temperature: 19°C (15-23°C) Deviations from OECD 405 (2012): - Acclimatisation period not stated - No topical anaesthetics or systemic analgesics were used - 7-day observation time (instead of 21) - Observation after 72 hours not daily - Observation only once per day in from 24-72 hours	New Zealand Albino rabbits, 3 males	Folpet technical, 95.6%	0.1 g, eyes were not washed in the 3-day post exposure period	<u>Individual 24-72 h means</u> Cornea opacity: 2.67, 0, 2.67 not reversible within 7 days Iris: 1, 0, 1 not reversible within 7 days Conjunctiva – redness: 3, 1.67, 3 not reversible within 2 days in 2/3 animals Conjunctiva – chemosis: 2.3, 0.67, 1 not reversible within 7 days in 2/3 animals	(R-7425) Study 1 (1993)
Non-GLP, EPA August 1978 Deviations from OECD 405 (2012): - Housing conditions (temperature, relative humidity): Not stated - 6 animals were treated - No topical anaesthetics or systemic analgesics were used - 13-day observation time (instead of 21) - Observation after 72 hours not daily - Observation only once per day from 24-72 hours - Unclear if fluorescein staining was used	New Zealand Albino Rabbit, 6 males and 3 females	Folpet technical, no purity reported	0.1 g, eyes were not washed in the 3-day post exposure period in 6 animals In 3 animals eyes were washed for one minute after 20 s of exposure	<u>Individual 24-72 h means for animals without eye washing</u> Cornea opacity: 2.67 (pannus present from day 10 onwards), 0, 0, 0, 2.33 (pannus present from day 13 onwards), 0.67 (pannus present from day 7 onwards) corneal opacity not reversible within 13 days (max follow-up) Iritis: 0, 0, 0, 0, 0, 0.33 not reversible within 13 days (max follow-up) Conjunctiva – redness: 3, 3, 3, 2.67, 3, 3 not reversible within 13 days (max follow-up) Conjunctiva – chemosis: 3, 3.3, 3, 2, 3.3, 3 not reversible within 13 days (max follow-up) One death (male) on day 8: The dead animal revealed hemorrhaging of the left lung and cecum as well as diarrhea. Autopsy results indicated death to be caused by an intestinal disorder and not by exposure to the test material. <u>Individual 24-72 h means for animals with eye washing</u>	(R-1737) Study 2 (1979)

				Cornea opacity: 0, 0, 0 Iritis: 0, 0, 0 Conjunctiva – redness: 1.66, 1.33, 1.33 (reversible after 48 hs) Conjunctiva – chemosis: 0.33, 0.33, 0.33 (reversible after 24 hs)	
GLP, EPA August 1978 Deviations from OECD 405 (2012): - Relative humidity: 49-73% - 6 animals were treated - No topical anaesthetics or systemic analgesics were used - Observation after 72 hours not daily - Observation only once per day from 24-72 hours - No fluorescein staining was used	New Zealand Albino rabbits, 9 males	Folpet technical, no purity reported	0.1 g, eyes were not washed in the 3-day post exposure period In 3 animals eyes were washed 30 s after exposure	<u>Individual 24-72 h means for animals without eye washing</u> Cornea opacity: 0, 0, 0, 0, 2.67 (pannus present at day 7-reversible until day 10), 0 reversible within 10 days Iritis: 0.67, 0, 0, 0, 1, 0 reversible within 4 days Conjunctiva – redness: 2.33, 2, 1.33, 2, 2.67, 2 reversible within 10 days Conjunctiva – chemosis: 1.33, 1, 0.33, 1.33, 1.33, 1 reversible within 4 days	(R-7091) Study 3 (1982)
GLP, US EPA Deviations from OECD 405 (2012): - 6 animals were treated - No topical anaesthetics or systemic analgesics were used - 14-day observation time (instead of 21) - Observation after 72 hours not daily - Observation only once per day in the first from 24-72 hours - No fluorescein staining was used	New Zealand White rabbits, 2 males, 4 females	Folpet technical, no purity reported	0.1 mL/87 mg for 3 days (eyes were not washed in the 3-day post exposure period)	<u>Individual 24-72 h means</u> Cornea (degree of opacity): 4, 3.7, 0, 0.7, 2, 0 reversible within 14 days; vascularisation of the cornea persisted in two animals until Day 14 Iritis: 1, 0.7, 0.3, 0, 0.3, 0 reversible within 7 days Conjunctiva – redness: 2, 2, 2, 1.3, 2, 1.3 reversible within 14 days, petechial haemorrhage of the nictitating membrane persisted in two animals until Day 14 Conjunctiva – chemosis: 2, 2, 1.7, 0.7, 2, 0.7 reversible within 14 days Conjunctiva – discharge: 2.3, 2, 1.3, 1, 2.7, 1 reversible within 7 days	(R-6511) Study 4 (1992)

Table 22: Summary table of serious eye damage/eye irritation *in vitro* studies

Method, guideline, deviations	**Dose levels duration of exposure, Species, strain, sex, no/group**	**Results** **-Observations and time point of onset** **-Mean scores/animal** **-Reversibility**	**Reference**
OECD TG 437 (Bovine Corneal Opacity and Permeability)		Study is currently being conducted. Results will be amended during public commenting.	(000107036/ 20273729)
OECD TG 492 (Reconstructed Human Cornea-like Epithelium)		Study is currently being conducted. Results will be amended during public commenting.	(000107037/ 20273731)

9.5.1 Short summary and overall relevance of the provided information on serious eye damage/eye irritation

There are four acute eye irritation studies available. None of the studies have a follow-up period until 21-days after exposure and two studies report eye effects up to the maximum follow-up day. Hence reversibility of eye effects within 21 cannot be shown by the available data. Overall, there is irritation observed in all studies, however, with different potencies. The conjunctiva seems to be more affected by treatment than corneal opacity or iritis parameters.

In Study 1 (1991), three rabbits were treated with test item and the eyes were not washed after treatment. One rabbit showed a diffuse crimson-red conjunctival appearance during the first 48 hours following instillation. Very slight or slight chemosis and discharge were observed during this time. At the 72-hour examination, injection of the conjunctival blood vessels and very slight discharge were evident. The eye of this rabbit was overtly normal on the seventh day after treatment. A diffuse crimson-red conjunctival appearance, slight or moderate chemosis and discharge and iritis were observed in the other two rabbits one hour following instillation. At the 24, 48 and 72-hour examinations, a beefy-red conjunctival appearance, slight or substantial discharge and very slight to moderate chemosis were observed. Iritis and slight to severe opacity were also evident during this time. On the seventh day, the eyes of these two rabbits showed injection of the conjunctival blood vessels or a crimson-red conjunctival appearance, very slight chemosis, iritis and severe opacity. Pannus formation was associated with the areas of severe opacity; due to the irreversible nature of this change, the animals were sacrificed, and the study terminated.

In study 2, the eyes for three animals were washed for one minute with lukewarm water 20 seconds after instillation, the eyes for six animals remained unwashed. Eyes washed 20 seconds after instillation showed conjunctival redness (scores after 24 hs: 3, 2, 2), chemosis (scores after 24 hs: 1, 1, 1) and discharge (scores after 24 hs: 1, 1, 1) 24 hours after instillation. Redness was the only sign of irritation seen at 48 hours (scores after 48 hs: 2, 2, 2) and, by 72 hours, all three eyes were normal. Eyes that were not washed after treatment exhibited corneal opacity, iritis, conjunctival redness and discharge. Two eyes returned to normal by day ten, while the remaining three eyes exhibited signs of severe irritation through day 13. One death occurred during the study. A male in the unwashed group died on day eight. Autopsy results indicated death to be caused by an intestinal disorder and not by exposure to the test material.

In Study 3 (1982), the eyes of three animals were rinsed for one minute after a 30-second exposure, while the eyes of six animals remained unwashed. For the rinsed eyes, no corneal opacity or iritis were observed. Only slight conjunctival irritation was observed one hour after treatment and all eyes were clear by 24 hours following treatment. For the unrinsed eyes, complete corneal opacity was observed in one eye, and iritis in two eyes within 72 hours after treatment. Moderate to severe conjunctival irritation was observed in most eyes during this period. And all eyes appeared normal by 14 days after treatment.

In Study 4 (1992), 0.1 mL of test item (about 87 mg) were used in six New Zealand white rabbits and the eyes were not washed after 1 hour of treatment. A dulling of the normal lustre of the corneal surface was noted in four treated eyes one hour after treatment. Areas of diffuse to opaque corneal opacity were noted in four treated eyes at the 24 and 48-hour observations. Translucent to opaque corneal opacity was noted in three treated eyes at the 72-hour observation. Diffuse or translucent corneal opacity was noted in two treated eyes at the 7-day observation. Vascularisation of the cornea was also noted in these two treated eyes at the 7 and 14-day observations. Iridial inflammation was noted in all treated eyes one hour after treatment, in four treated eyes at the 24-hour observation, in two treated eyes at the 48-hour observation and in one treated eye at the 72-hour observation. No other adverse iridial effects were noted. Moderate conjunctival irritation was noted in all treated eyes one and 24 hours after treatment with minimal to moderate conjunctival irritation at the 48 and 72-hour observations. Minimal conjunctival irritation was noted in two treated eyes at the 7-day observation. Petechial haemorrhage or haemorrhage of the nictitating membrane was noted in three treated eyes at the 24, 48 and 72-hour observations. Pale areas over the nictitating membrane were noted in one treated eye at the 48-hour observation and in two treated eyes at the 7-day observation. Four treated eyes appeared normal 7 or 14 days after treatment.

9.5.2 Comparison with the CLP criteria

According to the criteria shown in the Tables 3.3.1 and 3.3.2 of Annex I, Part 3 of CLP, substances can be allocated to one of two eye damage/irritation categories based mean responses.

The category 1 (Irreversible effects on the eye) applies if the following is observed in the available data package:

If, when applied to the eye of an animal, a substance produces:

- at least in one animal effects on the cornea, iris or conjunctiva that are not expected to reverse or have not fully reversed within an observation period of normally 21 days;

and/or

- at least in 2 of 3 tested animals, a positive response of:
- corneal opacity ≥ 3 and/or
- iritis > 1,5

calculated as the mean scores following grading at 24, 48 and 72 hours after installation of the test material.

The category 2 (Irritating to eyes) applies if the following is observed in the available data package:

If, when applied to the eye of an animal, a substance produces:

- at least in 2 of 3 tested animals, a positive response of:
- —corneal opacity ≥ 1 and/or
- — iritis ≥ 1, and/or
- — conjunctival redness ≥ 2 and/or
- — conjunctival oedema (chemosis) ≥ 2
- — calculated as the mean scores following grading at 24, 48 and 72 hours after installation of the test material, and which fully reverses within an observation period of 21 days

In 2 out of 4 acute eye irritation studies, the effects reversed within 14 days, however, in 2 out of 4 studies the eye effects did not reverse within the maximum study period, which was below 21 days.

The most pronounced corneal opacity is seen in study 4, with 2 of 6 animals exceeding corneal opacity of 3; the threshold for Irreversible effects on the eye of 1.5 for iritis in any of the studies, while the threshold for Irritating to eyes is clearly reached.

Since, reversibility of the effects was not demonstrated by the available data, Category 1 seems to be appropriate.

9.5.3 Conclusion on classification and labelling for serious eye damage/eye irritation

Folpet is proposed to be classified as serious eye damage, Category 1, according to the CLP classification criteria.

9.6 Respiratory sensitisation

No specific studies are available in the data set to address Respiratory sensitisation.

9.7 Skin sensitisation

Two Magnusson and Kligman skin sensitisation assays in Guinea pig are available for folpet, which indicated a similar skin sensitisation potency. Both studies also indicate signs of irritation, however, with different potency. There are some studies that report skin reactions after folpet exposure, but it is unclear whether the results are biased by its irritative properties.

If not stated otherwise in the summary tables, the studies are considered as fully reliable.

Table 23: Summary table of animal studies on skin sensitisation

Method, guideline, deviations from OECD 406 (1992) if any	**Species, strain, sex, no/group**	**Test substance,**	**Dose levels duration of exposure**	**Results**	**Reference**
GLP, 84/449/EEC method B.6, OECD 406 (1981), GPMT	Albino Dunkin-Harley Guinea pigs, 20/females/folpet group	Folpet technical, no purity reported	Induction Intradermal: 0.1% Topical: 50% (48 hrs, 1 week after intradermal induction) Topical challenge 25% (24 hrs, 2 weeks after topical induction) Topical Re-challenge 10% (1 week after challenge)	At 24-hour and 48-hour observation timepoints after challenge, 17/19 and 14/19 skin reactions. Erythema suspected to be irritation hence the re-challenge concentration was decreased. At 24-hour and 48-hour observation timepoints after re-challenge, 12/ 19 and 13/19 skin reactions. Skin reactions were considered as sensitisation rate 68% (13/19). Erythema could not be evaluated at all sides due to other dermal adverse reactions such as desquamation, oedema, scraps or other type of skin damage.	(R-5863) Study 1 (1991)
GLP, OECD 406	Albino Dunkin-Harley Guinea	Folpet technical,	Induction	At the 24-hour observation after challenge with 50% and 10%,	(R-7424)

Method, guideline, deviations from OECD 406 (1992) if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results	Reference
(1992), GPMT	pigs, 10/sex/folpet group	95.6%	Intradermal: 10% Topical: 50% (48 hrs, 1 week after intradermal induction) Topical challenge 10%, 50% (24 hrs, 2 weeks after topical induction)	19/20 and 14/15 skin reactions, respectively. At the 48-hour observation after challenge with 50% and 10%, 20/20 and 10/15 skin reactions, respectively.	Study 2 (1993)

Table 24: Summary table of human data on skin sensitisation

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
Peer-reviewed literature		Retrospective chart review of a small number of patients tested with sunscreens, antimicrobial agents, medications, fragrances, plants and plant derivates and pesticides (n=76)	Folpet was included in the pesticides patch test series. It elicited 3 positive photopatch test reactions (0.1% concentration diluent), captan elicited 2 positive photopatch test reactions (0.1% concentration diluent) and captofol elicited 1 positive photopatch test reaction (0.1% concentration diluent). Folpet, captan and captafol belong to the group of phthalimide fungicides. The total number of tests is unclear. Two the positive pesticide results were considered to be possibly relevant and 4 of unknown relevance, although it is unclear which active substance and how many subjects these relate to.	Victor et al. 2010
Peer-reviewed literature		Patch test of patients with chronic actinic dermatitis	Folpet was included in the photoallergen patch series. One patient (out of 9) had a positive patch test response to folpet.	Lim et al. 1998
Peer-reviewed literature		Patch test of 26 patients with suspected photoallergy	Two patients had reactions to folpet (0.1% concentration diluent), one to photopatch and one to both patch and photopatch tests. For the first the reactions were considered clinically relevant since she had apparently recently sprayed her property with ‘fungicides and pesticides’. It is not stated whether this included folpet.	Mark et al. 1999
Peer-reviewed		Case study and patch test with 45 year old female	Folpet exposure unknown, required gloves not worn when handling	Peluso et al. 1991

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
literature		agricultural worker with a 2-month history of eczema of the fingers of her right hand, which improved when she stopped work. Patch tests with the GIRDCA standard series and a pesticides series showed positive	pesticides. Dermatitis completely resolved when exposure towards pesticides was stopped. Positive reaction to folpet 0.1%. Unclear how sensitisation and irritation effects were distinguished.	
Peer-reviewed literature		Patch test of 122 farmers who regularly prepared and sprayed pesticides and a group of 63 printing press workers with no known exposure to pesticides were. Exposure assessment via interview.	None of the farmers reported frequent use of folpet. 13 (10.7%) were reported as being sensitised to folpet against 5 (7.9%) for the controls (printing press workers). Unclear how sensitisation and irritation effects were distinguished.	Guo et al. 1996
Peer-reviewed literature		Patch test of 652 subjects to establish the optimal test concentration, and the frequency of irritant and allergic reactions.	3 of 442 subjects showed irritation towards 0.1% Folpet and 6 of443 allergic reactions. 1 of 89 agricultural workers showed irritant and 1 of 89 allergic reactions. 1 of 30 Ex agricultural workers showed allergic reactions. It is unclear whether the cases with irritation and allergic reactions are in different patients.	Lisi et al. 1987

Table 25: Summary table of studies on skin sensitisation

Method, guideline, deviations	Dose levels duration of exposure, Species, strain, sex, no/group	Results -Observations and time point of onset -Mean scores/animal -Reversibility	Reference
OECD 442C (DPRA)		Study is currently being conducted. Results will be amended during public commenting.	(000107048/ 20273723)
OECD442D (KeratinoSens)		Study is currently being conducted. Results will be amended during public commenting.	(000107039/ 20273733)
OECD 442E (USens)		Study is currently being conducted. Results will be amended during public commenting.	(000107040/ 20273737)
GARD skin assay		Study is currently being conducted. Results will be amended during public commenting.	(000107041/ 1063-2003)

9.7.1 Short summary and overall relevance of the provided information on skin sensitisation

Two Magnusson and Kligman skin sensitisation assays in guinea pig are available for folpet, which indicated a similar skin sensitisation potency. Both studies also indicate signs of irritation, however, with different potency.

In vivo studies

In Study 1 (1991), twenty test and ten control animals were used for the main study. Based on the results of sighting tests, the concentrations of test material in arachis oil were 0.1% for the intradermal induction, 50% for topical induction, 25% for topical challenge, which was reduced to 10% for a re-challenge, as the concentration at the first challenge showed clear signs of skin irritation. Freund's Complete Adjuvant was used according to the test guideline.

After the topical induction. Moderate and diffuse redness was noted at eighteen treatment sites of test group animals one hour after patch removal. Scattered mild redness was noted at one treatment site at this time and at nine treatment sites at the 24-hour observation. Evaluation of the erythema was not possible at some treatment sites of test group animals due to bleeding open wounds, small superficial scattered scabs, dried blood, hardened light brown-coloured scabs and residual test material. One animal was found dead on day twelve.

After topical challenge. Moderate and diffuse redness was noted at three treatment sites of test group animals at the 24-hour observation and at one treatment site at the 48-hour observation. Scattered mild redness was noted at fourteen treatment sites at the 24-hour observation and at thirteen treatment sites at the 48-hour observation. At some treatment sites the reaction extended beyond the test site. An isolated incident of well-defined oedema was noted at the 24-hour observation and an isolated incident of desquamation was noted at the 48-hour observation. Scattered mild redness was noted at one treatment site of the control group animals at the 24 hour observations. Due to the suspected primary irritation, the concentration was reduced for a re-challenge.

After the topical re-challenge scattered mild redness was elicited by the test material at six treatment sites at the 24-hour observation and at two treatment sites at the 48-hour observation, with the reaction extending beyond the treatment site in some animals. Evaluation of the erythema was not possible at some treatment sites of test group animals due to desquamation, well-defined oedema, small superficial scattered scabs, superficial cracking of the epidermis, fur loss, fissuring, hyperkeratinisation, loss of skin elasticity and flexibility and hardened dark brown/black-coloured scabs.

At the re-challenge the test material produced a 68% (13/19) sensitisation rate and was classified as a strong sensitiser to guinea pig skin.

In Study 2 (1993), ten control animals and twenty test animals were used in the main study for treatments with propylene glycol, 50% and 10% folpet. Based on the results of sighting tests, the concentrations of test material in propylene glycole were 10% for the intradermal induction and 50% for topical induction. Before topical induction the animals were treated with 10% sodium lauryl sulfate because topical induction did not result in any dermal responses. The topical challenge used concentrations of 10% and 50%. Freund's Complete Adjuvant was used according to the test guideline.

In contrast to topical induction, intradermal induction resulted in moderate erythema, low incidences of eschar formation, pallor and discolouration. Challenge with 50% folpet resulted in eschar formation and/or oedema and exfoliation in all animals. Two animals showed fissuring and another loss of flexibility. Challenge with 10% folpet resulted in eschar formation and/or oedema in 9 of 20 animals. Moderate erythema was observed in one animal, slight in five and barely perceptible in four. Twelve animals showed exfoliation and a single animal showed fissuring.

Peer-reviewed literature

The available studies in the literature report cases of allergic reactions upon folpet challenge. However, the exposure for induction is not defined and only assessed in interviews. It is unclear for most studies how allergic reactions were distinguished from irritant reactions. Lisi et al. 1987 report some irritant reactions to a concentration of 0.1% folpet, hence, it is unclear whether sensitisation was actually observed. Due to the limitations of the available human studies classification is proposed to be performed based on the available animal data.

9.7.2 Comparison with the CLP criteria

According to the criteria shown in Annex I, Part 3 of CLP, substances can be allocated to one Skin Sensitisation category:

When an adjuvant type guinea pig test method for skin sensitisation is used, a response of at least 30 % of the animals is considered as positive.

Since both studies were adjuvant type Guinea pig test methods and Study 1 shows a sensitisation rate of 68% (13/19) and Study 2 a sensitisation rate of 100% (20/20), a classification for Skin Sensitisation Category 1 is applicable.

Furthermore in Study 1 ≥ 30 % responding at ≤ 0,1 % intradermal induction dose. Therefore, sub-category 1A applies. Thereby skin reactions were considered as sensitisation rate 68% (13/19). This corresponds to the potency of an extreme skin sensitiser. Therefore, an SCL of 0.001% should be set.

Due to the limitations of the available human studies classification is proposed to be performed based on the available animal data.

9.7.3 Conclusion on classification and labelling for skin sensitisation

Folpet is proposed to be classified for Skin Sensitisation, Category 1A with a SCL of 0.001%, according to the CLP classification criteria.

9.8 Germ cell mutagenicity

There is a substantial genotoxicity data package available for folpet. Overall, *in vitro* assays are positive, which may be due to folpet's direct reactivity with external cellular structures, which is also the reason for its fungicidal activity. folpet's genotoxic activity is partly reduced or abolished by adding S9 or by thiol sources such as GSH, as described in the review article of Arce et al. 2010 (summarized in Annex I) on genotoxic properties of folpet (and captan). This is expected with regard to folpet's toxicokinetic properties as described in Section 9. *In vivo* tests are consistently negative, also, when investigating the small intestine, which is important with respect to the observation of small intestinal tumours in mice, see Section 8.9, as this supports a non-genotoxic aetiology. For captan, which has similar irritative properties, is structurally related to folpet and has the same toxicophore, there is also a negative transgenic rodent assay available.

As folpet does not appear to enter the systemic compartment, see Section 9, it is instructive to also review *in vitro* genotoxic data related to its systemic metabolites. There is, however, the *caveat* that its metabolites can be dosed substantially higher because 1) they are not or are less cytotoxic than folpet and 2) similar doses are higher molar doses, as the molecular weight of the metabolites is less than that of folpet – the metabolites lack the trichloromethythio-side chain:

Folpet 296.558 g/mol

Phthalimide 147.131 g/mol

Phthalamic acid 165.146 g/mol

Phthalic acid 166.131 g/mol

[Molecular weight from ChemSpider (chemspider.com)]

Hence, equimolar doses for the metabolites are achieved by using roughly half the corresponding folpet dose. For example, the highest dose for folpet achieved in Study 9 (2017) of 500 µg/plate, corresponds to an equimolar dose of about 250 µg/plate for phthalimide.

If not stated otherwise in the summary tables, the studies are considered as fully reliable.

Table 26: Summary table of mutagenicity/genotoxicity tests in vitro with folpet

Method, guideline, deviations if any	Test system	Concentrations tested	Result	Reference
OECD 471 (1997)	S. typhimurium TA98, TA100, TA1535,	0.50, 1.58, 5.00, 15.8, 50.0, 158, 500 µg/plate	Positive (+/- S9) Lower potency under conditions of	(R-38829) Study 9

Method, guideline, deviations if any	Test system	Concentrations tested	Result	Reference
	TA1537 E.coli WP2 uvrA		metabolic activation.	(2017)
OECD 471 (1983) Deviations from OECD 471 (1997): Only one strain was tested	S. typhimurium TA100	2.5, 5.0, 10, 25, 50, 100, 200 µg with and without S9	Positive (+/- S9)	(R-7365) Study 10 (1993a)
OECD 471 (1983) Deviations from OECD 471 (1997): *E. coli* WP2 uvrA, or *E. coli* WP2 uvrA (pKM101), or *S. typhimurium* 102 were not included	S. typhimurium TA98, TA100, TA1535, TA1537	2.5, 7.9, 25, 79 250µg (folpet with PCMM<50 ppm) 10, 32, 100, 316 and 1000 µg (folpet with PCMM: 2200 ppm)	Positive (+/- S9)	(R-7208) Study 11 (1993b)
OECD 476 (2016)	Chinese hamster V79/HGPRT locus	0.0001, 0.0003, 0.0005, 0.0007 and 0.001 mM (-S9)) 0.005, 0.01, 0.02, 0.04 and 0.06 mM (+S9)	Positive (+/- S9) Lower potency under conditions of metabolic activation.	(R-38830) Study 12 (2018a)
No guideline stated Deviations from OECD 476 (2016): - no information is given on the amount of S9 added - only 105 cells instead of 2 x 106 were cultured during the expression period and plated for mutant selection - the highest concentration did not meet the conditions for cytotoxicity (i.e. 20 and 10% RS) - no HCD data are reported - no statistical analysis was performed Supplementary information (reliable with restrictions)	Chinese hamster V79/HGPRT locus	0.125, 0.25, 0.5, 1 and 2 µg/mL without S9; 3.125, 6.25, 12., 25 and 50 µg/mL with S9	Inconclusive (+/- S9)	(R-4340) Study 13 (1986)
OECD 473 (2016)	Human lymphocytes	10, 25 and 50 µM (-S9) 7, 20, 50 and 70 µM (+S9)	Positive (+/- S9) Lower potency under conditions of metabolic activation.	(R-38831) Study 14 (2018b)
OECD 473 (1983) - exposure time was 2 hours (instead of 3-6 hours) - only one dose group (3 µg/ml) met all three experimental conditions - only 100 (instead of 300)	Human lymphocytes	1, 2 and 3 µg/mL with and without S9	Inconclusive (+/- S9)	(R-4392) Study 15 (1987)

Method, guideline, deviations if any	Test system	Concentrations tested	Result	Reference
metaphases were scored - the highest concentration did not meet the conditions for cytotoxicity (reduction in MI for primary cultures of lymphocytes to 45± 5% of the concurrent negative control) - no measurements of cell proliferation have been reported (to assure that a sufficient number of treated cells have reached mitosis during the test) - no information regarding the length of the cell cycle is given in the study report - no trend test was performed - no HCD data are reported Supplementary information (reliable with restrictions)				
US EPA FIFRA, Subdivision F, 84-2 Deviations from OECD 473 (2016): - exposure time was 2 hours (instead of 3-6 hours) in the activated assay - exposure time was 10 and 20 hours (instead of 3-6 hours or an equivalent to about 1.5 normal cell cycle lengths) in the non-activated assay - no dose group met all three experimental conditions - only 100 (instead of 300) metaphases were scored - no measurements of cell proliferation have been reported (to assure that a sufficient number of treated cells have reached mitosis during the test) - no information regarding the length of the cell cycle is given in the study report - no trend test was performed - no HCD data are reported Supplementary information	Chinese Hamster ovary	0.08, 0.25 and 0.75 µg/mL without S9 0.8, 2.6 and 7.7 µg/mL with S9	Positive (+/- S9)	(R-5211) Study 16 (1989)

Method, guideline, deviations if any	Test system	Concentrations tested	Result	Reference
(reliable with restrictions)				
No guideline stated, publication, Cell transformation and cell cycle analysis	BALB/3T3 cells	10.0 µg/mL without S9	Cell transformation by folpet is associated with multiple defects in the signals involved in the regulated progression of the cell cycle and the induction of cell-cycle checkpoints	Santucci et al. (2003)
No guideline available, Genotoxicity screening assays	GreenScreen HCGADD45a-GFP CellCiphr p53 CellSensor p53RE-bla	GreenScreen HCGADD45a-GFP: 50, 100 and 200 µM GADD45a-GFP, CellCiphr p53: 10 concentrations from 0.39-200 µM CellSensor p53RE-bla: 15 concentrations from 1.2 nM to 92 µM	Negative in GreenScreen HC GADD45a-GFP and CellCiphr p53 Positive in CellSensor p53 assay.	Knight et al. (2009)

Table 27: Summary table of mutagenicity/genotoxicity tests in mammalian somatic or germ cells in vivo

Method, guideline, deviations if any	Test system	Relevant information about the study (as applicable)	Observations	Reference
OECD 474 (1983) Deviations from OECD 474 (2016): - acclimatisation period 4 days (instead of 5) - dose spacing too wide (factor 5 instead of maximal 4) - highest dose group was below the MTD - only one treatment (instead of ≥ 2) -samples of bone marrow were solely taken twice (within 24-48 hours) from negative control group and high dose group	Bone marrow	Oral gavage 10, 50 and 250 mg/kg	Negative (indirect evidence of target tissue exposure)	(R-3651) Study 1 (1985)
Not stated, chromosomal aberration Deviations from OECD 475 (2016): - Groups of four animals/sex/time period used, not at least five, as recommended - only 50 (instead of 200) metaphases were analysed/ animal - mitotic index was determined in 500 scored cells (instead of 1000)	Rat bone marrow	Oral gavage 150, 500, 1500 and 2000 mg/kg	Negative (indirect evidence of target tissue exposure)	(R-6133) Study 2 (1983)

Method, guideline, deviations if any	Test system	Relevant information about the study (as applicable)	Observations	Reference
Not stated, Comet assay Deviations from OECD 489 (2016): - Only one treatment (instead of 2) - Only 4 animals analysed (instead of 5)	Mouse duodenum	Oral gavage 1000 and 2000 mg/kg bw	Negative	(R-17100) Study 3 (2004)
Not stated, Mouse somatic cell mutation test Deviations from OECD 484 (1986): - Route of administration via diet (instead of via gavage or intraperitoneal) - Animals fed test diets from Days 8-12 inclusive (instead of single doses on days 8, 9 and 10)	C57B1/6 mouse (140-146 per group) peripheral mononuclear cells	days 8th to 12th of gestation, inclusive 0, 17, 300 and 965 mg/kg bw/d	Negative	(R-6138a) Study 4 (1985) Historical control data in statement of Anonymous 1997
Not stated, Halo and Comet assay Deviations from OECD 489 (2016): - Only 4 animals analysed (instead of 5) - No general clinical observation during the study period is reported	mouse duodenum cells	Oral gavage 2000 mg/kg	Negative	(FSR-IPL 071003) Study 5 (2008)
Not stated, Comet assay Deviations from OECD 489 (2016): - Only two dose groups (instead of 3) - Only 4 animals analysed (instead of 5) - No trend analysis was performed - No general clinical observation during the study period is reported	mouse duodenum cells	Oral gavage 1000 and 2000 mg/kg	Negative	(FSR-IPL 070604) Study 6 (2007)
Not stated, Dominant lethal test Deviations from OECD 478 (2016): - no information regarding the dose volume used - unclear if the highest dose was the MTD - no food consumption was measured - unclear on which day of pregnancy the females were sacrificed - results on weighing are not reported - < 400 implantation sites/ group - only 7 weeks instead of 8 for the mating period	Rat	Oral gavage for 5 days 50, 100, 200 mg/kg bw	Negative	(R-6121) Study 7 (1980)
Not stated, Dominant lethal test Deviations from OECD 478 (2016): - no information on animal housing and feeding conditions - no information regarding the dose volume used - no information on preparation of the animals	Rat	Intraperitoneal injection and oral gavage for 5 days 2.5, 5 and 10 mg/kg bw/d (i.p.) 50, 100 and 200	Inconclusive	(R-545) Study 8 (1982)

Method, guideline, deviations if any	Test system	Relevant information about the study (as applicable)	Observations	Reference
and animal weight - unclear if the highest dose was the MTD - no food consumption was measured - no positive control - females sacrificed on day 13 (instead of 14/15) - corpora lutea was not assessed - number of late deaths not reported - < 400 implantation sites/ group - post and pre-implantation loss as well as dominant lethal factor were not calculated Supplementary information (reliable with restrictions)		mg/kg bw/d (oral)		
Not stated, Dominant lethal test Deviations from OECD 478 (2016): - no information regarding laboratory proficiency or historical control data are reported - no information regarding animal housing or feeding is reported - no further information regarding methods and results of the positive control - no information regarding the dose volume used in the i.p study - No justification for the use of intraperitoneal injection as the administration route has been given - only 2 dose groups (instead of 3) - highest dose did affect the mating success - animals were not weighed (adults and foetuses) - no food consumption was measured - unclear on which day of pregnancy the females were sacrificed - < 400 implantation sites/ group - only 6 weeks instead of 8 for the mating period - corpora lutea were not counted (only calculated) - post implantation loss and dominant lethal factor were not calculated - no statistical analysis - Not reliable (due to major study deviations and IBT as the testing facility, where the study was conducted)	Male albino mice	Intraperitoneal injection (single dose) 0, 5 and 10 mg/kg bw/d	Negative	R-6073 Study 17 (1971)

Table 28: Summary table of other genotoxicity data for folpet

Method, guideline, deviations if any	Test system	Concentrations tested	Result	Reference
Ames	*S. typhimurium* TA97, TA98, TA100 and TA102	0, 1, 50, 100, 150, 200, 250, 300 and 400 µg/plate +/- S9	Positive (+/- S9)	Yu et al. 2006
Comet, in vitro	human peripheral mononuclear cells	0, 0.1, 1 and 10 µg/mL -S9	Positive	
Comet, in vivo rat, 5/group/sex - no positive control - very high doses when compared with other 90-day studies for folpet Supplementary information (reliable with restrictions)	peripheral mononuclear cells	0.0, 239, 717, 2150 mg/kg bw via diet for 90 days NB 90 d dietary studies for folpet LOAEL [F344]) 136 mg/kg bw/day NOAEL [SD] = 56 mg/kg bw/day	Negative	
Spermatogonial chromosomal aberration test	Mouse (5 males) testis cells	oral gavage for 5 days 1000, 2000 and 4000 mg/kg bw/day	Negative	
Mouse micronucleus	mouse bone marrow (5 each sex) polychromatic erythrocytes	oral gavage for 2 days 1000, 2000 and 4000 mg/kg bw	Negative	

Table 29: Summary table of mutagenicity/genotoxicity tests with folpet metabolites

Method, guideline, deviations if any	Test system	Concentrations/Doses tested	Result	Reference
Phthalimide				
Ames test (public literature) (not reliable)	TA98 +/- S9	400 and 2000 µg/plate	Negative	Riggin et al. 1983 (R-11350)
Chromosome aberration (public literature) (not reliable)	Human lymphocytes	0, 0.1, 1 and 10 µg/mL	Negative	Pilinskaya (1986)
Ames test (authority report of Japan) Original studies are not available, solely summary of Ministry of Health and Welfare (MHW), Japan Supplementary information (reliable with restrictions)	*S. Typh.* TA 98, TA100, TA1535, TA1537 Escherichia coli WP2 uvrA +/- S9	5000, 1250, 313, 78.1, 19.5, 4.88, 1.22, 0 µg/plate	Negative	MHW (1999)
Chrom. Aberration in vitro (authority	Chinese hamster	0, (313), 625, 1250,	Inconclusive*	

Method, guideline, deviations if any	Test system	Concentrations/Doses tested	Result	Reference
report of Japan) - No statistical analysis as required in the OECD TG 473 was performed. - No historical negative control data - precipitation was observed from the lowest concentration onwards Original studies are not available, solely summary of Ministry of Health and Welfare (MHW), Japan supplementary information (reliable with restrictions)	cells (CHL/IU)	2500, 5000 µg/mL	*Highest concentration increase in mutant frequency along with precipitation, no historical control data available	
Phthalic acid and phthalic anhydride				
Ames test (fully reliable) and Chrom. Aberration (supplementary information-reliable with restrictions) in vitro (public literature) Chromosome aberration test: - only 100 (instead of 300) metaphases were scored - the highest concentration did not meet the conditions for cytotoxicity (reduction in MI for primary cultures of lymphocytes to 45± 5% of the concurrent negative control) - no measurements of cell proliferation have been reported (to assure that a sufficient number of treated cells have reached mitosis during the test)	Salmon. Typh. TA 98, TA100, TA102, TA1535, TA1537 +/- S9	Phthalic acid: 0, 20, 100, 500, 2500, 12500 µg/plate	Negative	Lee & Lee (2007)
	CHO cells +/- S9	Phthalic acid: 0, 20, 100, 500, 2500, 12500 µg/plate	Negative	
Ames test (public literature) not reliable, very limited reporting	Salmon. Typh. TA98, TA100, TA1535, TA1537, TA1538, and TA2637	Phthalic acid: 100-2000 µg/plate	Negative	Agarwal et al. 1985
Ames test not reliable, very limited reporting	Salmon. Typh. TA97, TA98, TA100, TA102, and TA104	Phthalic acid: up to 10 mg/plate	No mutagenic activity	Sayato et al. 1987
Chrom. Aberration in vitro (public literature) supplementary information (reliable with restrictions)	CHO cells - S9	Phthalic acid (as sodium salt): 0, 10, 20, 50 mM	Negative	Phillips et al. 1982
Micronucleus test in vivo (public literature) supplementary information (reliable with	Bone marrow of male ICR mice	Phthalic acid: 0, 20, 100, 500, 2500, 12500 µg/kg bw	Negative	Lee & Lee 2007

Method, guideline, deviations if any	Test system	Concentrations/Doses tested	Result	Reference
restrictions)				
Chrom. Aberration in vitro (public literature) supplementary information (reliable with restrictions)	CHO cells - S9	Phthalic acid (as sodium salt): 0, 10, 20, 50 mM	Negative	Phillips et al. 1982
Dominant lethal test and Sperm head abnormality assay (public literature) - only 2 dose groups (instead of 3) - only 4 weeks instead of 8 for the mating period - animals were not weighed (adults and foetuses) - no food consumption was measured - < 400 implantation sites/ group - route of administration: i.p. - no information on corpora lutea - highest dose did affect the mating success - solely information regarding MTD is mortality and limited information regarding clinical signs - no information regarding positive controls is available - post and pre-implantation loss were not calculated - dominant lethal factor was not calculated appropriately supplementary information (reliable with restrictions)	Swiss albino mice	0,40, 80 mg/kg bw/d for 5 days i.p.	Inconclusive	Jha et al. 1998
	Swiss albino mice	Single dose of 0, 50, 100, 150, 200 and 300 mg/kg bw i.p.	Abnormal sperms at 50 mg/kg bw	
Ames test (public literature) - Only 108 cells were incubated with the test substances - E. coli WP2 uvrA, or E. coli WP2 uvrA (pKM101), or S. typhimurium TA102 was not tested - Reporting of cytotoxicity is limited supplementary information (reliable with restrictions)	Salmon. Typh. TA98, TA100, TA1535, TA1537	Phthalic anhydride: 3.3-3333 µg/plate (experiment 1) 1-666 µg/plate (experiment 2)	Ames	Zeiger et al. (1985)
Chrom. Aberration in vitro (public literature)	CHO cells - S9	Phthalic anhydride: 0, 6, 8, 10 mM	Chromosome aberrations at top dose	Hilliard et al. 1998

Method, guideline, deviations if any	Test system	Concentrations/Doses tested	Result	Reference
- Only 200 metaphases were scored - Data with metabolic activation not shown - Only one experimental condition with an exposure time of 3 hours and harvesting after 20 hours was tested supplementary information (reliable with restrictions)			(precipitation)	
Chrom. Aberration and sister chromatid exchange (SCE) in vitro (public literature) - Only 100 metaphases were scored - Unclear how many and which concentrations were tested - Only one trial - Only one experimental condition was tested (continuous exposure without S9 and 2 hours with S9) - Evaluation of the results in not appropriate - Reporting of the results is very limited not reliable	CHO cells - S9	Phthalic anhydride: 10-300 µg/ml in SCE; 30-300 µg/ml in CA	Negative	Galloway et al. 1987

9.8.1 Short summary and overall relevance of the provided information on germ cell mutagenicity

Folpet's genotoxic properties are well investigated *in vitro* and *in vivo*, considering gene mutation, clastogenicity, aneugenicity and DNA damage.

