Committee for Risk Assessment

RAC

Annex 1

Background document

to the Opinion proposing harmonised classification and labelling at EU level of

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

EC Number: 205-088-6
CAS Number: 133-07-3

CLH-O-0000007326-73-01/F

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

8 June 2023

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September 2023: latest version, replacing all previous versions

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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ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON FOLPET (ISO); N-(TRICHLOROMETHYLTHIO)PHTHALIMIDE

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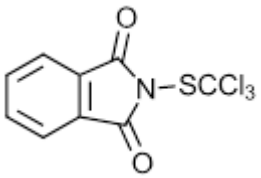
1 IDENTITY OF THE SUBSTANCE

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

RAC general comment

Folpet is a broad-spectrum contact fungicide derived from phthalimide and trichloromethylsulfenyl chloride and structurally related to captan and captafol, which also contain a trichloromethylthio (TCM) side-chain.

Toxicokinetics

Folpet has been extensively assessed in a series of guideline and non-guideline studies in rats and mice. *In vitro* data and two publications on human volunteers are also available.

In the rodent studies, radiolabels were incorporated in the aromatic ring, in the carbonyl group and in the TCM side-chain. 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 after oral or dermal exposure.** Radioactive recovery in the systemic compartment seems to be exclusively associated with its metabolites.

Radioactivity associated with folpet is rapidly absorbed, widely distributed and rapidly excreted predominantly via urine. Folpet and its metabolites do not show any potential for accumulation.

The TCM is the reactive site generating thiophosgene (figure below) both via hydrolysis and its rapid reaction with thiols. Thiophosgene is conjugated with one cysteine of glutathione (GSH), excreted as thiazolidine, disulphonic acid or mineralised to CO_2, HCl and H_2S. In a comparative study in rats and mice, the changes in GSH and glutathione S-transferase levels after exposure to folpet reflects this relationship. Initial bolus administration of folpet in rats and mice results in a transient depletion of GSH that is rapidly followed by a rebound as a homeostatic response. In this study, a greater depletion of GSH has been noted in mice especially from the duodenum.

The removal of the TCM side-chain by detoxification mechanisms yields to phthalimide, which is further metabolised to phthalamic acid, which may be converted to phthalic acid, all of them are rapidly excreted via urine (figure below).

Results of the comparative *in vitro* metabolism studies showed no relevant differences between humans and rats. Folpet's half-life in human blood is less than 5 seconds, and the half-life of thiophosgene in blood is less than 1 second.

Toxicokinetic publications reporting phthalimide and phthalic acid levels in plasma and urine of human volunteers seem to indicate similar metabolism in humans and rats as well as a lower dermal absorption fraction compared to oral absorption.

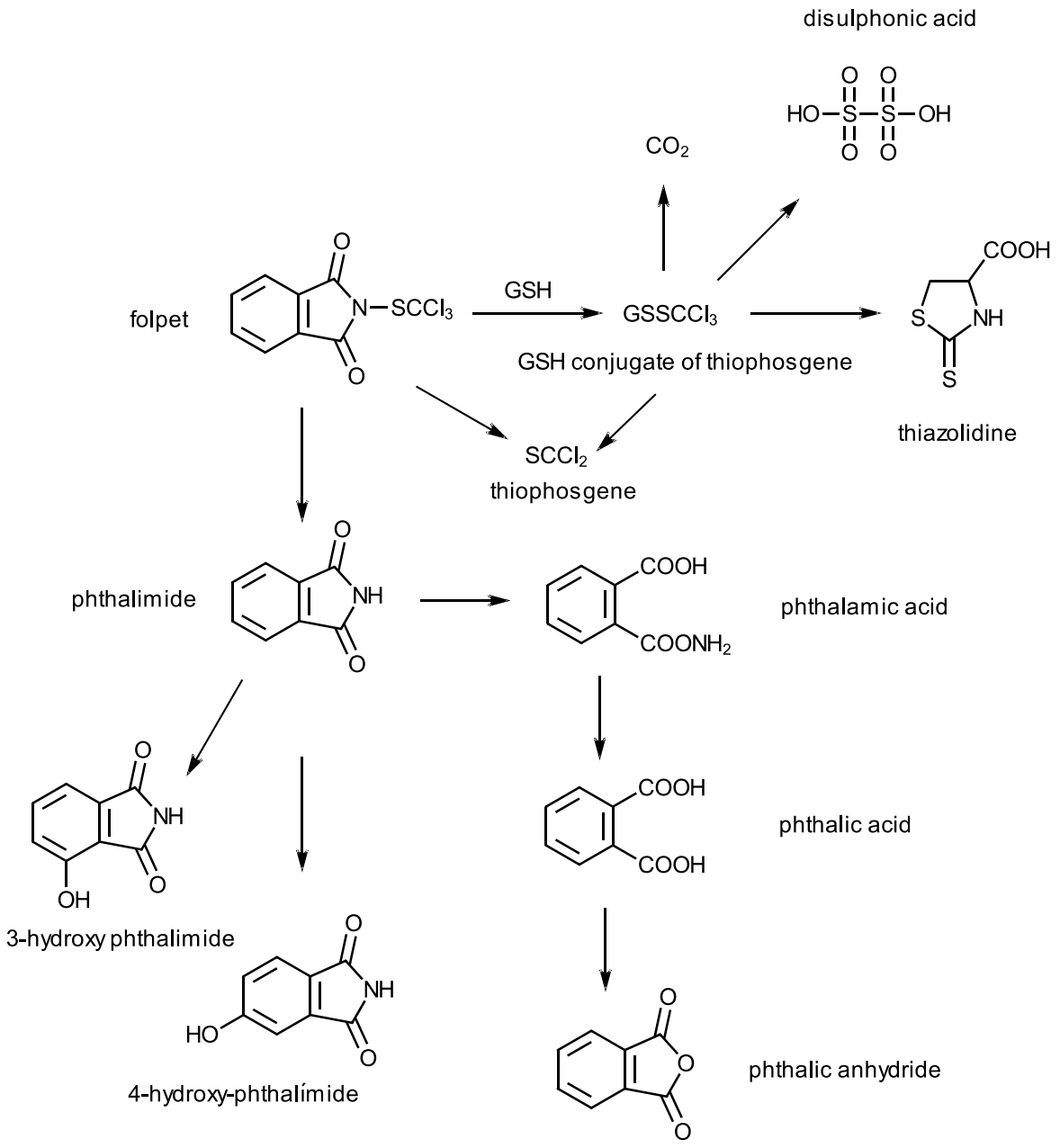


***Figure**: Generalised metabolic pathway for folpet in rodents following oral administration*

Overall, the toxicokinetic studies are crucial for understanding folpet's hazard profile of local irritation and adverse effects. There is no evidence for systemic exposure towards the intact parent molecule. The toxicophore TCM is highly reactive and severely irritating to any tissues such as mucous membranes (respiratory, digestive or ocular). Effects are therefore expected to occur at the first site of exposure, before entering the systemic compartment. Any observed systemic effects are therefore subsequent to primary irritating effects or driven by folpet's systemic metabolites.

The toxicological database for folpet is substantial. For each hazard class, several GLP compliant studies are available generally conducted according to contemporaneous OECD

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

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)

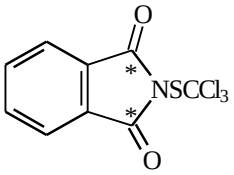
Folpet is a contact fungicide, it rapidly reacts with thiols upon which it is degraded. The key degradation product is thiophosgene which is highly reactive and also reacts with thiols and other functional groups.

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.

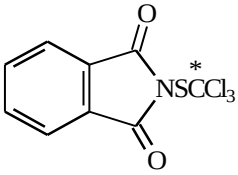
Radiolabels have been incorporated in the aromatic ring, in the carbonyl ring, and in the trichloromethylthio side-chain (TCM). The aromatic ring was shown to be the most stable, and the trichloromethylthio side-chain the least stable part of the molecule.

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.

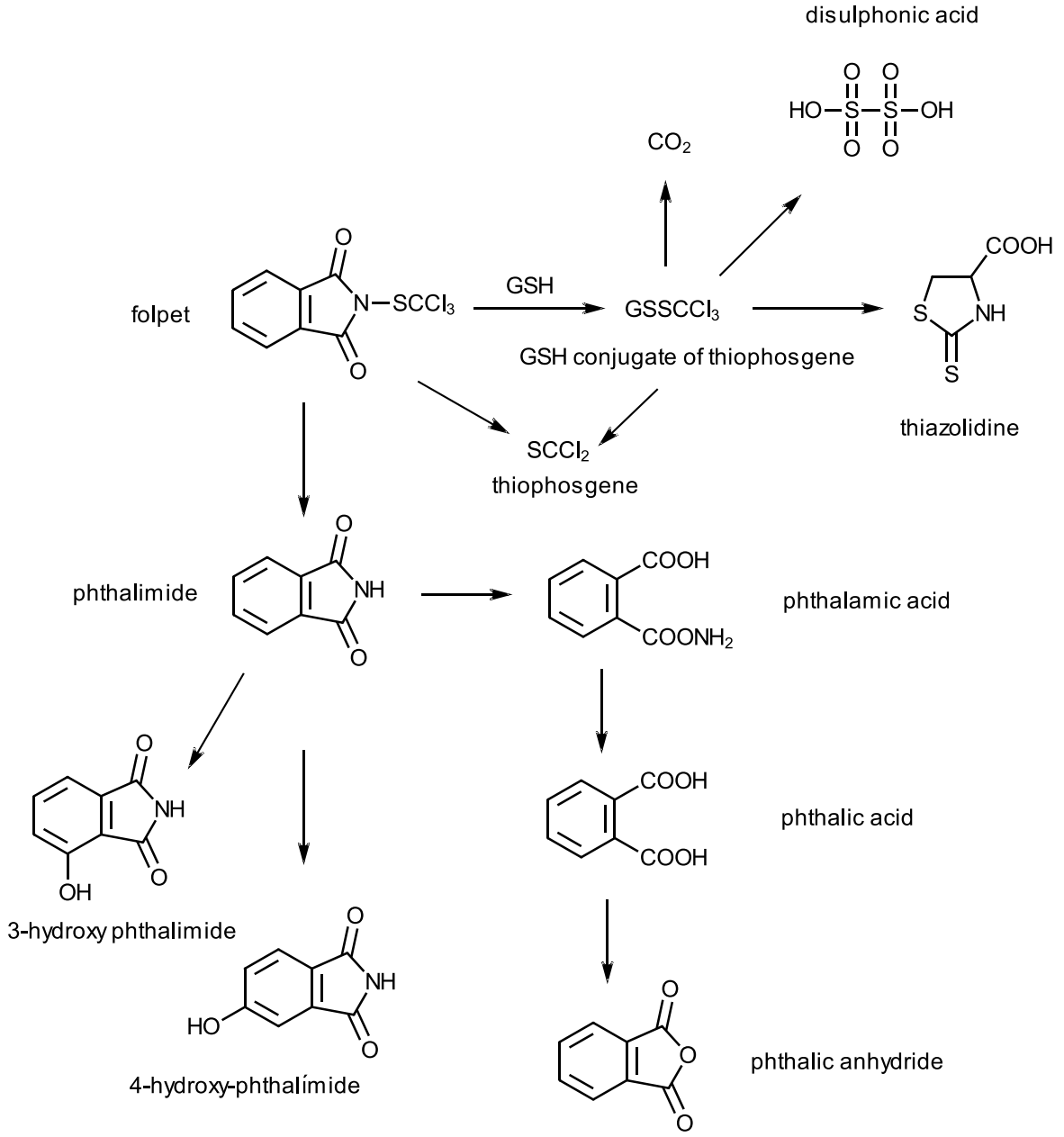


Figure 2: Generalised metabolic pathway for folpet in rodents following oral administration

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)

Method; Deviations from OECD 417 (2010)	**Results**	**Reference (Company reference number)**
- 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	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)

Method; Deviations from OECD 417 (2010)	**Results**	**Reference (Company reference number)**
time points of tissue distribution - only 3 animals/ sex/ dose for the different time points for blood sampling - tissue distribution was not measured in 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)
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.	Berthet et al. 2012a

Method	Results	Reference
	Folpet appears to be similarly metabolized in humans and rats.	
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 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.	Berthet et al. 2012b
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	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)

Method	Results	Reference
- Recovery was not reported (however, it was stated that all the values were corrected for 100%) Supplementary information (reliable with restrictions)		
No guideline stated, *in vivo* rat study Supplementary information (reliable with restrictions)	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. 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.	Shah et al. 1987
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.