In vitro assays with folpet consistently become positive. It is unclear, whether folpet is directly genotoxic or whether the assay outcomes are biased by direct interaction with external cellular structures. Folpet is used as a contact fungicide and rapidly reacts with proteins or freely available thiol sources or degradants, see Section 7. Folpet has also limited bactericidal properties, see Section 8.10, which may be due to the same mechanism. The addition of metabolic activation via S9 mix mitigates the potency of the genotoxic effects, which may be associated with an increased thiol-pool that detoxifies folpet. Arce et al. 2010 report that freely available thiols can detoxify folpet by binding and degrading the molecule.

Contrary to the *in vitro* studies, the *in vivo* studies consistently become negative. As described in Section 9, it is unlikely that folpet is systemically available. Hence, the systemic compartment is most likely only exposed to folpet's metabolites. Although the specific *in vitro* genotoxicity studies on folpet's metabolites have a lower quality than the most current studies for folpet, the *in vitro* studies for the metabolites are consistently negative. However, also *in vivo* studies that investigate genotoxicity at the first site of exposure are negative. Hence, either a) folpet cannot penetrate into fully viable cells, and only its metabolites/degradants penetrate, which are not genotoxic, or b) there is a larger thiol pool available *in vivo* than *in vitro* for detoxification or c) the *in vitro* assays are biased by the direct interaction of cell membranes with folpet and give incorrect *in vivo* predictions. Based on the available data all, of the three hypotheses appear plausible and may be interacting.

They indicate that the observed *in vitro* genotoxicity has no human relevance for hazard classification and labelling purposes.

9.8.2 Comparison with the CLP criteria

According to the criteria shown in the Table 3.5.1 of Annex I, Part 3 of CLP, substances can be allocated to one of two germ cell mutagenicity classes, as no human data, which are used as a qualifier for Category 1, are available.

> *The classification in Category 1B is based on:*
>
> - *positive result(s) from in vivo heritable germ cell mutagenicity tests in mammals; or*
> - *positive result(s) from in vivo somatic cell mutagenicity tests in mammals, in combination with some evidence that the substance has potential to cause mutations to germ cells. It is possible to derive this supporting evidence from mutagenicity/genotoxicity tests in germ cells in vivo, or by demonstrating the ability of the substance or its metabolite(s) to interact with the genetic material of germ cells; or*
> - *positive results from tests showing mutagenic effects in the germ cells of humans, without demonstration of transmission to progeny; for example, an increase in the frequency of aneuploidy in sperm cells of exposed people.*
>
> *The classification in Category 2 is based on positive evidence obtained from experiments in mammals and/or in some cases from in vitro experiments, obtained from:*
>
> - *somatic cell mutagenicity tests in vivo, in mammals; or*
> - *other in vivo somatic cell genotoxicity tests which are supported by positive results from in vitro mutagenicity assays.*
>
> *Note: Substances which are positive in in vitro mammalian mutagenicity assays, and which also show chemical structure activity relationship to known germ cell mutagens, shall be considered for classification as Category 2 mutagens.*

Based on a weight-of-evidence assessment folpet is considered not to be genotoxic to human germ cells.

- *Toxicokinetic considerations*: According to the data, folpet is not systemically available itself, only its metabolites reach the systemic compartment. Further, folpet is quickly degraded in human blood. Hence, an exposure of human germ cells towards folpet almost certainly cannot occur in any conceivable exposure scenario.
- *Gene mutation:*
  - Ames tests and mammalian gene mutation tests with folpet are all positive *in vitro*. While there are no direct appropriate follow-up tests with folpet available for gene mutation, the available *in vivo* Comet assays are all negative up to 2000 mg/kg bw both in systemic and target tissues at the first site of exposure. Further, the potency of the observed effects is reduced with metabolic activation, probably due to an increased thiol source, which detoxifies Folpet's irritative properties and degrades the molecule.
  - There is a negative *in vivo* mutagenicity assay, i.e. a transgenic rodent assay, available for captan, which has the same toxicophor/trichloromethiothio-side chain as folpet and the same genotoxic properties *in vitro*.
  - Folpet's systemic metabolites are consistently negative in Ames tests, which is relevant as human germ cells are most likely exposed towards folpet's metabolites and not folpet itself.
- *Clastogenicity, Aneugenicity*: Chromosome aberration and micronucleus tests that are positive *in vitro* and can be used to support germ cell genotoxicity are negative in the higher tier *in vivo* studies.

- *Germ cell mutagenicity*: Multiple dominant lethal tests are available for folpet, which, while not fully compliant with the current test guideline, directly investigate germ cell mutagenicity and are consistently negative.

9.8.3 Conclusion on classification and labelling for germ cell mutagenicity

Folpet is proposed to be not classified for germ cell mutagenicity, according to the CLP classification criteria.

9.9 Carcinogenicity

There are six long-term studies available for folpet, three in mice and three in rats, using two strains for each species.

If not stated otherwise in the summary tables, the studies are considered as fully reliable.

In mice (Studies 1-3), but not in rats (Studies 4-6), folpet treatment results in small intestinal tumours, duodenal carcinomas and adenomas. Benign papillomas in the non-glandular region of the stomach were also observed. The aetiology of the small intestinal tumour has been investigated in dedicated mode of action studies as being driven by cytotoxicity with subsequent regenerative proliferation.

Captan, which is structurally related with folpet and has the trichloromethylthio-side chain shows the same carcinogenicity, with small intestinal tumours in mice but none in rats. Also, the structurally very different hexavalent chromium results in small intestinal tumours in mice, which has however similar irritating effects as folpet and captan. Based on these observations, an adverse outcome pathway was proposed for small intestinal tumours involving chronic cytotoxicity and regenerative hyperplasia by Bhat et al. 2020, based on prior works of Thompson et al 2017, Chappell et al 2019 and Becker et al 2015, with captan, folpet and hexavalent chromium as lead substances.

The administration of folpet for 2 years to rats at dietary doses of 10 – 120 mg/kg bw/day produced decreased body weight and food consumption. Enzymatic activity and total protein levels were reduced at the higher dose levels. Hyperkeratosis of the non-glandular stomach and the oesophagus were present in animals treated at levels of 50 mg/kg bw/day and above.

In the chronic studies with mice further clinical signs were reported, such as dry, flaking skin, skin encrustations, reduced body weights and food consumption, hyperkeratosis and acanthosis of the epidermis, hyperplasia of the duodenal mucosa and of the jejunum.

It is very likely that all reported observations in rat and mice are associated with folpet's irritative properties and occur due to direct contact with folpet at the site of first exposure, which is plausible for the observed effects. The skin effects in the dietary studies are most likely related due to direct exposure towards folpet diet. A dermal repeated exposure study (Section 8.4/Study 3, 1988) shows that folpet results in similar skin effects in rats.

Table 30: Summary table of animal studies on carcinogenicity

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Mouse			
Not stated, conducted predominately according to OECD 451, Dietary carcinogenicity, for 112-113 weeks Deviations from OECD 451 (2009): Cervix, coagulating gland, lachrymal gland parathyroid, rectum, testis, vagina	Folpet Purity 93%, Treatment of 0, 1000, 5000 and 12000 ppm for 112-113 weeks	NOAEL= <1000 ppm (equivalent to 93 mg/kg/day) Carcinogenic effects <u>12000 ppm (1282 mg/kg bw/d)</u> Mucosal hyperplasia in jejunum (males 40%, females: 37%) and ileum (males:	(R-6036) Study 1 (1982)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
were not processed histopathologically	CD-1 mouse 80/sex/treatment group Control= 104/sex	10%), stat. sign. Mucosal hyperplasia in duodenum (68% males, 53% females), stat. sign. extramedullary haematopoiesis of the spleen (34% males, 35% females), stat. sign. in males ↓ haemosiderosis (16% males, 14% females), stat. sign. in females duodenal adenoma, jejunum: adenocarcinoma (males) bw ↓ (17% males, 16% females) <u>5000 ppm (502 mg/kg bw/d)</u> duodenal adenocarcinoma Mucosal hyperplasia in duodenum (43% males, 41% females), stat. sign. extramedullary haematopoiesis of the spleen (47% males, 44% females), stat. sign. bw ↓ (10%) <u>1000 ppm (93 mg/kg bw/d)</u> Mucosal hyperplasia in duodenum (33% males, 40% females), stat. sign.	
Not stated, conducted according to OECD 451 (1981), Dietary carcinogenicity, 2- year Deviations from OECD 451 (2009): Coagulating gland, peripheral nerve, trachea and vagina were not processed histopathologically; no information regarding spinal cord examination (number of levels)	Folpet Purity 89% Treatment of 0, 1000, 5000 and 10000 ppm (21 weeks) and 0, 1000, 3500 and 7000 ppm for remainder (total exposure 104 weeks) B6C3F1 mouse 52/sex/group	NOAEL= < 1000 ppm (equivalent to 123 mg/kg/day) Carcinogenic effects <u>7000 ppm (1264 mg/kg bw/d)</u> malignant lymphoma (females) stomach papilloma (females) duodenal adenoma and carcinoma clinical signs (e.g. dry flaking skin, erythema, reddish discolouration of the coat and weeping skin, in the early weeks of the study) bw ↓, stat. sign (please refer to table 3.9.1-6 in the Annex Human Health) slight reduction in the probability of survival to 104 weeks <u>3500 ppm (564 mg/kg bw/d)</u> duodenal adenoma and carcinoma bw ↓, stat. sign (please refer to table 3.9.1-6 in the Annex Human Health) slight reduction in the probability of	(R-3650) Study 2 (1985)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		survival to 104 weeks 1000 ppm (123 mg/kg bw/d) histopathological findings of non-neoplastic and neoplastic lesions in GI tract (forestomach, duodenum, jejunum)	
US EPA 83-2; in accordance with OECD 451, Dietary carcinogenicity, 2-year Deviations from OECD 451 (2009): - Histopathology performed on liver, stomach, duodenum, ileum and jejunum only. - Coagulating gland, lacrimal gland, peripheral nerve, vagina were not processed histopathologically no information regarding spinal cord examination (number of levels)	Folpet Purity 92.4% Treatment of 0, 150, 450 and 1350 ppm 98 and 104 weeks in male and female animals, respectively CD-1 mouse 52/sex/treatment group Control=100/sex	NOAEL= 450 ppm (equivalent to 47 mg/kg/day) Carcinogenic effects 1350 ppm (119.9 mg/kg bw/d) Duodenal adenoma (1 incidence in females), forestomach tumours in high dose animals Duodenum: Villous hyperplasia (females: 3/52), stat. sign. Stomach – non glandular region: Keratoacanthosis (females: 13/52), stat. sign. slight reduction in body weight in the high dose males up to Week 70 but the overall weight gain was similar to the controls	(R-6530) Study 3 (1994)
Rat			
Generally met the essential criteria of Directive 87/302/EEC Part B. Dietary toxicity and carcinogenicity, 2-year Deviations from OECD 452 (2009): - Initial male weight range marginally less than –20% of mean - no haematological and clinical chemistry examination after 3 months - MCV, MCH, MCHC was not calculated; prothrombin time, and activated partial thromboplastin time was not measured - adrenals., spleen, thyroid and uterus not weighted; testes and epididymides were not weighed separately - coagulating gland, gall bladder and lacrimal gland were not processed histopathologically; spinal cord was	Folpet Purity 89.5% Treatment of 0, 200, 800 and 3200 ppm for a minimum of 104 weeks Crl:CD(SD) rat 50/sex/group Additional groups of 10 /sex were sacrificed after 52 weeks of treatment	NOAEL= 800 ppm (equivalent to 40 mg/kg/day) No oncogenic effects 3200 ppm (161.8 mg/kg bw/d) Testes: Interstitial cell hyperplasia and cell tumour (no clear dose response) Stomach: Hyperkeratosis (males: 34/50, females: 37/50), erosion/ulceration in nonglandular region (males: 7/50, females: 8/50), submucosal oedema (males: 10/50, females: 8/50), submucosal inflammatory cell infiltrate (males: 13/50, females: 12/50), non sign. 200 ppm (9.9 mg/kg bw/d) Testes: Interstitial cell hyperplasia and cell tumour (no clear dose response) For further details incl. HCD please refer to the discussion below as well as to Annex I.	(R-6081) Study 4 (1985)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
only examined in two levels (thoracic and cervical)		Please refer also to Kidwell 2010 for additional data on HCD. No detailed information on time range of HCD or potential confounding factors is available. Therefore reliability of HCD is limited.	
US EPA Pesticide Assessment Guidelines, Subdivision F, Hazard evaluation: Human and domestic animals, pp 117-125 (1982), Dietary carcinogenicity, 2-year Deviations from OECD 452 (2009): - no ophthalmological examination was performed - only total and differential leukocyte count was measured regarding haematological parameters - no clinical chemistry analysis was performed - no urine analysis was performed - adrenals. epididymides, ovaries, spleen, thyroid and uterus not weighted - aorta, coagulating gland, epididymides, gall bladder, lacrimal gland, rectum, seminal vesicle and vagina were not processed histopathologically; spinal cord was only examined in two levels	Folpet Purity 89.5-91.1% Treatment of 0, 500, 1000 and 2000 ppm for 2 years Fisher rat 60/sex/group	NOAEL= 500 ppm (28 mg/kg/day) No oncogenic effects <u>2000 ppm (108.2 mg/kg bw/d)</u> Hyperkeratosis in the stomach non glandular epithelium (moderate, males: 60/60, females: 60/60), stat. sign. Thyroid: Follicular cell hyperplasia (males: 4/60), stat. sign. Cystic seminal vesicles (males: 4/60), stat. sign. Mammary gland: lobular (acinar) hyperplasia (females: 4/60), stat. sign, benign fibro-epithelial tumour (females) foci or areas of cellular alteration (basophilic cell type) in the liver (males. 59/60; females: 60/60), stat. sign. Malignant lymphoma c-cell adenoma (females) For further details incl. HCD please refer to the discussion below as well as to Annex I. Please refer also to Kidwell 2010 and the position paper of Anonymous (2016, R-37570) for additional data on HCD. No detailed information on time range of HCD or potential confounding factors is available. Therefore reliability of HCD is limited. <u>1000 ppm (56 mg/kg bw/d)</u> hyperkeratosis of the stomach non glandular epithelium (slight, males: 58/60, females: 58/60), non. sign. foci or areas of cellular alteration (basophilic cell type) in the liver (males. 26/60; females: 45/60), not sign.	(R-4330) Study 5 (1985)
generally met the essential criteria of Directive 87/302/EEC Part B. OECD 452 (1981) Dietary toxicity, 2-year Deviations from OECD 452 (2009) - it is not reported at which frequency the animals were checked for morbidity and mortality	Folpet Purity 91.1% Treatment of 0, 250, 1500 and 5000 ppm for 2 years Fisher rat	NOAEL= 250 ppm, equivalent to 12 mg/kg/day No oncogenic effects <u>5000 ppm (296.3 mg/kg bw/d)</u> bw, food and water consumption ↓ (please refer to tables 3.9.2-12-14 in Annex Human Health)	(R-4672) Study 6 (1989)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
- prothrombin time, and activated partial thromboplastin time were not measured - epididymides, thyroid and uterus not weighted - aorta, coagulating gland, epididymides, gall bladder, lacrimal gland, rectum, seminal vesicle and vagina were not processed histopathologically; spinal cord was only examined in two levels	20/sex/group	AP, ALT, Cholesterol, protein, albumin, globulin ↓, phosphorus ↑ (males) (please refer to table 3.9.2-16 in Annex Human Health) urine of all males was more concentrated and of a lower volume than the controls at the 3 month examination, stat. sign. - please refer to table 3.9.2-17 Hyperkeratosis in the non glandular epithelium of the stomach (moderate: males: 19/20, females: 17/20), non sign. and oesophagus (slight: males: 10/20, females: 9/20; moderate: males: 8/20, females: 11/20), stat. sign. Thyroid: Follicular cell hyperplasia (males: 6/20), stat. sign. <u>1500 ppm (83.21 mg/kg bw/d)</u> AP ↓ (males),phosphorus ↑ (males) (please refer to table 3.9.2-16 in Annex Human Health) Hyperkeratosis in the non glandular epithelium of the stomach (slight: males: 7/20, females: 14/20; moderate: males: 9/20, females: 6/20), non sign. urine of all males was more concentrated and of a lower volume than the controls at the 3 month examination, stat. sign. - please refer to table 3.9.2-17 <u>250 ppm (12 mg/kg bw/d)</u> urine of all males was more concentrated and of a lower volume than the controls at the 3 month examination, stat. sign. - please refer to table 3.9.2-17	

Table 31: Summary table of other studies relevant for carcinogenicity

Type of study/data	Relevant information about the study (as applicable)	Observations	Reference
Review	Carcinogenic mode of action of folpet in mice and evaluation of its relevance to humans		Cohen et al. 2010
Review	The weight-of-evidence suggests that folpet induces small intestine tumours by a nongenotoxic mode of action involving cytotoxicity and regenerative cell hyperplasia that exhibits a clear dose threshold. Folpet should be classified as "not likely to be carcinogenic to humans at doses that do not cause irritation response in the mucosal epithelium"		Kidwell, 2010
Position paper	Contemporary historical control data relating to Study 5 (1985), which allows a rough estimate of neoplasm incidence in F344 rats.		R-37570

Type of study/data	Relevant information about the study (as applicable)	Observations	Reference
			Anonymous 2016
Mechanistic feasibility study regarding effects of folpet on the duodenum 21 days, dietary	0 and 5000 ppm folpet for 21 days, dietary, male mice	Duodenum: hyperplasia of the crypts, positive PCNA staining	(R-7632) Study 7 (1994)
Extended mechanistic feasibility/preliminary study regarding effects of folpet on the duodenum 28 days, dietary	0 and 5000 ppm folpet or PCMM, male mice	Duodenum: hyperplasia of the crypts, positive PCNA staining and 2-fold induction of PCNA/cyclin dependent kinases in the whole duodenum	(R-7794) Study 8 (1994)
Mechanistic study regarding effects of folpet on the duodenum 28 days, dietary with 28 days recovery period	0 and 5000 ppm folpet or PCMM, Male mice, Crl:CD-1(ICR)BR	Duodenum: hyperplasia of the crypts, positive PCNA staining, ↑ thiol concentrations – biochemical effects reversible during recovery period	(R-8004) Study 9 (1995)
Mechanistic study regarding hyperplasia of folpet in the duodenum 28 days, dietary	0, 150, 450 and 5000 ppm, CD 1 mice (male and female)	NOAEL = 150 ppm (approximately equivalent to 22.5 mg/kg/day in males and 29 mg/kg/day in females) duodenal crypt hyperplasia (females) ↑ in the number of cells per crypt (females) thickening of the duodenal wall (males) 5000 ppm: Duodenal crypt hyperplasia, villi reduced in size and signs of fusion ↑ numbers of inflammatory cells in the lamina propria ↑ number of BrdU labelled cells per crypt, ↑ mean number of cells in the duodenal crypt ↓ in the villus to crypt height ratio	(R-9688) Study10 (1997)
Intestinal irritation study after 24-hour exposure with sacrifices after 1, 3 and 7 days	Study 1: 200 and 5000 ppm over a 24-hour period, dietary (900 mg/kg bw, oral gavage) Study 2: 0, 50, 200 and 5000 ppm over a 24-hour period, dietary (900 mg/kg bw, oral gavage), Female mice, ICR (CD-1 equivalent)	Folpet at 5000 ppm (dietary, equivalent to 1000 mg/kg bw) causes no or minimal (borderline) irritation in the duodenum Folpet at 900 mg/kg bw (gavage) causes minimal irritation in the stomach	(R-16283) Study 11 (1997)
Mechanistic study dietary administration for up to 28 days followed by a 17-day recovery period	6000 ppm (males: 894 mg/kg bw/d females: 1024 mg/kg bw/d) CD-1 mice (sacrifices on Day 8, 15, 29 and 18 of recovery period)	Macroscopic changes typified by distension of the caecum, thickening of the duodenum and roughened forestomach. Hypertrophy and hyperkeratosis in the forestomach as well as epithelial hyperplasia After recovery (17 days) lesions returned to normal or decreased in incidence and/or severity, indicating reversibility of the changes	(R-26473) Study 12 (2011)

Type of study/data	Relevant information about the study (as applicable)	Observations	Reference
Mechanistic study for GI-tract changes (same data as in Study 12 (2011))	See Study 12 (2011)	Reversible macroscopic and histologic changes in caecum and duodenum on Day 7 up to Day 28	Gordon et al. 2012
Application of Tailored Bradford-Hill Considerations for Evaluating Weight of Evidence to develop AOPs	AOP for non-genotoxic induction of cytotoxicity and regenerative hyperplasia by a threshold mechanism promotes duodenal tumours in mice based on hexavalent chromium, which is stated to be similar to observations for folpet and captan.		Becker et al. 2015
28-day study in B6C3F1 mice	180 ppm Cr(VI) in drinking water, 6000 and 12,000 ppm captan in feed, or 6000 and 16,000 ppm folpet, 20/sex/group	Villous enterocyte hypertrophy and mild crypt epithelial hyperplasia. Duodenal samples were generally indistinguishable from those of unexposed mice after 28-day recovery (satellite group). Changes in the villi and lack of observable damage to the crypt compartment suggest that toxicity was mediated in the villi, which is consistent with earlier studies on each chemical. These findings indicate that structurally diverse agents can induce similar (and reversible) phenotypic changes in the duodenum. These intestinal carcinogens likely converge on common pathways involving irritation and wounding of the villi leading to crypt regenerative hyperplasia that, under protracted high-dose exposure scenarios, increases the risk of spontaneous mutation and tumorigenesis.	Thompson et al. 2017
Gene expression analysis	Transcriptomic responses of tissues from Thompson et al. 2017, i.e. after treatment with 180 ppm Cr(VI) in drinking water, 6000 and 12,000 ppm captan in feed, or 6000 and 16,000 ppm folpet, 20/sex/group	Transcriptional responses were similar between all 3 agents; gene-level comparison identified 126/546 (23%) differentially expressed genes altered in the same direction, with a total of 25 upregulated pathways. These changes were related to cellular metabolism, stress, inflammatory/immune cell response, and cell proliferation, including upregulation in hypoxia inducible factor 1 (HIF-1) and activator protein 1 (AP1) signalling pathways, which have also been shown to be related to intestinal injury and angiogenesis/carcinogenesis. The similar molecular-, cellular-, and tissue-level changes induced by these 3 carcinogens can be informative for the development of an adverse outcome pathway for intestinal cancer.	Chappell et al. 2019
AOP	Adverse outcome pathway for small intestinal tumours in mice involving chronic cytotoxicity and regenerative hyperplasia: a case study with hexavalent chromium, captan, and folpet, based on Thompson et al. 2017,		Bhat et al. 2020

Type of study/data	Relevant information about the study (as applicable)	Observations	Reference
	Chappell et al. 2019 and folpet and captan publications Gordon 2007; Cohen et al. 2010, Arce et al. 2010 and Gordon et al. (2012) AOP [MIE, molecular/cellular] villous enterocyte cytotoxicity [KE1, tissue] sustained crypt cell proliferation/hyperplasia [KE2, tissue] mutation/transformation [AOP, individual] small intestinal tumours		

9.9.1 Short summary and overall relevance of the provided information on carcinogenicity

There are six long-term studies available for folpet, three in rat and three in mice, using two strains for each species.

The doses/dietary concentrations for the mouse studies decrease from Study 1 to 3. Folpet's carcinogenicity is limited to tumours of the small intestine in mice, primarily the proximal portion of the duodenum, as well as the jejunum, and the forestomach in both sexes and two strains. The treatment related small intestine tumours were seen in two mouse carcinogenicity studies (Study 1 1982 and Study 2 1985). Duodenal tumours were observed at dietary dose levels of ≥3500 ppm (525 mg/kg/day) in B6C3F1 mice (Study 2 1985) and ≥5000 ppm (502 mg/kg/day) in CD-l mice (Study 1 1982). Jejunal tumours occurred in male and female CD-l mice at 12000 ppm dietary levels (Study 1 1982*).* Forestomach tumours occurred in CD-1 male mice in one study (Study 1 1982) and in CD-l and B6C3F1 female mice in two other studies (Study 2 1985 and Study 3 1994). Study 3 (1994) was a non-guideline study designed to establish a threshold for tumour development in CD-l mice and to supplement the two previous mouse carcinogenicity studies.

Table 32: Gastrointestinal tumours in mice, there are no neoplastic findings associated with the gastrointestinal tract in rat

Study	Detail	Sex	ppm			
1	Study 1 (1982), n=80/sex/group, Control= 104/sex, CD-1		*0*	*1000*	*5000*	*12000*
2	Study 2 (1985), n= 52/sex/group, B6C3F1		*0*	*1000*	*3500*	*7000*
3	Study 3 (1994), n=52/sex/group, CD-1		*0*	*150*	*450*	*1350*
Duodenum						
1	Adenoma	M	1	2	3	14**
		F	0	2	4	18**
	Adenocarcinoma	M	0	2	10**	48**
		F	0	0	7*	40*
2	Carcinoma	M	0	3	17	24***
		F	0	1	5	18***
3	Benign B-adenoma	M	0	0	0	0
		F	0	0	0	1
Jejunum						
1	Adenoma	M	0	0	2	2

Study	Detail	Sex	ppm			
		F	0	0	0	3
	Adenocarcinoma	M	0	2	0	11**
		F	0	0	0	4
2	Carcinoma	M	0	0	0	1
		F	0	0	0	1
Stomach						
1	Papilloma	M	1	1	6	8
		F	1	5	6	1
2	Papilloma	M	0	2	3	2
		F	2	1	5	7**
3	Papilloma	M	0	0	0	1
		F	0	0	1	3*

Study 1: Chi-squared/Yates * ($p<0.05$), ** ($p<0.01$)
Study 2: Peto's test for trend ** ($p<0.01$), *** ($p<0.001$)
Study 3: Fischer's exact test * ($P<0.05$).

There was an increase of malignant lymphoma in Study 2, see Table 31. While the study do not report a historical control range, Haseman et al. 1984[2] report mean incidences (and standard deviation) of 12% (7.2%) and 25.1% (10%) for male and female B6C3F1 mice in the NTP studies, respectively, the strain used in Study 2. No detailed information on time range of HCD or potential confounding factors is available. Therefore reliability of HCD is limited. Malignant lymphomas are attributed to haematopoetic system (multiple organs). No further distinction is included in the study report. However, when the incidence of both malignant lymphoma and small intestinal tumours in mice in Study 2 (1985) are investigated, see Table 33: The apparent increase in the high dose could be attributable to the increased incidence of intestinal neoplasms. In the study report, malignant lymphomas are summarised from various organs. However, there is an increase of animals with both malignant lymphoma and small intestinal tumours justifying this assumption. Survival analysis by the method of Peto (1980) revealed a significant trend of increased rate of malignant lymphoma among females only. No such trend was observed for males.

Table 33: Assessing occurrence of both malignant lymphoma and small intestinal tumours in mice in Study 2 (1985)

Dietary concentration [ppm]	Number of animals	Malignant lymphoma (animal number)	Small intestinal tumours (Carcinoma in duodenum and jejunum) (animal number)	Animals with both malignant lymphoma and small intestinal tumours (animal number)
Males: Decedents week 1-52				
3500	1	0	0	0
Females: Decedents week 1-52				
1000	2	1 (304)	0	0
7000	1	0	0	0

[2] Haseman JK, Huff J, Boorman GA (1984) Use of historical control data in carcinogenicity studies in rodents. Toxicol Pathol 12(2):126-35 doi:10.1177/019262338401200203

Dietary concentration [ppm]	Number of animals	Malignant lymphoma (animal number)	Small intestinal tumours (Carcinoma in duodenum and jejunum) (animal number)	Animals with both malignant lymphoma and small intestinal tumours (animal number)
Males: Decedents week 53-78				
0	2	1 (39)	0	0
1000	2	2 (95, 72)	0	0
3500	2	0	0	0
7000	5	0	0	0
Females: Decedents week 53-78				
0	2	0	0	0
1000	1	0	0	0
3500	1	0	0	0
7000	0	0	0	0
Males: Decedents week 79-104				
0	7	2 (4, 25)		
1000	10	3 (85, 91, 102)	1 (100[b])	
3500	11	7 (111, 113, 115, 117, 119, 145, 147)	6[#] (115[b], 118, 119, 140[c], 145[a], 145[b])	3 (115, 119, 145)
7000	11	5 (165, 166, 168, 186, 207)	4 (161[b], 166, 202[b], 207[b])	1 (207)
Females: Decedents week 79-104				
0	8	6 (217, 226, 230, 237, 248, 249)	0	0
1000	7	5 (261, 267, 281, 298, 299)	0	0
3500	13	8 (316, 237, 329, 334, 335, 338, 340, 343)	1 (345)	0
7000	17	12 (366, 367, 372, 377, 388, 396, 399, 400, 405, 406, 415, 416)	5 (366[b], 391[a], 400b, 406, 414[b])	3 (366, 400, 406)
Males: Terminal kill				
0	43	10 (7, 14, 16, 24, 29, 31, 40, 42, 43, 51)	0	0
1000	40	6 (56, 74, 79, 82, 89, 101)	2 (92, 98)	0
3500	38	5 (107, 127, 129, 131, 153)	11 (114, 120[a], 121, 124[b], 127[b], 129[b], 130, 135[b],142, 151, 156[b])	2 (127, 129)
7000	36	4 (179, 194, 204, 205)	20[##] (157, 158[b], 160[b], 170[a], 172, 172[b], 177, 177[b], 178[b], 179[a], 180[a], 182[a], 184[b], 185[b], 188, 191, 195[c], 197[b], 200[a],	2 (179, 205)

Dietary concentration [ppm]	Number of animals	Malignant lymphoma (animal number)	Small intestinal tumours (Carcinoma in duodenum and jejunum) (animal number)	Animals with both malignant lymphoma and small intestinal tumours (animal number)
			205[b])	
Females: Terminal kill				
0	42	10 (209, 212, 220, 233, 236, 239, 242, 250, 257, 260)	0	0
1000	42	11 (272, 284, 285, 290, 292, 295, 300, 301, 306, 310, 311)	1 (264[b])	0
3500	38	9 (322, 325, 328, 339, 344, 348, 353, 355, 359)	4 (313, 337, 339, 342c)	1 (339)
7000	34	14 (365, 368, 369, 376, 379, 380, 384, 386, 389, 390, 394, 401, 403, 407)	14 (365[a], 368[b], 369[b], 373[a], 376[a], 378, 380[b], 384, 386, 394[b], 395[a], 404, 407[a], 409[b])	9 (365, 368, 369, 376, 380, 384, 386, 394, 407)
Males: Total No malignant lymphoma				
0	52	13		
1000	52	11		
3500	52	12		
7000	52	9		
Females: Total No malignant lymphoma				
0	52	16		
1000	52	16		
3500	52	19		
7000	52	26		

a: Infiltration through muscular layers (tunica muscularis) to varying depth
b: Penetration through muscular layers (tunica muscularis) to varying depth
c: Penetration through whole thickness of the wall including the tunica serosa
#: One animal with > 1 carcinoma
#: Two animals with > 1 carcinoma

In rat, there were also histopathological signs associated with inflammation in the gastrointestinal tract, such as hyperkeratosis in the non-glandular stomach and very prominently the oesophagus, where up to all animals were affected in the highest treatment groups. However, the intestine seems to be less affected than in mouse and there were also no neoplastic lesions. The toxicokinetic Study 4 (1991) in Section 7 explores possible reasons for this difference, which may be associated with achieved doses, available GSH pools and reliance on GSH to detoxify folpet's irritant properties.

Incidences of other observed tumours are not clearly dose-related or not consistent between studies, see Table 34.

Increases of benign fibro-epithelial tumours in the mammary gland (combined sex), Thyroid C-cell adenoma and malignant lymphoma in Study 5 in the highest treatment groups are not observed in either Study 4 or 6 or are without dose-response. It should, however, be noted that in Study 6 only 20 animals/ sex and group were exposed. There are only sparce historical control data available in the studies, as the study directors considered

published NTP data to be well-representing background incidences of neoplasms. It should be noted that historical control data provided by the same lab performing the study would be more appropriate.

The testicular (Leydig cell) tumours seen in the Sprague-Dawley rats (Study 4 1985) were considered not to be treatment related based on a lack of a dose response (please refer to Table 34); a comparison with Fisher F344 rats is not appropriate as such neoplasms occur commonly in that strain. In addition, there was no dose response in the corroborative non-neoplastic lesions.

Table 34: Systemic tumours in rat

Study	**Detail**	**Sex**	**ppm**				**HCD[1]**	**Assessment**
4	Study 4 (1985), n = 50/group, CD(SD)		*0*	*200*	*800*	*3200*		
5	Study 5 (1985), n = 60/group, F344		*0*	*500*	*1000*	*2000*		
6	Study 6 (1989), n=20/group, F344		*0*	*250*	*1500*	*5000*		
Mammary gland: Benign fibro-epithelial tumour								
4		M	0	0	1	0		The combined sex of Study 5 shows an increase of benign fibro-epithelial tumours in the highest dose group. However, this is within the HCD distribution, also in Study 6. However, historical control data provided by the same lab performing the study would be more appropriate.
		F	15	8	17	12		
5		M	2	1	3	3 [C]	1.32[2] or 2.2% (2.0)	
		F	7	5	8	12[C]	14.46[2] or 24.1% (10.1)	
6		M	0	0	0	0	0.44[2] or 2.2% (2.0) 11%[3]	
		F	1	0	2	2	4.82[2] or 24.1% (10.1) 11%[3]	
Malignant lymphoma								
4	Lymphocytic (unscheduled death)	M	0	0	10	0		The apparent increase in Study 5 is within the HCD distribution and not observed in either Study 4 or 6. However, historical control data provided by the same lab performing the study would be more appropriate.
		F	0	1	0	0		
	Histocytic (unscheduled death)	M	0	1	0	0		
		F	0	0	1	0		
	Lymphocytic (study termination)	M	0	1	0	0		
		F	1	0	0	0		
	Histocytic (study termination)	M	0	0	0	1		
		F	0	0	1	0		

Study	Detail	Sex	ppm				HCD[1]	Assessment
5		M	0	1	2	2[C]	1.32 [0-9.6][2] or 2.2% (3.4)	
		F	0	2	2	3[C]	0.9 [0-3.6][2] or 1.5% (2.2)	
6		M	1	1	1	0		
		F	1	0	0	1		
Thyroid: C-cell adenoma								
4		M	0	8	2	2	2.25 [1-3.5] or 4.5% [2-7][3]	The effect in Study 5 is not observed in males, where control group response is almost as high as in the high dose group response in females, or either Study 4 or Study 6.
		F	4	4	3	2		
5		M	6	2	2	2	2.7 [1.2-4.2][3] 3.06[2] or 5.1% (4.4)	
		F	4	0	2	8*	2.7 [1.2-4.2][3] 2.94[2] or 4.9% (4.1)	
6		M	1	2	1	1		
		F	0	0	0	1		
Testicular (Leydig cell) tumours								
4		M	1	5	4	8	6.06 [1.2-13.8][3] or 10.1% [2-23]	The increase in Study 4 is within HCD and there are no effects on reprotoxicity parameters, see Section 8.10. HCD are of limited reliability. Historical control data provided by the same lab performing the study would be more appropriate.
5		M	57	58	57	58	Typical for F440 rats	
6		M	17	20	19	20		

* statistically significantly different at p <= 0.05

C statistically significantly different at p <= 0.05, when sex are combined

[1] Mean [Range] or (SD), where used in the study report to dismiss findings. HCD expressed as incidence based on study specific observation number; e.g. HCD of 10% in a group of n= 50 corresponds to 5 neoplasms in a group.

[2] Haseman, Huff and Boorman. (1984) Toxicologic Pathology, 12, 126-135, No detailed information on time range of HCD or potential confounding factors is available. Therefore reliability of HCD is limited.

[3] As stated in Kidwell, 2010, 8 studies conducted before 1987, No detailed information on time range of HCD or potential confounding factors is available. Therefore reliability of HCD is limited.

Overall, folpet clearly induces tumours in the small intestine of mice, however, only at doses that also cause an irritation response in the mucosal epithelium, while other tumour incidences appear spurious because they are either within the biological variability and historical control data range or are not reproducible within the study package. This is supported by the toxicokinetic investigations which indicate that folpet is not systemically available. Furthermore, folpet does not show genotoxic potential *in vivo*.

The aetiology of the small intestinal tumours was investigated in multiple mode-of-action investigations. The first experiments (Section 7, Study 4 (1991)) investigated species differences between mouse and rat. It was concluded that the greater folpet intake of mice when compared to rat might exceed the irritation threshold required for tumorigenicity. Further, the mouse, more than the rat, relies on glutathione for the detoxification of folpet, therefore glutathione supply in the mouse may be inadequate to deal with such high doses. A biochemical threshold in the defensive capability of glutathione and its associated glutathione S-transferase might exist which is exceeded in the target tissue in the overexposed mouse.

It was never considered or discussed on whether anatomical differences may be another key factor. Since, the *duodenum* of mice has about half the diameter of that from rats, the mucosa is exposed towards more folpet per area for the same amount of diet at the same dietary concentration. Also, the likelihood of exposure is higher due to the smaller diameter. An anatomical driver would further support the non-relevance of the effect for human intestine, which has an at least 30-fold larger diameter than that of mice, i.e. about 5 cm in human as compared to 2.5-3 mm in rat[3] and accordingly about 1.25-1.5 in mouse.

The subchronic mechanistic studies 7-10 show that folpet exposure with concentrations that result in small intestinal tumours in mouse, result in an increase of proliferation markers PCNA and CDK and hyperplasia of crypts. This induction of proliferation, especially at biochemical level, was reversible when treatment was not continued, which highlights the need for continuous high exposure. In Study 10 (1997), folpet treatment with 5000 ppm resulted in enlarged crypts containing an increased number of cells. The villi were reduced in size and showed signs of fusion in some cases. Increased numbers of inflammatory cells in the *lamina propria* were seen in some animals. Folpet treatment resulted in a dose-dependent increase of BrdU-labelling index in the crypts, which indicates proliferation. Study 11 (2004) supports the previous hypothesis that continuous, repeated treatment is required to induce proliferation, as a 24-hour exposure up to 5000 ppm in diet or 900 mg/kg bw/day via gavage does not result in gastrointestinal irritation. Study 12 (2011) and Gorden et al. 2012, which describe the same study, show that subchronic folpet treatment with 6000 ppm results in macroscopic changes typified by distension of the caecum, thickening of the duodenum and roughened forestomach. Further, hypertrophy and hyperkeratosis are observed in the forestomach as well as epithelial hyperplasia. All these effects were reduced in incidence and/or severity in animals killed after 17 days of recovery compared to animals killed directly after 28 days of treatment with folpet, which shows that the effects are reversible.

Based on the published studies on folpet and prior work on hexavalent chromium, it was suggested that folpet, the structurally similar captan and hexavalent chromium have a common AOP (Becker et al. 2015).

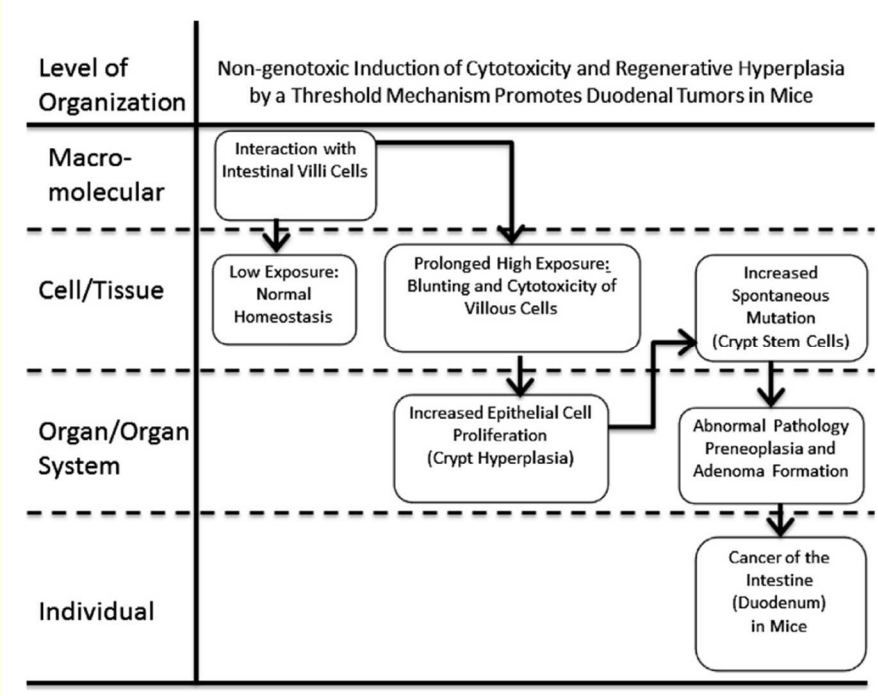


Figure 4: From Becker *et al.* 2015 "Depiction of the AOP of induction of cytotoxicity and regenerative hyperplasia by oral CrVI leading to duodenal tumours in mice." Becker et al. 2015 further states a similarity to effects observed for folpet and captan.