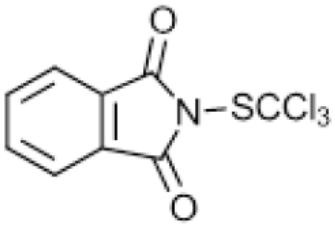
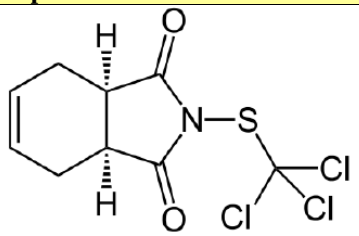
Overall conclusion with respect to toxicity

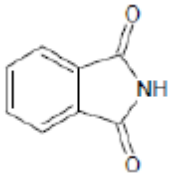
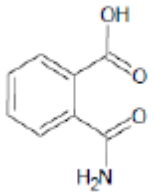
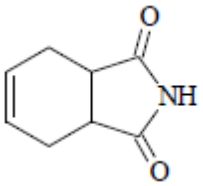
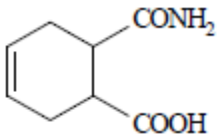
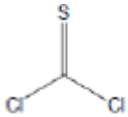
Together, folpet's metabolism is driven by rapid non-enzymatic reactions in rat and human. There is no evidence for systemic exposure towards the intact parent molecule, which incorporates the reactive toxicophore, i.e. the trichloromethylthio side-chain. Accordingly, irritative effects are expected 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	Rapid and extensive (app. 95 % within 48 h); ring-labelled captan is excreted mainly via urine (75%

	Folpet	**Captan**
	study measuring CO_2 traps is available with folpet.	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)
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 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	Rat, Sprague Dawley, 5/group, 10/group in control	Folpet Technical, Purity not reported	5000, 6500, 8500, 11200, 14800, 20000 or 26300 mg/kg	M: 19500 mg/kg F: 43800 mg/kg	(R-7902) Study 1 (1983)
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	M: 0.34 mg/L F: 1 mg/L Converted LC_{50} levels to 100% purity: M: 0.3 mg/L	(000092041) Study 1 (1988)

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**
			hours	F: 0.89 mg/L	
AEPA, Proposed Guidelines for Registering Pesticides in the U.S., Part II, August 22, 1978 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)	Rat, Sprague-Dawley-derived, 5/sex/group	Folpet technical, purity not stated MMAD (GSD) 2.4 (12), 2.3 (0.2), 5.3 (1.3), 2.0 (0.8), 3.0 (0.3) µm	0.64, 0.65, 0.67, 2.68, 3.61 mg/L for 4 hours	M: 1.38 mg/L F: 1.30 mg/L	(000039795) Study 2 (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	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)

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**
Supplementary information (reliable with restrictions)					
GLP, OECD 403 (1981) Deviations from OECD 403 (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)	Rat, CD strain (Sprague-Dawley derived), 5/sex/group	Folpet Technical, 98.99% MMAD: 4.6, 4.9, 5.2 µm	0, 0.8, 1.6, 1.99 mg/L for 4 hours	M: 1.54 mg/L F: 2.89 mg/L	(000009988) Study 6 (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.

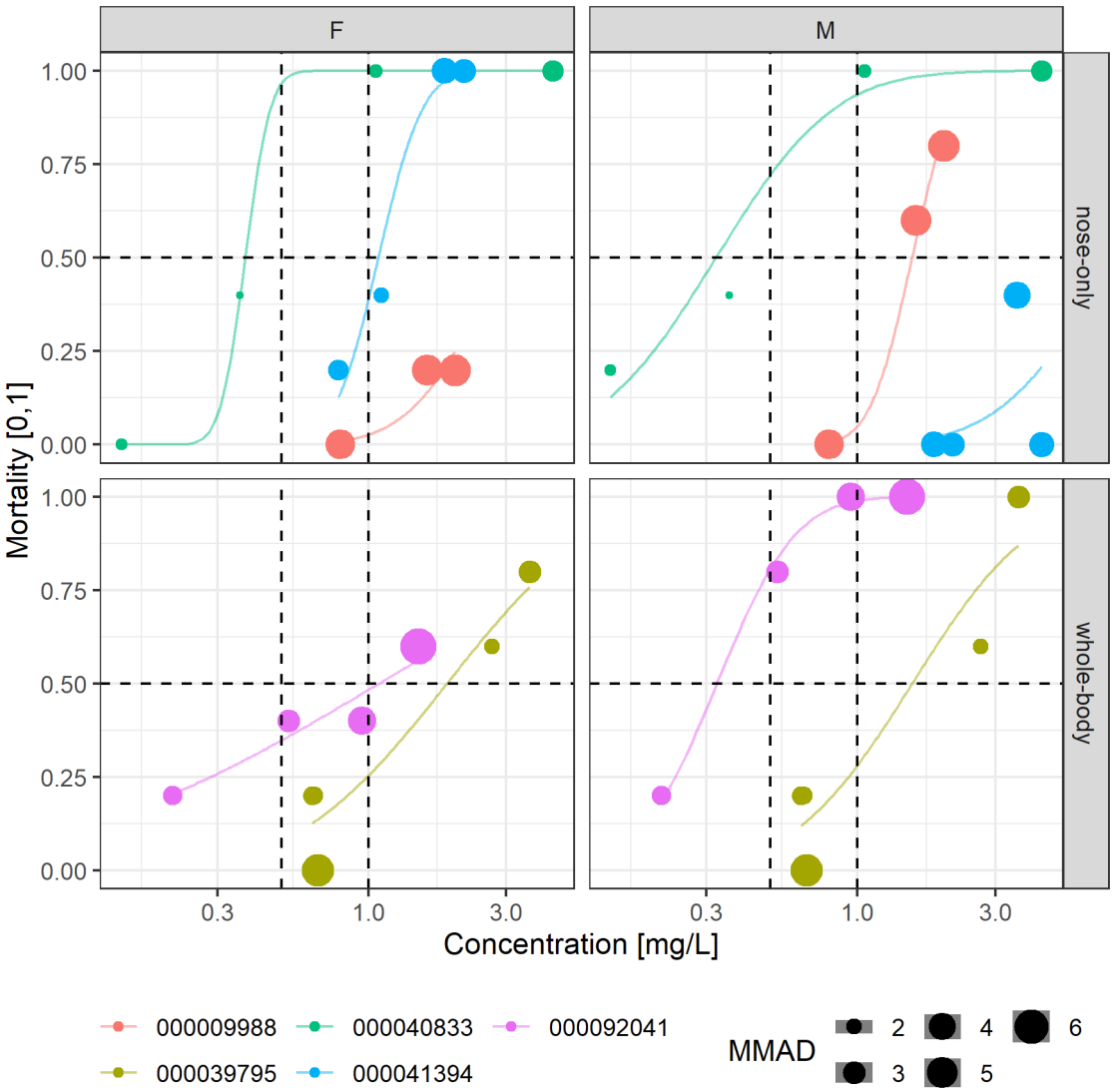


Figure 3: Overview of the observed mortalities, as a fraction between 0 (no mortality) and 1 (100% mortality in the group), in acute inhalation toxicity studies with folpet.

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.

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

Acute Oral Toxicity

No classification was proposed by the DS for acute oral toxicity based on the results from two acute oral studies in rats.

Acute Dermal Toxicity

No classification was proposed by the DS for acute dermal toxicity based on the results from two acute oral studies (one in rabbits and the other one in rats).