[3] Karali TT (1995) COMPARISON OF THE GASTROINTESTINAL ANATOMY, PHYSIOLOGY, AND BIOCHEMISTRY OF HUMANS AND COMMONLY USED LABORATORY ANIMALS. BIOPHARMACEUTICS & DRUG DISPOSITION 16(351-380)

Thompson *et al.* 2017 directly compared folpet, captan and hexavalent chromium in a subchronic mouse study and showed that villous enterocyte hypertrophy and mild crypt epithelial hyperplasia were induced by all compounds and was similarly reversible in all exposed mice. They concluded that "These intestinal carcinogens likely converge on common pathways involving irritation and wounding of the villi leading to crypt regenerative hyperplasia that, under protracted high-dose exposure scenarios, increases the risk of spontaneous mutation and tumorigenesis."

Chappell et al. 2019 investigated the gene expression profile of tissues from Thompson *et al.* 2017 and showed that all compounds induced similar molecular-, cellular-, and tissue-level changes, namely affecting cellular metabolism, stress, inflammatory/immune cell response, and cell proliferation, including upregulation in hypoxia inducible factor 1 (HIF-1) and activator protein 1 (AP1) signalling pathways, which have also been shown to be related to intestinal injury and angiogenesis/carcinogenesis.

Based on this Bhat *et al*. 2020 proposed a common AOP, see Figure 5, with folpet, captan and hexavalent chromium as lead compounds. The authors conclude "The extensive evidence for this AOP, along with the knowledge that human exposures are orders of magnitude below those associated with KEs in this AOP, supports its use for regulatory applications, including hazard identification and risk assessment." Bhat *et al.* 2020 concluded, "in the context of the WHO/IPCS human relevance framework, the WOE is sufficient to establish the MOA in animals, with qualitatively plausible KEs in humans. However, the KEs become quantitively implausible in humans after accounting for interspecies toxicokinetic differences [for Cr(IV)], as well as background levels of human exposure (for captan and folpet). Confidence in this AOP/MOA is high and the implications suggest that the AOP/MOA is unlikely to be quantitatively relevant to humans. As mentioned previously, the AOP/MOA may be relevant to rats, if sufficient exposures across dose and time are achieved, since the KEs (but not the AO) have been observed in rats exposed to Cr(VI). "

As the AOP's key events are dependent on exposure levels, a consideration of possible human exposure scenarios is helpful to assess the relevance of the observed effects for human hazard identification. The primary users of folpet are farmers that treat their crop with fungicidal products that contain folpet as the active ingredient. For this use, the relevant exposure route is dermal, which is not applicable for small intestinal tumours, due to its local mode of action. The studies in Section 7 demonstrate that folpet does not reach the small intestine via systemic circulation, but only due to direct contact from diet. Conversely, consumers may be exposed towards residues of intact folpet via diet (most likely external residues as folpet is not efficacious systemically). However, the carcinogenic mode of action is only applicable for continuous, chronic and irritating dietary concentrations of folpet, levels which do not occur in diet. The acceptable daily intake level (ADI) of 0.1 mg/kg bw/day, which is used in dietary risk assessment of folpet, is more than 400-fold lower than the chronic NOAEL for irritation in mice (47 mg/kg bw/day, for which no response in the mucosal epithelium in mice is observed, Study 3 (1994)). The ADI is not exhausted in dietary risk assessments, hence the margin of exposure is even higher. The current highest maximum residue levels for folpet (which include those of phthalimide, which lacks the trichloromethylthio-side chain, at a ratio of about 2:1) are for hops (400 ppm) and table grapes (20 ppm), according to the EU pesticides database. A dietary exposure of residue levels corresponding to 450 ppm, the NOAEL of Study 3 (1994), would be achieved by daily, life-long consumption of >1.1 kg raw/unprocessed hops or >22.5 kg grapes, assuming that the folpet residue would not react with the available increased thiol pool from the diet itself, which is very unlikely. Moreover, folpet is sensitive to hydrolysis and any processing of the raw agricultural commodity will significantly reduce its concentration in the food item. Together, there is no applicable human exposure scenario, where the molecular initiating event of the proposed AOP would be triggered.

Nevertheless, no differences in toxicokinetic behaviour of folpet between mice and human are evident and from a qualitative perspective the proposed MoA can be also established in humans.

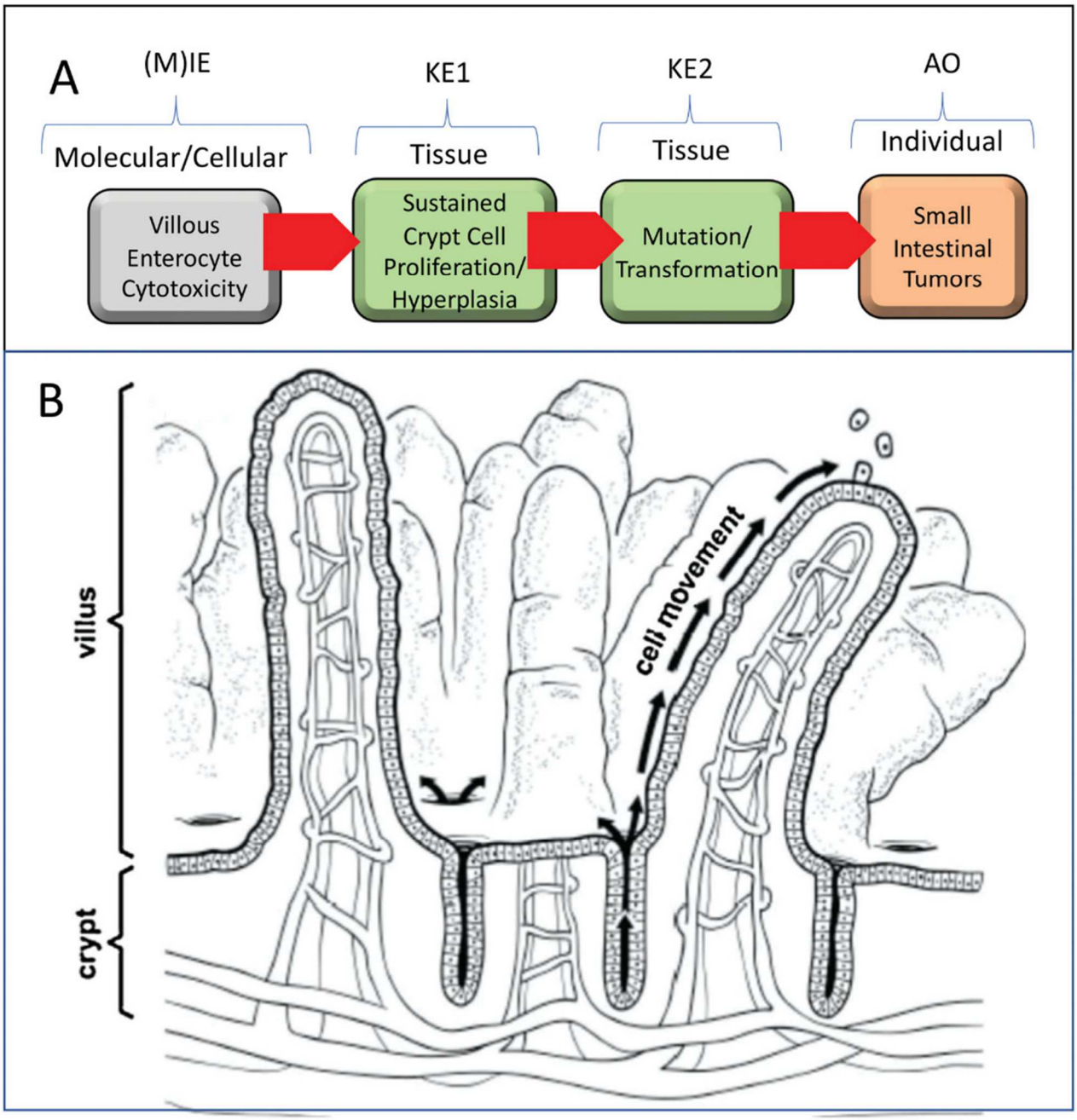


Figure 5: From Bhat *et al.* 2020 "Proposed AOP for cytotoxicity-mediated SI cancer in mice. (A) AOP diagram. See text for discussion regarding (M)IE. Future evolution of this AOP may better define the (M)IE associated with villous cytotoxicity. (B) Structure of intestinal mucosa. Villi are finger- or leaf-like projections into the lumen that are predominantly covered with mature, absorptive enterocytes, along with occasional mucus-secreting goblet cells. These cells live only for a few days, then die and slough into the lumen. The crypts, or glands of Lieberkühn, are tubular invaginations of the epithelium, lined largely with younger epithelial cells, which serve as a source of enterocytes to multiple villi. At the base of the crypts are stem cells, which divide continually and function as the source of all the epithelial cells in the crypts and on the villi. Adapted from O'Brien et al. (2013)."

9.9.2 Comparison with the CLP criteria

Classification as a carcinogen is made on the basis of evidence from reliable and acceptable studies and is intended to be used for substances which have an intrinsic property to cause cancer.

No epidemiologic studies in humans are available for the carcinogenic potential of folpet.

According to CLP criteria, substances which have induced benign and malignant tumours in well performed experimental studies on animals are considered also to be presumed or suspected human carcinogens unless there is strong evidence that the mechanism of tumour formation is not relevant for humans.

Folpet's intrinsic property is local acute irritation and not carcinogenicity; carcinogenicity in mice is only observed as a consequence of prior local repeated irritation.

Continuous high dose folpet treatment results in small intestinal tumours in mice only. The tumours are a consequence of a non-genotoxic mode of action secondary to cytotoxicity due to repeated, local acute irritation, i.e. occurs exclusively at irritating doses. The tumours do not occur in rats at irritating doses. There is a substantial dataset available on folpet, but also for other substances, which gives confidence in the mode of action.

Folpet shows irritating effects at the site of first exposure in the acute and repeated studies in all species. All or almost all rats treated with dietary concentrations of 4000 and 8000 ppm folpet for 90 days (see Section 8.12) or with 2000 ppm (Study 5 1985) or 5000 ppm (Study 6 1989) for 2 years develop hyperkeratosis in the oesophagus and the stomach. Rats treated with folpet via inhalation for 28 days (see Section 8.12) show laryngeal changes at all ambient exposure levels from 5-100 µg/L, due to a cumulation of irritative toxicity, and further signs associated with irritation in the respiratory system. In a repeated-dose dermal toxicity study with rats (see Section 8.12) and all chronic studies with mice (Studies 1-3) signs of substantial skin irritation are observed, for the latter most likely due to repeated dermal exposure towards diet. Together, the intrinsic property of folpet is local irritation at the first site of contact, which is plausible based on its fungicidal and toxicokinetic properties.

In the context of the WHO/IPCS human relevance framework a MoA analysis was conducted by Bhat *et al.* 2020 concluding that, the KEs become quantitively implausible in humans after accounting for background levels of human exposure. Nevertheless, the authors also concluded that the KEs are qualitatively plausible in humans.

Carcinogenicity attributable to oral administration of folpet has been demonstrated in a single species (mouse) and in a single target tissue (duodenum) in three independent studies. Without further investigations these data would show sufficient evidence of carcinogenicity in experimental animals. However, the underlying MoA has been identified and is initiated by local irritation at the first site of contact. Thereby, continuous irritating doses of folpet are needed for tumour formation limiting the strength of evidence.

Therefore, classification of folpet as Carc. 2, H351 should remain.

9.9.3 Conclusion on classification and labelling for carcinogenicity

No change in classification of folpet is warranted. Folpet is proposed to be classified for carcinogenicity, Cat. 2.

9.10 Reproductive toxicity

Folpet has been extensively investigated for its potential to induce reproductive and developmental toxicity. Three multigeneration studies (Study 1, 1986; Study 2, 1985; Study 3, 1967- not reliable) have been conducted to detect potential adverse effects on sexual function and fertility in the rat. None of the studies fully comply with the current version of OECD Test Guideline Number 416 (2001) but the most similar and the most relevant study, testing the highest dose level of folpet, is Study 1 (1986). Also, this study is the most appropriate to inform on effects on or via lactation.

Four studies of prenatal developmental toxicity in the rat have been conducted. Two (Study 4, 2007; Study 5, 2003) are recent, robust GLP studies conducted to OECD Test Guideline Number 414 (2001). These two studies investigated the same dose levels of folpet and therefore provide a solid basis for the evaluation of the potential of folpet to induce developmental toxicity in the rat. One of the earlier studies (Study 7, 1983) was

largely compliant with the current test guideline and the other (Study 6, 1985) met the requirement of the time, to dose through the period of major organogenesis only.

Three guideline compliant studies of prenatal developmental toxicity in the rabbit and one investigative study have been conducted (Study 11, 2006; Study 12, 1984; Study 13, 1985; Study 14, 1985). These studies collectively contribute to the weight of evidence assessment for adverse effects on development in the rabbit.

Additional data on folpet are available from the published literature. These publications also include reference to the metabolite phthalimide, and to the structurally similar chemical, captan and its metabolite THPI although the data are not described because of the unknown quality of the studies and small numbers of animals tested. They are further discussed in a review of the potential of folpet to induce developmental toxicity (Anonymous, 2018, R-39172). However, directly comparable regulatory standard studies of folpet (Study 11, 2006), phthalimide (Study 15, 2006), captan (Study 16, 2006) and THPI (Study 17, 2006) are described.

If not stated otherwise in the summary tables, the studies are considered as fully reliable.

All available data are described in the following sections.

9.10.1 Adverse effects on sexual function and fertility

Table 35: Summary table of animal studies on adverse effects on sexual function and fertility

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Two Generation (one litter/gen.) Oral (continuous in diet) US EPA 83-4 Deviations from OECD 416 (2001): - Nine Group 2 males and two Group 3 males received untreated diet for one or two days during the week before sacrifice. - Weighing during gestation was on days 0, 3, 6, 10, 14, 17 and 20 (instead of 0, 7, 14 and 20/21) - Epididymis, uterus, prostate, seminal vesicles, brain, liver, spleen, pituitary, thyroid, coagulating gland and adrenal glands were not weighed - Sperm parameters were not measured - Thyroid, cervix, coagulating gland were not processed histopathologically - Age of vaginal opening and balano-preputial separation was not measured - Organ weights were not determined for pups - Histopathology was not determined for pups	Folpet batch 631933 (technical grade), purity 91% 0, 250, 1500 or 5000 ppm Vehicle: laboratory animal diet	*Parental toxicity* <u>5000 ppm</u> F0: ↓ body weight (8.5% males, 5% females by end pre-pairing period); ↓ body weight gain: gestation (11%, days 1-20), lactation (16%, days 1-14); ↓ food consumption pre-pairing both sexes, gestation and lactation; ↑ hyperkeratosis of the non-glandular stomach - moderate (16/25 males, 12/25 females) to slight (9/25 males, 10/25 females) with single incidence of moderate and slight in control males only; ↑ basophilic renal tubules in males (7/25 slight [3/25 controls], 2/25 moderate [0/25 controls]) F1: ↓ body weight week 0 (24.5% males, 18.5% females); ↓ body weight gain weeks 0-14 (13% males, 5% females); ↓ body weight gain gestation (8%, days 1-20);↓ food consumption pre-pairing both sexes, gestation and lactation; ↑ hyperkeratosis of the non-glandular stomach – moderate (22/25 males, 24/25 females) slight (3/25 males,1/25 females) [none in controls];↑ oesophageal hyperkeratosis in females (11/25 slight, 13/25 moderate) [none in controls] <u>1500 ppm</u>	(R-4347) Study 1 (1986)

<table>
<tr>
<td>- Coagulating gland was not assessed for histopathology
- Detailed testicular histopathological examination was not performed
- A quantitative evaluation of primordial follicles was not conducted for F1 females
GLP
Rat, CD(SD)
25/sex/group</td>
<td></td>
<td>F0: ↑ slight hyperkeratosis of the non-glandular stomach (19/25 males, 23/25 females) [single incidence in control males]
F1: ↑ slight hyperkeratosis of the non-glandular stomach (21/24 males, 24/24 females) [none in controls]; ↑ oesophageal hyperkeratosis in females (15/24 slight) [none in controls]
<u>250 ppm</u>
No effects
NOEL 250 ppm on the basis of hyperkeratosis due to local contact irritation
Reproductive toxicity
No effects at any dose level
NOAEL 5000 ppm
Offspring toxicity
<u>5000 ppm</u>
F1: ↓ body weight gain from day 7 (27% days 7-21)
F2: ↓ body weight gain from day 7 (12% days 7-21)
<u>1500 ppm</u>
No effects
NOAEL 1500 ppm
Test item intake in mg/kg bw/day
<table>
<tr><td>ppm</td><td>250</td><td>1500</td><td>5000</td></tr>
<tr><td>M, F0</td><td>18.9</td><td>112.3</td><td>370.1</td></tr>
<tr><td>F, F0</td><td>22.5</td><td>133.4</td><td>435.6</td></tr>
<tr><td>M, F1</td><td>25.2</td><td>150.1</td><td>520.2</td></tr>
<tr><td>F, F1</td><td>28.4</td><td>168.3</td><td>565</td></tr>
</table>
</td>
<td></td>
</tr>
<tr>
<td>Two Generation (two litter/gen.)
Oral (continuous in diet)
Guideline not stated.
Deviations from OECD 416 (2001): dosing of F0 (P) males was carried out for 62 days prior to mating, not 10 weeks
Organs were not weighed
Estrus cyclicity was not examined
Number of implants was not determined
Sperm parameters were not measured
Age of vaginal opening and balano-preputial separation was not measured</td>
<td>Folpet batch SX-1388 (technical grade), purity 89.5%
0, 200, 800 or 3600 ppm
Vehicle: laboratory animal diet</td>
<td>Parental toxicity
<u>3600 ppm</u>
F0: ↓ slight body weight gain males (5.6%, days 1-155)
F1(b): ↓ slight body weight gain males (5.7%, days 1-155)
<u>800 ppm</u>
No effects
NOEL 800 ppm
Reproductive toxicity
No effects at any dose level
NOAEL 3600 ppm
Offspring toxicity</td>
<td>(R-6134)
Study 2 (1985)</td>
</tr>
</table>

<table>
<tr>
<td>Organ weights were not determined for pups
Histopathology was not determined for pups
Coagulating gland, thyroid, vagina and adrenals was not assessed for histopathology
Detailed testicular histopathological examination was not performed
A quantitative evaluation of primordial follicles was not conducted for F1 females
GLP
Rat, CrL:COBS/CD(SD)
30/sex/group</td>
<td></td>
<td><u>3600 ppm</u>
F1a: ↓ body weight day 21, gain days 0-21 (17%)
F1b: ↓ body weight day 21, gain days 0-21 (20%)
F2a: ↓ body weight days 14 &21, gain days 0-21 (19%)
F2b: ↓ body weight days 14 &21, gain days 0-21 (14.5%)
<u>800 ppm</u>
No effects
NOAEL 800 ppm

Test item intake in mg/kg bw/day
<table>
<tr><td>ppm</td><td>200</td><td>800</td><td>3600</td></tr>
<tr><td>M, F0</td><td>14.9</td><td>59.6</td><td>263.4</td></tr>
<tr><td>F, F0</td><td>18.1</td><td>72.9</td><td>314.5</td></tr>
<tr><td>M, F1b</td><td>22.3</td><td>90.8</td><td>421.6</td></tr>
<tr><td>F, F1b</td><td>23.4</td><td>94.8</td><td>436.3</td></tr>
</table></td>
<td></td>
</tr>
<tr>
<td>Three Generation (two litter/gen.)
Oral (continuous in diet)
Guideline not stated. Notable number of deviations from OECD 416 (2001) and deficiencies in reporting.
Non-GLP
Rat, Charles River
8 males/group
16 females/group
Not reliable (conducted at Industrial Bio-Test Laboratories)</td>
<td>Phaltan (the former name of folpet) (technical grade), purity 94.4%
0, 100, 500 or 1000 ppm
Vehicle: laboratory animal diet</td>
<td>Parental toxicity
<u>1000 ppm:</u> No effects: NOEL
Reproductive toxicity
<u>1000 ppm:</u> No effects: NOEL
Offspring toxicity
<u>1000 ppm:</u> No effects: NOEL</td>
<td>B3566
Study 3 (1967)</td>
</tr>
</table>

Human data on adverse effects on sexual function and fertility

None available

Other studies relevant for toxicity on sexual function and fertility

A number of endocrine assays have been conducted and are summarised in the following table.

Table 36: Summary table of endocrine assays

Assay	Results
Study 23 (2012a) : OPPTS 890.1150: Androgen Receptor Binding Assay (Rat Prostate)	Negative: Folpet does not interact with the androgen receptor.
Study 19, 2012a: OPPTS 890. 1400: Hershberger Assay	Negative: Folpet does not exhibit agonist or antagonist activity in castrated male rats.
Study 24 (2012): OPPTS 890. 1200: Human Recombinant Aromatase Assay	Equivocal
Study 25 (2012a): OPPTS 890. 1250: Estrogen Receptor Binding Assay	Negative: Folpet does not interact with the rat estrogen receptor.
Study 26 (2012c) OPPTS 890. 1300: Estrogen Receptor Transcriptional Activation Assay	Negative: Folpet is not an agonist to hERα in the HeLa-9003 model.
Study 27 (2012): OPPTS 890.1550: Steroidogenesis Assay	Negative
Study 22, 2012d: OPPTS 890.1600: Uterotrophic Assay	Negative: Folpet did not affect uterine weight (i.e. show estrogen activity) in ovariectomized rats.
Study 21 (2012b): OPPTS 890.1450: Pubertal Assay in Male Rats	Negative: Folpet does not adversely pubertal development in male rats.
Study 20 (2012b): OPPTS 890.1500: Pubertal Assay in Female Rats	Negative: Folpet does not adversely pubertal development in female rats.

The results of the assays have been considered in a weight of evidence analysis according to the EFSA/ECHA Guidance for the identification of endocrine disruptors. It was concluded that folpet does not meet the ED criteria for humans.

There are no other studies relevant for toxicity on sexual function and fertility. The short-term toxicity studies (Section 8.12) in rats and mice did not elicit any adverse effect on the reproductive organs and provide no indication of a potential adverse effect of folpet on sexual function and fertility. In the dog, at dose levels which induced marked toxicity resulting in generally poor condition and reduced body weight (1800 and 4000 mg/kg bw/day), testes weights were decreased with microscopic testicular degeneration and prostatic atrophy (Section 8.12/Study 7 1985). The males given 4000 mg/kg bw/day (4-fold higher dose than current TG recommendations) were killed *in extremis* after 4 weeks. In a 52-week study, dose levels of 650 and 1300 mg/kg bw/day also caused the dogs to be in poor general condition, with reduced body weight (Section 8.12/Study 8 1988). At the highest dose (greater than the TG recommended limit dose), decreased testis weight, tubular testicular degeneration associated with no spermatozoa in the epididymides and a single incidence of prostatic gland atrophy were observed. The effects on the testis of the dog are considered to be a consequence of the marked toxicity induced by the selected dose levels and not a direct effect of folpet on the testis.

The results of the short-term toxicity studies do not indicate an adverse effect of folpet on sexual function and fertility.

9.10.2 Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility

Three multigeneration studies in the rat have been conducted (Study 1, 1986; Study 2, 1985; Study 3, 1967-not reliable). None of the studies have been conducted to current OECD Test Guideline Number 416 (2001). However, the key study is considered to be Study 1 (1986). Whilst not fully compliant with the current test guideline, the deviations are considered unlikely to alter the conclusions reached. At the highest dose tested, 5000 ppm (approx. equivalent to 370.1-435.6 mg/kg bw/day) parental toxicity was observed as reduced body

weight and food consumption. Hyperkeratosis in the stomach and oesophagus due to local contact irritation were observed at 5000 ppm and at 1500 ppm (approx. equivalent to 112.3-133.4 mg/kg bw/day). Toxicity in the offspring was observed only at 5000 ppm as reduced body weight gain from post-natal day 7. There was no effect on the number and size of the pups at birth or on pup viability during lactation. The NOAELs for parental and offspring toxicity were 250 and 1500 ppm (equivalent to approx. 25.2-28.4 and 150.1-168.3 mg/kg bw/day, respectively). There was no effect on sexual function or fertility at any dose level tested and the NOAEL was 5000 ppm (approx. equivalent to 370.1-435.6 mg/kg bw/day).

Lower dose levels were used in the older studies of Study 2 (1985) and Study 3 (1967- not reliable) and no adverse effect on sexual function or fertility was observed.

9.10.3 Comparison with the CLP criteria

Toxicological result	**CLP Criteria**
No effect on sexual function or fertility at the highest dose level tested (5000 ppm approx. equivalent to 370 and 435.6 mg/kg bw/day in males and females, respectively) which reduced body weight and food consumption in parental animals and reduced pup growth from postnatal day 7. Folpet is not indicated to be a reproductive toxicant.	**Category 1A: Known human reproductive toxicant.** Classification largely based on evidence from humans. **Category 1B: Presumed human reproductive toxicant.** Classification largely based on data from animal studies providing clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect is considered not to be a secondary non-specific consequence of other toxic effects. **Category 2: Suspected human reproductive toxicant.** Classification based on evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility or development, and where the evidence is not sufficiently convincing to place the substance in Category 1. Such effects shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect is considered not to be a secondary non-specific consequence of the other toxic effects.

9.10.4 Adverse effects on development

Table 37: Summary table of regulatory animal studies on adverse effects on development

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Rat prenatal Developmental Toxicity Studies			
Developmental toxicity OECD 414 (2001) GLP Oral (gavage) Rat, Rj Han:SD	Folpet technical batch 200612071, purity 98.0% 0, 20, 100 or 800 mg/kg bw/day on gestation days 6-20	*Maternal toxicity* <u>800 mg/kg bw/day</u>: 2/24 deaths; 2/24 dyspnea, 4/24 loud breathing; ↓ body weight gain (17% days 6-21); ↓ body weight gain adjusted for gravid uterus weight (25% days 6-21); ↓ food consumption (7.7% days 6 to 9). <u>100 mg/kg bw/day:</u> ↓ body weight gain (9% days 6-21); ↓ body weight gain adjusted for gravid uterus weight (20% days 6-21); ↓ food consumption (7.7% days 6 to 9).	(000101015) Study 4 (2007)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
24 mated females/group 20 litters/group used for foetal examination	Vehicle: CMC	20 mg/kg bw/day: No effects Maternal NOAEL 20 mg/kg bw/day *Developmental toxicity* 800 mg/kg bw/day: ↑ post-implantation loss (7.85% compare to 3.6% in controls and 9.8% at 20 mg/kg/bw/day); ↓ number live fetuses (92.2% compare to 94.4% in controls and 90.2% at 20 mg/kg/bw/day. Differences from control not statistically significant. These effects were not considered adverse 100 mg/kg bw/day: No effects Developmental NOAEL 800 mg/kg bw/day	
Developmental toxicity OECD 414 (2001) GLP Oral (gavage) Rat, CD(SD) 22 mated females/group	Folpet batch 91330206, purity 93.7% 0, 20, 100 or 800 mg/kg bw/day on gestation days 6-19 Vehicle: CMC+ Tween 80	*Maternal toxicity* 800 mg/kg bw/day: ↑ salivation post dosing days 13-19; ↓ body weight gain (9% days 6-20); ↓ body weight gain adjusted for gravid uterus weight (21% days 6-20); ↓ food consumption (days 6 to 8 and 15 to 17). 100 mg/kg bw/day: No effects 20 mg/kg bw/day: No effects Maternal NOEL 100 mg/kg bw/day *Developmental toxicity* 800 mg/kg bw/day: Slight increase in visceral and skeletal abnormalities with unclear treatment relationship 100 mg/kg bw/day: No effects Developmental NOEL 100 mg/kg bw/day	(R-14259) Study 5 (2003)
Developmental toxicity US EPA 83-3 Deviations from OECD 414 (2001): -No information on light/dark cycle is included in the study report - Dosage only from Day 6-15 (instead of the day prior to scheduled kill) GLP Oral (gavage) Rat, CD(SD)	Folpet batch 631729 (technical grade), purity 91.1% 0, 150, 550 or 2000 mg/kg bw/day on gestation days 6-15 Vehicle: CMC+ acetic acid	*Maternal toxicity* 2000 mg/kg bw/day: 1/22 death day 16; multiple haemorrhagic ulcerations of gastric mucosa; clinical signs of soft faeces, staining of body fur and perianal staining; ↓ body weight gain (28% days 6-20), ↓ food consumption days 7-9 (43%), 10-13 (33%) 550 mg/kg bw/day: ↓ body weight gain (18% days 6-20); ↓ food consumption days 7-9 (16%) 150 mg/kg bw/day: No effects Maternal NOEL 150 mg/kg bw/day *Developmental toxicity* 2000 mg/kg bw/day: ↓ foetal body weight (7%); ↑ incidence of skeletal variations - ↓ ossification of cranial bones, sternebrae, pubes, metacarpals and metatarsals 550 mg/kg bw/day: ↓ foetal body weight (4%); ↑ incidence of skeletal variations - ↓ ossification of cranial bones, sternebrae, pubes, metacarpals and metatarsals	(R-3653) Study 6 (1985) incl. HCD (1987)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
22 mated females/group		150 mg/kg bw/day: ↑ litter incidence of angulated ribs and reduced ossification of the interparietal (the latter not clearly dose-related). Developmental NOAEL <150 mg/kg bw/day	
Developmental toxicity Predates test guidelines but complies largely to OECD 414 (2001) Deviations from OECD 414 (2001): Food consumption was recorded weekly (instead at 3-day intervals) No QA statement Oral (gavage) Rat, Crl:COBS/CD(SD)BR 25 mated females/group	Folpet batch SX-1388 (technical grade), purity 89.5% 0, 10, 60 or 360 mg/kg bw/day on gestation days 6-19 Vehicle: CMC + Tween 80	*Maternal toxicity* 360 mg/kg bw/day: Clinical signs of rales, dyspnea, salivation, chromorrhinorrhea, chromodacryorrhoea, decreased motor activity, soft/liquid faeces, staining of body fur; ↓ body weight gain (28% days 6-20); ↓ food consumption (11% days 6-13, 15% days 13-20) 60 mg/kg bw/day: Clinical signs of rales, ↓ body weight gain (10% days 6-20) 10 mg/kg bw/day: No effects Maternal NOEL 10 mg/kg bw/day *Developmental toxicity* 360 mg/kg bw/day Incomplete ossification in the pelvis, pubis and/or ischium 60 mg/kg bw/day No effects Developmental NOEL 60 mg/kg bw/day	(R-6117) Study 7 (1983)
Preliminary developmental toxicity Non-guideline Non-GLP Oral (gavage) Rat, Rj Han:SD 7 mated females/group supplementary information (reliable with restrictions)	Folpet technical batch 200612071, purity 98.0% 0, 20, 100 or 800 mg/kg bw/day on gestation days 6-20 Vehicle: CMC	*Maternal toxicity* 800 mg/kg bw/day: loud breathing 1/7 for 4 days, ↓ overall body weight gain (-15%), ↓ mean gravid uterus weight and mean net weight change. 100 mg/kg bw/day: No effects 20 mg/kg bw/day: No effects Maternal NOEL 100 mg/kg bw/day. Same dose levels selected for the definitive evaluation (Davies, 2007) *Developmental toxicity (limited evaluation)* 800 mg/kg bw/day: No effect on numbers of implantations or foetuses, no external foetal malformations or variation	(000101035) Study 8 (2007)
Preliminary developmental toxicity Non-guideline Non-GLP Oral (gavage)	Folpet batch 91330206, purity 93.7% 0, 20, 100 or 800 mg/kg bw/day on gestation days 6-19	*Maternal toxicity* 800 mg/kg bw/day: ↑ salivation post dosing days 13-19; ↓ body weight gain (40% days 6-8); ↓ body weight gain adjusted for gravid uterus weight (37%); ↓ food consumption (days 6 to 11 and 15 to 19). 100 mg/kg bw/day: No effects	(R-14258) Study 9 (2002)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Rat, CD(SD) 6 mated females/group supplementary information (reliable with restrictions)	Vehicle: CMC+ Tween 80	20 mg/kg bw/day: No effects Maternal NOEL 100 mg/kg bw/day. Same dose levels selected for the definitive evaluation (Myers, 2003) *Developmental toxicity (limited evaluation)* 800 mg/kg bw/day: No effects	
Preliminary developmental toxicity Non-guideline Non-GLP Oral (gavage) Rat, CD(SD) 6 mated females/group supplementary information (reliable with restrictions)	Folpet batch 631729, purity 88.6% 0, 10, 65, 420 or 2750 mg/kg bw/day on gestation days 6-15 Vehicle: CMC+ acetic acid	*Maternal toxicity* 2750 mg/kg bw/day: clinical signs of soft faeces and perianal scouring; ↓ body weight gain (40% days 6-20), ↓ food consumption days 7-9 (47.5%), 10-13 (41%). 420 mg/kg bw/day: ↓ body weight gain (15% days 6-20) ↓ food consumption days 7-9 (11%) 65 mg/kg bw/day: no clearly stated effects *Developmental toxicity (uterine contents and weights only)* 2750 mg/kg bw/day: ↑ post-implantation loss; ↓ foetal weight, ↓ in crown rump length and placenta weights (markedly depressed in litters) 420 mg/kg bw/day: no clearly stated effects	(R-18200) Study 10 (1985)
Rabbit Prenatal Developmental Toxicity studies			
Developmental toxicity OECD 414 (2001) Deviations from OECD 414 (2018): 14 hours light/10 hours dark (instead of 12 hours light/ 12 hours dark) GLP Oral (gavage) Rabbit, New Zealand White 25 mated females/group	Folpet (Folpan Technical) batch 601 385 79, purity 95.8% 0, 10, 30 or 60 mg/kg bw/day on gestation days 6-28 Vehicle: CMC+ Tween 80	*Maternal toxicity* 60 mg/kg bw/day: body weight loss (-0.13kg cf. +0.03kg controls days 6-10 and -0.03kg cf. +0.26kg days 6-29)*; ↓ body weight adjusted for gravid uterus weight (4% day 29)*; ↓ food consumption throughout treatment period; observations of thin, few or pale faeces and little water drunk. 30 mg/kg bw/day: body weight loss (-0.03kg cf. +0.03kg controls days 6-10)*; ↓ body weight gain (69% days 6-29)*; ↓ food consumption throughout treatment period; observations of thin, few or pale faeces. 10 mg/kg bw/day: ↓ body weight gain (46% days 6-29)*; ↓ food consumption from day 12; observation of few or pale faeces. Maternal NOAEL < 10 mg/kg bw/day *Developmental toxicity* 60 mg/kg bw/day: ↑ late resorption (0.9 cf. 0.1 controls) and ↑ post-implantation loss (12.3% cf. 4.6% controls); ↓ fetal weight (18%); ↑ small/misshapen/oval lens with lenticular irregularities/opaque areas (8 fetuses/2 litters, 0 incidence controls); ↑ extra ribs (80% fetuses cf. 57% controls); 20 thoracolumbar vertebrae (51% fetuses cf. 29% controls); fetal immaturity (reduced ossification, atelectatic lungs).	(R-18200) Study 11 (2006)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		30 mg/kg bw/day: ↓ fetal weight (7%); ↑ extra ribs (73% fetuses cf. 57% controls); 20 thoracolumbar vertebrae (50% fetuses cf. 29% controls); fetal immaturity (reduced ossification, atelectatic lungs). 10 mg/kg bw/day: No effects. Developmental NOAEL 10 mg/kg bw/day	
Developmental toxicity EPA –FIFRA (1978) Deviations from OECD 414 (2001): - no mating but artificial insemination - only 11 animals in the high dose group for foetal examination (14 were pregnant) - soft tissue alterations of the head-except brain (including eyes, nasal passages and tongue) were not assessed GLP Oral (gavage) Rabbit, New Zealand White (DLI:NZW) 20 mated females/group (only 11 high dose with litters at term; 16 control litters)	Folpet batch SX-1338 (technical grade), purity 89.5% 0, 10, 20 or 60 mg/kg bw/day on gestation days 6-28 Vehicle: CMC + Tween 80	*Maternal toxicity* 60 mg/kg bw/day: 1/20 dead day 27 (gastric ulceration); ↓ body weight gain (21% days 6-29)*; body weight loss (days 6-29) when adjusted for gravid uterus weight*; ↓ food consumption throughout treatment period. 20 mg/kg bw/day: ↓ body weight loss (days 6-29) before and after adjustment for gravid uterus weight*; ↓ food consumption occasionally significant. 10 mg/kg bw/day: No effects Maternal NOAEL 10 mg/kg bw/day *Developmental toxicity* 60 mg/kg bw/day: 4 fetuses (3 live, 1 dead) from 3 litters with hydrocephalus (significant for fetal incidence but not litter incidence). No increase in visceral or skeletal variations. 20 mg/kg bw/day: Single incidence of hydrocephalus. 10 mg/kg bw/day: No effects. Developmental NOAEL 10 mg/kg bw/day	(R-6136) Study 12 (1984)
Developmental toxicity Non-standard design GLP Oral (gavage) Rabbit, New Zealand White (DIa Hra:NZW) 20 mated females/group	Folpet batch SX-1338 (technical grade), purity 89.5% 0 mg/kg bw/day on gestation days 7-18; 60 mg/kg bw/day on gestation days 7-9, 10-12, 13-15 or 16-18	Study conducted to further investigate an apparent dose-dependent increase in incidence of hydrocephalic fetuses (Study 12, 1984). 60 mg/kg bw/day given for 3 day periods (days 7-9, 10-12, 13-15 or 16-18) to investigate any association with hydrocephalus and maternal exposure for a specific period of development. Internal hydrocephalus was observed in one fetus (treatment period days 10-12) and in a second fetus (treatment period days 16-18); no-treatment-related increase in incidence for a specific period. Irregular shaped fontanelle was statistically significantly increased at foetal level when folpet was administered between Days 13-15.	(R-6183) Study 13 (1985)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
	Vehicle: CMC + Tween 80		
Developmental toxicity US EPA 83-3 Deviations from OECD 414 (2001): - age of the animals was not reported - light dark cycle was 14 hours light, 10 hours dark (instead of 12/12) - only 14 animals/ dose group (high dose group only 12) - dosing only from day 7-19 - soft tissue alterations of the head-except brain (including eyes, nasal passages and tongue) were not assessed GLP Oral (gavage) Rabbit, New Zealand White (HY/CR) 14 mated females/group	Folpan batch 631729 (folpet technical grade), purity 91.1% 0, 10, 40 or 160 mg/kg bw/day on gestation days 7-19 Vehicle: CMC	*Maternal toxicity* 160 mg/kg bw/day: clinical signs of soft faeces, few or no faeces; ↓ body weight gain (60% days 7-29)*, ↓ gravid uterus weight (19%); ↓ food consumption (approx. 50% during dosing period). 40 mg/kg bw/day: ↓ body weight gain (15% days 7-29)*; ↓ gravid uterus weight (16%) 10 mg/kg bw/day: No effects Maternal NOAEL 10 mg/kg bw/day *Developmental toxicity* 160 mg/kg bw/day: ↑ post-implantation loss; ↓ slight fetal body weight (not statistically significant); ↓ skeletal ossification; ↑ extra ribs 40 mg/kg bw/day: ↓ skeletal ossification; ↑ extra ribs 10 mg/kg bw/day: No effects Developmental NOAEL 10 mg/kg bw/day	(R-3684) Study 14 (1985)

*: According to section 3.7.2.4.4 of the CLP Regulation: "In rabbits, the body weight gain may not be useful indicators of maternal toxicity because of normal fluctuations in body weight during pregnancy."