Acute Inhalation Toxicity

The DS proposed to classify folpet for acute inhalation toxicity, Category 2, with an ATE of 0.39 mg/L (dusts and mists), based on the lowest LC_{50} obtained among the rat acute inhalation toxicity studies considered relevant for classification (nose-only exposure).

Comments received during consultation

One Member State Competent Authority (MSCA) and an Industry representative (IND) supported the DS's proposal.

The IND also commented that it may be instructive to indicate that folpet fully qualifies for the "split-entry approach" proposed by Pauluhn (2008) for irritant particles as generated by folpet. Consequently, for folpet products containing larger particle sizes, in the form they are placed on the market, a refined approach could be followed (dedicated testing or, preferably, by other new approach methods which consider particle size in the hazard characterisation) instead of generic studies.

The DS disagreed with the "split-entry approach" for folpet-based products pointing out that folpet is used per spraying, where nozzles could have an impact on particle size, and highlighting some uncertainties on whether criteria for the "split-entry approach" are actually met by folpet.

RAC does not support a split entry approach. RAC underlines that CLP is an hazard-based Regulation and Annex VI is dedicated to substance classification and not to formulated products. For acute inhalation toxicity of dusts and mists, testing with mass median aerodynamic diameters (MMAD) in the range of 1.0 to 4.0 µm is explicitly required under CLP Regulation to ensure comprehensive respiratory tract exposure in order to appropriately address inhalation hazard of the substance and subsequent labelling to communicate recommended measures. While, not all forms in formulated products and life cycle according to different uses can be anticipated, the works from Canal-Raffin *et al*. (2007) provide one example not supporting the split-entry approach. The authors showed that the majority (> 75%) of the particles of two commercial forms of folpet Folpan 80WG® and Myco 500® had a size under 5 µm under their typical application conditions whatever the granulometry of the formulated product was.

Assessment and comparison with the classification criteria

Acute Oral Toxicity

The purity of the tested substance is not reported for any of the two available studies. In the first study (study 1, 1983) predating OECD TG 401 (1987), groups of five rats per sex were given a single oral dose of folpet at doses of 5000, 6500, 8500, 11200, 14800, 20000 or 26300 mg/kg bw. There were no mortalities at 5000 mg/kg bw. The acute oral LD_{50} of folpet was 19500 mg/kg bw in females and 43800 mg/kg bw in males. In the GLP compliant second study (study 2, 1992), conducted in accordance with OECD TG 401 (1987), no death occurred in the five male and five female rats dosed at a level of 2000 mg/kg bw. The acute oral median lethal dose (LD_{50}) of folpet was greater than 2000 mg/kg bw.

Since the LD_{50} values in the two available studies exceed 2000 mg/kg bw, RAC agrees with the DS's proposal of **no classification for acute oral toxicity.**

Acute Dermal Toxicity

Two acute dermal studies are available. In the first study (study 1, 1982) similar to OECD TG 402 (1981), conducted in five rabbits per sex, no death occurred after a 24-hour dermal application of 5000 mg/kg bw of folpet on abraded skin in five males and five females. The acute dermal LD_{50} of folpet was greater than 5000 mg/kg bw. In the GLP compliant second study (study 2, 1991), conducted in accordance with OECD TG 402 (1981), no death occurred in the five males and five females rats after a 24-hour dermal application of 2000 mg/kg bw. The acute dermal LD_{50} of folpet was greater than 2000 mg/kg bw.

Since the LD_{50} values in the two available studies exceed 2000 mg/kg bw, RAC agrees with the DS's proposal of **no classification for acute dermal toxicity.**

Acute Inhalation Toxicity

The available studies for acute inhalation toxicity are summarized in the table below.

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

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels, duration of exposure	Value LD_{50}	Reference
GLP, Acute Inhalation – Rat US EPA OPP 81.3. Deviations from OECD TG 403 (2009): - No justification for whole-body exposure - MMAD partly exceeding the recommended range (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 Deviations from OECD TG 403 (2009): - No justification for whole body exposure - No justification for 0.64 mg/L geometric standard deviation (GSD) exceeding the recommended range (1.5-3) and for 0.67 mg/L MMAD exceeding the recommended range Supplementary information (reliable with restrictions)	Rat, Sprague-Dawley-derived, 5/sex/group	Folpet technical, purity not stated MMAD(GSD) 2.4 (12), 2.3 (0.2), 5.3 (1.3), 2.0 (0.8), 3.0 (0.3) µm	0.64, 0.65, 0.67, 2.68, 3.61 mg/L for 4 h	M: 1.38 mg/L F: 1.30 mg/L Death within 4-5 d	000039795 Study 2, 1979
GLP, OECD TG 403 (1981) Nose-only exposure	Rat, Sprague-Dawley derived, 5/sex/group	Folpet technical, purity not stated	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

		MMAD: 1.7, 1.6, 1.8, 2.8 µm			
GLP, EPA Guideline No. 83-1 (equivalent to OECD TG 403 (1981)) Nose-only exposure	Rat, CD strain (Sprague-Dawley derived), 5/sex/group	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 Time of death ≤ 2 d	000041394 Study 4, 1993
GLP, EPA Guideline No. 83-1 (equivalent to OECD TG 403 (1981)) Nose-only exposure Deviations from OECD TG 403 (2009): - Only one concentration tested - MMAD 14.3 µm and GSD 5.8 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 No death	000041392 Study 5, 1993
GLP, OECD TG 403 (1981) Nose-only exposure Deviations from OECD TG 403 (2009): - Particle size distribution only measured once - MMAD exceeding the recommended range	Rat, CD strain (Sprague-Dawley derived), 5/sex/group	Folpet Technical, 98.99% MMAD: 4.6, 4.9, 5.2 µm	0, 0.8, 1.6, 1.99 mg/L for 4 hours	M: 1.54 mg/L F: 2.89 mg/L Time of death ≤ 2 day	000009988 Study 6, 1993

Six GLP compliant acute inhalation toxicity studies are available for folpet (studies 1 and 2 with whole-body mode of exposure and studies 3 to 6 with nose-only mode of exposure). All studies were performed before the revised OECD TG 403 (2009) came into force. Considering the particle size range diameter recommended in the OECD TG 403 (2009) to achieve a respirable particle size (i.e. MMAD ranging from 1 to 4 µm), studies 3 and 4 fully fulfil this criterion for all tested concentrations while studies 1 and 2 do not for all tested concentrations. In the study 6, the MMAD slightly exceeds the recommended range for all tested doses while in the study 5 performed with non-micronized test material, the MMAD is clearly higher than the recommended MMAD.

Across studies, the LC_{50} values range from 0.30 mg/L (for males in study 1, converted value to 100% purity) to above 4.35 mg/L (for males in study 4).