Table 38: Summary table of other animal studies on adverse effects on development

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Published Rat Prenatal Developmental Toxicity Studies				
Developmental toxicity No reference to guidelines Non GLP	Folpet technical grade Purity: not reported Vehicle:	Limited data reported. Small groups of animals used. 10 pregnant rats dosed with 100 mg/kg bw/day gestation days 6-15	100 mg/kg bw/day: ↓ body weight gain days 6-15 500 mg/kg bw/day gestation days 8-10: no maternal effects Study not appropriate to derive NOAEL No significant increase in the number of	Kennedy, Fancher & Calandra. (1968) Toxicology and Applied Pharmacology,

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Oral (gavage) Rat: Charles River & Sprague Dawley Supplementary information due to major limitations (e.g. it is not clear if the number of animals dosed is similar to the number of animals pregnant, effects on maternal toxicity were only marginally reported, only one dose group for phthalimide, dosing period too short) (reliable with restrictions)	corn oil	4 pregnant rats dosed with 500 mg/kg bw/day gestation days 8-10	foetal abnormalities. Internal structure appeared normal. Well-defined skeletal development generally observed.	13(3), 420-430
Published Rabbit Prenatal Developmental Toxicity Studies				
Developmental toxicity No reference to guidelines Non GLP Oral (capsule) Rabbit: Dutch belted and New Zealand White Supplementary information due to major limitations (e.g. it is not clear if the number of animals dosed is similar to the number of animals	Folpet technical grade Purity: not reported	Limited data reported. Small groups of animals used. Susceptibility of rabbit to thalidomide confirmed. Dose administered gestation days 6-16 for the Dutch Belted rabbit and gestation days 6-18 for the NZW. Folpet dosed to NZW at 18.75, 37.5 and 75 mg/kg bw/day to 5-7 rabbits/dose	NZW rabbit 75 mg/kg bw/day: maternal death, ↓ body weight gain*. ↑ resorptions (61.5%), ↓ foetal body weight 37.5 mg/kg bw/day. ↓ body weight gain*, ↑ resorptions (31.4%) 18.75 mg/kg bw/day: no maternal effects. No abnormal foetuses. Internal structure appeared normal. No structural skeletal deformities. Dutch rabbit 75 mg/kg bw/day: ↓ body weight gain* 1/65 foetuses abnormal with microphthalmia. Study not appropriate to derive NOAEL	Kennedy, Fancher & Calandra. (1968) Toxicology and Applied Pharmacology, 13(3), 420-430

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
pregnant, effects on maternal toxicity were only marginally reported, only one dose group for phthalimide, dosing period too short) (reliable with restrictions)				
Developmental toxicity No reference to guidelines Non GLP Oral (gavage) Rabbit: New Zealand White Supplementary information (Only 6 animals/ dose level, only one dose level tested, dosing period was too short, stability of folpet in water was not tested) (reliable with restrictions)	Folpet (Phaltan) Purity: not reported	Limited data reported. Small group of animals used. 80 mg/kg bw/day given to 6 NZW rabbits on gestation days 7-12	No adverse effect on the foetus.	Fabro, Smith, & Williams (1966). Fd. Cosmet. Toxicol. Vol. 3, pp587-590.
Developmental toxicity No reference to guidelines Non GLP Oral (capsule) Rabbit: New Zealand White Not reliable (due to very limited reporting- only the abstract of the 8th annual meeting is available).	Folpet Purity: not reported	No data reported. Number of animals used not known 150 & 75 mg/kg bw/day given to NZW rabbits on gestation days 6-16	No adverse effects on the foetus.	McLaughlin, Reynaldo, Lamar & Marliac (1969). Toxicology and Applied Pharmacology 14(3), 641. Abstract only

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Other Published Prenatal Developmental Toxicity Studies				
Hamster Developmental toxicity No reference to guidelines Non GLP Oral (gavage) Golden Syrian hamsters Group size 2-13. Not reliable (Only 5 animals/ dose level, severe maternal toxicity resulting in only 1-3 litters evaluated at high dose levels, only foetuses with external abnormalities were evaluated for bone defects, not possible to determine litter incidences)	Folpet Vehicle: CMC Purity: not reported	Poor quality experiment of limited value (JMPR, 2004). Single dose (400 - 1000 mg/kg bw) on GD 7 or GD 8, or daily dose of 200, 300, 400 or 500 mg/kg bw/day on GD 6 to GD 10.	Maternal mortality after all single doses except 500 mg/kg bw/day. Some abnormal foetuses reported at maternally lethal doses but with no distinctive pattern of anomaly.	Robens (1970) Toxicology and Applied Pharmacology, 16, 24–34 and related position paper R-17845 (Anonymous 2004)
Mouse Developmental toxicity No reference to guidelines Non GLP Oral (gavage), subcutaneous injection, inhalation CD-1 mice 7-8/group Supplementary information (Only one dose level/route, severe	Folpet technical grade purity 87% Gavage vehicle corn oil:acetone 9:1 SC vehicle: DMSO 100 mg/kg/day oral & SC gestation days 6-15	Limited study using small numbers of animals and single dose levels.	Inhalation: 10% maternal mortality Oral & SC: No maternal effects No foetal toxicity	Courtney (1983) U.S. EPA Report No. EPA-600/1-83-017

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
maternal toxicity when administered by inhalation route, age of mice unknown) (reliable with restrictions)	Inhalation 624 mg/m^3/day, 4 hr/day on gestation days 6-13			
Non-human primate Developmental toxicity No reference to guidelines Non GLP Oral Primates: Rhesus monkeys and stump tailed macaques 7 pregnant females/group Not reliable (No information regarding purity or origin of the test substances, low number of animals/ dose group, age of animals not determined, dosing period is too short, Caesarean section at the half point of gestation, maternal effects were not reported, lack of reporting for foetal effects, e.g. weight, sex, … , no negative	Folpet Purity: not reported 0, 10, 25 or 75 mg/kg bw/day on gestation days 21-34 Vehicle: cream of coconut	Limited study. Folpet administered to pregnant rhesus monkeys and stump-tailed macaques to assess the teratogenic potential. Susceptibility of animal model to thalidomide confirmed. Justification for dosing period described. Dosing during the period of foetal limb development. Foetal viscera examined grossly and skeletons following staining with Alizarin Red S. No maternal data reported	No evidence for teratogenic potential of folpet in nonhuman primates at highest dose tested of 75 mg/kg bw/day.	Vondruska, Fancher & Calandra (1971) Toxicology and Applied Pharmacology, 18(3), 619-624

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
control group)				
Supporting Rabbit Prenatal Developmental Toxicity Studies				
Phthalimide Developmental toxicity OECD 414 (2001) GLP Oral (gavage) Rabbit, New Zealand White 25 mated females/group	Phthalimide batch 53825203, purity 100% 0, 5, 15 or 30 mg/kg bw/day on gestation days 6-28 Vehicle: CMC + Tween 80	Study of folpet's primary and systemically available metabolite, phthalimide. Phthalimide (MW 147.1) dosed at 30 mg/kg bw/day as the molar equivalent of 60 mg/kg bw/day folpet (MW 296.6). The dose rates used are therefore precisely comparable with Study 11 (2006) and Study 12 (1984).	*Maternal toxicity* 30 mg/kg bw/day: No effects *Developmental toxicity* 30 mg/kg bw/day: No effects	(R-18201) Study 15 (2006)
Captan Developmental toxicity OECD 414 (2001) GLP Oral (gavage) Rabbit, New Zealand White 25 mated females/group	Captan batch 601 385 40 purity 95.1% 0, 10, 20 or 45 mg/kg bw/day on gestation days 6-28 Vehicle: CMC + Tween 80	Study of captan, a chemical structurally similar to folpet.	*Maternal toxicity* NOEL< 10 mg/kg bw/day: dose-related effect ↓ in body weight* & food consumption with most severe effect at 45 mg/kg bw/day. *Developmental toxicity* 45 mg/kg bw/day: 1 total resorption; ↑ early resorptions therefore ↑ post-implantation loss; ↓ foetal weight; ↑ minor skeletal observations NOEL 20 mg/kg bw/day	(R-18199) Study 16 (2006)
THPI Developmental toxicity OECD 414 (2001) GLP Oral (gavage) Rabbit, New Zealand White 25 mated females/group	THPI batch S17363, purity 98.4% 0, 5, 10 or 22.5 mg/kg bw/day on gestation days 6-28 Vehicle: CMC + Tween 80	Study of captan's primary and systemically available Dosed at 22.5 mg/kg bw/day, the molar equivalent of 45 mg captan/kg bw/day, the highest dose tested by Study 16 (2006).	*Maternal toxicity* 22.5 mg/kg bw/day: No effects *Developmental toxicity* 22.5 mg/kg bw/day: No effects	(R-18202) Study 17 (2006)

*: According to section 3.7.2.4.4 of the CLP Regulation: "In rabbits, the body weight gain may not be useful indicators of maternal toxicity because of normal fluctuations in body weight during pregnancy."

9.10.5 Short summary and overall relevance of the provided information on adverse effects on development

The developmental toxicity of folpet was investigated primarily in rats and rabbits. In addition, published studies of folpet are available for mice, hamsters and non-human primates.

Rat Studies

There are two key studies in the rat Study 4 (2007) and Study 5 (2003). These are robust GLP studies conducted to OECD Test Guideline Number 414 (2001). Both studies investigated the same dose levels. At the highest dose tested, 800 mg/kg bw/day, maternal toxicity in form of clinical signs (dyspnea and loud breathing) and adjusted as well as unadjusted body weight gain (17 and 25% respectively) was demonstrated. Some maternal toxicity (decreased adjusted body weight gain of 20%) was also observed at 100 mg/kg bw/day in Study 4 (2007) but not in Study 5 (2003). The clear NOAEL for developmental toxicity was 100 mg/kg bw/day. At 800 mg/kg bw/day (Study 4, 2007), the developmental findings were minimal and of questionable toxicological significance. There were no adverse effects of folpet on foetal development in either study.

Two earlier, prenatal developmental toxicity studies in the rat (Study 6, 1985; Study 7, 1983) were conducted. Study 7 (1983) was similar in design to the more recent study (Study 5, 2003) and investigated a highest dose of 360 mg/kg bw/day. At this dose, clear maternal toxicity in form of clinical signs, decreased body weight gain (28%) and food consumption (11-15%) was demonstrated and the intermediate dose of 60 mg/kg bw/day caused some maternal effects (rales and -11% body weight gain). The NOAEL for developmental effects was 60 mg/kg bw/d based on incomplete ossification in the pelvis, pubis and/or ischium at 360 mg/kg bw/d. The severity of the maternal toxicity seen in Study 7 (1983) is greater than seen in Study 5 (2003). The reasons for this are uncertain.

Study 6 (1985) used the highest dose levels of all but dosed only through the period of major organogenesis (gestation days 6-15). At the highest dose marked maternal toxicity (1/22 mortality, ulcerations of gastric mucosa; clinical signs and decreased body weight gain of 28% and decreased food consumption of 33-43%) was observed. Some maternal toxicity (decreased body weight gain of 18% and decreased food consumption of 16%) was also observed at 550 mg/kg bw/d. This was associated at both dose levels with a consequential effect on foetal body weight but not viability. Evidence of reduced ossification was consistent with the reduced foetal body weight. At the lowest dose of 150 mg/kg bw/day, there was no maternal toxicity, no reduction in foetal body weight and no clear reduction in foetal ossification. An increased incidence of reduced ossification of the interparietal bone was an isolated finding, not clearly dose-related and was considered not to be of toxicological significance. Angulated ribs were observed in a low incidence of foetuses in all test groups but not in the control group although the study report does describe an historical control incidence of 5 foetuses in 131 litters. The incidence of affected foetuses was 5, 4 and 6 in the test groups (150, 550 and 2000 mg/kg bw/day groups respectively) in 20-22 litters. The litter incidence was 0, 2.84, 6.49 and 6.51%. The report concludes that the NOAEL for developmental toxicity is <150 mg/kg bw/day.

In summary, the four guideline studies described above show some inconsistency in defining the NOAEL for maternal toxicity. However, the studies are consistent in concluding that folpet has no adverse effect on foetal development in the rat.

The three multigeneration studies also demonstrate that folpet has no adverse effect on foetal development in the rat as determined from the offspring born.

Reference to evaluation of developmental toxicity in the rat is made in a publication (Kennedy, 1968) with limited reliability. There it is concluded that the dose of 100 mg/kg bw/day administered on gestation days 6-15 reduced maternal body weight but did not induce developmental effects.

Table 39: Summary of the conclusions obtained from rat developmental toxicity studies

Dose level of folpet	Reference	Maternal toxicity	Developmental toxicity
2000 mg/kg bw/day	Study 6 (1985)	Yes: 1/22 death day 16 (multiple haemorrhagic ulcerations of gastric mucosa); clinical signs of soft faeces, staining of body fur and perianal staining; ↓ body weight gain (28% days 6-20), ↓ food consumption	Yes: ↓ foetal body weight (7%); ↑ incidence of skeletal variations (anterior fontanelle large, angulated ribs) - stat. significant, ↓ ossification of cranial bones (supraoccipital, interparietal, parietal, squamosal) - stat. significant, ↓ ossification of sternebrae 1-4 and pubic bones- stat. significant, ↓ ossification of metacarpals (fewer than three metacarpal bones unossified on one or both manus) and metatarsals (Metatarsal V unossified bilaterally and fewer than three metatarsal bones unossified on one or

		days 7-9 (43%), 10-13 (33%)	both pedes- the latter non statistically significant) Assumed to occur secondary to maternal toxicity
800 mg/kg bw/day	Study 4 (2007)	Yes: 2/24 deaths; 2/24 dyspnea, 4/24 loud breathing; ↓ body weight gain (17% days 6-21); ↓ body weight gain adjusted for gravid uterus weight (25% days 6-21); ↓ food consumption (7.7% days 6 to 9).	No: Cited effects (↑ post-implantation loss and ↓ number live foetuses) not clearly treatment-related, not stat. significant These effects were not considered adverse
800 mg/kg bw/day	Study 5 (2003)	Yes: ↑ salivation (15/22) post dosing days 13-19; ↓ body weight gain (9% days 6-20); ↓ body weight gain adjusted for gravid uterus weight (21% days 6-20); ↓ food consumption (days 6 to 8 and 15 to 17).	Yes?: Slight increase in visceral (e.g. on umbilical artery) and skeletal abnormalities (e.g. incomplete ossification of vertebral centrum and thoracic vertebrae). These effects were within HCD (see Annex) and not stat. significant. Assumed to occur secondary to maternal toxicity
550 mg/kg bw/day	Study 6 (1985)	Yes: ↓ body weight gain (18% days 6-20); ↓ food consumption days 7-9 (16%)	Yes: ↓ foetal body weight (4%); ↑ incidence of skeletal variations (anterior fontanelle large, angulated ribs)- stat. significant, ↓ ossification of cranial bones (supraoccipital, interparietal, parietal, squamosal- the latter non statistically significant), ↓ ossification of sternebrae 1-4, pubic bones- stat. significant, ↓ ossification of metacarpals (fewer than three metacarpal bones unossified on one or both manus) and metatarsals (Metatarsal V unossified bilaterally and fewer than three metatarsal bones unossified on one or both pedes- the latter non statistically significant) Assumed to occur secondary to maternal toxicity
360 mg/kg bw/day	Study 7 (1983)	Yes: Clinical signs of rales, dyspnea, salivation, chromorrhinorrhea, chromodacryorrhoea, decreased motor activity, soft/liquid faeces, staining of body fur; ↓ body weight gain (28% days 6-20); ↓ food consumption (11% days 6-13, 15% days 13-20)	Yes: Incomplete ossification in the pelvis, pubis and/or ischium- not stat. significant Assumed to occur secondary to maternal toxicity

150 mg/kg bw/day	Study 6 (1985)	No	Yes: ↑ litter incidence of angulated ribs (low incidences, but stat. significant)
100 mg/kg bw/day	Study 4 (2007)	Yes: ↓ body weight gain (9% days 6-21); ↓ body weight gain adjusted for gravid uterus weight (20% days 6-21); ↓ food consumption (7.7% days 6 to 9).	No
100 mg/kg bw/day	Study 5 (2003)	No	No
60 mg/kg bw/day	Study 7 (1983)	Yes: Clinical signs of rales, ↓ body weight gain (10% days 6-20)	No
20 mg/kg bw/day	Study 4 (2007)	No	No
20 mg/kg bw/day	Study 5 (2003)	No	No
10 mg/kg bw/day	Study 7 (1983)	No	No

Overall, the weight of evidence shows that folpet, when administered to the pregnant rat at doses up to and including 2000 mg/kg bw/day does not alter foetal development in the absence of maternal toxicity. folpet is not teratogenic in the rat.

Rabbit Studies

Four studies of prenatal developmental toxicity in the rabbit (Study 11, 2006; Study 12, 1984, Study 13, 1985; Study 14, 1985) have been conducted and together provide a thorough evaluation of the potential of folpet to adversely affect foetal development in the rabbit. Supplementary information, including more recently obtained historical control data in support of Study 12 (1984) and Study 13 (1985) is included in a comprehensive review of the potential of folpet to induce developmental toxicity (Anonymous, 2018, R-39172).

Study 11 (2006) was similar in design to that of Study 12 (1984) in that the period of treatment was from gestation day 6 to day 28 according to OECD Test Guideline Number 414 (2001). The dose levels were similar i.e. 0, 10, 30 or 60 mg/kg bw/day cf. 0, 10, 20 or 60 mg/kg bw/day (Study 12, 1984). Also, a larger group size, 25 rabbits cf. 20 rabbits was used. In Study 12 (1984), only 11 high dose females and 16 control females had litters at term; 22-25 litters per group had live foetuses at term in the more recent study. For these reasons, Study 11 (2006) should be regarded as the most robust of all. However, slightly more severe maternal toxicity in form of body weight loss, reduced food consumption (44-69%), thin physique, altered faeces and little water drunk was demonstrated at 60 mg/kg bw/day (Study 11, 2006), compared to the earlier study (Study 12, 1984) in form of mortality (1/20), body weight loss and reduced food consumption (26-46%), the reasons for which are uncertain.

There was no evidence of treatment-related malformation at 60 mg/kg bw/day (Study 11, 2006). Lens malformations observed at the highest dose of 60 mg/kg bw/d were associated with general immaturity of the foetuses and marked maternal toxicity. No foetal malformation was observed in Study 14 (1985) at the highest dose level tested of 160 mg/kg bw/day which induced maternal toxicity in form of clinical signs (soft faeces, few or no faeces) and reduced food consumption (approx. 50% during dosing period). In Study 12 (1984) increased incidences of hydrocephali were observed at the highest dose group and one single incidence at the mid dose group. The report author concluded that folpet was not a unique hazard to the conceptus since the small increase in the incidence of foetal anomalies was observed only at maternally toxic doses. Maternal toxicity at 60 mg/kg bw/d was marked (1/20 dead, body weight loss and statistically significant reduced food consumption of 26-46%) and maternal effects at 20 mg/kg bw/ included also body weight loss, but less pronounced (occasionally significant) reduced food consumption (5-33%).

A subsequent study (Study 13, 1985) was undertaken to investigate the possible association of hydrocephalus with a specific window of development using a pulse dosing regimen. A chemically-induced foetal

malformation would be expected to correlate with a particular window of exposure during gestation. The window for the induction of hydrocephaly is indicated to be early during organogenesis i.e. days 7-8 (Anonymous, 2018, R-39172). Study 13 (1985) demonstrated that there was no specific window during which administration of a maternally toxic dose of folpet induced developmental effects; hydrocephalus was observed in one foetus from treatment period gestation days 10-12 and in one from treatment period gestation days 16-18. Additionally, review of extensive historical control data (1980-1991) from the performing laboratory indicates that a higher frequency of occurrence of hydrocephaly was prevalent in the rabbit colony at the time of the studies (1984-1985) and that the incidence observed in the folpet studies was consistent with the control incidence (Anonymous, 2018, R-39172).

In summary, the four studies described above show that the maternal NOAEL is <20 mg/kg bw/day. Collectively, these studies demonstrate that folpet has no adverse effect on foetal development in the rabbit at dose levels that did not induce significant maternal toxicity.

Reference to evaluation of developmental toxicity in the rabbit is made in a publication (Kennedy, 1968) with limited reliability. There the dose of 75 mg/kg bw/day administered on gestation days 6-18 did induce maternal mortality, reduced maternal and foetal body weight and increased post-implantation loss but provided no evidence of treatment-related foetal abnormality.

Table 40: Summary of the conclusions obtained from rabbit developmental toxicity studies

Dose level of folpet	Reference	Maternal toxicity	Developmental toxicity
160 mg/kg bw/day	Study 14 (1985)	Yes: Clinical signs of soft faeces (5/12), few or no faeces (9/12); ↓ gravid uterus weight (19%); ↓ food consumption (approx. 50% during dosing period). [↓ body weight gain (40% days 7-29)]*	Yes: ↑ post-implantation loss (21.8% vs 14.4 in control, stat. significant); ↓ slight foetal body weight (7% not statistically significant); ↓ skeletal ossification (fewer than 16 caudal vertebral centra ossified, reduced/irregular ossification of hyoid bone, reduced/irregular ossification among sternebrae 1-4, reduced ossification of long bone epiphyses)- stat. significant; ↑ extra ribs and vertebrae (13 thoracic vertebrae and 13 pairs of thoracic ribs, 13th lumbar rib present bilaterally)- stat. significant Assumed to occur secondary to maternal toxicity (No hydrocephaly)
60 mg/kg bw/day	Study 11 (2006)	Yes: ↓ food consumption throughout treatment period (approx. 50% during dosing period)- stat. sign.; observations of thin physique (13/25), few (24/25) or pale faeces (24/25) and little water drunk (11/25) [body weight loss (-0.13kg cf. +0.03kg controls days 6-10 and -0.03kg cf. +0.26kg days 6-29);	Yes: ↑ late resorption (0.9 vs 0.1 in control- stat. sign.), ↑ post-implantation loss (12.3 vs. 4.6 in control- stat. sign.); ↓ foetal weight (18%- stat. significant); ↑ small/misshapen/oval lens with lenticular irregularities/opaque areas (malformation)- not stat. sign.; ↑ extra ribs- not stat. sign.; 20 thoracolumbar vertebrae- not stat. sign.; foetal immaturity (reduced ossification of epiphyses, astragalus, metacarpals/phalanges and atelectatic lungs)- not stat. sign. Assumed to occur secondary to maternal toxicity (No hydrocephaly)

		↓ body weight adjusted for gravid uterus weight (4% day 29)] *	
60 mg/kg bw/day	Study 12 (1984)	Yes: 1/20 dead day 27 (gastric ulceration); ↓ food consumption throughout treatment period (approx. 50% during treatment period) (↓ body weight gain (79% days 6-29); body weight loss (days 6-29) when adjusted for gravid uterus weight)- stat. sign.] *	Yes: Hydrocephaly- stat. significant at foetal incidence (please refer to the discussion above and to Anonymous, 2018, R-39172 in Annex Human Health) In the same animals- stat. significant at foetal incidence: lungs did not float in water, stomach not completely distended contained dark green semi-solid material, fontanelle irregularly shaped, fontanelle moderately enlarged
60 mg/kg bw/day	Study 13 (1985)- pulse dose	Yes?: mortality (1/20 in group dosed Days 7-9) (occasional soft or liquid faeces- unclear relationship to treatment as it was generally present after completion of the dosage period) [↓ body weight and body weight loss during dosing period- the latter stat. sign.]*	Internal hydrocephalus was observed in one foetus (treatment period days 10-12) and in a second foetus (treatment period days 16-18); no-treatment-related increase in incidence for a specific period, not stat. sign. (please refer to the discussion above and to Anonymous, 2018, R-39172 in Annex Human Health) Irregular shaped fontanelle was statistically significantly increased at foetal level when folpet was administered between Days 13-15.
40 mg/kg bw/day	Study 14 (1985)	Yes?: Soft faeces (2/14), white mucous excrement (2/14), ↓ gravid uterus weight (16%)- stat. sign. [↓ body weight gain (15% days 7-29)- not stat. sign.]*	Yes: ↓ skeletal ossification- stat. sign. (fewer than 16 caudal vertebral centra ossified, reduced/irregular ossification among sternebrae 1-4); ↑ extra ribs and vertebrae (13 thoracic vertebrae and 13 pairs of thoracic ribs, 13th lumbar rib present bilaterally)- stat. sign. No hydrocephaly
30 mg/kg bw/day	Study 11 (2006)	Yes: ↓ food consumption throughout treatment period (approx.. 35% through treatment period))- stat. sign; thin physique (4/25), few (13/25) or pale (5/25) faeces [body weight loss (-0.03kg cf. +0.03kg controls days 6-10);	Yes: ↓ foetal weight (7%)- not stat. sign.; ↑ extra rib- not stat. sign.; 20 thoracolumbar vertebrae- not stat. sign.; foetal immaturity (reduced ossification of epiphyses, astragalus, metacarpals/phalanges and atelectatic lungs)- not stat. sign. No hydrocephaly Assumed to occur secondary to maternal toxicity

		↓ body weight gain (69% days 6-29)]*	
20 mg/kg bw/day	Study 12 (1984)	Yes: ↓ food consumption occasionally stat. significant (approx.. 20% during treatment period) [↓ body weight loss (days 6-29) before and after adjustment for gravid uterus weight- stat. sign.]*	Yes: Single incidence of hydrocephalus, cleft palate and fontanelle irregularly shaped (same animal). With questionable dose- response skull skeletal findings: ↑ parietals contained holes- stat sign. at foetal level, frontals contained holes- not stat. sign.
10 mg/kg bw/day	Study 11 (2006)	Yes ↓ food consumption from day 12- stat. sign. (approx.. 20% during treatment period); observation of few (9/25) or pale (4/25) faeces [↓ body weight gain (46% days 6-29)- stat. sign.]*	No
10 mg/kg bw/day	Study 12 (1984)	No	No
10 mg/kg bw/day	Study 14 (1985)	No	No

*: According to section 3.7.2.4.4 of the CLP Regulation: "In rabbits, the body weight gain may not be useful indicators of maternal toxicity because of normal fluctuations in body weight during pregnancy."

There are two other publications (Fabro, 1966- limited reliability; McLaughlin, 1969- not reliable) relating to the evaluation of the developmental toxicity of folpet in the rabbit, but the reported details are extremely sparse and the number of animals tested is very small or not specified. Nevertheless, these studies do not indicate any adverse effect of folpet on foetal development in the rabbit. Fabro (1966) gave Phaltan, by oral gavage, at 80 mg/kg bw/day to 6 NZW rabbits on days 7-12 of gestation: no adverse effects on the foetus were observed. McLaughlin (1969) gave folpet orally by gelatine capsule to an unspecified number of rabbits on gestation days 6-16. The dose levels were 75 and 150 mg/kg bw/day. No evidence of teratogenicity was detected.

In a non-GLP study from the published literature (Robens, 1970), which is not reliable, the teratogenic effects of a number of derivatives of phthalimide, including folpet, were tested in groups of 2 - 8 pregnant golden hamsters. Folpet was given as a single dose of 400, 500, 600, 700, 800, 900 or 1000 mg/kg bw on day 7 or day 8 of gestation, or at a daily dose of 200, 300, 400 or 500 mg/kg bw/day on gestation days 6 to 10. Maternal mortality occurred after all single doses except 500 mg/kg bw and after repeated doses of 300mg/kg bw/day or more. No malformations were reported in the groups receiving repeated doses. Some malformed foetuses were observed in the groups treated with a single dose of folpet but these doses induced maternal lethality. There was no dose–response relationship. The study did not provide any clearly meaningful data on the effects of folpet on the developing hamster foetus.

In another non-GLP study from the published literature with limited reliability, pregnant mice were exposed to folpet by oral gavage, by subcutaneous injection or by inhalation (Courtney, 1983). A dose of 100 mg/kg bw/day was administered by gavage or subcutaneous injection on gestation days 6-15. The inhalation route provided daily average concentrations approximating 624 mg/hr/m^3 for folpet, 4/hr/day on gestation days 6-13. There was approximately 10% maternal mortality by the inhalation route only. No foetal toxicity was observed.

Folpet has also been evaluated for teratogenicity in the primate (Vondruska, 1971, not reliable). Folpet was administered to pregnant rhesus monkeys and stump-tailed macaques during the period of foetal limb

development (gestation days 21-34). No evidence of teratogenicity was observed at the highest dose tested of 75 mg/kg bw/day.

Folpet rapidly degrades in the presence of thiol-containing components, thus the systemic compartment and the developing foetus is exposed to folpet's metabolites. A prenatal developmental toxicity study of folpet's primary and systemically available metabolite, phthalimide, in the rabbit has also been conducted (Study 15, 2006). Phthalimide has a structure similar to thalidomide which is a known teratogenic substance in the rabbit. The dose rates used were precisely comparable with those of folpet by Study 11 (2006) and Study 12 (1984). This study demonstrated that phthalimide is not a developmental toxicant in the rabbit up to the doses tested. Also relevant to the investigation of the potential of folpet to induce developmental effects in the rabbit is consideration of the structurally similar chemical captan and its metabolite tetrahydrophthalimide (THPI). These two chemicals were also investigated (Study 16, 2006; Study 17, 2006) and found not to be developmental toxicants in the rabbit.

The four rabbit prenatal developmental toxicity studies (Study 11, 2006; Study 15, 2006; Study 16, 2006; Study 17, 2006) were undertaken in the same facility, using the New Zealand White rabbit from one accredited closed colony and conducted to the same study design (essentially OECD TG 414, 2001). All four studies were reported by the same Study Director. This series of studies provides a directly comparable set of data without the inherent variation between different laboratories and methodologies, different rabbit colonies and year of study conduct. The particular strength of these four studies relates to the consistent interpretation of the foetal observations based on the understanding and knowledge of spontaneously occurring foetal malformation and variation in the rabbit obtained from one supplier at a similar point in time (Anonymous, 2018, R-39172). The results of the four comparable studies, clearly demonstrate that neither folpet and its metabolite phthalimide, nor the structurally similar chemical captan and its metabolite THPI are developmental toxicants in the rabbit.

9.10.6 Comparison with the CLP criteria

In the classification system, adverse effects on development of the offspring include any effect which interferes with normal development of the conceptus, either before or after birth, and resulting from exposure of either parent prior to conception, or exposure of the developing offspring during prenatal development, or postnatally, to the time of sexual maturation.

Toxicological results key studies [and other studies]	**CLP Criteria**
Rat developmental toxicity No developmental toxicity was observed in the first key study (Study 4). In the other key study (Study 5) questionable developmental findings, which were within reliable HCD were detected. Furthermore, these effects are assumed to occur secondary to maternal toxicity. [Delayed ossification (Studies 6 and 7) as well as reduced foetal weight (Study 6) can be assumed to occur secondary to maternal toxicity. Skeletal variations at mid and high dose in Study 6 might be attributed to maternal toxicity, while at low dose, they were within reliable HCD.] **Rabbit developmental toxicity** Developmental effects were observed in three reliable rabbit studies (Studies 11, 12 and 14).	**Category 1A: Known human reproductive toxicant.** Classification largely based on evidence from humans. **Category 1B: Presumed human reproductive toxicant.** Classification largely based on data from animal studies providing clear evidence of an adverse effect on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect is considered not to be a secondary non-specific consequence of other toxic effects. **Category 2: Suspected human reproductive toxicant.** Classification based on evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. Such effects shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect is considered not to be a secondary non-specific consequence of the other toxic effects.

Toxicological results key studies [and other studies]	CLP Criteria
Severe maternal toxicity (e.g. reduced food consumption, clinical signs, body weight loss and low incidences of mortality) occurred in these studies at 60 and 160 mg/kg bw/d. Developmental effects (increased post implantation loss and late resorptions, reduced foetal weight, reduced skeletal ossification, increased incidences of extra ribs and vertebra) and lens malformations (Study 11) observed at these dose levels are assumed to occur secondary to maternal toxicity. Increased incidences of hydrocephali was observed in Study 12 at a dose level of 60 mg/kg bw/d and a single incidence at 20 mg/kg bw/d. In a pulse dose study (No. 13), conducted to clarify the findings of hydrocephali, no-treatment-related increase in its incidence for a specific period was observed. Furthermore, HCD showed a peak in hydrocephali at the time the study was conducted. For detailed discussion please refer also to the Human Health Annex, Section 3.10.8.1 (Review Anonymous 2018, R-39172). All HCD included in Studies 11, 12, 13 and 14) as well as in the review can be considered as fully reliable. Reduced skeletal ossification and increased incidences of extra ribs and vertebrae [partly within HCD (Study 14)] observed in Studies 11 and 14 might be also attributed to maternal toxicity and associated foetal immaturity. **Overall conclusion** No classification as developmental toxicant is required for folpet.	

9.10.7 Adverse effects on or via lactation

Table 41: Summary table of animal studies on effects on or via lactation

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Two Generation (one litter/gen.)	Folpet batch 631933	*Parental toxicity* 5000 ppm (250 mg/kg bw/day)	Study 1 (1986)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Oral (continuous in diet) US EPA 83-4 Complies largely to OECD 416 (2001) but with no developmental landmarks, no sperm analysis, only limited organ weights and histopathology in adults, none in pups. GLP Rat, CD(SD) 25/sex/group	(technical grade), purity 91% 0, 250, 1500 or 5000 ppm Vehicle: laboratory animal diet	F0: ↓ body weight (8.5% males, 5% females by end pre-pairing period); ↓ body weight gain: gestation (11%, days 1-20), lactation (16%, days 1-14); ↓ food consumption pre-pairing both sexes, gestation and lactation; ↑ hyperkeratosis of the non-glandular stomach - moderate (16/25 males, 12/25 females) slight (9/25 males, 10/25 females) single incidence of moderate and slight in control males only; ↑ basophilic renal tubules in males (7/25 slight [3/25 controls], 2/25 moderate [0/25 controls]) F1: ↓ body weight week 0 (24.5% males, 18.5% females); ↓ body weight gain weeks 0-14 (13% males, 5% females); ↓ body weight gain gestation (8%, days 1-20);↓ food consumption pre-pairing both sexes, gestation and lactation; ↑ hyperkeratosis of the non-glandular stomach – moderate (22/25 males, 24/25 females) slight (3/25 males,1/25 females) [none in controls];↑ oesophageal hyperkeratosis in females (11/25 slight, 13/25 moderate) [none in controls] <u>1500 ppm (35 mg/kg bw/day)</u> F0: ↑ slight hyperkeratosis of the non-glandular stomach (19/25 males, 23/25 females) [single incidence in control males] F1: ↑ slight hyperkeratosis of the non-glandular stomach (21/24 males, 24/24 females) [none in controls]; ↑ oesophageal hyperkeratosis in females (15/24 slight) [none in controls] <u>250 ppm (12.5 mg/kg bw/day)</u> No effects NOEL 250 ppm (12.5 mg/kg bw/day) on the basis of hyperkeratosis due to local contact irritation *Reproductive toxicity* No effects at any dose level NOAEL 5000 ppm (250 mg/kg bw/day) *Offspring toxicity* <u>5000 ppm (250 mg/kg bw/day)</u> F1: ↓ body weight gain from day 7 (27% days 7-21) F2: ↓ body weight gain from day 7 (12% days 7-21) <u>1500 ppm</u> (35 mg/kg bw/day) No effects NOAEL 1500 ppm (35 mg/kg bw/day)	
Two Generation (two litter/gen.) Oral (continuous in diet) Guideline not stated.	Folpet batch SX-1388 (technical grade), purity 89.5%	*Parental toxicity* <u>3600 ppm</u> F0: ↓ slight body weight gain males (5.6%, days 1-155) F1(b): ↓ slight body weight gain males (5.7%, days 1-155) <u>800 ppm</u>	(R-6134) Study 2 (1985)

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr><td>Deviations from OECD 416 (2001): dosing of F0 (P) males was carried out for 62 days prior to mating, not 10 weeks
Organs were not weighed
Estrus cyclicity was not examined
Number of implants was not determined
Sperm parameters were not measured
Age of vaginal opening and balano-preputial separation was not measured
Organ weights were not determined for pups
Histopathology was not determined for pups
Coagulating gland, thyroid, vagina and adrenals was not assessed for histopathology
Detailed testicular histopathological examination was not performed
A quantitative evaluation of primordial follicles was not conducted for F1 females
GLP
Rat, CrL:COBS/CD(SD)
30/sex/group</td><td>0, 200, 800 or 3600 ppm
Vehicle: laboratory animal diet</td><td>No effects
NOEL 800 ppm
Reproductive toxicity
No effects at any dose level
NOAEL 3600 ppm
Offspring toxicity
<u>3600 ppm</u>
F1a: ↓ body weight day 21, gain days 0-21 (17%)
F1b: ↓ body weight day 21, gain days 0-21 (20%)
F2a: ↓ body weight days 14 &21, gain days 0-21 (19%)
F2b: ↓ body weight days 14 &21, gain days 0-21 (14.5%)
<u>800 ppm</u>
No effects
NOAEL 800 ppm

Test item intake in mg/kg bw/day
<table>
<tr><td>ppm</td><td>200</td><td>800</td><td>3600</td></tr>
<tr><td>M, F0</td><td>14.9</td><td>59.6</td><td>263.4</td></tr>
<tr><td>F, F0</td><td>18.1</td><td>72.9</td><td>314.5</td></tr>
<tr><td>M, F1b</td><td>22.3</td><td>90.8</td><td>421.6</td></tr>
<tr><td>F, F1b</td><td>23.4</td><td>94.8</td><td>436.3</td></tr>
</table>
</td><td></td></tr>
</table>

Human data on adverse effects on or via lactation

None available

Other studies relevant for effects on or via lactation

Decreased pup survival in a mouse spot test (Study 4, Section 8.8) was observed at the highest dose level, in the presence of severe maternal toxicity (mortality).

9.10.8 Short summary and overall relevance of the provided information on effects on or via lactation

Only Study 1 (1986) is considered to be the key reproduction study and the one that uses the highest dose of folpet and which induces clear parental toxicity. The highest dose of 5000 ppm reduced maternal body weight gain and food consumption during gestation and lactation. However, there was no effect on the number and size of the pups at birth and there was no indication of impaired nursing behaviour or decreased pup viability during lactation particularly during the early postnatal period. Pup body weight gain was reduced from postnatal day 7 probably due to direct contact with the diet containing folpet. There was no evidence of an adverse effect of folpet on or via lactation; no adverse effect of folpet due to transfer of the chemical in the milk or on the quality of the milk was indicated.

In study 2 reduced body weight and body weight gain could be observed at 3600 ppm. Reduced body weight was evident not earlier than Day 21 for the F1 generation and not earlier than Day 14 for the F2 generation. No effects on body weight of the dams was observed in this study.

Decreased pup survival during lactation period in a mouse spot test (Study 4, Section 8.8) could be associated to severe maternal toxicity (mortality).

9.10.9 Comparison with the CLP criteria

The classification is intended to indicate when a substance may cause harm due to its effects on or via lactation and is independent of consideration of the reproductive or developmental toxicity of the substance. There were no effects to warrant classification of folpet for effects on or via lactation.