Lethality seems to be associated with differences in achieved particle sizes as illustrated in figure 3 in the CLH report. The clinical effects and macroscopic findings, typical to exposure towards irritant particles (respiratory rate, laboured breathing, increased lung weight, swollen lungs and oedema) are further described in the STOT SE section.

The DS considered only studies 3 and 4 as relevant for classification purposes (i.e. studies with nose-only exposure and in the required test guideline particle size range) and to a lesser

extend study 6, which had a slightly higher MMAD. However, RAC considers that studies with whole-body exposure are also relevant for classification purposes and are therefore taken into consideration.

While in four out of the six studies, the LC_{50} values exceed 0.5 mg/L, in two studies the LC_{50} are within the range of 0.05 mg/L < ATE ≤ 0.5 mg/L defined under CLP Regulation as acute toxicity estimates (ATE) range for acute inhalation toxicity Category 2 for dusts and mists:

- In study 1 (whole-body), the LC_{50} for males is 0.30 mg/L and (converted to 100% purity)
- In study 3 (nose-only exposure), the LC_{50} is 0.39 mg/L for males and 0.43 mg/L for females (purity not stated).

Therefore, in accordance with the criteria laid down in the CLP Regulation, RAC supports the DS's proposal to classify folpet as **Acute Toxicity, Category 2 (H330; fatal if inhaled)**. An **ATE of 0.30 mg/L** is proposed based on the lowest LC_{50} in males from study 1 (1988).

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>
</table></td><td>(R-6508)

Study 1 (1991)</td></tr>
</table>

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
		3 Erythema 0.3 Oedema 0 4 Erythema 0 Oedema 0 5 Erythema 0 Oedema 0 6 Erythema 0 Oedema 0	
GLP, OECD 404 (1981)	0.5 g for 4 hours Folpet, 95.6% New Zealand White rabbits, 3 females	No dermal response (erythema or oedema) **Animal No.** / **Mean scores (24 - 72 h)** 1 Erythema 0 Oedema 0 2 Erythema 0 Oedema 0 3 Erythema 0.3 Oedema 0	(R-7394) Study 2 (1993)

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 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	Folpet 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	Dose and exposure time dependent increase in skin irritation potency. Due to the severity of the skin effects one of the highest 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: mg/kg bw/d: 0 | 1 | 10 | 30 | 30/20 M: 0.5/0 | 1.33/0.17 | 1.5/0.67 | 2.17/1 | 2.67/1.5 F: 0.17/0 | 0.17/0 | 0.5/0.17 | 0.83/0.17 | 0.5/0.33 Day 9 erythema/oedema scores:	(R-5452) Study 3 (1988)

<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></td><td></td><td>
<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></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 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

[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"

(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.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

The DS initially proposed to classify folpet for skin irritation Category 2 (H315) based on irritation observed from the second day of exposure in a 4-week dermal toxicity study in rats and similar reactions observed in other animal studies. Following comments from the Consultation, the DS revised their proposal, considering that the results from the two available acute skin corrosion/irritation assays in rabbits do not fulfil the criteria for classification,

Comments received during consultation

One MSCA disagreed with the DS's proposal considering that there is not sufficient evidence for skin damage following up to 4-hour exposures and mentioned that skin effects reported for the repeated dose studies could be addressed by labelling with EUH066.

IND disagreed with the DS's proposal because the skin irritation was observed in rodent studies with repeated exposure, rodents seems more sensitive species due to different skin morphology/stratum corneum thickness, and captan, a structurally related substance with the same underlying toxic mode of action (MoA), also showed no relevant skin irritation. They added that two available *in vitro* tests (i.e. study reports submitted during the consultation) modelling human skin also supported a no-classification proposal.

The DS, in the light of the new *in vitro* studies, considered the arguments provided by both MSCA and IND, and revised their proposal from Skin Irrit. 2 to the labelling element EUH066. They considered the EUH066 is more appropriate than classification as skin irritant based on the erythema/oedema observed from day 2 in a 4-week dermal rat study.

Additional key elements

A GLP compliant *In Vitro* Skin Corrosion: Reconstructed human epidermis (RHE) test and a GLP compliant *In Vitro* Skin Irritation: Reconstructed Human Epidermis test performed according to and OECD TGs 431 and 439, respectively, have been submitted during the consultation and are further discussed here after.

Assessment and comparison with the classification criteria

Two GLP compliant acute dermal irritation/corrosion studies are available. In the first study (Study 1, 1991) similar to OECD TG 404, conducted in six rabbits, after a 4-hour dermal exposure to 0.5 g folpet moistened with 0.6 mL of distilled water, a mean score of 0 (24-72 h) for both erythema and oedema was obtained in all animals but one. This animal showed only mild oedema, which was reversible within 24 hours, with a mean 0.3 (24-72 h). In the second study (study 2, 1993) conducted according with OECD TG 404, there were no dermal responses in any of the 3 rabbits exposed to 0.5 g folpet to moistened skin for 4 hours.

In the two GLP compliant *in vitro* assays, using a human 3D skin model, submitted during consultation, folpet was non-corrosive in the OECD TG 431 test (study 1, 2022) and non-irritant in the OECD TG 439 test (study 2, 2022), which corroborates the results obtained in the rabbit studies (summary table in Supplemental information).

On the other hand, irritation was observed in other animal studies than the investigation of skin corrosion/irritation.

In the dermal acute toxicity performed in rabbits, non-suppurative dermatitis and mild hyperkeratosis were observed in 4 females as well as mild acanthosis in one female. However, the protocol of this test (dose of 5000 mg/kg bw, 24-hour exposure and abraded skin, study 1, 1982) calls for a very high dose to be applied compared to that of OECD TG 404 and it is considered inadequate to investigate irritating potential under CLP criteria.

In the first GPMT (study 1, 1991) after topical induction of folpet 50% w/w in arachis oil for 48 hours, 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 redness. However, erythema could not be scored because of other adverse skin reactions. It is noteworthy that no irritation was observed in the second GPMT after topical induction of folpet 50% w/v in propylene glycol (study 2, 1993).

In rats, while no skin irritation was observed in the acute dermal toxicity study, significant skin irritation was observed in a 4-week dermal toxicity study in rats (study 3, 1988). In this study, folpet was applied at a volume of 2 mL/kg bw in mineral oil to unabraded skin on the back (two alternating skin sites) of six male and six female Sprague-Dawley rats at dose levels of 0, 1, 10 and 30 (two groups) mg/kg bw/d for six hours/day for five days per week for 4 weeks (total of 21 applications). The dose level for the male animals in one of the high dose groups was reduced to 20 mg/kg bw/d on day 6 and dosing was discontinued in all high dosed males after day 13 allowing animals of this group to recover, before sacrifice on day 15. The animals showed erythema (mean score 3.67/1.67 in males/females on day 9), oedema (mean score 2.33/0.67 in males/females on day 9), scabs and sloughing from 10 mg/kg bw/d onwards. Incidence and severity increased in both sexes with dose and duration of the study and were more pronounced in males including lacerations at the high dose level.