9.10.10 Other data on rabbit

Three studies were conducted to investigate folpet's ability to interact with the gastrointestinal tract of rabbits. As known from the repeated oral exposure studies with rat and mouse, folpet induces irritation in the gastrointestinal tract. For rabbits, reduced food consumption, water intake and thin, few or pale faeces were observed. Rabbits rely on caecotrophy, i.e. they need to orally take up partly digested material from the anus to inoculate their gastrointestinal system and assure nutrient supply (Anonymous, 2016, R-37533). This is why rabbits do not tolerate antimicrobials, which is well known as "gastrointestinal stasis" or also "antibiotic toxicity" in rabbits. Gastrointestinal stasis is characterized by reductions in food and water intake, reductions in faecal mass and consistency, and malnutrition that consequently (specifically due to the dehydration) leads to severe symptomology, moribundity and (if the condition persists untreated), fatality. This condition is typically seen in rabbits in attempts to characterise teratology of antibiotics (which are per definition anti-microbial). The gastrointestinal symptomology described above is not seen in rats, dogs, monkeys and humans. In most cases it is not even seen in rabbits when the same systemic dose (plasma level) is applied via the dermal route. This indicates that this symptomology is not only species- but also route-specific. The mode of action of antimicrobials in caecotrophs such as rabbits is eradication of the gastrointestinal flora (either with overgrowth of pathogenic bacteria like *Clostridium difficile* and internal poisoning, or without), which leads to strongly reduced excretion which in turn leads to malnutrition and strong toxicity as a consequence. It is known from clinical experience in humans with antibiotics, and the lack of similar effects in dogs, rodents and monkeys that this mode of action is only active in rabbits among traditional test species.

Anonymous (2005a) investigated the antimicrobial activity of folpet on bacteria of the rabbit microbiome. It shows inhibitory potential on *Bacteroides sp., Enterococcus faecalis* and *Candida albicans.* A corresponding study (Anonymous, 2005b) shows that folpet's systemic metabolite phthalimide is inactive. This demonstrates

that the antimicrobial activity is associated with the trichloromethylthio-side chain, which is associated with folpet's local toxicity and its fungicidal effect. Note, folpet was found in the faeces of orally dose rats, see Section 7.

In Study 18 (2016), both male and female rabbits treated for 9 days orally (gavage) with folpet at 0, 10, 30 and 90 mg/kg bw/d, show a dose-dependent decreased water intake, food consumption and body weight. Also, faecal output is decreased dose-dependently (up to no excretion at all) and towards harder consistency. No consistent effects that would indicate a role of overgrowth of pathogenic bacteria or reductions in gastrointestinal motility were observed, which may be a consequence of not investigating a time course. Similar observations were also made for the dams in Study 11 (2006).

On the basis of the above results it is confirmed that folpet has a species-specific mode of action in rabbits identical to the mode of action of antimicrobials and antibiotics in cecotrophs. This mode of action reduces the faecal output which in turn leads to malnutrition and dehydration in cecotrophic species such as rabbits and in consequence to toxicity. This mode of action has not been observed in any other mammalian species.

Hence rabbits appear to be overly sensitive towards folpet exposure, which explains the maternal toxicity observed in the developmental toxicity studies.

Table 42: Clinical observations from Study 18 (2016) relating to gastrointestinal stasis, n=10/group

Findings	**Males**				**Females**			
	mg/kg bw/day							
	0	**10**	**30**	**90**	**0**	**10**	**30**	**90**
Faeces slightly dry		1						
Faeces small		2	5	9		1	1	10
Faeces partly small		1	5	6		1	1	
Faeces hard			1	5				
Faeces partly hard				1				
No Faeces				1				1
Slightly abnormal breathing		1						
Moderately abnormal breathing			2					
Reduced water consumption			1	4				4
No water consumption								1
No food consumption								3

Table 43: Clinical observations from Study 11 (2006) relating to gastrointestinal stasis

Observations	Control	[10 mg/kg bw/day]	[30 mg/kg bw/day]	[60 mg/kg bw/day]
Physique				
Thin	2/25	1/25	4/25	13/25
Behaviour				
Little water drunk	3/25	8/25	5/25	11/25
Little diet eaten	0/25	0/25	0/25	1/25
Excreta				
Few faeces	3/25	9/25	13/25	24/25
Pale faeces	0/25	4/25	5/25	24/25

Table 44: Summary table of other studies relevant for developmental toxicity

Type of study/data	Relevant information about the study (as applicable)	Observations	Reference
Overall weight-of-evidence analysis considering data from folpet and its primary metabolite phthalimide and the related captan and its respective primary metabolite THPI and historical control data.			R-39172 Anonymous (2018)
Due to folpet's irritancy, toxicological studies with rabbits, which rely on caecotrophy for digestion, should be treated with caution when used in human and/or other mammalian risk assessments.			R-37533 Anonymous (2016)
Determination of minimum inhibitory concentrations against selected micro-organisms representative of rabbit gut microflora	*Bacteroides sp., Enterococcus faecalis* and *Candida albicans* Folpet: 2000, 1000, 500, 200, 100, 50, 20, 10 and 2 µg/ml	Folpet demonstrated antimicrobial activity MIC for folpet was 5, 50 and 200 µg/mL for *Candida albicans, Bacteroides sp.* and *Enterococcus faecalis*	R-18665 Anonymous 2005a
Minimum inhibitory concentrations against selected micro-organisms representative of the rabbit gut micro-flora	*Bacteroides sp., Enterococcus faecalis* and *Candida albicans* Phthalimide: 1000, 500, 200, 100, 50, 20, 10, 5, 2 or 1 µg/ml NB: equimolar concentrations to Folpet used in Anonymous, 2005a	Phthalimide demonstrated no antimicrobial activity MIC > 1000 µg/ml	R-18734 Anonymous 2005b
Mechanistic study oral (gavage) administration over 9 days	New Zealand White rabbit Folpet: 0, 10, 30 and 90 mg/kg/day	Clinical signs: faeces consistence) ↓body weight gain (at LOAEL) Bacterial flora was not significantly affected dose-dependent on faeces consistency, daily faeces weight, body weight and food consumption (may represent a beginning gastrointestinal stasis)	Allingham (2016) Study 18 (2016)

Type of study/data	Relevant information about the study (as applicable)	Observations	Reference
		LOAEL of 10 mg/kg bw/d	

9.10.11 Conclusion on classification and labelling for reproductive toxicity

Folpet is proposed to be not classified for reproductive toxicity.

9.11 Specific target organ toxicity-single exposure

STOT-SE should be considered where there is clear evidence of toxicity to a specific organ especially when it is observed in the absence of lethality. For this, observations from the acute studies can be used. There are further 24-hour feeding studies for folpet, which are acute scenarios.

Folpet shows irritative properties at all sites of first exposure in all investigated species, i.e. skin, eye, gastrointestinal and the respiratory tract. The only consistent systemic effects appear to be subsequent to prior local and acute irritation, e.g. mortality due to oedema in the respiratory tract or reduced food consumption in oral studies. Folpet exposure induces skin irritation in rat and mouse. The effects cumulate over time and are also transient, as the potency decreases with recovery periods. This clearly shows that the initiating toxicity is irritation.

The toxicophore for the effects is most probably the trichloromethylthio-side chain, which quickly reacts at the site of first exposure and is also mediating folpet's fungicidal efficacy. Folpet is not systemically available, see Section 9.

Hence, folpet is not specifically targeting a single organ but is an irritant compound affecting all epithelia at the site of exposure. This leads to similar toxic effects, however, due to its rapid reaction and degradation, the effects are of different potency depending on the route of exposure. While the respiratory system in test guideline studies is continuously exposed for 4 hours and degraded folpet is replenished with newly inhaled and deposited material, the various sections of the gastrointestinal tract only receive a bolus dose. Further, a transitional functional disturbance of respiratory epithelia is more relevant in short-term scenarios than in the gastrointestinal tract. Hence, folpet is acutely toxic via the inhalation route but not via the oral route. The underlying toxic effect is however the same.

Folpet does not qualify for a STOT-SE classification, either due to its acute classification proposals (eye, skin, inhalation) or because the effects are not potent enough after single exposure (gastrointestinal tract).

If not stated otherwise in the summary tables, the studies are considered as fully reliable.

Table 45: Summary table of animal studies on STOT SE (partly already summarised in other sections)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results**	**Reference**
Acute studies	The acute study package shows signs of irritation in eye, respiratory tract and skin. The effects on eye result in a classification proposal for serious eye damage. The effect in respiratory tract result in oedema with mortality. The effects in the acute skin irritation studies are not pronounced enough to warrant an acute classification. However, irritation is pronounced upon intradermal injection, as observed in the skin sensitisation studies and in a repeated dose dermal toxicity study in rats Skin irritation is thus clearly a hazard associated with folpet exposure and a classification for acute skin irritation is accordingly proposed. No abnormalities were observed in the gastrointestinal tract of the acute oral toxicity studies.		Section 8.1-8.7
Intestinal irritation study after 24-hour exposure with sacrifices after 1, 3 and 7 days	Study 1: 200 and 5000 ppm over a 24-hour period, dietary (900 mg/kg bw, oral gavage) 3 mice/group Study 2: 0, 50, 200 and 5000 ppm over a 24-hour period, dietary (900 mg/kg bw, oral gavage), 15 mice/group Female mice, ICR (CD-1 equivalent)	Folpet at 5000 ppm (dietary) causes no or minimal irritation in the duodenum Folpet at 900 mg/kg bw (gavage) causes minimal irritation in the stomach	Section 8.9/Study 11 (1997)
24-hour, feeding with 13 days recovery	0, 313, 1250, 5000 ppm Wistar rat	No adverse effects, NOAEL 5000 ppm/386.9 mg/kg bw/day Reduced food consumption in the highest treatment level	(000081258) Study 1 (2015)

9.11.1 Short summary and overall relevance of the provided information on specific target organ toxicity – single exposure

Most of the studies that provide information on a potential STOT-SE classification are part of the acute study package, which are summarized in Section 8.1-8.7. The studies clearly show signs of irritation after dermal and inhalation exposure and instillation in the eye. There are only limited signs of irritation after single oral exposure, which may be due to the short contact of folpet to the gastrointestinal tract epithelia after bolus gavage application.

The 24-hour feeding study in mouse (Section 8.9/Study 11 (1997)) showed in a pre-study using 3 mice/group, at 900 mg/kg/bw by gavage (actual 1430 mg/kg bw) or 5000 ppm (845 mg/kg bw) in the diet, 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.

A second trial was conducted with 15 animals/group but histopathology was only conducted on 5 animals; 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 (actual 815 mg/kg bw) were judged "minimal." There were no effects after dietary exposure (5000 ppm/1060 mg/kg bw).

The 24-hour feeding study in rats shows no irritation/histopathological findings in the gastrointestinal tract.

Table 46: Macroscopic and microscopic findings in the stomach and duodenum of mice treated with folpet (trial 2 of 2) in Section 8.9/Study 11 (1997)

Finding*	**Dose level**					
	0	**50 ppm (diet)** **10 mg/kg bw**	**200 ppm (diet)** **44 mg/kg bw**	**500 ppm (diet)** **123 mg/kg bw**	**5000 ppm (diet)** **1060 mg/kg/day**	**900 mg/kg (gavage)** **815 mg/kg bw**
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

9.11.2 Comparison with the CLP criteria

Substances can be allocated into one of three hazard categories for STOT-SE.

CLP criteria acc to Regulation 1272/2008 **and CLP guidance[4]**	**Assessment**
Category 1: Substances that have produced significant toxicity in humans or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following single exposure Substances are classified in Category 1 for specific target organ toxicity (single exposure) on the basis of:	There is no evidence for a primary target organ in the data package, all epithelia, i.e. sites of first exposure are affected. Effects on the respiratory tract are more pronounced (mortality after acute inhalation exposure) than via other

[4] ECHA (2017) Guidance on the Application of the CLP Criteria, vol Version 5.0 – July 2017, Reference: ECHA-17-G-21-EN Cat.Number: ED-02-17-754-EN-N edn. European Chemicals Agency

a. reliable and good quality evidence from human cases or epidemiological studies; or *b. observations from appropriate studies in experimental animals in which significant and/or severe toxic effects of relevance to human health were produced at generally low exposure concentrations. Guidance dose/concentration values are provided below (see 3.8.2.1.9) to be used as part of weight-of-evidence evaluation.* *Category 2: Substances that, on the basis of evidence from studies in experimental animals can be presumed to have the potential to be harmful to human health following single exposure Substances are classified in Category 2 for specific target organ toxicity (single exposure) on the basis of observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were produced at generally moderate exposure concentrations. Guidance dose/concentration values are provided in order to help in classification. In exceptional cases, human evidence can also be used to place a substance in Category 2.* The regulation further notes: *Attempts shall be made to determine the primary target organ of toxicity and to classify for that purpose, such as hepatotoxicants, neurotoxicants. The data shall be carefully evaluated and, where possible, secondary effects should not be included (e.g. a hepatotoxicant can produce secondary effects in the nervous or gastro-intestinal systems).*	routes, however, the underlying toxicity is the same, i.e. irritation at the first site of contact.
There is also Category 3 for transient target organ effects: *This category only includes narcotic effects and respiratory tract irritation. These are target organ effects for which a substance does not meet the criteria to be classified in Categories 1 or 2 indicated above. These are effects which adversely alter human function for a short duration after exposure and from which humans may recover in a reasonable period without leaving significant alteration of structure or function.* *The criteria for classifying substances as Category 3 for respiratory tract irritation are:* *(a) respiratory irritant effects (characterised by localised redness, oedema, pruritis and/or pain) that impair function with symptoms such as cough, pain, choking, and breathing difficulties are included. This evaluation will be based primarily on human data;* *(b) subjective human observations could be supported by objective measurements of clear respiratory tract irritation (RTI) (such as electrophysiological responses, biomarkers of inflammation in nasal or bronchoalveolar lavage fluids);* *(c) the symptoms observed in humans shall also be typical of those that would be produced in the exposed population rather than being an isolated idiosyncratic reaction or response triggered only in individuals with hypersensitive*	Folpet is acutely toxic via direct interaction at the first site of contact and is proposed to be classified for the respective and more relevant acute hazard classes. While the effects for folpet clearly indicate respiratory irritant effects, the effects are not specific for the respiratory tract. Classification for the acute hazard classes characterizes and communicates the hazard appropriately. • Folpet is proposed to be classified for acute inhalation toxicity category 2 due to mortality by oedema, which is induced by irritation. Hence the respiratory irritation is the effect resulting in mortality and is not distinct from the acute toxicity. • Folpet is proposed to be classified for serious eye damage category 1 • Folpet is proposed to be classified for skin irritation category 2

airways. Ambiguous reports simply of 'irritation' shall be excluded as this term is commonly used to describe a wide range of sensations including those such as smell, unpleasant taste, a tickling sensation, and dryness, which are outside the scope of classification for respiratory irritation; *(d) there are currently no validated animal tests that deal specifically with RTI, however, useful information may be obtained from the single and repeated inhalation toxicity tests. For example, animal studies may provide useful information in terms of clinical signs of toxicity (dyspnoea, rhinitis etc) and histopathology (e.g. hyperedmia, edema, minimal inflammation, thickened mucous layer) which are reversible and may be reflective of the characteristic clinical symptoms described above. Such anima studies can be used as part of weight of evidence evaluation;* *(e) this special classification would occur only when more severe organ effects including in the respiratory system are not observed.* According to the CLP guidance, *there are two hazard classes for single exposure toxicity: 'Acute toxicity' and 'STOT-SE'. These are independent of each other and both may be assigned to a substance or a mixture if the respective criteria are met. Acute toxicity refers to lethality and STOT-SE to non lethal effects. However, care should be taken not to assign both classes for the same toxic effect, essentially giving a 'double classification', even where the criteria for both classes are fulfilled. In such a case the most appropriate class should be assigned.*	
Acute toxicity classification is generally assigned on the basis of evident lethality (e.g. an LD50/LC50 value) or where the potential to cause lethality can be concluded from evident toxicity (e.g. from fixed dose procedure). STOT-SE should be considered where there is clear evidence of toxicity to a specific organ especially when it is observed in the absence of lethality.	Folpet's hazard properties are independent of a specific target organ Folpet is irritating to all sites of first exposure, i.e. eye, skin, the gastrointestinal and respiratory tract
Furthermore, specific toxic effects covered by other hazard classes are not included in STOT-SE. STOT-SE should only be assigned where the observed toxicity is not covered more appropriately by another hazard class. For example, specific effects caused after a single exposure like corrosion of skin or effects on the reproductive organs should be used for classification for skin corrosion or reproductive toxicity, respectively, but not for STOT-SE.	Acute toxicity classifications describe folpet's hazard properties appropriately. • All irritation occurs due to the same mode of action. • Irritation effects in the respiratory tract are sufficiently characterized by the Acute inhalation toxicity category 2 classification proposal. While acute respiratory irritation (ARI) is likely, based on the available data, which would potentially justify a STOT-SE Category 3 classification, the Acute inhalation toxicity category 2, is based on the same underlying irritation effect which results in oedema and subsequent mortality. The classification proposal of category 2 is more

	protective than a STOT-SE 3 classification and requires the application of personal protective equipment, which in practice protects against respiratory irritation. Further, the respiratory tract is no specific target organ. • Irritation effects in the eye are sufficiently characterized by the serious eye damage category 1 classification proposal. Further, the eye is no specific target organ. • Irritation effects in the skin do not occur upon single exposure in the acute irriation studies in rabbits but are seen in multiple independent studies. Accordingly, a classification for skin irritation is proposed, which sufficiently characterizes the skin effects. • Irritation effects in the gastrointestinal tract occur only after repeated exposure or after a gavage bolus of 815 mg/kg bw in the stomach of mice, which were assessed to be minimal and do not occur not after dietary exposure towards a higher total dose of 1060 mg/kg bw. Irritation does not occur at the highest tested dose in a 24-hour feeding study in rat, 386.9 mg/kg bw. Further, the gastrointestinal tract is no specific target organ.

Together, a classification for STOT-SE is not considered to be appropriate. Folpet's hazard profile is sufficiently described and communicated by the proposed acute classifications.

9.11.3 Conclusion on classification and labelling for STOT SE

Folpet is proposed to be not classified for STOT-SE.

9.12 Specific target organ toxicity-repeated exposure

The purpose of STOT-RE is to identify the primary target organ(s) of toxicity. As stated under 8.11 STOT-SE, folpet generally shows irritative properties at all sites of first exposure and is not targeting specific organs.

For the oral route, all repeated exposure studies show gastrointestinal tract irritation which results in small intestinal tumours in mice and hyperkeratosis of the non-glandular stomach and of the oesophagus in rats. Clinical signs associated with gastrointestinal effects are observed in dog.

For the inhalation route, a 28-day study shows histopathological changes in the larynx.

The repeated exposure studies show the same effects as the single exposure studies, which is expected as they occur due to the same underlying toxicity: irritation, i.e. direct interaction with the sites of first exposure. Due to the rapid reaction and degradation of folpet, the effects have to be considered as repeated local acute irritation events. Similarly, as for the acute toxicity classifications, there is an apparent potency difference between the exposure routes, which probably occurs due to the experimental method. Effects in the respiratory tract occur at lower concentrations than for the other exposure routes. One explanation for this might be the continuous exposure of the respiratory system over repeated 6 hour treatment periods in combination with the availability of newly inhaled and deposited un-degraded material. Due to the specific toxicity, the toxic effects cumulate and both acute and repeated-exposure inhalation studies can be directly compared by using Haber's rule or more refined dosimetry. However, no further data (e.g. repeated dose inhalation ADME, histopathological evaluations in acute inhalation studies) are available proving this hypothesis and for example

skin irritation seems to be mediated by the vehicle used, as studies according to OECD TG 404 did not exhibit skin irritation potential.

If not stated otherwise in the summary tables, the studies are considered as fully reliable.

Table 47: Summary table of animal studies on STOT RE: oral route

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results**	**Reference**
21-28 day studies			
21-day feeding study in rat No guideline stated. Similar to Directive 92/69/EEC B. Deviations from OECD 407 (2008): - 21 days (instead of 28) - Animals were clinically observed for 5 days per week - no haematology and clinical chemistry parameters were measured - no organs were weighed - no histopathology was performed Supporting information (reliable with restrictions)	Folpet Purity 93.5% 1000, 3000, 5000, 12000 ppm 21 d 5/sex/group SD rats	NOAEL = 5000 ppm (585 mg/kg bw/d) <u>12000 ppm (585 mg/kg bw/d)</u> Bw ↓ (9-16%) Food consumption ↓ (partly due to palatability) (up to 29%) <u>5000 ppm (351 mg/kg bw/d)</u> Food consumption ↓ due to palatability (up to 17%)	(R-6116) Study 1 (1979)
28-d feeding study in mice Non guideline stated. Deviations to OECD 407 (2008) - 30 days (instead of 28) - No detailed clinical observations - no haematology and clinical chemistry parameters were measured - no organs were weighed - no histopathology (except on gross lesions) was performed - no individual data were provided Supporting information (reliable with restrictions)	Phaltan technical Purity 91% 2000, 5000, 10000, 16000, 20000 ppm 28-d 12/sex/group B6C3F1 mouse	NOAEL = 2000 ppm/280 mg/kg bw/day (m), 5000 ppm/ 700 mg/kg bw/day (f) <u>20000 ppm (2780 mg/kg bw/d)</u> bw ↓ (22-27%) Body weight loss <u>16000 ppm (2350 mg/kg bw/d)</u> bw ↓ (20-22%) bw gain ↓ (99% males, 94% females) <u>10000 ppm (1350 mg/kg bw/d)</u> bw ↓ (up to 10%) bw gain ↓ (50% males, 38% females) <u>5000 ppm (690 mg/kg bw/d) (males)</u> bw ↓ (up to 10%) bw gain ↓ (48%)	(R-6119) Study 2 (1978)

Four-week feeding mice No guideline stated, similar to Directive 92/69/EEC B.7. Deviations from OECD 407 (2008): - no haematology and clinical chemistry parameters were measured - adrenals, testes epididymides, prostate + seminal vesicles with coagulating glands as a whole, thymus, and brain were not weighed - no histopathology was performed Supporting information (reliable with restrictions)	Folpet Purity 88.6% 0, 1000, 5000, 10000 ppm 8/sex/group B6C3F1	NOAEL = 1000 ppm/180 mg/kg bw/day <u>10000 ppm (1768 mg/kg bw/d)</u> bw ↓ (approx. 20% males, approx. 10% females) <u>5000 ppm (873.5 mg/kg bw/d)</u> bw ↓ (approx. 10%)	(R-1777) Study 3 (1981)
Four week feeding dogs pilot study No guideline stated. Similar to Directive 92/69/EEC B.7 Deviations from OECD 409 (1998): - only 2 animals/ sex - a measure of clotting potential such as clotting time, prothrombin time, or thromboplastin time were not evaluated - volume was not measured for urinalysis - epididymides, uterus and thymus were not weighed - spinal cord (lumbar) and oesophagus were not processed for histopathology Supporting information (reliable with restrictions)	Folpet Purity 89.5% 20, 60, 180, 540 mg/kg/day Capsule 2/sex/group Beagle dog	Due to small number of animals no NOAEL can be set <u>540 mg/kg bw/d</u> bw loss (1.55-2 kg) total protein (28% males, 25% females) and related albumin (35% males, 42% females) and A/G ratios ↓ cholesterol ↓ (22% males, 29% females) calcium (15% males, 20% females)↓ chloride ↑ (9% males) GGTP levels ↑ (males) alkaline phosphatase ↓ (45% males) Blood urea nitrogen levels were ↓ (49% males) <u>180 mg/kg bw/d</u> bw loss (1-1.1 kg) Blood urea nitrogen levels ↓ (45% males) <u>40 mg/kg bw/d</u> bw loss (0.05-0.2 kg) <u>20 mg/kg bw/d</u> bw gain ↓ (67-90%)	(R-6135) Study 4 (1983)
13-52 week studies			
13-week dietary study rat No guideline stated, similar to Directive 87/302/EEC Part B Deviations from OECD 408 (1998): - no information regarding acclimatisation period provided	Folpet Purity ≥ 88.6% 0, 2000, 4000, 8000 ppm	LOAEL = 2000 ppm / 136 mg/kg bw/day <u>8000 ppm (533 mg/kg bw/d</u> bw gain ↓ (21% males, 14% females) food consumption ↓ (males: 14%, females: 10%)	(R-1800) Study 5 (1982)

- no ophthalmoscopy was performed - haemotological parameters was measured only in control and high dose animals - a measure of blood clotting time/potential was not evaluated - total cholesterol, blood urea nitrogen and albumin, were not measured - epididymides, uterus, thymus and brain were not weighed - spinal cord, trachea, aorta, caecum, ileum, jejunum, sciatic nerve, prostate, a section of bone marrow (and/or a fresh bone marrow aspirate) and skin were not processed histopathologically	20/sex/group F344 rats	AST ↓ (15% males, 13% females) ALT ↓ (64% males, 65% females) AP ↓ (40% males, 42% females) Males: Slight number of focal atrophic basophilic tubules in the kidney up to 5 foci (11/20, stat. sign) up to 10 foci (8/20, stat. sign.); hyperkeratosis in the oesophagus (moderate 18/20, stat. sign.); hyperkeratosis (moderate 20/20, stat. sign.), elongation of rete pegs (10/20, stat. sign.) and acanthosis (slight 3/20, not sign. to moderate 17/20, stat. sign) in the stomach Females: hyperkeratosis in the oesophagus (slight 19/20 stat. sign.); hyperkeratosis (slight 1/20, moderate 18/20, stat. sign., marked 1/20), elongation of rete pegs (19/20, stat. sign.) and acanthosis (moderate 18/20, stat. sign.) in the stomach <u>4000 ppm (270 mg/kg bw/d</u> bw gain ↓ (10% males) food consumption ↓ (males: 7%) AST ↓ (17% males) ALT ↓ (19% males, 15% females) AP ↓ (30% males, 34% females) Males: Slight number of focal atrophic basophilic tubules in the kidney up to 5 foci (14/20, stat. sign) up to 10 foci (1/20); hyperkeratosis in the oesophagus (slight 20/20, stat. sign.); hyperkeratosis (slight 1/20, moderate 19/20, stat. sign.), elongation of rete pegs (6/20, stat. sign.) and acanthosis (slight 11/20 to moderate 8/20, stat. sign) in the stomach Females: hyperkeratosis in the oesophagus (slight 16/20 stat. sign.); hyperkeratosis (moderate 20/20, stat. sign.), elongation of rete pegs (18/20, stat. sign.) and acanthosis (moderate 8/20, stat. sign.) in the stomach <u>2000 ppm (136 mg/kg bw/d)</u> AP ↓ (30% males, 34% females) Males: hyperkeratosis in the oesophagus (slight 2/20, stat. sign.); hyperkeratosis (slight 6/20 to moderate 14/20, stat. sign.), elongation of rete pegs (4/20) and acanthosis (slight 15/20, stat. sign., to moderate 5/20) in the stomach Females: hyperkeratosis in the	

<table>
<tr><td></td><td></td><td>oesophagus (slight 8/20 stat. sign.); hyperkeratosis (moderate 20/20, stat. sign.), elongation of rete pegs (11/20, stat. sign.) and acanthosis (slight 10/20 to moderate 10/20, stat. sign.) in the stomach</td><td></td></tr>
<tr><td>13-week dietary rat
No guideline stated. Similar to Directive 87/302/EEC Part B
Deviations from OECD 408 (1998):
- temperature range of animal housing from 17.8-26.7°C (instead of 22±3°C)
- no information regarding light/dark cycle provided
- the initial weight range exceeded ±20% of mean for males (lower limit was —28% of initial overall mean)
- no ophthalmological examination was performed
- blood clotting time/potential was not evaluated
- urea was not measured in clinical chemistry parameters
- adrenals, uterus, spleen and thymus were not weighed
- histopathology was only performed for control and high dose animals (except for liver, kidney and heart where all dose groups were examined)
- cervical spinal cord, parathyroid, aorta, female mammary gland, peripheral nerve and skin were not processed for histopathological analysis</td><td>Folpet
Purity 92.8%
0, 300, 1000, 3000, 10000 ppm
20/sex/group
90 d
CD rats</td><td>NOAEL = 1000 ppm / 56 mg/kg bw/day
<u>10000 ppm (614 mg/kg bw/d)</u>
bw ↓ (13% males, week 13), stat. sign.
bw gain ↓ (22% males, 24% females), stat. sign.
Protein, albumin, globulin ↓, stat. sign.
Stomach (non-glandular):<table><tr><td></td><td>male</td><td>fema</td></tr><tr><td>Pleocellular</td><td>9/10</td><td>10/1</td></tr><tr><td>Submucosal</td><td>10/10</td><td>10/1</td></tr><tr><td>Acanthosis</td><td>10/10</td><td>9/10</td></tr><tr><td>Hyperkeratosis</td><td>10/10</td><td>3/10</td></tr><tr><td>Focal erosion</td><td>2/10</td><td>5/10</td></tr><tr><td>Focal ulceration</td><td>1/10</td><td>2/10</td></tr></table>LDH ↓ week 6: (54% males), LDH week 13↑ (114% females), stat. sign.
Brain weight ↓ (males 7%), stat. sign.
<u>3000 ppm (169 mg/kg bw/d)</u>
Protein (week 6: 6%), albumin (week 13: 11%), globulin (week 6: 13%) ↓ (females), stat. sign.
LDH ↓ week 6: (62% males), LDH week 13↑ (118% females), stat. sign.
Brain weight ↓ (males 7%), stat. sign.</td><td>(R-6118)
Study 6 (1981)</td></tr>
<tr><td>90-day capsules dog
No guideline stated.
Deviations from OECD 409 (11988)
- no urinalysis was performed
- epididymides, uterus and thymus were not weighed</td><td>Folpet
Purity 89.8-91.1%
0, 790, 1800, 4000 mg/kg bw/d
4/sex/group
90 d
Beagle dogs</td><td>LOAEL = 790 mg/kg bw/day
<u>4000 mg/kg bw/d</u>
Mortality (4/4 males, 1/4 females)
Clinical signs (vomiting and diarrhoea)
bw ↓ (please refer to table 3.12.2.15 in Appendix human health), occasionally stat. sign.
food consumption ↓↓ (please refer to table 3.12.2.16 in Appendix human health), occasionally stat. sign.
Heart weight rel. to bw (-17% females), stat. sign.
Liver weight rel. to bw ↑ (46% females), not stat. sign.
<table><tr><td>Thymus</td></tr></table></td><td>(R-3654)
Study 7 (1985)</td></tr>
</table>

	males	females
Slight atrophy	0	1
Moderate/marked atrophy	4	1
with depletion of lymphocytes	4	2
Fibrosis	4	1
Bone marrow		
Slight depletion	0	3
Moderate depletion	1	1
Marked depletion	3	0
Replacement by watery fat	3	4
Replacement by connective tissue	1	0

Testis

Slight testicular degeneration	0
Moderate testicular degeneration	0
Marked testicular degeneration	4
Cryptochid	0
Partial aspermia	0
No spermatogenesis	1

<u>1800 mg/kg bw/d</u>

Clinical signs (vomiting and diarrhoea)

bw ↓ (please refer to table 3.12.2.15 in Appendix human health), occasionally stat. sign.

food consumption ↓ (please refer to table 3.12.2.16 in Appendix human health), occasionally stat. sign.

Bilirubin ↓ (40%, males, 27% females, week 12), stat. sign.

Calcium ↓ (6%, week 12, males), stat. sign.

β globulin ↓ (25% week 12, males), stat. sign.

Brain weight absolute ↓ (13% males), not stat. sign.

Testes weight absolute ↓ (36% males), not stat. sign.

Liver weight rel. to bw ↑ (32% males, 17% females), not stat. sign.

Testis

Slight testicular degeneration	2
Moderate testicular degeneration	2
Marked testicular degeneration	0
Cryptochid	1
Partial aspermia	1
No spermatogenesis	1

<u>790 mg/kg bw/d</u>

bw ↓ (please refer to table 3.12.2.15 in

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<td></td>
<td></td>
<td>Appendix human health), not stat. sign

food consumption ↓ (please refer to table 3.12.2.16 in Appendix human health), occasionally stat. sign.

Bilirubin ↓ (50%, week 6; 44%, week 12, males), stat. sign.

Calcium ↓ (5%, week 12, males), stat. sign.

β globulin ↓ (14% week 12, males), stat. sign.

Brain weight absolute ↓ (9% males), not stat. sign.

Testes weight absolute ↓ (32% males), not stat. sign.

Liver weight rel. to bw ↑ (14% males, 22% females), not stat. sign.

Testis
<table>
<tr><td>Slight testicular degeneration</td><td>1</td></tr>
<tr><td>Moderate testicular degeneration</td><td>1</td></tr>
<tr><td>Marked testicular degeneration</td><td>0</td></tr>
<tr><td>Cryptochid</td><td>1</td></tr>
<tr><td>Partial aspermia</td><td>0</td></tr>
<tr><td>No spermatogenesis</td><td>0</td></tr>
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<tr>
<td>1-year capsules dog.

No guideline stated. Similar to Directive 87/302/EEC Part B

Deviations from OECD 452 (2009):

- dosing only 6 days/ week (instead of 7)

- activated partial thromboplastin time was not measured

- epididymides, uterus and spleen were not weighed

- lacrimal gland, seminal vesicles and vagina were not examined histopathologically</td>
<td>Folpet

Purity 82.7-91.3%

325, 650, 1300 mg/kg bw/d

5/sex/group

Beagle dogs</td>
<td>LOAEL = 325 mg/kg bw/day

relative adrenal weight↑ (males)
relative liver weight ↑

<u>1300 mg/kg bw/d</u>

Clinical signs (vomiting, diarrhoea and salivation)- please refer to table 3.12.2-21 in Appendix human health

Bw loss (males), bw gain ↓, please refer to table 3.12.2-23 in Appendix human health

bw ↓ (males: stat. sign. from Week 8 onwards, females: not stat. sign.), please refer to table 3.12.2-22 in Appendix human health

food consumption ↓, occasionally stat. sign., please refer to table 3.12.2-24 in Appendix human health

changes in clinical chemistry (e.g. protein, ALB, urea, cholesterol ↓, Na ↑ females)

testes weight ↓ (26%), stat. sign.

thyroid weight rel. to bw ↑ (39% females), stat. sign.

<u>650 mg/kg bw/d</u>

Clinical signs (vomiting, diarrhoea</td>
<td>(R-4663)

Study 8 (1988)</td>
</tr>
</table>

		and salivation)- please refer to table 3.12.2-21 in Appendix human health bw gain ↓, please refer to table 3.12.2-23 in Appendix human health bw ↓ (males: not stat. sign., females: stat. sign. from Week 18 onwards), please refer to table 3.12.2-22 in Appendix human health changes in clinical chemistry (e.g. protein, ALB, urea, cholesterol ↓, Na ↑ females) please refer to table 3.12.2-26 in Appendix human health thyroid weight rel. to bw ↑ (44% males), stat. sign. <u>325 mg/kg bw/d</u> Clinical signs (vomiting, diarrhoea and salivation)- please refer to table 3.12.2-21 in Appendix human health bw gain ↓, please refer to table 3.12.2-23 in Appendix human health Pt time ↓ (-9% females), stat. sign. with unclear dose-response relationship packed cell volume, haemoglobin concentration and erythrocyte counts ↓ (females) after week 12, stat. sign. with unclear dose-response relationship changes in clinical chemistry (e.g. protein, ALB, urea ↓, Na ↑ females) please refer to table 3.12.2-26 in Appendix human health	
1-year capsules dog No guideline stated. Similar to Directive 87/302/EEC Part B. Deviations from OECD 452 (2009) - mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean and corpuscular haemoglobin concentration (MCHC) were not calculated - volume was not measured for urinalysis - epididymides and uterus were not weighed - superficial lymph nodes, rectum, lacrimal gland, spinal cord (lumbar), seminal vesicles and vagina were not examined histopathologically	Folpet Purity 89.5% 10, 60, 120 mg/kg bw/d 6/sex/group Beagle dogs	NOAEL = 10 mg/kg bw/day <u>120 mg/kg bw/d</u> bw gain ↓ (62% males, 42% females), not stat. sign. bw ↓ (males), not stat. sign., please refer to table 3.12.2.28 in Appendix human health food consumption (early weeks)↓, stat. sign., please refer to table 3.12.2.30 in Appendix human health cholesterol ↓ in females occasionally stat. significant, please refer to table 3.12.2.31 in Appendix human health protein and albumin/globulin ↓, occasionally stat. significant, please refer to tables 3.12.2.32-34 in Appendix human health brain weight absolute ↓ (9% males),	(R-6035) Study 9 (1986)

		stat. sign. left adrenal weight rel. to bw (32%) and absolute (50%) ↓ (males), stat. sign. <u>60 mg/kg bw/d</u> bw gain ↓ (40% males, 31% females), not stat. sign. bw ↓ (males), not stat. sign., please refer to table 3.12.2.28 in Appendix human health food consumption (early weeks)↓, stat. sign., please refer to table 3.12.2.30 in Appendix human health cholesterol ↓ (males), not stat. significant with questionable dose response, please refer to table 3.12.2.31 in Appendix human health protein and albumin/globulin ↓, occasionally stat. significant, please refer to tables 3.12.2.32-34 in Appendix human health	
13- weeks neurotoxicity study in rat The study generally met requirements of OECD 424 (1997), although the study pre-dates the Guideline Deviations from OECD 424 (1997) - ophthalmological examination not conducted - Haematology and clinical biochemistry not examined - Histopathology: only whole brain (transverse section), spinal cord and sciatic nerves (transverse and longitudinal sections each) examined - Functional observations: limited to auditory or visual stimulation, mechanical measurement of motor activity.	Folpet Purity 88.6% 0, 2500, 5000 and 10000 ppm 10/sex/group CD rats	NOAEL : 2500 ppm (males : 181 mg/kg bw/d) <u>10000 ppm (701 mg/kg bw/d)</u> bw gain ↓ (22% males, 27% females), stat. sign bw ↓ (18% males, 8% females), stat. sign. <u>≥ 5000 ppm (363 mg/kg bw/d)</u> bw gain ↓ (males, 19%), stat. sign bw ↓ (males, 13-14`%) stat. sign. No neurotoxic effects	R-1791 Study 12
Other studies			
Reproduction toxicity	Please refer to table 33 in section 8.10.1 as well as table 35 in section 8.10.4.		
Carcinogenicity mouse	Please refer to table 28 in section 8.9		
Carcinogenicity rat			

Table 48: Summary table of animal studies on STOT RE: dermal route

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results**	**Reference**
4-week rat No guideline stated. Similar to Directive 92/69/EEC B.9. Deviations from OECD 410 (1981): Groups of six animals of each sex were used (instead of 10)	Folpet Purity 89.2% 0, 1,10, 30 mg/kg bw/day 4-weeks 6/sex/group SD rats	bw gain ↓ (males), probably due to a significant decrease in food efficiency during week 2 and 3 (from 10 mg/kg bw/d onwards) changes in haematological and clinical chemistry parameters, probably due to skin reactions at the top dose level (females) Local effects: erythema, oedema, scabs, sloughing (from 10 mg/kg bw/d onwards) and lacerations (highest dose) NOAEL: 1 mg/kg bw/d (males), 10 mg/kg bw/d (females) For local effects LOAEL: 1 mg/kg bw/d	(R-5452) Study 10 (1988)- see also Study 3 Section 8.4

Table 49: Summary table of animal studies on STOT RE: inhalation route

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results**	**Reference**
US EPA 870.3465, OECD 412 (1981) Deviations from OECD 412 (2009): - Humidity during dosing was slightly too low (12-60% instead of 30-70% - Bodyweight was recorded weekly (instead of twice weekly) - No clinical pathology was performed (i.e. haematology and clinical chemistry) - Lymph nodes, oesophagus, ovaries, stomach, thyroid and uterus were not examined histo-pathologically	Folpet Purity 96.8% 0, 5, 25, 100 µg/L 6 h/day, 5 days/week for four weeks 5/sex/group SD rats	bw gain ↓ probably due to reduced food consumption: 100 µg/L metaplasia, keratinization, hyperplasia, fibrosis of the mucosa in the larynx: All dose levels degeneration/atrophy of olfactory epithelium, metaplasia of the respiratory epithelium in the nasal turbinates: 1 male at 25 µg/L, high incidences at 100 µg/L Thinning/ atrophy of the respiratory epithelium in the nasal turbinates, inflammatory cells/debris in the lumen, acute/subacute inflammation of mucosa at 100 µg/L. At that concentration there was also significantly increased lung weights that correlated with slight subacute to chronic peribronchiolar inflammation. Squamous/squamoid metaplasia epithelium of the trachea 100 µg/L Mixed inflammatory cells were present within the mucosa in all groups, including animals from the control group, but were increased in severity (minimal to slight in controls versus slight to moderate) in both sexes in folpet technical exposed animals. NOAEC = 25 µg/L Local effects LOAEC: 5 µg/L	(R-22661) Study 11 (2008) Weber (2012)
Publication	Captan and folpet do not have specific toxicity in the respiratory tract but are irritating to all bio-membranes, as observed in their toxicological data package and which is also reflected by their fungicidal activity. Hence, it seems that the effects in the repeated exposure inhalation toxicity studies are neither specifically targeting the respiratory tract nor toxicity with a different etiology than that of acute toxicity. Thus, a classification for STOT-RE does not appropriately reflect captan's and folpet's hazard profile, which is driven by local acute irritation and cumulative exposure time.		Kluxen and Koenig (unpublished but accepted manuscript submitted to Regulatory Toxicology and Pharmacology)

9.12.1 Short summary and overall relevance of the provided information on specific target organ toxicity – repeated exposure

Folpet's toxicological profile in the short-term studies are in line with what is observed in the acute, and repeated exposure reproductive toxicity and chronic studies.

No severe systemic effects were observed in a repeated dermal study (Study 10, 1988).

No neurotoxic effects could be observed (Study 12).

All repeated oral exposure studies show an early decrease in food consumption with a subsequent effect on body weight. For dietary studies this may be associated with palatability because folpet has a distinct chemical smell; animals in some studies and at higher treatment levels completely cease feeding for some time but often feeding returns to normal or supersedes normal intake with longer study duration. However, a reduction in food consumption is also observed upon gavage application and in dogs that are treated with capsules. Here a reduction in food consumption seems to be associated with folpet's irritative properties, which affect the gastrointestinal tract. In dogs for example, emesis and diarrhoea increased substantially with increasing treatment levels.

In dogs the only consistent severe systemic effects are found for high treatment levels in Study 7 (1985) and Study 8 (1988): Testes weight decrease at about 790 and 1300 mg/kg bw/day. In Study 7 (1985) this decrease was accompanied by histopathological findings (i.e. testicular degeneration). While a corresponding 1-year dog study for captan (Anonymous, 1988/ R-5284) showed a tendency of decreased testes in the highest dose (300 mg/kg bw/day), the effect is neither pronounced nor statistically significant. However, these effects occurred only at dose levels far above the guidance values for oral studies, triggering STOT-RE classification (i.e. 10 mg/kg bw/d for STOT-RE 1 and 100 mg/kg bw/d for STOT-RE 2, based on effects observed in a 90-day rat repeated-dose study). Effects below this guidance values are not considered relevant for supporting classification for specific target organ toxicity following repeated exposure (i.e. clinical observations, changes in body weight and food consumption, changes in clinical chemistry and haematological parameters).

In rat studies, histopathological findings in the oesophagus and non-glandular stomach were observed which may be associated with the local acute irritation properties of the compound. In one 90 day study these effects started at a dose of 136 mg/kg bw/d (Study 5, 1982), while in the other (Study 6, 1981) no histopathological effects were observed at 169 mg/kg bw/d. Also in the generational study (Study 1, 1986) slight hyperkeratosis of the non-glandular stomach and oesophagus was observed at a dose level of 112 mg/kg bw/d. These effects were observed above the guidance values of 100 mg/kg bw/d for oral studies, triggering STOT-RE 2 classification. No adverse effects were observed at a dose level of 56 mg/kg bw/d in the 90 d rat studies (Study 6, 1981) and 19 or 91 mg/kg bw/d in the generational study (Study 1, 1986 and Study 2, 1985, respectively).

According to Haber's rule, the adjusted standard guidance value for studies of longer duration would be 12.5 mg/kg bw/d triggering STOT-RE 2 classification. In long term rat studies, the only effect at this dose level was that urine of males was more concentrated and of a lower volume than the controls was observed at the 3 month examination (Study 6, 1989). This effects was not repeated at other examination time points and is not considered supporting STOT-RE classification in terms of severity of effects. In long term mouse studies no adverse effects were observed at a dose level 47 mg/kg bw/d.

Body weight and food consumption are also decreased in the dermal and the inhalation study, probably due to the general distress of severe irritation at the sites of first exposure.

For the inhalation route, a 28-day study shows squamous/squamoid metaplasia, epithelial hyperplasia, mucosal fibrosis and inflammation in the larynx. Changes were generally more pronounced in the anterior larynx than the posterior larynx but were present at all exposure levels down to a concentration of 5 µg/L. Further, squamous metaplasia in the nasal turbinates, along with inflammation, degeneration, atrophy, and ulceration of the epithelium in the nasal cavity occured. Metaplasia was also observed in the trachea at the same dose level as peribronchiolar subacute/chronic inflammation in the lung was observed. Captan, which has the same toxicophore, results in similar observations in the available 90-day inhalation study, however, also in mortality (5/10 males exposed to 12.98 µg/L captan). Overall, the observations are in-line with the exposure towards an

irritant particle. The respiratory rate is affected as expected for respiratory irritation[5][6] and the histopathology shows inflammation, e.g. an influx of inflammatory cells and increased lung weights. In addition, rhinitis, laryngitis, bronchitis and alveolitis have all been diagnosed in the rat inhalation study packages for both folpet and captan.

Fibrosis, especially in the larynx, is a typical finding subsequent to irritation and inflammation in the respiratory system of rats[7][8]. Subsequent proliferation and keratinization are also typical signs of inflammation and critical for the healing process[9]. The test item induced irritative effects are accordingly also repaired. Following a 4-week recovery period in the 13 week inhalation exposure captan study the lung and nasal passage effects had resolved, but the laryngeal effects (squamous metaplasia and squamous hyperplasia) were still present in the high dose group (low and mid dose groups were not examined). The lack of full recovery in all animals might be attributed to the short recovery period following exposure, as described in the study report, and not due to an inability to recover. Nevertheless, also permanent adverse effects occurred in the respective study in form of mortalities at a dose level of 12.98 µg/L. These mortalities occurred not earlier than week 5, while the duration for the repeated inhalation toxicity study with folpet was 28 days. Partly recovery was also observed in the 4-week dermal toxicity studies for folpet, which demonstrates that the irritation depends on acute irritation insults occurring repeatedly.

It is generally accepted that the rat is very sensitive with respect to inhalation toxicity[10][11]. Particularly, squamous metaplasia is considered to have no toxicological relevance for human health[12]. Therefore, findings in the rat larynx are often considered adaptive responses not relevant for human hazard identification, according to the CLP guidance. The extreme sensitivity of the rat larynx to irritant particulates is considered to arise from anatomical, airflow, epithelial cell type and possibly clearance rates. In rat larynx, the cartilage associated with the ventral pouch is U-shaped and larynx and trachea form a relatively straight line from the nasal turbinates, which enhances the deposition of aerosols. In contrast, in humans the U-shaped pouch is absent, and the larynx is more sharply angled to the oro-nasal cavity[13]. Often degeneration of the original epithelial cells with subsequent regeneration hyperplasia and squamous metaplasia occurs[14], which was observed in the available study package for folpet, which on the one hand could be considered as an adaptive response to inhalation of irritants. On the other hand, laryngeal effects (squamous metaplasia and squamous hyperplasia) were still present in the recovery period in the 13 week inhalation exposure study conducted with the folpet sibling captan, where additionally mortalities were observed at a dose level of 12.98 µg/L.

In a supplementary 8-day inhalation study in pregnant mice (Section 8.10/Courtney et al. (1983)) there were 5/15 mortalities after 8 days exposure for 4 h/day upon a treatment concentration of 624 mg/m^3 [µg/L]. Due

[5] Alarie Y (1981) Toxicological evaluation of airborne chemical irritants and allergens using respiratory reflex reactions In: Leong B (ed) Inhalation toxicology and technology Ann Arbor Science Publishers, Inc, Ann Arbor, MI p207-231

[6] Castranova V, Frazer DG, Manley LK, Dey RD (2002) Pulmonary alterations associated with inhalation of occupational and environmental irritants. International immunopharmacology 2(2-3):163-72 doi:10.1016/s1567-5769(01)00169-2

[7] Renne R, Brix A, Harkema J, et al. (2009) Proliferative and nonproliferative lesions of the rat and mouse respiratory tract. Toxicol Pathol 37(7 Suppl):5S-73S doi:10.1177/0192623309353423

[8] Weber K, Germann P-G, Iwata H, Hardisty J, Kaufmann W, Rosenbruch M (2009) Lesions in the Larynx of Wistar RccHan: WIST Rats. J Toxicol Pathol 22(4):229-246 doi:10.1293/tox.22.229

[9] Landén NX, Li D, Ståhle M (2016) Transition from inflammation to proliferation: a critical step during wound healing. Cell Mol Life Sci 73(20):3861-3885 doi:10.1007/s00018-016-2268-0

[10] Hayes AW (2014) Hayes' Principles and Methods of Toxicology, 6th edn. CRC Press, Taylor & Francis Group, Boca Raton

[11] Mowat V, Alexander DJ, Pilling AM (2017) A Comparison of Rodent and Nonrodent Laryngeal and Tracheal Bifurcation Sensitivities in Inhalation Toxicity Studies and Their Relevance for Human Exposure. Toxicol Pathol 45(1):216-222 doi:10.1177/0192623316678695

[12] Osimitz TG, Droege W, Finch JM (2007) Toxicologic significance of histologic change in the larynx of the rat following inhalation exposure: a critical review. Toxicol Appl Pharmacol 225(3):229-37 doi:10.1016/j.taap.2007.08.027

[13] Kaufmann W, Bader R, Ernst H, et al. (2009) 1st international ESTP expert workshop: "Larynx squamous metaplasia". A re-consideration of morphology and diagnostic approaches in rodent studies and its relevance for human risk assessment. Exp Toxicol Pathol 61(6):591-603 doi:10.1016/j.etp.2009.01.001

[14] Lewis D (1991) Morphological assessment of pathological changes within the rat larynx. Toxicol Pathol 19:352-7

to the rapid degradation and reaction of folpet, one can use Haber's rule to translate this concentration to a corresponding acute inhalation toxicity exposure. The repeated exposure corresponds to a single exposure concentration of 624 µg/L/day × 8 day = 4992 µg/L. This concentration exceeds the lowest LC_{50} in non-pregnant female rats of 0.43 mg/L (Section 8.3/Study 3 (1991)) almost 12 times.

Similar calculations are proposed by Kluxen and Koenig (unpublished), which discuss that the lowest cumulative concentration of 0.005 mg/L in Study 11 (2011) corresponds to a single exposure concentration of 0.15 mg/L (0.005 mg/L × 4 weeks × 5 days/week × 1.5 [6 h to 4 h ratio]), which corresponds to about half the lowest LC_{50} of 0.39 mg/L in Study 3 (1991). Hence, assuming cumulative toxicity, which is supported by the study package, the lowest tested repeated exposure inhalation toxicity study concentration was very high, considering the specific mode of action of folpet and the sensitivity of the rat's respiratory system towards irritants. It may be questioned, whether adverse effects caused by irritation observed in repeated exposure studies are specific target organ toxicity distinct from acute effects or whether they occur due to the same aetiology, being already covered by classification for irritation and acute toxicity.

However, no repeated dose inhalation ADME studies are available proving this assumed cumulative toxicity of folpet in the respiratory tract. Furthermore, oral ADME studies show that > 90% of folpet was excreted within 24 hours and half-life of folpet in blood (*in vitro*) was extremely short (seconds) raising the level of uncertainty towards exposure to cumulated folpet deposits in the respiratory tract.

According to the ECHA "Guidance on the Application of the CLP Criteria" (2017) one way to distinguish if the severe effect is a reflection of true repeated exposure toxicity or whether it is in fact just acute toxicity (i.e. corrosivity) is to consider the dose level which causes the toxicity. If the dose is more than half an order of magnitude lower than that mediating the evident acute toxicity (corrosivity) then it could be considered to be a repeated-dose effect distinct from the acute toxicity.

Currently folpet is classified as Eye Irritant Cat. 2 and Acute Tox. Cat. 4 (inhalation) (LC50 = 1.54 mg/L in males). Acute inhalation toxicity is proposed to modify to Acute Tox 2 based on a lower LC50 value of 0.39 mg/L in males from a fully reliable study (i.e. Study 3) in this CLH report. Clinical signs were noted in animals exposed to 0.14 mg/L (lowest dose) in the same acute inhalation study. In a repeated dermal toxicity study erythema and oedema were recorded at 1 mg/kg bw/d (lowest dose level). There is no information on the irritation/corrosive potential of folpet at lower dose levels. However, there is a factor of > 25 between the dose causing adverse effects in the larynx and the dose level causing clinical signs in acute inhalation toxicity studies. Therefore, the effects in the larynx could be considered relevant for considering classification as STOT-RE.

9.12.2 Comparison with the CLP criteria

Substances can be allocated into one of three hazard categories for STOT-RE. Where the same target organ toxicity of similar severity is observed after single and repeated exposure to a similar dose, it may be concluded that the toxicity is essentially an acute (i.e. single exposure) effect with no accumulation or exacerbation of the toxicity with repeated exposure.

In the case of folpet, study package suggests that the primary toxicity is local acute irritation at the first site of contact. Due to the direct interaction and rapid degradation, the effect is increasing with dose and exposure time and might not occur specifically due to repetition. However, no histopathological evaluations of the respiratory system were performed after single exposure, making a direct comparison of effects difficult.

CLP criteria acc to Regulation 1272/2008 and CLP guidance[15]	**Assessment**
Category 1 Substances that have produced significant toxicity in humans or that, on the basis of evidence from	No human data is available.

[15] ECHA (2017) Guidance on the Application of the CLP Criteria, vol Version 5.0 – July 2017, Reference: ECHA-17-G-21-EN Cat.Number: ED-02-17-754-EN-N edn. European Chemicals Agency

studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following repeated exposure. *Substances are classified in Category 1 for target organ toxicity (repeat exposure) on the basis of:* *— reliable and good quality evidence from human cases or epidemiological studies; or* *— observations from appropriate studies in experimental animals in which significant and/or severe toxic effects, of relevance to human health, were produced at generally low exposure concentrations.* *Guidance dose/concentration values are Guidance dose/concentration values are provided below (see 3.9.2.9), to be used as part of a weight-of- evidence evaluation*	Larynx Effects in the larynx, i.e. squamous/squamoid metaplasia with keratinization (moderate: 5/5 males and 5/5 females), epithelial hyperplasia (minimal: 1/5 females, slight: 2/5 males, moderate: 1/5 males), mucosal fibrosis (minimal: 1/5 males and 1/5 females, slight: 2/5 males and 1/5 females) and inflammation (slight: 1/5 males and 1/5 females, moderate: 4/5 males and 4/5 females) in the larynx, occur in a 4-week study at 0.005 mg/L in rat, similar effects, with a slight progression in severity were observed at 0.025 mg/L. Both concentrations are below the guidance value of 0.06 mg/L (modified for 28-d studies). Please refer to table 3.12.3-3 in the Annex Human Health. Nasal turbinates 1/5 male animals showed squamous/squamoid metaplasia in the respiratory epithelium and/or degeneration/atrophy of the olfactory epithelium at 0.025 mg/L in rat, which is below the guidance value of 0.06 mg/L (modified for 28-d studies). Trachea and lung Inflammatory cells were observed in the trachea starting at a concentration of 0.005 mg/L (slight 1/5 males and 1/5 females; moderate 1/5 males and 1/5 females). However, increased incideces and severity were observed only at 0.1 mg/L but not at 0.025 mg/L. Please refer to table 3.12.3-5 in the Annex Human Health. All these effects progressed at the next higher dose levels. In a 90 day inhalation exposure study with the folpet sibling captan laryngeal effects (squamous metaplasia and squamous hyperplasia) were still present in the recovery period. Only the highest dose level of 12.98 µg/L was included in the recovery assessment. In the same study mortalities were observed at a dose level of 12.98 µg/L which is below the guidance value of 0.02 mg/L. Therefore, STOT-RE 1 classification seems appropriate.
Category 2 - Substances that, on the basis of evidence from studies in experimental animals can be presumed to have the potential to be harmful to human health following repeated exposure. *Substances are classified in category 2 for target organ toxicity (repeat exposure) on the basis of observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were produced at generally moderate exposure concentrations.* *Guidance dose/concentration values are provided below (see 3.9.2.9) in order to help in classification. In*	Oesophagus/Gastrointestinal tract All repeated exposure studies show findings in the gastrointestinal tract, which might be related to irritative effects, which results in small intestinal tumours in mice and hyperkeratosis of the non-glandular stomach and of the oesophagus in rats. None of these effects is below the guidance value triggering STOT-RE 2 classification (i.e. 100 mg/kg bw/d for oral studies, based on 90 day rat studies).

exceptional cases human evidence can also be used to place a substance in Category 2 (see 3.9.2.6).	
Note Attempts shall be made to determine the primary target organ of toxicity and classify for that purpose, such as hepatotoxicants, neurotoxicants. One shall carefully evaluate the data and, where possible, not include secondary effects (a hepatotoxicant can produce secondary effects in the nervous or gastro-intestinal systems).	The effects associated with folpet exposure are not specific to any target organ. The effects in the repeated exposure studies might be the consequence of multiple local acute irritation events that occur at all exposed epithelia.

Classification for target organ toxicity (repeated exposure) STOT-RE 1 seems appropriate, considering the effects in the respiratory tract after repeated inhalation exposure to folpet. This is supported by persistent effects and mortality observed after exposure (≤ 0.02 mg/l) to its sibling captan.

9.12.3 Conclusion on classification and labelling for STOT RE

Folpet is proposed to be classified for STOT-RE Category 1.

9.13 Aspiration hazard

This hazard class not assessed in this dossier.

10 EVALUATION OF ENVIRONMENTAL HAZARDS

Robust study summaries (all studies submitted) are provided in Annex III (Environmental Fate & Behaviour) to this CLH report.

10.1 Rapid degradability of organic substances

Table 50: Summary of relevant information on rapid degradability

Method	Results	Remarks	Reference
Ready Biodegradability, OECD 301B	Cumulative TCO_2 production by a mixtures containing 10 mg C/L of folpet technical was equivalent to 35 % and 46 % (mean = 41 %) of the TCO_2 (106.4 mg CO_2) over the 29 day period. Degradation was slow but progressive throughout and a degradation plateau was not attained. Test material: Folpet technical GLP: Yes Study considered valid Relevant for classification regarding degradability in the aquatic environment (*DT50* in the aquatic environment based on CO_2 production > 16 days; major	Results in this study based on technical folpet (*DT50* based on CO_2 production > 16 days) are somewhat in contradiction to Anonymous (1998), who considers folpet (radio labelled) readily biodegradable (*DT50* based on CO_2 production < 16 days). The reasons for this discrepancy are unknown. Notice that degradation products of folpet have not been assessed in this study. However, based on aquatic hydrolysis studies (Anonymous, 1988b), studies on the aerobic mineralisation in surface water (Anonymous, 2016g, Anonymous, 2016j) and water sediment studies (Anonymous, 1999, Anonymous, 2007c), phthalimide, phthalamic and phthalic acid, 2-	Anonymous, 1994

Method	Results	Remarks	Reference
	degradation products assumed to be formed in this study do not meet the criteria for classification as hazardous to the aquatic environment – please see point 9.4 to 9.7)	cyanobenzoic acid and benzamide are considered major degradation products under the conditions of this study.	
Ready Biodegradability, OECD 301B	Mean cumulative $^{14}CO_2$ production by mixtures containing in total 10 mg/L of radiolabelled and unlabelled folpet (1:9) was equivalent to 13 % AR after four days of incubation and 63 % AR at day 14; 73 % degradation was achieved by the end of the study at day 28. Test material: [U-phenyl-^{14}C]-folpet (radiopurity 99.4 %) and unlabelled folpet (purity 100 %) in a 1:9 ratio GLP: Yes Study considered valid Relevant for classification regarding degradability in the aquatic environment (*DT50* in the aquatic environment based on CO_2 production ≤ 16 days, major degradation products assumed to be formed in this study do not meet the criteria for classification as hazardous to the aquatic environment – please see point 9.4 to 9.7)	Results in Anonymous (1998) are not fully in line with result obtained in Anonymous (1994) for unknown reasons. See above. Notice that degradation products of folpet have not been assessed in this study. However, based on aquatic hydrolysis studies (Anonymous, 1988b), studies on the aerobic mineralisation in surface water (Anonymous, 2016g, Anonymous, 2016j) and water sediment studies (Anonymous, 1999, Anonymous, 2007c), phthalimide, phthalamic and phthalic acid, 2-cyanobenzoic acid and benzamide are considered major degradation products under the conditions of this study.	Anonymous, 1998
Aquatic hydrolysis, OECD 111	The hydrolysis of radiolabelled folpet increased with pH, the first-order half-lives being 2.9 hrs at pH 5, 1.3 hrs at pH 7, and 59 secs at pH 9. Test material: [carbonyl-^{14}C]-folpet radiopurity 99.6 % GLP: Yes Study considered valid Relevant for classification regarding degradability in the aquatic environment (*DT50* in the aquatic environment ≤ 16 days,	Legacy study broadly in line with OECD 111	Anonymous, 1988b

Method	Results	Remarks	Reference
	degradation products do not meet the criteria for classification as hazardous to the aquatic environment – please see point 9.4 to 9.7)		
Aquatic hydrolysis, OECD 111	Radiolabelled folpet was recovered as 47 to 52 % AR at 1 hr at pH 5 and pH 7, but was not found at pH 9 (0 % AR) as anticipated from its very short half-life at this pH. At 24 hrs, only low levels of folpet were observed at pH 5 (14.9 % AR) and pH 7 (1.1 % AR). Test material: [Trichloromethyl-^{14}C]-folpet radiopurity 99.2 % GLP: Yes Study considered valid Relevant for classification regarding degradability in the aquatic environment (*DT50* in the aquatic environment ≤ 16 days, degradation products do not meet the criteria for classification as hazardous to the aquatic environment – please see point 9.4 to 9.7)	Legacy study broadly in line with OECD 111	Anonymous, 1992b
Aquatic hydrolysis, OECD 111	Refer to summary on water, water-sediment and soil degradation data provided below (Chapter 9.1.4.3). Test material: Phthalimide unlabelled purity 99.1 % GLP: Yes Study considered valid	Study conducted with folpet metabolite phthalimide	Anonymous, 2015i
Aquatic hydrolysis, OECD 111	Refer to summary on water, water-sediment and soil degradation data provided below (Chapter 9.1.4.3). Test material: Phthalimide unlabelled purity 99.7 % GLP: Yes Study considered valid	Study conducted with folpet metabolite phthalimide	Anonymous, 2016f
Aerobic mineralisation in surface water, OECD 309	Radiolabelled folpet applied at either 10.5 or 100.8 µg/L disappeared completely within one hour of incubation. Test material:		Anonymous, 2016g

Method	Results	Remarks	Reference
	[U-phenyl-^{14}C]-folpet radiopurity 98.09 % GLP: Yes Study considered valid Relevant for classification regarding degradability in the aquatic environment (*DT50* in the aquatic environment ≤ 16 days, degradation products do not meet the criteria for classification as hazardous to the aquatic environment – please see point 9.4 to 9.7)		
Aerobic mineralisation in surface water, OECD 309	Radiolabelled folpet was found to degrade rapidly (DT50 of approx. 0.3 hrs) in a system consisting of natural water incubated in the dark at 20 ± 2 °C. Test material: [U-phenyl-^{14}C]-folpet radiopurity 98.09 % GLP: Yes Study considered valid Relevant for classification regarding degradability in the aquatic environment (*DT50* in the aquatic environment ≤ 16 days, degradation products do not meet the criteria for classification as hazardous to the aquatic environment – please see point 9.4 to 9.7)		Anonymous, 2015j
Water/sediment study, OECD 308	In a silty clay water/sediment system radiolabelled folpet declined rapidly over the period of the study from 79.7 % AR at 5 minutes to 2.1 % AR at 4 hrs. In the sandy loam water/sediment system folpet declined rapidly over the period of the study from 80.2 % AR after 5 minutes to 11.2 % AR after 4 hrs. Test material: [U-phenyl-^{14}C]-folpet radiopurity > 99.3 % GLP: Yes Study considered valid Relevant for classification regarding degradability in the aquatic environment		Anonymous, 1999

Method	Results	Remarks	Reference
	(*DT50* in the aquatic environment ≤ 16 days, degradation products do not meet the criteria for classification as hazardous to the aquatic environment – please see point 9.4 to 9.7)		
Water/sediment study, OECD 308	Radiolabelled folpet was rapidly degraded in the water phase of two water/sediment systems. No folpet was detectable at one day after application and it did not transfer to the sediment. Test material: [U-phenyl-^{14}C]-folpet radiopurity 98.04 % GLP: Yes Study considered valid Relevant for classification regarding degradability in the aquatic environment (*DT50* in the aquatic environment ≤ 16 days, degradation products do not meet the criteria for classification as hazardous to the aquatic environment – please see point 9.4 to 9.7)		Anonymous, 2007c
Direct photochemical degradation, OECD 316	Recovery of radiolabelled folpet after 8 hrs of irritation (sterile buffer solution at pH 3) was 34.2 % under natural sunlight and 15.3 % under UV light (350 nm), respectively. Dark and irradiated samples behaved in a very similar manner. Test material: [U-phenyl-^{14}C]-folpet radiopurity 97.7 % GLP: Yes Study considered valid	Legacy study broadly in line with OECD 316	Anonymous, 1989b
Aerobic transformation in soil, OECD 307	Refer to summary on water, water-sediment and soil degradation data provided below (Chapter 9.1.4.3). Test material: [U-phenyl-^{14}C]-folpet, radiopurity 98 % GLP: Yes Study considered valid Relevant for classification regarding degradability in the	Legacy study broadly in line with OECD 307	Anonymous, 1991a

Method	Results	Remarks	Reference
	aquatic environment (*DT50* in soil ≤ 16 days, degradation products do not meet the criteria for classification as hazardous to the aquatic environment – please see point 9.4 to 9.7).		
Aerobic transformation in soil, OECD 307	Refer to summary on water, water-sediment and soil degradation data provided below (Chapter 9.1.4.3). Test material: [U-phenyl-^{14}C]-folpet, radiopurity 97.4 % GLP: Yes Study considered valid Relevant for classification regarding degradability in the aquatic environment (*DT50* in soil ≤ 16 days, degradation products do not meet the criteria for classification as hazardous to the aquatic environment – please see point 9.4 to 9.7).	Legacy study broadly in line with OECD 307	Anonymous, 2001a
Aerobic transformation in soil, OECD 307	Refer to summary on water, water-sediment and soil degradation data provided below (Chapter 9.1.4.3). Test material: [U-phenyl-^{14}C]-folpet radiopurity 99.3 % GLP: Yes Study considered valid Relevant for classification regarding degradability in the aquatic environment (*DT50* in soil ≤ 16 days, degradation products do not meet the criteria for classification as hazardous to the aquatic environment – please see point 9.4 to 9.7).		Anonymous, 2007a
Aerobic transformation in soil, OECD 307	Refer to summary on water, water-sediment and soil degradation data provided below (Chapter 9.1.4.3). Test material: [U-phenyl-^{14}C]-folpet radiopurity 98.5 % GLP: Yes Study considered valid Relevant for classification		Anonymous, 2015b

Method	Results	Remarks	Reference
	regarding degradability in the aquatic environment (*DT50* in soil ≤ 16 days, degradation products do not meet the criteria for classification as hazardous to the aquatic environment – please see point 9.4 to 9.7).		
Aerobic transformation in soil, OECD 307	Refer to summary on water, water-sediment and soil degradation data provided below (Chapter 9.1.4.3). Test material: [U-phenyl-^{14}C]-phthalamic acid radiopurity 95.3 % GLP: Yes Study considered valid	Study performed with folpet metabolite phthalamic acid	Anonymous, 2015e
Aerobic transformation in soil, OECD 307	Refer to summary on water, water-sediment and soil degradation data provided below (Chapter 9.1.4.3). Test material: [U-phenyl-^{14}C]-phthalic acid radiopurity 95.2 % GLP: Yes Study considered valid	Study performed with folpet metabolite phthalic acid	Anonymous, 2012a
Aerobic transformation in soil, OECD 307	Refer to summary on water, water-sediment and soil degradation data provided below (Chapter 9.1.4.3). Test material: [U-phenyl-^{14}C]-phthalimide radiopurity 99.8 % GLP: Yes Study considered valid	Study performed with folpet metabolite phthalimide	Anonymous, 2016d
Anaerobic transformation in soil, OECD 307	Refer to summary on water, water-sediment and soil degradation data provided below (Chapter 9.1.4.3). Test material: [U-phenyl-^{14}C]-folpet radiopurity 98 % GLP: Yes Study considered valid	Legacy study broadly in line with OECD 307	Anonymous, 1991b
Soil photolysis, OECD draft (2002)	Refer to summary on water, water-sediment and soil degradation data provided below (Chapter 9.1.4.3). Test material: [U-phenyl-^{14}C]-folpet radiopurity 98.8 % GLP: Yes Study considered valid		Anonymous, 2014

Method	Results	Remarks	Reference
Soil photolysis, OECD draft (2002)	Refer to summary on water, water-sediment and soil degradation data provided below (Chapter 9.1.4.3). Test material: [U-phenyl-^{14}C]-folpet radiopurity 100 % GLP: Yes Study considered valid		Anonymous, 2015c
Field dissipation study (US-EPA guidelines)	Refer to summary on water, water-sediment and soil degradation data provided below (Chapter 9.1.4.3). Test material: Formulated folpet GLP: Yes Study considered valid Relevant for classification regarding degradability in the aquatic environment (*DT50* in soil ≤ 16 days, degradation products do not meet the criteria for classification as hazardous to the aquatic environment – please see point 9.4 to 9.7).		Anonymous, 1991d
Field dissipation study (US-EPA guidelines)	Refer to summary on water, water-sediment and soil degradation data provided below (Chapter 9.1.4.3). Test material: Formulated folpet GLP: Yes Study considered valid Relevant for classification regarding degradability in the aquatic environment (*DT50* in soil ≤ 16 days, degradation products do not meet the criteria for classification as hazardous to the aquatic environment – please see point 9.4 to 9.7).		Anonymous, 1991e
Field dissipation study (US-EPA guidelines)	Refer to summary on water, water-sediment and soil degradation data provided below (Chapter 9.1.4.3). Test material: Formulated folpet GLP: Yes Study considered valid Relevant for classification		Anonymous, 2000a

Method	Results	Remarks	Reference
	regarding degradability in the aquatic environment (*DT50* in soil ≤ 16 days, degradation products do not meet the criteria for classification as hazardous to the aquatic environment – please see point 9.4 to 9.7).		

10.1.1 Ready biodegradability

The low solubility of folpet and its slow dissolution rate are significant factors in the **ready biodegradability** of folpet, investigated in two studies. At environmental exposure concentrations folpet is classified as readily biodegradable. The conclusion of the first study submitted (Anonymous, 1994) consider folpet "inherently degradable".

For further details please refer to the study reports below.

[Study 1]

Reference:	**Folpet technical: assessment of its ready biodegradability**
Author(s), year:	Anonymous, 1994
Report/Doc. Number:	94/MAK/186/0048, R-7491
Guideline(s):	OECD 301B
GLP:	Yes
Validity:	Yes
Status:	**Previously submitted**

In a preliminary Closed Bottle test (OECD 301D) folpet technical at a nominal concentration of 10 mg C/L (27.5 mg/folpet technical/L) did not significantly inhibit degradation of the reference material sodium benzoate. In this preliminary test, folpet technical alone showed no significant evidence of biodegradation.

In the modified Sturm test, folpet technical was added to two vessels containing inoculated mineral salts medium (the inoculum was a sample of active sludge from a local domestic sewage treatment works collected the day before the test and was aerated in the laboratory for 4 hours before the test). The nominal test concentration of folpet technical was 10 mg C/L. Control vessels comprised two inoculated mineral salts medium alone and one containing inoculated salts medium and the reference material sodium benzoate (10 mg C/L). Test and control vessels were aerated for 29 days with air that had been treated to remove carbon dioxide. The *p*H of control, reference and test mixtures was measured at the start of the study, and after 28 days, and was in the range of 7.2 - 7.7. Temperatures ranged from 19.6 °C to 24.4 °C.

Table 51: Biodegradability of sodium benzoate and folpet technical in modified Sturm test, in terms of % TCO_2

Time (days)	Sodium benzoate reference (% TCO_2)[(a)]	Folpet technical (% TCO_2)[(a)]	
		Culture 1	Culture 2
1	2	0	0
2	19	0	0
4	44	4	1
5	58	7	3
7	70	11	12
11	83	16	25
15	90	20	31
20	93	25	37
25	95	29	41
28	96	33	44
29	97	35	46

(a) From blank corrected CO_2 production

The day 29 results for the degradation of sodium benzoate reference material (97 % TCO_2) and for cumulative CO_2 production (21.2 mg and 25.7 mg in the two cultures) fulfil the validity criteria.

Cumulative TCO_2 production by the mixtures containing 10 mg C/L of folpet technical was equivalent to 35 % and 46 % (mean = 41 %) of the TCO_2 (106.4 mg CO_2) over the 29 day period. Degradation was slow but progressive throughout and a degradation plateau was not attained.

Substances are considered to be readily biodegradable in this test if CO_2 production is equal to or greater than 60 % of the theoretical value within 10 days of the level first achieving 10 %. Folpet technical cannot therefore be considered readily biodegradable, but because significant degradation occurred, it can be considered to be inherently degradable.

Comments (RMS AT):

- It may be noted that results in this study based on technical folpet are somewhat in contradiction to Anonymous (1998), who considers folpet (radio labelled) readily biodegradable. The reasons for this discrepancy are unknown.

[Study 2]

Reference:	**[^{14}C]-Folpet: Assessment of ready biodegradability - Modified Sturm test**
Author(s), year:	Anonymous, 1998
Report/Doc. Number:	MAK512/984038, R-10488
Guideline(s):	OECD 301B
GLP:	Yes
Validity:	Yes
Status:	**Previously submitted**

The ready biodegradability of [U-phenyl-^{14}C]-folpet (radiochemical purity 99.4 %) and unlabelled folpet (100 % purity) in a 1:9 ratio was investigated in a modified Sturm test. The folpet test sample was deposited on the walls of two culture vessels and mineral salts medium inoculated with active sludge (obtained the previous day from a local domestic waste sewage treatment works) was then added to each vessel to give a nominal [^{14}C]-folpet concentration of 1 mg/L (10 mg/L of total folpet). Control vessels comprised of two containing inoculated mineral salts medium alone and one containing inoculated mineral salts and the reference material sodium benzoate (10 mg C/L).

Test, control and reference mixtures were aerated for 28 days with air that had been treated to remove carbon dioxide and the unlabelled and $^{14}CO_2$ evolved was trapped from the respective vessels. The test was conducted at temperatures that were nominally in the range 20 °C to 24 °C. The *p*H of all test and control mixtures was 7.5 at the start of the test, and ranged from 7.7 to 7.8 at the end of the test. The distribution of radioactivity in the mixtures containing [^{14}C]-folpet was measured at the end of the study.

Table 52: Biodegradability of sodium benzoate and [^{14}C]-folpet in a modified Sturm test

Time (days)	Sodium benzoate reference (% TCO_2)[a]	[^{14}C]-folpet (% AR $^{14}CO_2$)	
		Culture 1	Culture 2
1	2	0	0
2	22	0	1
4	44	12	14
6	62	30	33
9	75	49	51
12	82	58	60
14	84	62	63
19	86	67	68
22	88	69	71
26	90	71	73
28	91	72	74

[a] From blank corrected CO_2 production.

Sodium benzoate was degraded to 22 % of the theoretical value after two days, to 62 % after six days, and 91 % by the end of the test on day 28. Cumulative levels of CO_2 production in the controls after 28 days (49.8 and 49.2 mg CO_2) were within the acceptable range for this assay system. These results confirmed that the inoculum was viable and that the test was valid.

Mean cumulative $^{14}CO_2$ production by mixtures containing [^{14}C]-folpet was equivalent to 13 % AR after four days of incubation and 63 % AR at day 14; 73 % degradation was achieved by the end of the study at day 28.

The total recovery was 92.4 to 96.7 % AR. Levels of 15.8 to 18.5 % AR were found in the sewage solids which suggested that the degradation of folpet had resulted in the incorporation of radiolabel into bacterial cells. Low levels (4.0 to 4.6 % AR) were found in the test medium and analysis showed that this comprised soluble $^{14}CO_2$ and residual ^{14}C-containing materials which may have been water-soluble degradation intermediates of folpet, such as phthalamic acid or phthalic acid. The levels on the walls of the test vessels (< 0.3 % AR) were considered insignificant.

Table 53: Summary of distribution of radioactivity (% AR) at end of [^{14}C]-folpet biodegradation study

Sample	NaOH traps ($^{14}CO_2$)	Vessel filtrate	Acidified vessel filtrate		Acetone rinse	Sewage solids	Total
			NaOH[a] trap	Filtrate[a]			
Culture 1	71.9	4.6	1.2	2.6	0.1	15.8	92.4
Culture 2	73.9	4.1	0.8	2.4	0.3	18.5	96.7

[a] After overnight aeration

A substance can be considered readily biodegradable in this test if CO_2 production is equal to or greater than 60 % of the theoretical value within 10 days of the level achieving 10 %. Folpet can therefore be considered to be readily biodegradable according to this criterion.

Due to the rapid aerobic soil degradation and transient nature of the significant major metabolites phthalimide, phthalamic acid and phthalic acid the ready biodegradability of these components has not been classified.

Comments (RMS AT):

- As already noted, results in Anonymous (1998) are not fully in line with result obtained in Anonymous (1994) for unknown reasons.

10.1.2 BOD5/COD

No data

10.1.3 Hydrolysis

Folpet rapidly **hydrolyses** in sterile water and the rate of hydrolysis rapidly increases with *p*H. Hydrolysis *DT50* values are 2.9 hrs at *p*H 5, 1.3 hrs at *p*H 7, and only 59 sec at *p*H 9. At *p*H 5 the predominant degradate of phenyl labelled folpet is phthalimide but there is a shift towards phthalic acid which becomes the predominant degradate at *p*H 9. Phthalimide is considered stable to hydrolysis at *p*H 4 (*DT50* = 125 and 141 days, two studies), whereas it is rapidly hydrolysis with a *DT50* of 2.3 days at *p*H 7 and a *DT50* of 0.05 days at *p*H 9. Phthalic acid is considered stable (final degradate) under conditions of hydrolysis. Phthalamic acid was not detected above 2.5 % AR in the hydrolysis studies.

For further details please refer to the study reports below.

[Study 1]

Reference:	**Hydrolysis of [^{14}C]-folpet**
Author(s), year:	Anonymous, 1988b
Report/Doc. Number:	PTRL 124, R-5235
Guideline(s):	OPP 161-1 Hydrolysis Studies (1982)
GLP:	Yes
Validity:	Yes
Status:	**Previously submitted**

The hydrolysis of [carbonyl-^{14}C]-folpet (radiochemical purity 99.6 %, containing 0.4 % [^{14}C]-phthalimide) was investigated at *p*H 5, 7 and 9 in sterile buffer solutions in the dark according to EPA guidelines in a 1988 study. Concentrations were 1.20 (*p*H 5), 1.11 (*p*H 7) and 1.01 mg/l (*p*H 9), and the temperature was 25 ± 1 °C. Duplicate samples were taken at 0, 1.0, 3.0, 5.0, 9.5 and 24 hours at *p*H 5 and 0, 0.5, 1.0, 2.0, 3.0, 4.0 and 8.0 hours at *p*H 7. Duplicate samples were also taken at 15 to 30, 70 to 71, 131 to 147, 191 to 196, 366 to 371 and 611 to 613 seconds at *p*H 9.