While this study provides evidence of skin irritation in rats after repeated exposure to folpet, the results cannot be extrapolated to a single 4 h dermal exposure scenario (as defined in CLP criteria). The same limitation applies to chronic toxicity studies in mouse where skin effects were observed due to contact with diet with high folpet concentration.

Comparison with criteria

The rabbit acute irritation studies, with a single 4 h dermal exposure, showed no skin irritation relevant for classification. The *in vitro* studies also indicated that folpet does not induce irritation in models with human-like epithelia.

On the other hand, some studies not specifically designed to address skin corrosion/irritation (OECD TG 402; OECD TG 410 and OECD TG 406), provided some indication of skin irritation after folpet administration. However, the exposure pattern in those studies is very maximizing compared to that of OECD TG 404 (longer exposure and/or repeated exposure).

Consequently based on the reliable studies dedicated to answer to the CLP criteria, RAC considers that no classification is warranted for skin acute irritation.

In order to signal the skin effects reported in the repeated dose studies, **RAC concurs with the DS's proposal to add the supplementary label EUH066.**

Supplemental information - In depth analyses by RAC

The available *in vitro* studies on acute skin corrosion/irritation are summarized in the table below.

***Table**: Summary table of acute skin corrosion/irritation in vitro 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>OECD TG 431 (Reconstructed Human Epidermis, corrosion)
Deviation: none</td><td>Folpet
Purity:95.8%
25 mg
EpiDerm™
Triplicate
Moistened Milli-Q water (25 µL)
3 or 60 minutes
Negative control: Milli-Q water
Positive control: KOH</td><td>Cytotoxicity expressed as the reduction of mitochondrial dehydrogenase activity measured by formazan production from MTT in a plate reader at the end of the treatment (OD_{570}).
<table><tr><td></td><td colspan="2">Corrosion</td></tr><tr><td></td><td>3 min</td><td>1 hour</td></tr><tr><td>Viability (% of control)</td><td>95</td><td>91</td></tr><tr><td>Interpretation</td><td colspan="2">Corrosive: < 50% 3 min
Corrosive: ≥ 50% 3 min & < 15% 1 h
Non-corrosive: ≥ 50% 3 min & ≥ 15% 1 h</td></tr></table>→ Non-corrosive</td><td>000107034/
20273724
Study 1, 2022</td></tr>
<tr><td>OECD TG 439 (Reconstructed Human Epidermis, irritation)</td><td>Folpet
Purity:95.8%
12.9 to 14.6 mg</td><td>Cytotoxicity expressed as the reduction of mitochondrial dehydrogenase activity measured by formazan production from MTT in a plate reader at the end of the treatment (OD_{570}).
<table><tr><td>Irritation</td></tr></table></td><td>000107035/
20273726
Study 2, 2022</td></tr>
</table>

Deviation: none	EPISKIN-SM™ Triplicate Moistened Milli-Q water (5 µl) 15 minutes Negative PBS Positive control: SDS	15 min Viability (% of control): 106 Interpretation: Irritation: < 50%; No irritation: ≥ 50% → Non-irritant	

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	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	<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	(R-1737) Study 2 (1979)

- 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			animals eyes were washed for one minute after 20 s of exposure	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> 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	New Zealand White rabbits, 2	Folpet technical, no purity reported	0.1 mL/87 mg for 3 days (eyes were not	<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	(R-6511) Study 4 (1992)

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	males, 4 females		washed in the 3-day post exposure period)	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	

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.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

The DS proposed to revise the current classification as eye irritation, Category 2 to serious eye damage, Category 1 due to irreversible eye effects at the end of the observation period in two out of the four available acute eye irritation studies in rabbits.

Comments received during consultation

One MSCA agreed with the DS's proposal.

IND agreed with this conclusion based on available vertebrate studies but mentioned two available *in vitro* tests (i.e. study reports submitted during the public consultation) supporting a classification for Category 2 that may indicate a lower sensitivity of human tissue against folpet induced irritation, which they considered biologically plausible as human cornea has a different morphology than rabbit cornea.

The DS disagreed considering that *in vitro* results could not overrule positive reliable *in vivo* results and challenged the adequacy of the proposed *in vitro* studies which is only applicable to neat non-surfactant liquids (solid suspensions or solids are outside the applicability domain) according to the OECD TG 467 (please refer to RCOM for detailed answer).

A GLP compliant Bovine Corneal Opacity and Permeability (BCOP) test and a GLP compliant Reconstructed human Cornea-like Epithelium (RhCE) test performed according to OECD TGs 437 and 492 respectively were submitted during the consultation and are further discussed below.

Assessment and comparison with the classification criteria

The available *in vivo* studies are summarized in the table below.

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

Method, guideline, deviations from OECD TG 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 TG 405 Room temperature: 19°C (15-23°C) Deviations from OECD TG 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	NZW 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 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 TG 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	NZW 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**	(R-1737) Study 2, 1979

per day from 24-72 hours - Unclear if fluorescein staining was used				**within 13 days (max follow-up**) <u>Individual 24-72 h means for animals with eye washing</u> Cornea opacity:0, 0, 0 Iritis:0, 0, 0 Conjunctiva – redness: 1.66, 1.33, 1.33 (reversible after 48h) Conjunctiva – chemosis: 0.33, 0.33, 0.33 (reversible after 24h)	
GLP, EPA August 1978 Deviations from OECD TG 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	NZW 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, 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 No eye effects at 24-72 h for animals with eye washing	(R-7091) Study 3, 1982
GLP, US EPA Deviations from OECD TG 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	NZW 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; **persistent corneal vascularisation in 2 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, **persistent petechial haemorrhage of the nictitating membrane in 2 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

Four *in vivo* studies are available, performed according to OECD TG 405 or similar.

In Study 1 (GLP compliant, 1991) performed on three New Zealand White (NZW) male rabbits, one rabbit had a mean 24-72 h score of 1.67 and 0.67 for conjunctival redness and chemosis respectively. The eye was returned to normal by day seven. In the two other rabbits, at the 24, 48 and 72 h examinations, a beefy-red conjunctival appearance, slight or substantial discharge and very slight to moderate chemosis were observed. Iritis and slight to severe

corneal opacity were also evident. 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 corneal opacity associated with pannus formation. Due to the irreversible nature of this change, the animals were sacrificed, and the study terminated.