The recovery of radioactivity was 92 to 104 % AR, with mean values for duplicates of 98.9 ± 2.9 %, 98.8 ± 4.4 % AR (*p*H 5), 97.4 ± 6.1 %, 94.9 ± 5.1 % AR (*p*H 7), and 95.2 ± 1.3 %, 96.2 ± 1.3 % AR (*p*H 9). No volatile hydrolysis products were formed.

At *p*H 5, folpet decreased from 89.7 % AR to 0.5 % AR during the study over 24 hours. Phthalimide increased from 6.2 % AR to 91.5 % AR during the study, accumulating to a 10:1 ratio relative to the other hydrolysates, phthalamic acid and phthalic acid, which do not exceed a combined level of 10 % AR.

Table 54: The distribution of radioactivity (% AR) in the hydrolysis of [carbonyl-^{14}C]-folpet at *p*H 5 (numbers shaded in grey indicate exceedance of 5 % AR).

HAT (hrs)	Folpet	Phthalimide	Phthalamic acid	Phthalic acid	Unknowns[(a)]	Total
0	89.7	6.2	0.0	0.0	0.3	96.2
1	76.7	16.0	0.3	3.1	2.1	98.0
3	49.3	37.7	0.7	6.8	1.6	96.1
5	28.5	59.7	0.9	5.9	1.7	96.5
9.5	9.7	80.7	0.5	8.0	3.9	102.8
24	0.5	91.5	0.6	8.5	3.4	103.7

(a) Total for between 2 and 9 unknowns, dependent on sample. Largest unknown was 1.7 % AR.

At *p*H 7, folpet decreased from 90.6 % AR to 3.0 % AR during the eight hours of the study. Phthalimide increased from 1.7 % AR to 44.4 % AR, the ratio with phthalamic acid and phthalic acid becoming approximately 1:1, the combined level of these two metabolites being 48.7 % AR at 24 hours.

Table 55: The distribution of radioactivity (% AR) in the hydrolysis of [carbonyl-^{14}C]-folpet at *p*H 7 (numbers shaded in grey indicate exceedance of 5 % AR).

HAT (hrs)	Folpet	Phthalimide	Phthalamic acid	Phthalic acid	Unknowns[(a)]	Total
0	90.6	1.7	0.0	0.4	1.9	94.5
0.5	60.1	11.0	0.7	11.2	1.3	84.3
1	50.5	21.5	1.9	21.0	3.1	97.9
2	26.8	33.4	2.2	32.9	3.6	98.8
3	24.6	34.0	1.9	32.1	6.2	98.7
4	17.3	37.8	0.6	40.6	2.9	99.1
8	3.0	44.4	2.5	46.2	3.8	99.9

(a) Total for between 2 and 7 unknowns, dependent on sample. Largest unknown was 4.4 % AR.

At *p*H 9, folpet rapidly decreased to 0.3 % AR during the 611 to 613 seconds of the study. Phthalimide and phthalamic acid were also very unstable at this *p*H, the combined level not exceeding 17 % AR. Phthalic acid predominated, increasing to 78.4 % AR by the end of the study.

Table 56: The distribution of radioactivity (% AR) in the hydrolysis of [carbonyl-^{14}C]-folpet at *p*H 9 (numbers shaded in grey indicate exceedance of 5 % AR).

SAT[(a)] (seconds)	Folpet	Phthalimide	Phthalamic acid	Phthalic acid	Un Unknowns[(b)]	Total
15 – 30	59.5	11.3	2.0	18.0	3.2	94.0
70 – 71	47.2	8.5	1.2	36.3	1.8	94.9
131 – 147	27.2	11.5	1.4	51.8	3.9	95.7
191 – 196	16.0	13.0	0.4	63.1	3.9	96.4
366 – 371	4.4	15.7	1.6	71.8	3.1	96.6
611 – 613	0.3	14.5	0.7	78.4	3.0	96.8

(a) It was not possible to obtain a time zero value because of the rapid hydrolysis.
(b) Total for 2 to 5 unknowns, dependent on sample. Largest unknown was 3.9 % AR.

The hydrolysis of folpet increased with *p*H, the first-order half-lives being 2.6 hours at *p*H 5, 1.1 hours at *p*H 7, and 67 seconds at *p*H 9. Kinetic analysis suggested that hydrolysis takes place both from folpet and phthalimide at higher *p*H values and folpet only at low *p*H values.

Comments (RMS AT):

- RMS AT recalculated hydrolysis rate for folpet applying SFO kinetics according to pertinent guidance: *DT50/90* (*p*H 5) = 2.9/9.8 hrs, *DT50/90* (*p*H 7) = 1.3/4.4 hrs and *DT50/90* (*p*H 9) = 59/196 sec. Fits to SFO were excellent in each case (data not shown).
- Linking phthalimide to folpet via $P_{SFO} \rightarrow M_{SFO}$ degradation pathway did not yield reliable fitting results for phthalimide (RMS AT assessment). Nevertheless, based on Anonymous (2015i) phthalimide is considered to show *p*H dependent hydrolysis similar to folpet.
- Phthalic acid is considered stable to hydrolysis at all *p*H values.

[Study 2]

Reference:	**Hydrolysis of [^{14}C-trichloromethyl]-folpet at pH 5, 7 and 9**
Author(s), year:	Anonymous, 1992b
Report/Doc. Number:	PTRL 371W, R-5235a
Guideline(s):	OPP 161-1 Hydrolysis Studies (1982)
GLP:	Yes
Validity:	Additional information only (refer to comment section)
Status:	**Previously submitted**

The hydrolysis of [trichloromethyl-^{14}C]-folpet (radiochemical purity 99.2 %) was investigated in sterile buffer solutions in the dark at *p*H 5, 7 and 9. The nominal concentration in solution was 1 mg/l, and the temperature was 19.3 – 22.5 °C. Duplicate samples were taken at 1 hour and 24 hours.

Table 57: Distribution of radioactivity (% AR) in hydrolysis of [trichloromethyl-^{14}C]-folpet (numbers shaded in grey indicate exceedance of 5 % AR).

pH	HAT (hrs)	Folpet	Unknown 1	Unknown 2	$^{14}CO_2$	Air sampling	Other unknowns[(a)]	Total recovery
5	1	47.0	3.9	25.5	0.7	0.4	5.8	83.2
	24	14.9	0.3	0.0	1.6	0.1	0.3	17.2
7	1	52.0	17.3	0.0	18.3	1.5	1.0	90.1
	24	1.1	14.5	0.0	26.6	0.9	1.8	44.9
9	1	0.0	8.8	51.8	13.7	0.7	16.0	91.0
	24	0.0	36.0	3.7	21.5	0.9	4.5	66.6

(a) These unknowns comprised of several peaks all < 10 % AR

The mean overall recoveries at *p*H 5, 7 and 9 were 83.2 – 91.0 % AR at 1 hour, but decreased to 17.2 – 66.6 % AR after 24 hours. Addition of barium chloride to the solution at *p*H 7 and *p*H 9 indicated that $^{14}CO_2$, the

terminal product of degradation, was formed in substantial amounts. However, the $^{14}CO_2$ was mainly in solution (≈ 15 % AR) and only small amounts were evolved into the volatile traps (≈ 13 % AR). It was postulated that the short exposure times contributed to the low levels of diffusion observed. Air sampling of the head-space above the solution afforded only about 1 % AR. Losses for the *p*H 5 hydrolysis were most severe, probably due to the presence of free $^{14}CO_2$, whereas at higher *p*H sodium carbonate formation helps retain $^{14}CO_2$ in solution. However, after one hour of exposure, half-lives at *p*H 5 and pH 7 were reached, and surpassed for *p*H 9, and at that point 83 to 91 % AR was still in solution.

Folpet was recovered as 47 to 52 % AR at 1 hour at *p*H 5 and *p*H 7, but was not found at *p*H 9 (0 % AR) as anticipated from its very short half-life at this *p*H. At 24 hours, only low levels of folpet were observed at *p*H 5 (14.9 % AR) and *p*H 7 (1.1 % AR).

Two unknown compounds, Unknown 1 and Unknown 2, were detected in solution. Unknown 2 was noted at high levels (25 % AR at *p*H 5, 52 % AR at *p*H 9) at 1 hour. Both unknowns must contain the functions derived from the thio(trichloromethyl)group since the hydrolysis of [carbonyl-^{14}C]-folpet did not produce these metabolites (Anonymous, 1988b).

Allowing the 1 hour sample at *p*H 9 to stand in a freezer for one week resulted in the disappearance of Unknown 2. The corresponding radiocarbon was also lost from solution, and some Unknown 1 volatilised or decomposed during this period. Reaction at *p*H 9 for 24 hours showed a significant decrease in Unknown 2 relative to the 1 hour sample (51.8 % AR to 3.7 % AR), and at *p*H 9 Unknown 1 was the major product. Since ≈ 30 % AR was lost between 1 and 24 hours and Unknown 2 was ≈ 30 % AR after 1 hour, it was suggested that Unknown 2 was the major source of volatiles.

When the hydrolysate solution at *p*H 9 was refrigerated for one week, the level of Unknown 1 remained unchanged, but upon acidification Unknown 1 decreased substantially.

At *p*H 7, only Unknown 1 was observed at 1 hour and after 24 hours was still the major component. At *p*H 5, Unknown 2 was the major degradate at 1 hour, but degraded after 24 hours.

Based on these results, it was postulated that Unknown 1 was the primary degradate, probably the trichloromethylsulfenic acid salt, which on changes of *p*H and exposure time, degrade to the volatile trichloromethylmercaptan (Unknown 2) which in turn may degrade to thiophosgene ($CSCl_2$), carbon oxysulphide (COS) and ultimately CO_2.

Comments (RMS AT):

- As already indicated by RMS Italy for first Annex I listening, mass balance of the two sampling points (1 and 24 hrs) was partly far below 90 % AR, particularly for the 24 hrs sample. Thus there is indeed some uncertainty about the maximum occurrence of unknown metabolite fractions formed in the study. Although this may invalidate the study, the RMS AT would like to highlight that this study is the only study available with trichloromethyl labelled folpet in aquatic systems. Based on the somewhat limited information available from this study, significant formation of degradation products deriving from the thio(trichloromethyl) sidechain of folpet (e.g. trichloromethylsulfenic acid or trichloromethylmercaptan as postulated in this study) in other aquatic systems cannot be excluded.

10.1.4 Other convincing scientific evidence

10.1.4.1 Field investigations and monitoring data (if relevant for C&L)

No data

10.1.4.2 Inherent and enhanced ready biodegradability tests

No data

10.1.4.3 Water, water-sediment and soil degradation data (including simulation studies)

Under conditions of **aerobic mineralisation in surface water** (two studies) folpet dissipates with *DT50* values < 1 hr (pH ~ 8). Similar to hydrolysis, the metabolites phthalimide (max. 51.6 % AR), phthalamic acid (max. 73.7 % AR) and phthalic acid (max. 76.9 % AR) are formed in significant amounts. Dissipation of phthalimide was rapid as well with *DT50* values in a range from 0.4 – 1.2 days. Mineralisation to CO_2 was significant reaching 68.2 % AR after 60 days in one of the low dose experiments.

Folpet rapidly degraded in two **water/sediment studies** with *DT50* values in the range of 0.01 to 0.02 days in the total systems. Folpet was extensively metabolised to phthalimide (max. 31.8 % AR), phthalamic acid (max. 42.7 % AR), phthalic acid (max. 41.3 %), 2-cyanobenzoic acid (41.6 % AR), benzamide (max. 10.2 % AR) and finally to carbon dioxide (max. 80 % AR after 99 days). Levels of carbon dioxide from mineralisation increased rapidly throughout the period of the studies. The level of unextractable residue in the sediment reached a maximum (26.3 % AR) by 14 days declining thereafter.

It is noted that the fate of the thio(trichloromethyl) side chain of folpet in viable aquatic systems in largely unknown as there is no study available with trichloromethyl labelled folpet in such systems.

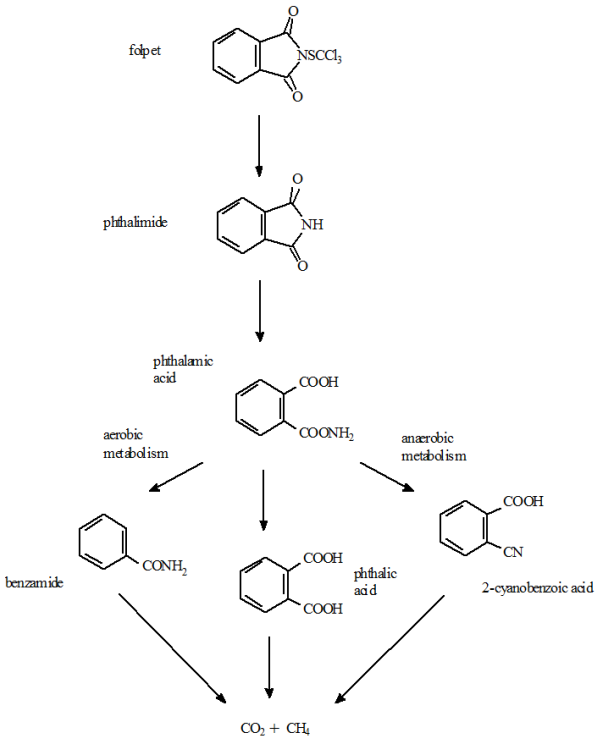

Figure 6: Proposed route of degradation of phenyl labelled folpet in aquatic systems

Table 58: Summary on maximum occurrence (% AR) of identified metabolites in aquatic laboratory studies conducted with folpet

Compound	Aquatic hydrolysis	Aquatic photolysis	Aerobic mineralisation in surface water (low/high dose)	Water/sediment		
				Water phase	Sediment phase	Total system
Folpet	na	na	na	na	0.1	na
Phthalimide	91.5	56.3	51.6 / 42.0	31.3	5.9	31.8

Phthalamic acid	2.5	2.6[(a)]	60.7 / 73.7	42.7	1.7	42.7
Phthalic acid	78.4	2.6[(a)]	54.4 / 76.9	37.5	3.8	41.3
2-cyanobenzoic acid	no	no	no	41.6	0.8	41.6
Benzamide	no	no	no	10.2	0.6	10.2
Trichloromethylsulfenic acid	36.0[(b)]	ni	ni	ni	ni	ni
Trichloromethylmercaptan	51.8[(c)]	ni	ni	ni	ni	ni

(a) Sum of phthalamic and phthalic acid
(b) Indicatively assign to unknown metabolite fraction 'Unknown 1'
(c) Indicatively assign to unknown metabolite fraction 'Unknown 2'
na denotes not applicable
no denotes not observed
ni denotes not investigated (no study available with trichloromethyl labelled folpet)

The rate of degradation of folpet and its metabolites in aquatic systems has been assessed in laboratory studies and is summarised in the tables below.

Table 59: Summary on degradation of folpet at conditions of aquatic hydrolysis

***p*H**	**Temp**	***DT50***	***DT90***	**χ^2 err. (%)**	**Kinetic model**	**Reference**
4	25 °C	2.9 hrs	9.8 hrs	1.7	SFO	Anonymous, 1988b
7	25 °C	1.3 hrs	4.4 hrs	10.9	SFO	
9	25 °C	59 sec	196 sec	11.5	SFO	

Table 60: Summary on degradation of phthalimide at conditions of aquatic hydrolysis

***p*H**	**Temp (°C)**	***DT50* (days)**	***DT90* (days)**	**χ^2 err. (%)**	**Kinetic model**	**Reference**
4	25 °C	125	415	0.3	SFO	Anonymous, 2015i
7	25 °C	2.3	7.6	0.4	SFO	
4	25 °C	141	468	0.6	SFO	Anonymous, 2016f
7	25 °C	2.3	7.8	0.6	SFO	
9	25 °C	0.05	0.2	4.4	SFO	

Table 61: Summary on degradation of folpet in aerobic water

Water	***p*H**	**Application**	***DT50* water**	***DT90* water**	**χ^2 err. (%)**	**Kinetic model**	**Reference**
Rhineland-Palatinate	8.2	Low dose	< 1 hr	< 1 hr	na	SFO	Anonymous, 2016g
		High dose	< 1 hr	< 1 hr	na	SFO	
Carsington	6.8	Low dose	0.3 hrs	1.1 hrs	7.0	SFO	Anonymous, 2015j
		High dose	0.3 hrs	1.0 hrs	3.2	SFO	

na denotes not applicable

Table 62: Summary on degradation of phthalimide in aerobic water

Water	***p*H**	**Application**	***DT50* water (d)**	***DT90* water (d)**	**χ^2 err. (%)**	**Kinetic model**	**Reference**
Rhineland-Palatinate	8.2	Low dose	1.2	4.1	9.3	$P_{SFO} \rightarrow M_{SFO}$	Anonymous, 2016g
		High dose	0.8	2.7	5.2	$P_{SFO} \rightarrow M_{SFO}$	
Carsington	6.8	Low dose	0.4	1.4	13.2	$P_{SFO} \rightarrow M_{SFO}$	Anonymous, 2015j
		High dose	0.7	2.4	12.6	$P_{SFO} \rightarrow M_{SFO}$	

na denotes not applicable

Table 63: Summary on degradation of folpet in water/sediment (modelling & persistence endpoints)

Water / sediment system	***p*H[(a)] water / sediment**	***DegT50* system (d)**	***DegT90* system (d)**	**Kinetic model**	***DisT50* water (d)**	**Kinetic model**	***DisT50* sed. (d)**	**Kinetic model**	**Reference**
Row Pond	8.1/6.8	0.02	0.05	SFO	-	-	-	-	Anonymous, 1999
Emperor Lake	7.1/5.9	0.02	0.07	SFO	-	-	-	-	
Row Pond	8.8/6.0	0.01	0.04	SFO	-	-	-	-	Anonymous, 2007c
Emperor Lake	7.1/5.9	0.04	0.10	SFO	-	-	-	-	
Geometric mean (n = 4)		**0.02**	**0.06**	**SFO**	-	-	-	-	-

(a) Water in sediment

Table 64: Summary on degradation of phthalimide in water/sediment (modelling & persistence endpoints)

Water / sediment system	*p*H[(a)] water / sediment	*DegT50* system (d)	*DegT90* system (d)	Kinetic model	*DisT50* water (d)	Kinetic model	*DisT50* sed. (d)	Kinetic model	Reference
Row Pond	8.1/6.8	0.4	1.4	SFO[(b)]	-	-	-	-	Anonymous, 1999
Emperor Lake	7.1/5.9	0.5	1.6	SFO[(b)]	-	-	-	-	
Row Pond	8.8/6.0	0.8	2.7	SFO[(b)]	-	-	-	-	Anonymous, 2007c
Emperor Lake	7.1/5.9	1.6	5.3	SFO[(b)]	-	-	-	-	
Geometric mean (n = 4)		**0.7**	**2.4**	**SFO**	-	-	-	-	-

(a) Water in sediment
(b) Decline fit

Table 65: Summary on degradation of phthalamic acid in water/sediment (modelling & persistence endpoints)

Water / sediment system	*p*H[(a)] water / sediment	*DegT50* system (d)	*DegT90* system (d)	Kinetic model	*DisT50* water (d)	Kinetic model	*DisT50* sed. (d)	Kinetic model	Reference
Row Pond	8.1/6.8	2.8	9.4	SFO[(b)]	-	-	-	-	Anonymous, 1999
Emperor Lake	7.1/5.9	6.0	19.9	SFO[(b)]	-	-	-	-	
Row Pond	8.8/6.0	12.0	39.8	SFO[(b)]	-	-	-	-	Anonymous,
Emperor Lake	7.1/5.9	13.5	44.8	SFO[(b)]	-	-	-	-	2007c
Geometric mean (n = 4)		**7.2**	**24.0**	**SFO**	-	-	-	-	-

(a) Water in sediment
(b) Decline fit

Table 66: Summary on degradation of phthalic acid in water/sediment (modelling and persistence endpoints)

Water / sediment system	*p*H[(a)] water / sediment	*DegT50* system (d)	*DegT90* system (d)	Kinetic model	*DisT50* water (d)	Kinetic model	*DisT50* sed. (d)	Kinetic model	Reference
Row Pond	8.1/6.8	0.4	1.4	SFO[(b)]	-	-	-	-	Anonymous, 1999
Emperor Lake	7.1/5.9	5.6	18.5	SFO[(b)]	-	-	-	-	
Row Pond	8.8/6.0	4.4	16.0	SFO[(b)]	-	-	-	-	Anonymous, 2007c
Emperor Lake	7.1/5.9	6.1	20.3	SFO[(b)]	-	-	-	-	
Geometric mean (n = 4)		**2.8**	**9.6**	**SFO**	-	-	-	-	-

(a) Water in sediment
(b) Decline fit

Table 67: Summary on degradation of 2-cyanobenzoic acid in water/sediment (modelling and persistence endpoints)

Water / sediment system	*p*H[(a)] water / sediment	*DegT50* system (d)	*DegT90* system (d)	Kinetic model	*DisT50* water (d)	Kinetic model	*DisT50* sed. (d)	Kinetic model	Reference
Row Pond	8.1/6.8	0.2	0.6	SFO[(b)]	-	-	-	-	Anonymous, 1999
Emperor Lake	7.1/5.9	0.6	1.8	SFO[(b)]	-	-	-	-	
Row Pond	8.8/6.0	4.2	16.0	SFO[(b)]	-	-	-	-	Anonymous, 2007c
Emperor Lake	7.1/5.9	22.7	75.4	SFO[(b)]	-	-	-	-	
Geometric mean (n = 4)		**1.8**	**6.0**	**SFO**	-	-	-	-	-

(a) Water in sediment
(b) Decline fit

Table 68: Summary on degradation of benzamide in water/sediment (modelling and persistence endpoints)

Water / sediment system	*p*H[(a)] water / sediment	*DegT50* system (d)	*DegT90* system (d)	Kinetic model	*DisT50* water (d)	Kinetic model	*DisT50* sed. (d)	Kinetic model	Reference
Row Pond	8.1/6.8	0.6	2.1	SFO[(b)]	-	-	-	-	Anonymous, 1999
Emperor Lake	7.1/5.9	nr	-	-	-	-	-	-	
Row Pond	8.8/6.0	no	-	-	-	-	-	-	Anonymous, 2007c
Emperor Lake	7.1/5.9	no	-	-	-	-	-	-	
Worst case (n = 1)		**0.6**	**2.1**	**SFO**	-	-	-	-	-

(a) Water in sediment
(b) Decline fit
no denoted not observed
nr denotes no reliable fit

The **rate of degradation/dissipation** of folpet and its metabolites phthalimide, phthalamic acid and phthalic acid **in aerobic soil** has been assessed **in laboratory studies** and is summarised in the tables below.

Table 69: Summary of aerobic degradation rates for folpet (persistence endpoints) – laboratory studies

Soil name	Soil type (USDA)	pH ($CaCl_2$)	T (°C)	Water content	*DegT50* (d)	*DegT 90* (d)	χ^2 err (%)	Kinetic model	Ref.
Valdosta	Sandy loam	5.4[a]	25	75-80% FC	2.3	24.2	13.1	HS[b]	Anonymous, 1991a
Warsop	Loamy sand	4.3	20	40% MWHC	10.0	33.2	4.2	SFO	Anonymous, 2007a
Farditch	Silt loam	5.9	20	40% MWHC	4.8	15.9	5.2	SFO	
			10	40% MWHC	16.8	55.8	3.3	SFO	
Chapel hill	Clay/clay loam	7.3	20	40% MWHC	1.4	4.6	5.8	SFO	
Calke	Sandy loam	5.1	20	pF2-2.5	2.1	18.0	3.1	DFOP	Anonymous, 2015b
Empingham	Clay	7.4	20	pF2-2.5	1.8	9.3	5.1	FOMC	
Brierlow	Loam/silt loam	5.3	20	pF2-2.5	2.1	14.2	14.8	FOMC	
Ingleby	Loamy sand	4.0	20	pF2-2.5	20.6	99.8	6.6	DFOP	
				Worst case	**20.6**	**99.8**		**DFOP[c]**	

(a) Matrix unknown
(b) HS break point fixed to 6.43 days
(c) DFOP-k_1 = 0.652 d^{-1}, DFOP-k_2 0.020 d^{-1}, g = 0.241

Table 70: Summary on soil temperature (f_T) and soil moisture correction factors (f_{WC}) to obtain reference conditions (20 °C, pF2)

Soil name	Soil type (USDA)	pH ($CaCl_2$)	T (°C)	Intended study WC	f_T[e]	MWHC %	Study WC	WC at pF2	f_{WC}[f]	$f_T \times f_{WC}$	Ref.
Valdosta	Sandy loam	5.4[a]	25	75-80% FC	1.61	27[b]	9.4	12.6[c]	0.82	1.31	Anonymous, 1991a
Warsop	Loamy sand	4.3	20	40% MWHC	1.00	39.5	15.8	9.4	1.00	1.00	Anonymous, 2007a
Farditch	Silt loam	5.9	20	40% MWHC	1.00	96.6	38.6	37.6	1.00	1.00	
			10	40% MWHC	0.39	96.6	38.6	37.6	1.00	0.39	
Chapel Hill	Clay/clay loam	7.3	20	40% MWHC	1.00	90.9	36.4	46.0	0.85	0.85	
Calke	Sandy loam	5.1	20	pF2-2.5	1.00	Study conducted at pF2-2.5			1.00	1.00	Anonymous, 2015b
Empingham	Clay	7.4	20	pF2-2.5	1.00				1.00	1.00	
Brierlow	Loam/silt loam	5.3	20	pF2-2.5	1.00				1.00	1.00	
Ingleby	Loamy sand	4.0	20	pF2-2.5	1.00				1.00	1.00	

(a) Matrix unknown
(b) FOCUS default MWHC for this soil type
(c) Water content at pF2.5 (from study report)
(d) FOCUS default water content at pF2 for this soil type
(e) $f_T = Q_{10}^{[(T_{act} - T_{ref})/10]}$ with $Q_{10} = 2.58$
(f) $f_{WC} = (WC_{study} / WC_{pF2})^{0.7}$

Table 71: Summary of aerobic degradation rates for folpet (modelling endpoints) – laboratory studies

Soil name	Soil type (USDA)	pH ($CaCl_2$)	T (°C)	Water content	*DegT50* (d)	*DegT 90* (d)	*DegT50* (d) 20 °C, pF2	χ^2 err (%)	Kinetic model	Ref.
Valdosta	Sandy loam	5.4[a]	25	75-80% FC	2.3	24.2	9.6[b]	13.1	HS[c]	Anonymous, 1991a
Warsop	Loamy sand	4.3	20	40% MWHC	10.0	33.2	10.0	4.2	SFO	Anonymous, 2007a
Farditch	Silt loam	5.9	20	40% MWHC	4.8	15.9	4.8	5.2	SFO	
			10	40% MWHC	16.8	55.8	nc	3.3	SFO	
Chapel hill	Clay/clay loam	7.3	20	40% MWHC	1.4	4.6	1.2	5.8	SFO	
Calke	Sandy loam	5.1	20	pF2-2.5	2.2	25.7	7.7[d]	6.1	FOMC	Anonymous, 2015b
Empingham	Clay	7.4	20	pF2-2.5	1.8	9.3	2.8[d]	5.1	FOMC	
Brierlow	Loam/silt loam	5.3	20	pF2-2.5	2.1	14.2	4.3[d]	14.8	FOMC	
Ingleby	Loamy sand	4.0	20	pF2-2.5	20.6	99.8	34.1[e]	6.6	DFOP	
				Geometric mean (*p*H < 6, 20 °C studies, n = 6)			**9.0**			
				Geometric mean (*p*H ≥ 6, 20 °C studies, n = 2)			**1.8**			
				pH-dependency: y/n			y[f]			

(a) Matrix unknown
(b) HS-*DegT90* divided by 3.32
(c) HS break point fixed to 6.43 days
(d) FOMC-$DegT_{90}$ divided by 3.32

(e) Slow phase DFOP rate (k_2)
(f) On basis of Kendall's tau-b test (refer to text below)
nc denotes not calculated

Table 72: Summary of aerobic degradation rates for phthalimide (persistence & modelling endpoints) – laboratory studies

Soil name	Soil type (USDA)	pH (Ca-Cl2)	T (°C)	Water content	*DegT50* (d)	*DegT90* (d)	*ff*[(b)]	*DegT50* (d) 20 °C, pF2	χ^2 (%)	Kinetic model	Ref.
Valdosta	Sandy loam	5.4[(a)]	25	75-80% FC	5.6	18.5	1.00	7.3[(g)]	19.6	H-S	Anonymous, 1991a
Warsop	Loamy Sand	4.3	20	40% MWHC	3.2	10.4	na	3.2	20.5	SFO[(c)]	Anonymous, 2007a
Farditch	Silt loam	5.9	20	40% MWHC	4.5	15.1	na	4.5	31.7	SFO[(c)]	
			10	40% MWHC	0.9	2.9	1.00	nc	29.5	S-S	
Chapel Hill	Clay/clay loam	7.3	20	40% MWHC	0.4	1.3	0.21	0.3	18.2	S-S	
Calke	Sandy loam	5.1	20	pF2-2.5	1.3	4.2	0.94	1.3	29.6	F-S	Anonymous, 2015b
Empingham	Clay	7.4	20	pF2-2.5	2.5	8.2	0.53	2.5	12.0	F-S	
Brierlow	Loam/silt loam	5.3	20	pF2-2.5	1.1	3.8	0.98	1.1	27.0	F-S	
Ingleby	Loamy sand	4.0	20	pF2-2.5	4.8	15.8	0.72	4.8	17.3	D-S	
Calke	Sand	5.2	20	pF2	0.4	1.2	na	0.4	4.8	SFO[(d)]	Anonymous, 2016d
Elmton	Sandy clay loam	7.2	20	pF2	0.1	0.3	na	0.1	2.5	SFO[(d)]	
Ingleby	Sand	4.6	20	pF2	1.1	3.7	na	1.1	6.2	SFO[(d)]	
				Arithmetic mean (20 °C studies, n = 6)			**0.73**	**-**			
				Geometric mean (20 °C studies, n = 11)			**-**	**1.3**			
				pH-dependency: y/n			-	n[(f)]			

(a) Matrix unknown
(b) From parent
(c) Decline fit starting from maximum occurrence
(d) Phthalimide applied
(f) On basis of Kendall's tau-b test (refer to text below)
(g) *DegT50* is 9.0 days if normalized to 20 °C without moisture correction; worst-case persistence endpoint used for soil exposure
nc denotes not calculated
S-S denotes $P_{SFO} \rightarrow M_{SFO}$ pathway fit (folpet applied)
F-S denotes $P_{FOMC} \rightarrow M_{SFO}$ pathway fit (folpet applied)
D-S denotes $P_{DFOP} \rightarrow M_{SFO}$ pathway fit (folpet applied)
H-S denotes $P_{HS} \rightarrow M_{SFO}$ pathway fit (folpet applied, HS breakpoint fixed to 6.43 days)

Table 73: Summary of aerobic degradation rates for phthalamic acid (persistence & modelling endpoints) – laboratory studies

Soil name	Soil type (USDA)	pH (Ca-Cl2)	T (°C)	Water content	*DegT50* (d)	*DegT90* (d)	*ff*[(b)]	*DegT50* (d) 20 °C, pF2	χ^2 err (%)	Kinetic model	Ref.
Valdosta	Sandy loam	5.4[(a)]	25	75-80% FC				no			Anonymous, 1991a
Warsop	Loamy Sand	4.3	20	40% MWHC				nr			Anonymous, 2007a
Farditch	Silt loam	5.9	20	40% MWHC				no			
		5.9	10	40% MWHC				nr			
Chapel hill	Clay/clay loam	7.3	20	40% MWHC				nr			
Calke	Sandy loam	5.1	20	pF2-2.5				no			Anonymous, 2015b
Empingham	Clay	7.4	20	pF2-2.5				no			
Brierlow	Loam/silt loam	5.3	20	pF2-2.5				no			
Ingleby	Loamy sand	4.0	20	pF2-2.5				no			
Kenslow	Loam	5.0	20	pF2-2.5	1.6	5.4	na	1.6	4.9	SFO[(d)]	Anonymous, 2015d
Hareby	Clay	7.5	20	pF2-2.5	0.8	2.7	na	0.8	12.8	SFO[(d)]	
Calke	Sand	5.2	20	pF2	1.7	5.7	0.12	1.7	23.8	S-S[(e)]	Anonymous, 2016d
Elmton	Sandy clay loam	7.2	20	pF2	0.4	1.3	1.00	0.4	13.9	S-S[(e)]	
Ingleby	Sand	4.6	20	pF2	2.6	8.8	0.08	2.6	30.1	S-S[(e)]	
				Arithmetic mean (20 °C studies, n = 3)			**0.40**	-			
				Geometric mean (20 °C studies, n = 5)			-	**1.2**			
				pH-dependency: y/n			-	n[(f)]			

(a) Matrix unknown
(b) From phthalimide
(c) Decline fit from maximum occurrence
(d) Phthalamic acid applied
(e) Phthalimide applied
(f) On basis of Kendall's tau-b test (refer to text below)

na denotes not applicable
no denotes not observed
nr denotes no reliable fit (sporadic findings << 5 % AR)

Table 74: Summary of aerobic degradation rates for phthalic acid (persistence & modelling endpoints) – laboratory studies

Soil name	Soil type (USDA)	pH ($CaCl_2$)	T (°C)	Water content	*DegT50* (d)	*DegT90* (d)	*ff*[(b)]	*DegT50* (d) 20 °C, pF2	χ^2 err (%)	Kinetic model	Ref.
Valdosta	Sandy loam	5.4[(a)]	25	75-80% FC	nr						Anonymous, 1991a
Warsop	Loamy sand	4.3	20	40% MWHC	Phthalic acid co-eluting with benzamide						Anonymous, 2007a
Farditch	Silt loam	5.9	20	40% MWHC							
			10	40% MWHC							
Chapel hill	Clay/clay loam	7.3	20	40% MWHC							
Calke	Sandy loam	5.1	20	pF2-2.5	nr						Anonymous, 2015b
Empingham	Clay	7.4	20	pF2-2.5	nr						
Brierlow	Loam/silt loam	5.3	20	pF2-2.5	nr						
Ingleby	Loamy sand	4.0	20	pF2-2.5	nr						
Kenslow	Loam	5.0	20	pF2-2.5	1.8	6.0	0.84	1.8	8.0	S-S	Anonymous, 2015e
Hareby	Clay	7.5	20	pF2-2.5	0.3	1.1	0.46	0.3	12.5	S-S	
Bruch West	Sandy loam	7.4	20	40% MWHC	0.1	0.3	na	0.1	1.7	SFO[(d)]	Anonymous, 2012a
Li10	Sandy loam	7.3	20	40% MWHC	0.1	0.2	na	0.1	0.9	SFO[(d)]	
LUFA 5M	Loamy sand	6.3	20	40% MWHC	0.3	0.9	na	0.2	3.8	SFO[(d)]	
Calke	Sand	5.2	20	pF2	1.1	3.8	na	1.1	5.0	SFO[(c)]	Anonymous, 2016d
Elmton	Sandy clay loam	7.2	20	pF2	0.2	0.6	1.00	0.2	9.2	S-S-S[(e)]	
			Arithmetic mean (20 °C studies, n = 3)				**0.77**	**-**			
			Geometric mean (20 °C studies, n = 5)				-	**0.3**			
			pH-dependency: y/n				**-**	n[(f)]			

(a) Matrix unknown
(b) From phthalamic acid
(c) Decline fit from maximum occurrence
(d) Phthalic acid applied
(e) Phthalimide applied (pathway fit)
(f) On basis of Kendall's tau-b test (refer to text below)
S-S denotes $P_{SFO} \rightarrow M_{SFO}$ pathway fit (phthalamic acid applied)
na denotes not applicable
nc denotes not calculated
no denotes not observed
nr denotes no reliable fit (sporadic findings << 5 % AR)

In order to address *p*H dependent degradation in soil for folpet the RMS AT suggests dividing the degradation dataset of folpet into 2 sections, one with soil *p*H values < 6 and one with *p*H values ≥ 6 (measured in $CaCl_2$). This allows calculating reliable geomean degradation rates for folpet under acidic and neutral to alkaline conditions, respectively, to be used in the exposure assessment.

The **rate of degradation** of folpet in **anaerobic soil** is rapid as well, but slower than in aerobic soil (*DegT50* of 15.8 days).

Studies on **field soil dissipation** of folpet and metabolites are not required since they are not triggered based on the result from aerobic soil laboratory studies with the active substance or the metabolites. In the laboratory studies, *DegT50* and *DegT90* values at 20 °C and pF2 for folpet and metabolites are far below the respective trigger values of 60 and 200 days. However, for first Annex I inclusion 3 field studies with folpet conducted in the US (applied to bare soil or citrus) were submitted. These studies confirm results already observed in the laboratory and do not alter the exposure assessment.

10.1.4.4 Photochemical degradation

In an **aquatic photolysis** study (Anonymous, 1989b) carbonyl labelled folpet was exposed to natural sunlight and UV light (350 nm) in a buffer solution of *p*H 3. The RMS AT notes that this study is not necessarily fully in line with current guidance (OECD 316) recommending exposure to a xenon arc lamp. However, exposure to natural sunlight is considered acceptable as well by OECD 316. After 8 hrs folpet has decreased to 34.2 %

and 38.4 % under sunlight irradiated and dark conditions, respectively. Based on the 8-HAT samples no significant differences in metabolite patterns were observed. Based on this study, the overall impact of irradiation on the dissipation of folpet in water, which is largely governed by hydrolysis, is considered negligible.

No quantum yield was determined for folpet.

Soil photolysis is not considered to significantly contribute to the overall dissipation/degradation of folpet in soil.

10.2 Environmental transformation of metals or inorganic metals compounds

Not applicable

10.2.1 Summary of data/information on environmental transformation

Not applicable

10.3 Environmental fate and other relevant information

Adsorption in soil of folpet, phthalimide, phthalamic acid and phthalic acid has been assessed in OECD 106 batch studies and is summarised in the tables below.