In Study 2 (non-GLP, 1979), the eyes of three NZW rabbits were washed for 1 minute 20 seconds after instillation, while the eyes of six NZW rabbits remained unwashed. Eyes washed 20 seconds after instillation showed conjunctival redness, chemosis and discharge 24 hours after instillation, and by 72 hours after instillation, all three eyes were normal. Eyes that were not washed after treatment exhibited corneal opacity, iritis, conjunctival redness and discharge. While two eyes returned to normal by day ten, signs of severe irritation in 3/6 rabbits persisted until the end of the treatment period (day 13). A male in the unwashed group died on day eight due to an intestinal disorder.

In Study 3 (GLP compliant, 1982), the eyes of three NZW rabbits were rinsed for one minute after a 30 second exposure, while the eyes of six NZW rabbits 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. All eyes appeared normal by 14 days after treatment.

In Study 4 (GLP compliant, 1992), 0.1 mL of test item (about 87 mg) were used in six NZW rabbits without washing. Eye irritation was noted in all animals, which was reversible after 7 or 14 days in four of the six animals treated. Corneal opacity with an average 24-72 h score of ≥ 3 was noted in two of six animals and three animals showed an average score > 1. Reversible iritis was noted for all animals but no animal showed a score > 1. Eye effects (corneal vascularisation and/or petechial haemorrhage of the nictitating membrane) did not fully reverse by the end of the study in two animals.

Overall, *in vivo* studies consistently show irritation potential. While, none of the four studies has a 21-day follow-up period, three of them reported eye effects up to the maximum follow-up day. One study was terminated after 7 days due to irreversibility of eye changes (pannus formation associated with severe opacity in the cornea) in 2/3 animals. In two other studies, reversibility of effects on the cornea, iris or conjunctiva was not demonstrated at termination (13 days and 14 days after exposure in 3/6 and 2/6 animals respectively).

Two GLP compliant *in vitro* assays (BCOP OP-KIT assay (OECD TG 437) and EpiOcularTM assay (OECD TG 492)) submitted during the consultation provide inconclusive results (see in-depth analyses by RAC in the Supplemental information below).

Comparison with the criteria

In all *in vivo* studies, the threshold for irritating to eyes is clearly reached. The most pronounced corneal opacity was seen in study 4, with 2/6 animals exceeding corneal opacity mean score of 3. While none of the four studies had a 21-day observation period, in three of them eye effects did not reverse up to termination.

The inconclusive results obtained in the *in vitro* assays do not challenge the positive results obtained in the reliable *in vivo* studies.

Based on the irreversibility of the effects, in accordance with the criteria laid down in the CLP Regulation, RAC supports the DS's proposal to **classify folpet for serious eye damage Category 1 (H318; Causes serious eye damage).**

Supplemental information - In depth analyses by RAC

The available *in vitro* studies are summarized in the table below.

Table: *Summary table of serious eye damage/eye irritation in vitro 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>OECD TG 437 (Bovine Corneal Opacity and Permeability)

OP-KIT

Deviation:

In the 2nd exp 1 of negative control eye excluded (cornea translucent after treatment IVIS 4.8)

No impact since the 2 other ones met the acceptability criteria</td><td>Folpet

Purity:95.8 %

Bovine cornea

Triplicate

4 hours

<u>First experiment</u>

329.3 to 398.5 mg (cornea completely covered)

<u>Second experiment</u>

About 300 mg + physiological saline to obtain ≈20%w/v

-ve control: physiological saline

+ve control: Imidazole 20%</td><td>Opacity determined by the amount of light transmission through the cornea measured by opacitometer.

Permeability determined by the amount of sodium fluorescein dye that crosses into the posterior chamber measured by a 96-well microtiter plate (optical density at 490 nm, OD_{490}).

IVIS with OP-KIT = mean opacity value (read-out OP-KIT) + (15 x mean permeability OD_{490} value)

<table><tr><th></th><th colspan="2">Corrosion (BCOP)</th></tr><tr><th></th><th>Mean IVIS, dry application</th><th>Mean IVIS, application as 20%</th></tr><tr><td>Folpet</td><td>-0.9</td><td>8.5</td></tr><tr><td>Negative control</td><td>-0.5</td><td>1.9</td></tr><tr><td>Positive control</td><td>135</td><td>175</td></tr><tr><td>Interpretation</td><td colspan="2">≤ 3 No category
< 3 to ≤ 55 No prediction can be made
> 55 Category 1</td></tr></table>
→ First experiment: No category

→ Second experiment: No stand-alone prediction can be

made</td><td>000107036 /

20273729</td></tr>
<tr><td>OECD TG 492 (Reconstructed Human Cornea-like Epithelium)</td><td>Folpet

Purity:95.8 %

Folpet

Purity:95.8 %

63 to 66.2</td><td>Cytotoxicity expressed as the reduction of mitochondrial dehydrogenase activity measured by formazan production from MTT determined spectrophotometrically at 570 nm with a plate reader at the end of the treatment (OD570).

<table><tr><th></th><th>Irritation (RhCE)</th></tr><tr><th></th><th>Mean viability (%control)</th></tr><tr><td>Folpet</td><td>7.3</td></tr></table></td><td>000107037 /

20273731</td></tr>
</table>

	mg EpiOcular™ Duplicate Moistened with Ca2+/Mg2+-free DPBS 6 hours -ve control: Milli-Q water +ve control: Methyl acetate	Negative control: 100 Positive control: 19 Interpretation: > 60% No category; ≤ 60% No prediction can be made → No prediction can be made	

Two GLP compliant *in vitro* assays were submitted during consultation.

In the BCOP OP-KIT assay (OECD TG 437), the first experiment (test item tested dry) did not indicate any irritation potential of folpet, contrary to *in vivo* results. In the second experiment, folpet was applied on the top of the corneas (± 300 mg to completely cover the cornea) and physiological saline was added to obtain a 20% w/v concentration in order to facilitate an interaction with cell membranes. The addition of water resulted in an increased In Vitro Irritancy Score (IVIS), however still below 55, which does not allow any prediction.

It is noteworthy that while solids are within the applicability domain of OECD TG 437, a high false negative rate was identified for solids during the validation of the method. Furthermore, when used to identify chemicals as inducing serious eye damage Cat. 1, the BCOP OP-KIT test method (used in the submitted test) has a higher false negative rate in comparison with the BCOP laser light-based opacitometer (LLBO) method (40% vs 30%) (Adriaens, 2021).

In the EpiOcular™ assay (OECD TG 492), using reconstructed human cornea-like epithelium (RhCE), again no prediction can be made since the viability is lower than 60%.

Overall, the *in vitro* tests are inconclusive. Furthermore, according to the OECD TG 467, the combination of the BCOP and EpiOcular™ assays is used for the defined approaches 1 (DAL-1) for eye hazard identification of neat non-surfactant liquids. Solid suspensions or solids are outside the applicability domain of DAL-1 and therefore is not applicable to folpet.