Table 75: Summary on soil adsorption for folpet

Soil name	Soil type (USDA)	OC (%)	pH ($CaCl_2$)	K_f (L/kg)	K_{foc} (L/kg)	1/*n* (-)	Reference
RefeSol 03-G	Clay loam	4.1	5.8	33.7	821	0.98	Anonymous, 2016e
Ingleby Acid	Sandy loam	3.1	3.7	27.9	899	0.98	
Kenslow	Loam	4.0	4.9	33.3	832	1.00	
Warsop	Loamy sand	1.7	4.2	15.5	910	0.98	
			Arithmetic mean (n = 4)		-	**0.98**	-
			Geometric mean (n = 4)		**865**	-	-
			pH-dependency: y/n		n[(a)]	-	-

(a) On basis of Kendall's tau-b test

Table 76: Summary on soil adsorption for phthalimide

Soil name	Soil type (USDA)	OC (%)	pH ($CaCl_2$)	K_f (L/kg)	K_{foc} (L/kg)	1/*n* (-)	Reference
EURO-Soil 1	Clay[(a)]	1.30	5.1	5.0	385	0.89	Anonymous, 2000b
EURO-Soil 3	Loam[(a)]	3.45	5.2	2.5	72	0.88	
EURO-Soil 5	Loamy sand[(a)]	9.25	3.2	15.6	169	0.84	
LUFA 2.1[(b)]	*Sand[(a)]*	*0.56*	*6.0*	*1.2*	*214*	*0.52*	
LUFA 2.2[(b)]	*Loamy sand[(a)]*	*2.19*	*5.8*	*2.7*	*123*	*0.58*	
Hareby	Clay	1.9	7.6	1.07	56	0.93	Anonymous, 2015f
Quilen	Loam	2.6	7.1	7.17	276	0.85	
South Witham	Sandy clay loam	3.4	7.4	1.97	58	0.91	
			Arithmetic mean (n = 6)		-	**0.88**	-
			Geometric mean (n = 6)		**127**	-	-
			pH-dependency: y/n		n[(c)]	-	-

(a) Not reported according to which classification
(b) Results for these soils were excluded from the calculation of mean values since the results are not considered to be reliable due to the low Freundlich exponents
(c) On basis of Kendall's tau-b test

Table 77: Summary on soil adsorption for phthalamic acid

Soil name	Soil type (USDA)	OC (%)	pH ($CaCl_2$)	K_f (L/kg)	K_{foc} (L/kg)	1/*n* (-)	Reference
Kenslow	Loam	3.2	5.0	1.3	40.6[(a)]	0.97	Anonymous, 2015g
Hareby	Clay	1.9	7.6	0.09	4.8	0.95	
Quilen	Loam	2.6	7.1	0.05	1.8	0.88	

	Arithmetic mean (pH > 5.0, n = 2)	-	**0.92**	-
	Geometric mean (pH > 5.0, n = 2)	**2.9**	-	-
	pH-dependency: y/n	n[(b)]	-	-

(a) Probably an outlier (excluded from averaging)
(b) On basis of Kendall's tau-b test (including all values)

Table 78: Summary on soil adsorption for phthalic acid

Soil name	Soil type (USDA)	OC (%)	pH ($CaCl_2$)	K_f (L/kg)	K_{foc} (L/kg)	1/*n* (-)	Reference
Kenslow	Loam	3.2	5.0	22.5	702[(a)]	0.91	Anonymous, 2015h
Hareby	Clay	1.9	7.6	0.21	11.1	0.98	
Quilen	Loam	2.6	7.1	0.17	6.5	0.97	
LUFA 2.1	Sand	0.52	5.2	0.18	34	0.93	Anonymous, 2011
Li 10	Loamy sand	0.88	6.0	0.08	9	0.85	
Nierswalde "Wildacker"	Silt loam	1.63	6.5	0.72	44	0.89	
Große Erde	Loamy sand	0.92	6.8	0.03	3	0.97	
Fiorentini Poggio Renatico	Silt loam	1.83	7.5	0.08	4	0.96	
	Arithmetic mean (pH > 5.0, n = 7)			-	**0.94**	-	
	Geometric mean (pH > 5.0, n = 7)			**10.2**	-	-	
	pH-dependency: y/n			n[(b)]	-	-	

(a) Probably an outlier (excluded from averaging)
(b) On basis of Kendall's tau-b test (including all values)

Soil column leaching experiments with phenyl labelled folpet were conducted within two studies. As folpet degrades rapidly in soil, the experiments were conducted using aged folpet residues. In the two studies, only minor amounts of radioactivity were found in the leachates (up to 2.7 %) which could either not be further characterised or mainly consist of the major soil metabolite phthalic acid. In the soil columns, the major part of the radioactivity remained in the upper parts. Overall, aged folpet and its metabolites are unlikely to significantly leach through soil.

10.4 Bioaccumulation

Table 79: Summary of relevant information on bioaccumulation

Method	Test substance	Results	Remarks	Reference
USEPA Test Method CG-1400 (shake flask)	Folpet Batch #: S/31 (512) Puritiy: 98.8%	log P_{OW} = 3.107 (25°C)	Acceptable EU agreed endpoint GLP: No	Anonymous (1987c)
Fish bioaccumulation test US EPA 72-6	[^{14}C]-Folpet Batch #:078F9213 Purity: > 98%	*Lepomis macrochirus* $BCF_{kinetic}$= 56 (measured) CT_{50} = 0.63 d Depuration after 14 days greater than 93%	Acceptable EU agreed endpoint GLP: Yes	Anonymous (1989a)
EEC A.8 OECD 107 (shake flask)	Phthalimide Batch #: 19189959 Purity: 99.9%	pH 4 log P_{OW} = 0.62 (20°C) pH 7 log P_{OW} = 0.62 (20°C) pH 10 log P_{OW} = -0.33 (20°C)	Acceptable GLP: Yes	Anonymous (2015a)
EEC A.8 OECD 107 (shake flask)	Phthalimide Batch #: 516-032-00 Purity: 99.1%	pH 7 log P_{OW} = 0.74 (20°C)	Acceptable GLP: Yes	Anonymous (2015b)
EEC A.8 OECD 107 (shake flask)	Phthalic acid Batch #: BCBH4681V Purity: 99.8%	pH 3.6 log P_{OW} = -0.05 (20°C) pH 5 log P_{OW} = -1.28 (20°C)	Acceptable GLP: Yes	Anonymous (2015c)
EEC A.8 OECD 107 (shake flask)	Phthalic acid Batch #: BCBM7315V Purity: 99.6%	pH 3.3 log P_{OW} = 0.27 (21.7°C)	Acceptable GLP: Yes	Anonymous (2015c)
EEC A.8 OECD 107 (shake flask)	Phthalamic acid Batch #: 10151680 Purity: 99%	pH 3.5 log P_{OW} = -0.71 (20°C) pH 6.8 log P_{OW} = -2.9 (20°C)	Acceptable GLP: Yes	Anonymous (2015b)

Method	Test substance	Results	Remarks	Reference
EEC A.8 OECD 107 (shake flask)	Phthalamic acid Batch #: MKBP5498V Purity: 98.2%	log P_{OW} < 0 (20°C)	Acceptable GLP: Yes	Anonymous (2015d)
EEC A.8 OECD 117 (HPLC)	2-cyanobenzoic acid Batch #: BGBB6262V Purity: 98.6%	pH 2.4 log P_{OW} = 0.89 (20°C)	Acceptable GLP: Yes	Anonymous (2015)
EEC A.8 OECD 117 (HPLC)	2-cyanobenzoic acid Batch #: BGBB6262V Purity: 98.6%	log P_{OW} < 0 (20°C)	Acceptable GLP: Yes	Anonymous (2015e)
EEC A.8 OECD 117 (HPLC)	Benzamide Batch #: MKBR3448V Purity: 99.8%	log P_{OW} = 0.21 (20°C)	Acceptable GLP: Yes	Anonymous (2015f)

10.4.1 Estimated bioaccumulation

No estimated data on bioaccumulation and partition coefficients are available.

10.4.2 Measured partition coefficient and bioaccumulation test data

The octanol-water-partitioning coefficient (log P_{OW}) for the active substance folpet and its relevant metabolites were experimentally determined. The measured log P_{OW} values were considered acceptable. The log P_{OW} of the active substance folpet was determined to be 3.107. The log P_{OW} determined for the metabolites was clearly below 1.

Considering that the log P_{OW} of folpet is greater than 3 a fish bioconcentrations study (Anonymous, 1989a) was conducted in which the bioconcentrations fator and the bioaccumulation potential of [^{14}C]-labelled folpet were measured in bluegill (*Lepomis macrochirus*). The steady-state bioconcentration factor (BCF) in whole fish was 56. Uptake residues were rapidly elimated from whole fish within the 14-day depuration phase.

However, during the peer-review of the active substance the validity and reliability of the fish bioconcentration study (Burgess, 1989a) was challenged because of several deficiencies identified in the studies.

In the fish bioconcentration study by Anonymous (1989a) all validity criteria according to the current test guideline (OECD 305, 2012) were met. However, the study was conducted with only one test concentration instead of two test concentrations. Further deficiencies were the lack of a dilution control group, the lack of information on the lipid content of the fish and the lack of detailed information on the environmental test conditions (TOC).

The studies were considered valid and reliable and it was agreed on a kinetic BCF of 56 for the active substance folpet.

Overall, the results of the studies ($BCF_{kinetic}$= 56) and the log P_{OW} of 3.1 indicate a low concern on the bioaccumulation of folpet in aquatic animals.

10.5 Acute aquatic hazard

Robust study summaries (all studies submitted) are provided in Annex II (Ecotoxicology) to this CLH report.

Table 80: Summary of relevant information on acute aquatic toxicity

Method	Species	Test material	Test condition	Exposure time	Results [1] [mg a.s./L]	Reference
US EPA 72-1	*Salmo gairdneri* Rainbow trout	Folpet Batch #: BN 230080 Purity: 90.3%	Flow-through	96 h	LC_{50} = 0.015 mm	Anonymous (1988a)
US EPA 72-1	*Lepomis macrochirus* Bluegill sunfish	Folpet Batch #: BN 230080 Purity: 90.3%	Flow-through	96 h	LC_{50} = 0.047 mm	Anonymous (1988b)
US EPA 72-1	*Cyprinodon variegatus* Sheepshead minnow	Folpet Batch #: BN 230080 Purity: 90.3%	Flow-through	96 h	LC_{50} = 0.0655 mm	Anonymous (1989a)
US EPA 72-1	*Oncorhynchus mykiss* Rainbow trout	Phthalimide Batch #: 01831CT Purity: 98%	Static	96 h	LC_{50} = 49 mm	Anonymous (1988c)
US EPA 72-1	*Lepomis macrochirus* Bluegill sunfish	Phthalimide Batch #: 01831CT Purity: 98%	Semi-static	96 h	LC_{50} = 38 mm	Anonymous (1989)
OECD 203 (1992) EC C.1 (1992)	*Oncorhynchus mykiss* Rainbow trout	Phthalamic acid Batch #: 1011562 Purity: 103%	Static	96 h	LC_{50} > 100 nom	Anonymous (2007g)
OECD 203 (1992) EC C.1 (1992)	*Oncorhynchus mykiss* Rainbow trout	Phthalic acid Batch #: 10805 BU Purity: 99.7%	Static	96 h	LC_{50} > 73 mm	Anonymous (1999a)
OECD 203 (1992) EC C.1 (1992)	*Oncorhynchus mykiss* Rainbow trout	Phthalic acid Batch #: 1276625 Purity: 100%	Static	96 h	LC_{50} > 100 nom	Anonymous (2007a)
OECD 203 (1992) EC C.1 (1992)	*Oncorhynchus mykiss* Rainbow trout	Benzamide Batch #: 9758305 477 Purity: > 98%	Static	96 h	LC_{50} > 100 nom	Anonymous (2000b)
OECD 203 (1992) EC C.1 (1992)	*Oncorhynchus mykiss* Rainbow trout	Benzamide Batch #: 1141565 Purity: 99.7%	Static	96 h	LC_{50} > 100 nom	Anonymous (2007b)
OECD 203 (1992) EC C.1 (1992)	*Oncorhynchus mykiss* Rainbow trout	2-cyanobenzoic acid Batch #: 1012130	Static	96 h	LC_{50} > 100 nom	Anonymous (2007h)

		Purity: > 97%				
US EPA 72-2	*Daphnia magna* Waterflea	Folpet Batch #: BN 230080 Purity: 90.3%	Flow-through	48 h	EC_{50} = 0.02 $_{mm}$	Anonymous (1988)
FIFRA 72-3	*Americamysis bahia*	Folpet Batch #: BN 230080 Purity: 90.3%	Flow-through	96 h	LC_{50} = 0.16 $_{mm}$	Anonymous (1989c)
US EPA 72-2	*Daphnia magna* Waterflea	Phthalimide Batch #: PT01831CT Purity: 98%	Static	48 h	EC_{50} = 39 $_{mm}$	Anonymous (1989)
OECD 202 (2004)	*Daphnia magna* Waterflea	Phthalamic acid Batch #: 516-014-00 Purity: 98.3%	Static	48 h	EC_{50} > 100 $_{nom}$	Anonymous (2016c)
OECD 202 (2004) EC C.2 (1992)	*Daphnia magna* Waterflea	Phthalamic acid Batch #: 1011562 Purity: 103%	Static	48 h	EC_{50} > 100 $_{nom}$	Anonymous (2007i)
OECD 202-1 (1984) EC C.2 (1992)	*Daphnia magna* Waterflea	Phthalic acid Batch #: 10805 BU Purity: 99.7%	Static	48 h	EC_{50} > 100 $_{nom}$	Anonymous (1999b)
OECD 202 (2004) EC C.2 (1992)	*Daphnia magna* Waterflea	Phthalic acid Batch #: 1276625 Purity: 100%	Static	48 h	EC_{50} > 100 $_{nom}$	Anonymous (2007j)
OECD 202-1 (1984) EC C.2 (1992)	*Daphnia magna* Waterflea	Benzamide Batch #: 9758305 477 Purity: > 98%	Static	48 h	EC_{50} > 102 $_{mm}$	Anonymous (2000e)
OECD 202 (2004) EC C.2 (1992)	*Daphnia magna* Waterflea	Benzamide Batch #: 1141565 Purity: 99.7%	Static	48 h	EC_{50} > 100 $_{nom}$	Anonymous (2007l)
OECD 202 (2004)	*Daphnia magna* Waterflea	2-cyanobenzoic acid Batch #: 534-013-00 Purity: 97%	Static	48 h	EC_{50} = 110 $_{nom}$	Anonymous (2016d)
OECD 202 (2004) EC C.2 (1992)	*Daphnia magna* Waterflea	2-cyanobenzoic acid Batch #: 1012130 Purity: > 97%	Static	48 h	EC_{50} > 100 $_{nom}$	Anonymous (2007k)
OECD 201 (2011) EC C.3 (2009)	*Raphidocelis subcapitata* Green algae	Folpet Batch #: 95138213 Purity: 96.6%	Static	72 h	E_rC_{50} > 0.161 $_{mm}$ E_yC_{50} = 0.089 $_{mm}$	Anonymous (2016e)
OECD 201 (2011)	*Raphidocelis subcapitata* Green algae	Phthalimide Batch #: SZBD221XV Purity: 99.9%	Static	72 h	E_rC_{50} > 46 $_{mm}$ E_yC_{50} > 46 $_{mm}$	Anonymous (2015)

OECD 201 (2011) EC C.3 (2009)	*Raphidocelis subcapitata* Green algae	Phthalamic acid Batch #: 516-014-00 Purity: 98.3%	Static	72 h	E_rC_{50} > 100 $_{nom}$ E_yC_{50} > 100 $_{nom}$	Anonymous (2016g)
OECD 201 (2006) EC C.3 (1992)	*Raphidocelis subcapitata* Green algae	Phthalamic acid Batch #: 1011562 Purity: 103%	Static	72 h	E_rC_{50} > 100 $_{nom}$ E_yC_{50} = 57.1 $_{nom}$	Anonymous (2007n)
OECD 201 (2011) EC C.3 (2009)	*Raphidocelis subcapitata* Green algae	Phthalic acid Batch #: 516-033-00 Purity: 99.5%	Static	72 h	E_rC_{50} = 56.8 $_{nom}$ E_yC_{50} = 49.3 $_{nom}$	Anonymous (2016f)
OECD 201 (2006) EC C.3 (1992)	*Raphidocelis subcapitata* Green algae	Phthalic acid Batch #: 1276625 Purity: 100%	Static	72 h	E_rC_{50} > 98 $_{im}$ E_yC_{50} > 98 $_{im}$	Anonymous (2007o)
OECD 201 (2011) EC C.3 (2009)	*Raphidocelis subcapitata* Green algae	Benzamide Batch #: 534-015-00 Purity: 99.3%	Static	72 h	E_rC_{50} > 100 $_{nom}$ E_yC_{50} > 100 $_{nom}$	Anonymous (2016h)
OECD 201 (2006) EC C.3 (1992)	*Raphidocelis subcapitata* Green algae	Benzamide Batch #: 1141565 Purity: 99.7%	Static	72 h	E_rC_{50} > 100 $_{nom}$ E_yC_{50} > 100 $_{nom}$	Anonymous (2007q)
OECD 201 1984) EC C.3 (1992)	*Raphidocelis subcapitata* Green algae	Benzamide Batch #: 9758305 Purity: > 98%	Static	72 h	E_rC_{50} > 100 $_{nom}$ E_yC_{50} > 100 $_{nom}$	Anonymous (2000h)
OECD 201 (2011) EC C.3 (2009)	*Raphidocelis subcapitata* Green algae	2-cyanobenzoic acid Batch #: 534-013-00 Purity: 97%	Static	72 h	E_rC_{50} > 100 $_{nom}$ E_yC_{50} > 100 $_{nom}$	Anonymous (2016i)
OECD 201 (2006) EC C.3 (1992)	*Raphidocelis subcapitata* Green algae	2-cyanobenzoic acid Batch #: 1012130 Purity: > 97%	Static	72 h	E_rC_{50} > 100 $_{nom}$ E_yC_{50} > 100 $_{nom}$	Anonymous (2007p)

[1] Indicate if the results are based on mean measured (mm), initial measured (im) or on nominal (nom) concentrations.

10.5.1 Acute (short-term) toxicity to fish

Acute toxicity studies with three different fish species were submitted by the applicants. The 96 h acute tests were conducted with the active substance folpet under flow-through test conditions. The endpoints derived from the studies were in the same range. The lowest endpoint was derived from the study with the rainbow trout (Anonymous, 1988a). The 96 h LC_{50} of 0.015 mg a.s./L based on mean measured concentrations was determined.

10.5.2 Acute (short-term) toxicity to aquatic invertebrates

Acute toxicity tests with *Daphnia magna* and *Americamysis bahia* were conducted with the active substance folpet. The daphnids were observed to be more sensitive than the mysid shrimps. The 48 h EC_{50} was determined to be 0.02 mg a.s./L based on mean measured concentrations.

10.5.3 Acute (short-term) toxicity to algae or other aquatic plants

A toxicity study with the green algae *Raphidocelis subcapitata* was conducted with the active substance folpet. The study were conducted under static test conditions; hence, no appropriate exposure could be maintained throughout the study duration of 72 hours.

Due to the lack of analytical verification of the test substance it was agreed during the peer-reivew of the active substance folpet to express the endpoint based on geometric mean measured concentrations. As a pragmatic approach it was agreed to use the LOQ/2 to calculate the mean measured concentrations for the test concentrations below the LOQ.

For the active substance folpet the relevant endpoint was derived form a study by Anonymous (2016e). The 72 h E_rC_{50} for the green algae was > 0.161 mg a.s./L, based on mean measured concentrations.

10.5.4 Acute (short-term) toxicity to other aquatic organisms

No toxicity data are available on other groups of aquatic organisms.

10.6 Long-term aquatic hazard

Robust study summaries (all studies submitted) are provided in Annex II (Ecotoxicology) to this CLH report.

Table 81: Summary of relevant information on chronic aquatic toxicity

Method	Species	Test material	Test condition	Exposure time	Results [mg a.s./] [1]	Remarks	Reference
ASTM (1983)	*Pimephales promelas* Fathead minnow	Folpet Batch #: BN 230080 Purity: 90.3%	Flow through	35 d (ELS)	Hatchability: EC_{10} = 0.0234 mm NOEC = 0.011 mm	NOEC based on statistically significant effects on reproduction (hatchability) and survival.	Anonymous (1989)
ASTM (1983)	*Pimephales promelas* Fathead minnow	Folpet Batch #: 61330799 Purity: 93.2%	Flow-through	33 d (ELS)	Growth: NOEC = 0.00881 mm	NOEC based on statistically significant effects on growth (mean fish length and wet weight).	Anonymous (1995)
OECD 201 (2011) EC C.3 (2009)	*Raphidocelis subcapitata* Green algae	Folpet Batch #: 95138213 Purity: 96.6%	Static	72 h	NOE_rC = 0.058 mm NOE_yC = 0.058 mm	-	Anonymous (2016e)

[1] Indicate if the results are based on mean measured (mm), initial measured (im) or on nominal (nom) concentrations.
ELS…Early life stage test

10.6.1 Chronic toxicity to fish

Two early life stage (ELS) toxicity tests with the fathead minnow (*Pimephales promelas*) were conducted under flow-through test conditions. The ELS studies were conducted with the active substance folpet. Statistically significant effects were observed on the survival of fry fish, the hatchability (reproduction) and the growth of fish. The lowest chronic endpoint was derived form the study by Anonymous (1995) with a NOEC of 0.00881 mg a.s./L.

10.6.2 Chronic toxicity to aquatic invertebrates

No valid reproduction study with *Daphnia magna* or other aquatic invertebrates is available.

10.6.3 Chronic toxicity to algae or other aquatic plants

A toxicity study with the green algae *Raphidocelis subcapitata* was conducted with the active substance folpet. The study were conducted under static test conditions; hence, no appropriate exposure could be maintained throughout the study duration of 72 hours.

Due to the lack of analytical verification of the test substance it was agreed during the peer-reivew of the active substance folpet to express the endpoint based on geometric mean measured concentrations. As a pragmatic approach it was agreed to use the LOQ/2 to calculate the mean measured concentrations for the test concentrations below the LOQ.

For the active substance folpet the relevant endpoint was derived form a study by Anonymous (2016e). The 72 h NOEC for the green algae was 0.058 mg a.s./L, based on mean measured concentrations.

10.6.4 Chronic toxicity to other aquatic organisms

No toxicity data are available on other groups of aquatic organisms.

10.7 Comparison with the CLP criteria

10.7.1 Acute aquatic hazard

- The most sensitive endpoint for fish is LC_{50} = 0.015 mg/L for *Salmo gairdneri*. The study was conducted with the active substance folpet under flow-through conditions.

  The most sensitive endpoint for aquatic invertebrates was *Daphnia magna* with an EC_{50} of 0.02 mg/L. The study was conducted with the active substance folpet under flow-through conditions.

  The most sensitive endpoint for algae was derived for green algae (*Raphidocelis subcapitata*) conducted with the active substance folpet under static conditions. The E_rC_{50} was determined to be greater than 0.161 mg a.s./L.

  Aquatic toxicity studies with the relevant metabolites phthalamide, phthalamic acid, phthalic acid, benzamide and 2-xyanobenzoic acid are available but are not considered relevant for classification (LC_{50}/EC_{50} clearly > 1 mg/L).

- Based on the acute toxicity of folpet to fish and daphnids, the active substance is classified as acute aquatic hazard, category 1 (CLP criteria $LC_{50}/EC_{50} \leq 1$ mg/L). A M-factor of 10 is required considering the high acute toxicity to fish and daphnids (CLP criteria $0.01 < LC_{50} \leq 0.1$ mg/L).

10.7.2 Long-term aquatic hazard (including bioaccumulation potential and degradation)

- Based on the fish bioaccumulation study *(*Burgess, 19889) with *L. macrochirus* a BCF (whole fish) of 56 was determined, which indicate a low potential to bioaccumulate in the aquatic food chain. The substance folpet does not meet the CLP criterion (BCF ≥ 500) based on the measured fish BCF. In addition, the log P_{OW} of folpet is 3.107 which is below the CLP criterion of log P_{OW} > 4.

- The active substance folpet is readily biodegradable (Anonymous, 1994 and 1998) and rapidly degradable.
  Folpet rapidly hydrolyses in water and the rate of hydrolysis rapidly increases with pH. Hydrolysis DT_{50} values are 2.9 h at pH 5, 1.3 h at pH 7, and only 59 sec at pH 9. At pH 5 the predominant degradate of phenyl labelled folpet is phthalimide but there is a shift towards phthalic acid which becomes the predominant degradate at pH 9. Phthalimide is considered stable to hydrolysis at pH 4 (DT_{50} = 125 and 141 days), whereas it is rapidly hydrolysis with a DT_{50} of 2.3 days at pH 7 and a DT_{50} of 0.05 days at pH 9. Phthalic acid is considered stable (final degradate) under conditions of hydrolysis. Phthalamic acid was not detected above 2.5 % AR in the hydrolysis studies.
  Under conditions of aerobic mineralisation in surface water folpet dissipates with DT_{50} values < 1 hr (pH ~ 8). Similar to hydrolysis, the metabolites phthalimide (max. 51.6 % AR), phthalamic acid (max. 73.7 % AR) and phthalic acid (max. 76.9 % AR) are formed in significant amounts. Dissipation of phthalimide was rapid as well with DT_{50} values in a range from 0.4 – 1.2 days.
  Folpet rapidly degraded in two water/sediment studies with DT_{50} values in the range of 0.01 to 0.02 days in the total systems. Folpet was extensively metabolised to phthalimide (max. 31.8 % AR), phthalamic acid (max. 42.7 % AR), phthalic acid (max. 41.3 %), 2-cyanobenzoic acid (41.6 % AR), benzamide (max. 10.2 % AR) and finally to carbon dioxide (max. 80 % AR after 99 days).

- Based on the chronic toxicity of folpet to fish (*Pimephales promelas*, NOEC = 0.00881 mg a.s./L), the active substance is classified as chronic aquatic hazard, category 1 (CLP criteria for rapidly degradable substances NOEC ≤ 0.01 mg/L). A M-factor of 1 is required considering the toxicity to fish (CLP criteria $0.001 <$ NOEC ≤ 0.01 mg/L for rapidly degradable substances).

10.8 CONCLUSION ON CLASSIFICATION AND LABELLING FOR ENVIRONMENTAL HAZARDS

Hazard pictogram		Environment
Hazard class and category:	Hazardous to the aquatic environment, Acute Hazard Category 1, M-factor 10, Chronic Hazard Category 1, M-factor = 1	
Signal word	Warning!	
Hazard statement:	H400	Very toxic to aquatic life
	H410	Very toxic to aquatic life with long lasting effects
Precautionary statements - Prevention	P273	Avoid release to the environment
Precautionary statements - Response	P391	Collect spillage
Precautionary Statement Disposal	P501	Proper disposal of contents/container

11 EVALUATION OF ADDITIONAL HAZARDS

11.1 Hazardous to the ozone layer

Hazard class not assessed in this dossier.

11.1.1 Short summary and overall relevance of the provided information on ozone layer hazard

11.1.2 Comparison with the CLP criteria

11.1.3 Conclusion on classification and labelling for hazardous to the ozone layer

No harmonised classification is proposed by the RMS due to data lacking.

12 ADDITIONAL LABELLING

None.

13 REFERENCES

Reference list with unpublished studies is provided in a separate confidential annex to this CLH report.

Data point	Author(s)	Year	Title Owner Report No. Source (where different from owner) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
CLH, Section 7	Austria	2019	(D)RAR: Renewal Assessment Report prepared according to the Commission Regulation (EU) N° 1107/2009, Folpet, Volume 3, B.2 (AS)	N	N		public	Submitted for the purpose of renewal
IIA, 5.8.1/2 CLH 3.8.5.5	Agarwal, D.K.; Lawrence, W.H.; Nunez, L.J. Autian, J:	1985	MUTAGENICITY EVALUATION OF PHTHALIC ACID ESTERS AND METABOLITES IN SALMONELLA TYPHIMURIUM CULTURES Journal of Toxicology and Environmental Health 16: 61-69 None GLP Published	N	N			Submitted for the purpose of renewal
IIA, 5.1.1/1 or IIA, 5.1.2/03 CLH 2.3.1	Berthet, A.; Bouchard, M.;Danuser, B.	2012a	Toxicokinetics of Captan and Folpet biomarkers in orally exposed volunteers Journal of Applied Toxicology 32:194-201 None GLP Published	Y	N		-	Submitted for the purpose of renewal
IIA, 5.1.2/2 or 5.1.2/04 CLH 2.3.2	Berthet, A.; Bouchard, M.;Vernez, D.	2012b	Toxicokinetics of Captan and Folpet biomarkers in dermally exposed volunteers Journal of Applied Toxicology 32:202-209 None GLP Published	Y	N		-	Submitted for the purpose of renewal

Data point	Author(s)	Year	Title Owner Report No. Source (where different from owner) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
IIA, 5.1.2/01 CLH 2.3.3	Canal-Raffin, M., Receveur, M., Martinez, B., Titier, K., Ohayon, C., Baldi, I., Molimard, M., Moore, N., Brochard, P.	2008	Quantification methods of Folpet degradation products in plasma with HPLC-UV/DAD: Application to an in vivo toxicokinetic study in rats Journal of Chromatography, 865, 106-113 GLP/GEP: no Published: yes	N	N		-	Submitted for the purpose of renewal
CLH 3.9.4.13	Chappell, G. A., Rager, J. E., Wolf, J., Babic, M., LeBlanc, K. J., Ring, C. L., Harris, M. A. and Thompson, C. M.	2019	Comparison of Gene Expression Responses in the Small Intestine of Mice Following Exposure to 3 Carcinogens Using the S1500+ Gene Set Informs a Potential Common Adverse Outcome Pathway Toxicol Pathol. 2019;47(7):851-64					
IIA, 5.8.2/02 CLH 3.9.4.1	Cohen, S.M., Gordon, E.B., Singh, P., Arce, G.T., Nyska, A.	2010	Carcinogenic mode of action of Folpet in mice and evaluation of its relevance to humans, R-27324 Crit Rev Toxicol, 40, 531-545 GLP/GEP: no Published: yes	N	N			Submitted for the purpose of renewal
IIA, 5.6.2 CLH 3.10.6.2	Courtney K.D., Andrews J.E., Stevens J.T., Farmer J.D	1983	Inhalation teratology studies of Captan and Folpet in mice Health Effects Research Laboratory, EPA-600/1-83-017 GLP : no	Y	N	New data for active ingredient, not previously submitted nor evaluated	ADM	Submitted for the purpose of renewal
IIA, 5.6.2 CLH 3.10.5.2	Fabro S., Smith R.L., Williams R.T.	1966	Embryotoxic activity of some pesticides and drugs related to phthalimide Fd Cosmet. Toxicol. Vol 3, pp 587-590, R-9970	Y	N			Submitted for the purpose of renewal

Data point	Author(s)	Year	Title Owner Report No. Source (where different from owner) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
IIA, 5.8.1/7 CLH 3.8.5.11	Galloway, S.M.; Armstrong, M.J.; Reuben, C.; Colman, S.; Brown, B.; Cannon, C., Bloom, A.D.; Nakamura, F.; Ahmed, M.; Duk, S.; Rimpo, J.; Margolin, B.H.; Resnick, M.A.; Anderson, B.; Zeiger, E.	1987	Chromosome Aberations ans Sister Chromatid Exchanges in Chinese Hamster Ovary Cells: Evaluations of 108 Chemicals Environmental and Molecular Mutagenesis 10: 1-35 Non GLP Published	N	N			Submitted for the purpose of renewal
IIA, 5.8.2/1 CLH 3.9.4.10	Gordon, E.; Cohen, S.M; Singh, P.	2012	Folpet-induced short term cytotoxic and proliferative changes in the mouse duodenum Toxicology Mechanisms and Methods 22: 54-59 None GLP Published	Y	N			Submitted for the purpose of renewal
CLH 3.7.2.5	Guo YL, Wang B-J, Lee C-C, Wang J-D	1996	Prevalence of dermatoses and skin sensitisation associated with use of pesticides in fruit farmers of southern Taiwan. Occup Environ Med 53:427-431					
IIA, 5.9.3/01	Gutiérrez-Fernández, D., Fuentes-Vallejo, M. S., Rueda-Ygueravides, M. D., Bartolome-Zavala, B., Foncubierta, F. A., & León, J. A.	2006	Contact urticaria to phthalic anhydride , not available Journal of investigational allergology & clinical immunology, 17, 422-423 GLP/GEP: no Published: yes	N	N			Submitted for the purpose of renewal
IIA, 5.2.7/01	Heisler, E.	1983	Acute toxicological study of Folpet after intraperitoneal application to the rat. Pharmatox GmbH, Project No. 1-4-113-83 (Company file: R-3593). Not GLP, Unpublished.	Y	N		ADM	DAR (2004)

Data point	Author(s)	Year	Title Owner Report No. Source (where different from owner) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
IIA, 5.1.2/05	Heredia-Ortiz, R., Berthet, A., Bouchard, M.	2013	Toxicokinetic modeling of Folpet fungicide and its ring-biomarkers of exposure in humans. J Appl Toxicol, 33, 607-617 GLP/GEP: no Published: yes	N	N		-	Submitted for the purpose of renewal
IIA, 5.8.1/6 CLH 3.8.5.10	Hilliard, C.A.; Armstrong, M.J.; Bradt, C.I.; Hill, R.B.; Greenwood, S.K.; Galloway, S.M.	1998	Chromosome Aberrations In Vitro Related to Cytotoxicity of Nonmutagenic Chemicals and Metabolic Poisons Environmental and Molecular Mutagenesis 31:316–326 None GLP Published	N	N		-	Submitted for the purpose of renewal
IIA 5.8.1 CLH 3.8.5.8	Jha A.M., Singh A.C. and Bharti M	1998	Germ cell mutagenicity of phthalic acid in mice Mutation Research 422 (1998) pp 207-212 R-11020 GLP: no Published: yes	Y	N			Submitted for the purpose of renewal
IIA, 5.6.2/07, 5.8.1 CLH 3.10.4.1 and 3.10.5.1	Kennedy, G., Fancher, O. E., and Calandra, J. C	1968	An investigation of the teratogenic potential of Captan, Folpet, and difolatan, Toxicology and Applied Pharmacology 13, 420-430 R-169 / CA69.105298	Y	N			Submitted for DAR (2004) but not included in DAR (2004)

Data point	Author(s)	Year	Title Owner Report No. Source (where different from owner) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
IIA, 5.5 CLH 3.9.4.2	Kidwell, J.	2010	US EPA cancer assessment document - Second evaluation of the carcinogenic potential of Folpet EPA, US Environmental Protection Agency U.S. EPA Report-no. PC code 081601 GLP/GEP: no Published: no	N	N		-	Submitted for the purpose of renewal
CLH 3.12.3.2	Kluxen F.M., Koenig C.M.	2021	Inhalation Toxicity of Captan and Folpet accepted manuscript submitted to Regulatory Toxicology and Pharmacology					
IIA, 5.4.1/1 CLH 3.8.2.10	Knight, A.W.; Little, S.; Houck, K.; Dix, D.; Judson, R.; Richard, A.; McCarroll, N.; Akerman, G.; Yang, C.; Birrell, L.;Walmsley, R.M.	2009	Evaluation of high-throughput genotoxicity assays used in profiling the US EPA ToxCastTM chemicals Regulatory Toxicology and Pharmacology 55:188-199 None GLP Published	N	N			Submitted for the purpose of renewal
IIA, 5.8.1/4 CLH 3.8.5.4	Lee, K. H.; Lee, B. M.	2007	Study of Mutagenicities of Phthalic Acid and Terephthalic Acid Using In Vitro and In Vivo Genotoxicity Tests Journal of Toxicology and Environmental Health 70: 1329-1335 None GLP Published	N/Y	N			Submitted for the purpose of renewal
CLH 3.7.2.2	Lim HW, Cohen D, Soter NA	1998	Chronic actinic dermatitis: results of patch and photopatch tests with Compositae, fragrances, and pesticides. J Am Acad Dermatol 38(1):108-11 doi:10.1016/s0190-9622(98)70549-3					

Data point	Author(s)	Year	Title Owner Report No. Source (where different from owner) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
CLH 3.7.2.6	Lisi P, Caraffini S, Assalve D	1987	Irritation and sensitization potential of pesticides. Contact Dermatitis 17(4):212-8					
CLH 3.7.2.3	Mark KA, Brancaccio RR, Soter NA, Cohen DE	1999	Allergie Contaet and Photoallergie Contaet Dermatitis to Plant and Pestieide Allergens.					
IIA, 5.6.2 CLH 3.10.5.3	McLaughin J., Reynaldo E.F:, Lamar J.K., Marliac J.P	1969	Teratology studies in rabbit with Captan, Folpet and thalidomide Toxicology and Applied Pharmacology 14 (3), 641 R-238	Y	N			Submitted for the purpose of renewal
IIA 5.8.1 CLH 3.8.5.3	Ministry of Health and Welfare (MHW), Japan	1999	Toxicity Testing Reports of Environmental Chemicals 7, 97-124 GLP: no Published: yes	N	N			Submitted for the purpose of renewal
CLH 3.7.2.4	Peluso AM, Tardio M, Adamo F, Venturo N	1991	Multiple sensitization due to bis-dithiocarbamate and thiophthalimide pesticides. Contact Dermatitis 25(327)					
IIA, 5.8.1/5 CLH 3.8.5.7	Phillips, B.J.; James, T.E.B.	1982	Genotoxicity studies of di(2-ethylhexyl)phthalate and its metabolites in CHO cells Mutation Research 102: 297-304 None GLP Published	N	N			Submitted for the purpose of renewal
IIA, 5.8.1/05 CLH 3.8.5.2	Pilinskaya, M.A.	1986	Study of the cytogenetic activity of ceptain metabolites of a number of pesticides representing several classes of chemical compounds , R-11352 Tsitologiya i Genetika, Journal, 20, 143-145 GLP/GEP: no Published: yes	N	N			Addendum to the DAR (2008)

Data point	Author(s)	Year	Title Owner Report No. Source (where different from owner) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
IIA, 5.8.1/06 CLH 3.8.5.1	Riggin, R.M., Margard, W.L., Kinzer, G.W.	1983	Characterization of impurities in commercial lots of sodium saccharin produces by the shermin-williams process. II. Mutagenicity , R-11350 Fd Cosmet. Toxicol., 21, 11-17 GLP/GEP: no Published: yes	N	N			Addendum to the DAR (2008)
IIA, 5.6.2 CLH 3.10.6.1	Robens J.	1970	Teratogenic activity of several phthalimide derivates in the golden hamster Toxicology and applied pharmacology, 16(1), 24-34, R-9965	Y	N			Submitted for the purpose of renewal
IIA, 5.4.1/01 CLH 3.8.2.9	Santucci, M.A., Mercatali, L., Brusa, G., Pattacini, L., Barbieri, E., Perocco, P.	2003	Cell-Cycle deregulation on BALB/c 3T3 cells transformed by 1,2-Dibromoethane and Folpet Pesticides Environmental and Molecular Mutagenesis, 41, 315 - 321 GLP/GEP: no Published: yes	N	N		-	Submitted for the purpose of renewal
CA 5.8.1 CLH 3.8.5.6	Sayato Y., Nakamuro K., Ueno H.,	1987	Mutagenicity of products formed by ozonation of naphthoresorcinol in aqueous solutions, Mutation Research 189 pp:217-222 GLP/GEP: no Published: yes	N	N		Public	Submitted for the purpose of renewal
IIA, 5.8.2/02 CLH 2.4.2	Shah, P.V., Fisher, H.L., Sumler, M.R., Monroe, R.J., Chernoff, N., Hall, L.L.	1987	Comparison of the penetration of 14 pesticides through the skin of young and adult rats. Journal of Toxicology and Environmental Health, 21: 353-366 (Company file: R-10023). Not GLP, Published.	Y	N			DAR (2004)

Data point	Author(s)	Year	Title Owner Report No. Source (where different from owner) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
CLH 3.9.4.12	Thompson, C. M., Wolf, J. C., McCoy, A., Suh, M., Proctor, D. M., Kirman, C. R., Haws, L. C., and Harris, M. A.	2017	Comparison of Toxicity and Recovery in the Duodenum of B6C3F1 Mice Following Treatment with Intestinal Carcinogens Captan, Folpet, and Hexavalent Chromium Toxicologic Pathology 45(8) 1091-1101					
IIA, 5.6.2 CLH 3.10.6.3	Vondruska, J. F., Fancher, O. E., & Calandra, J. C.	1971	An investigation into the teratogenic potential of Captan, Folpet, and Difolatan in nonhuman primates Toxicology and applied pharmacology, 18(3), 619-624, R-0268	Y	N			Submitted for the purpose of renewal
CLH 3.7.2.1	Victor FC, Cohen DE, Soter NA	2010	A 20-year analysis of previous and emerging allergens that elicit photoallergic contact dermatitis. J Am Acad Dermatol 62(4):605-10 doi:10.1016/j.jaad.2009.06.084					
IIA, 5.4.2/3 CLH 3.8.4.1	YU Zhong-bo; WU Nan-xiang; Tao He; LI Xin-wei; GU Liu-jin; ZHANG Xing	2006	Mutagenic Research on Folpet CARCINOGENESIS, TERATOGENESIS & MUTAGENESIS 18: 475-478 None GLP Published	N/Y	N		-	Submitted for the purpose of renewal
IIA, 5.8.1/3 CLH 3.8.5.9	Zeiger, E.; Haworth, S.; Mortelmans, K.; Speck, W.	1985	Mutagenicity Testing of Di(2-ethylhexyl)phthalate and Related Chemicals in Salmonella Environmental Mutagenesis, 7, 213-232 None GLP Published	N	N			Submitted for the purpose of renewal
CLH 7	Anonymous	2019	Renewal Assessment Report RMS:AT Unpublished	N	N			

14 ANNEXES

Annex I: Human Health

Annex II: Ecotoxicology I (AS)

Annex III: Environmental Fate & Behaviour

Confidential Annex