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 (1992), GPMT	Albino Dunkin-Harley Guinea pigs, 10/sex/folpet group	Folpet technical, 95.6%	Induction Intradermal: 10% Topical: 50% (48 hrs, 1 week after intradermal induction) Topical challenge 10%, 50% (24 hrs, 2 weeks after topical induction)	At the 24-hour observation after challenge with 50% and 10%, 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.	(R-7424) 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	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	Victor et al. 2010

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
		(n=76)	(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.	
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 literature		Case study and patch test with 45 year old female 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	Folpet exposure unknown, required gloves not worn when handling pesticides. Dermatitis completely resolved when exposure towards pesticides was stopped. Positive reaction to folpet 0.1%. Unclear how sensitisation and irritation effects were distinguished.	Peluso et al. 1991
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 $\geq$ 30 % responding at $\leq$ 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.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

Based on two Magnusson and Kligman skin sensitisation assays, the DS proposed to classify folpet for skin sensitisation. Sub-categorisation 1A and a SCL of 0.001% were proposed based on the results obtained in one of the two studies; the other study did not allow potency discrimination due to high concentration for intradermal induction.

Comments received during consultation

IND principally agreed with the assessment based on the available vertebrate studies but did not agree with classifying folpet as an extreme skin sensitizer considering that the underlying *in vivo* studies are not designed to identify potency. Industry mentioned relevant *in vitro* tests (submitted during the consultation) modelling human tissue including a GARDskin assay which allows an estimated LLNA EC3 of 3.27% for folpet indicating only moderate sensitizing potential in line with the unconvincing evidence for sensitization in exposed humans.

The DS disagreed because potency categorisation is feasible based on one of the two GPMT studies. The DS also considered that the new provided *in vitro* data are not appropriate to address potency categorisation since potency extrapolation from GARDskin assay is not validated according to OECD Guideline 497 (please refer to RCOM for details).

Additional key elements

GLP compliant ARE-Nrf2 luciferase KeratinoSens™ test (OECD TG 442D), U937 cell line activation Test (U-SENS™) (OECD TG 442E), Genomic Allergen Rapid Detection (GARD™) for assessment of skin sensitisers (GARD™skin) (similar to OECD TG 442E) as well as a GARD™skin dose-response assay (adapted from OECD TG 442E) have been submitted during the public consultation and are further discussed here after.

Assessment and comparison with the classification criteria

In the literature, cases of allergic reactions upon folpet challenge are reported. While not numerous, all diagnostic clinical studies among dermatitis patients reported cases with positive results from patch testing with folpet at 0.1%. In the largest one, allergic reactions were noted in 6 patients out of 442 (1.4%) (Lisi, 1987). In the other published clinic data, involving smaller samples of patients, the incidence of allergic positive responses to 0.1% folpet ranged from 3.8 to 10% (Guo, 1996; Lim, 1998; Mark, 1999) which is considered relatively high frequency of occurrence according to the Guidance on the Application of the CLP Criteria (CLP guidance, 2017). However, the exposure to folpet is not specifically reported hampering the calculation of an exposure index.

Two CLP-compliant Magnusson and Kligman skin sensitisation assays in Guinea pig (GPMT) are available for folpet, which both indicate clear sensitizing properties. In Study 1 (1991), the concentrations of folpet in arachis oil were 0.1% for the intradermal induction, 50% for topical induction, 25% for topical challenge, and 10% for a re-challenge (to clarify between irritating and sensitizing effects observed during the first challenge). During the re-challenge, a total of 13 out of 19 animals (68%) showed sensitisation responses.

In Study 2 (1993), the concentrations of folpet in propylene glycol were 10% for the intradermal induction, 50% for topical induction, 50% for topical challenge, and 10% for a re-challenge (to clarify between irritation and sensitizing). During challenge and re-challenge, a total of 20/20 and 15/20 animals respectively showed sensitisation responses. This test does not allow potency discrimination due to a high concentration used for intradermal induction.

Table: *Summary of skin reactions in the two GPMTs with folpet*

		No. skin reactions		Total number of animals affected (%)
		24 hours	48 hours	
Study 1 0.1%	Test group 1st challenge 25%	17/19	14/19	17/19 (89)
	Test group 2nd challenge 10%	12/19	13/19	13/19 (68)
Study 2 10%	Test group 1st challenge 50%	19/20	20/20	20/20 (100)
	Test group 2nd challenge 10%	14/20	10/20	15/20 (75)

The four GLP compliant submitted during the consultation to investigate the potential of folpet to induce certain key events in the skin sensitization AOP further support that folpet is a skin sensitizer but do not allow sub-categorisation (see in-depth analyses by RAC below).

Comparison with the criteria

Human, animal and *in vitro* data provide consistent evidence that folpet is a skin sensitizer.

Due to the limited number of the human data and their limitation regarding exposure estimation, the classification and sub-categorisation are firstly based on the available animal studies.

In the two GPMTs, a positive response was observed in more than 30% of the animals a classification for Skin Sensitisation 1 is applicable which is supported by *in vitro* tests applying the 2 out of 3 defined approach (OECD TG 497) and human data.

In the first GPMT (study 1), more than 30% animals responded at 0.1% intradermal induction concentration. Since criteria for subcategory 1A are fulfilled, RAC supports the DS's proposal to **classify folpet for Skin Sensitization Category 1A**.

Since the incidence of sensitised animals (68%) in Study 1 exceeded 60% corresponding to the potency of an **extreme skin sensitiser**, RAC concurs with DS's proposal that a **SCL of 0.001%** should apply**.**

Supplemental information - In depth analyses by RAC

The *in vitro* studies on skin sensitisation are summarized in the table below.

Table: *Summary table of in vitro 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 TG 442C (DPRA) KE 1 peptide/protein binding	Folpet Purity:95.8%	Assay terminated : no suitable assay compatible solvent found to dissolve the test items at the desired concentration	Mentioned in Kluxen, 2022
GLP, OECD TG 442D KeratinoSens™	Folpet Purity:95.8%	Acceptance criteria fulfilled Results:	Study report, 2021 in confidential

<table>
<tr>
<td>KE 2 Keratinocyte response</td>
<td>KeratinoSens™ cells
(ARE-Nrf2) Luciferase
2 exp:
0.03 to 63 µM
DMSO</td>
<td><u>Experiment 1</u>: $EC_{1.5}$ = 0.05 µM Imax = 2.39
Plateau at lower concentrations (up to 7.8 µM), clear dose response after.
<u>Experiment 2:</u> $EC_{1.5}$ = 9.6 µM Imax = 30.87
Clear dose-response
No cytotoxicity in both exp.
Positive→ skin sensitiser (with complementary information)</td>
<td>attachment Submitted docs.zip</td>
</tr>
<tr>
<td>GLP, OECD TG 442E U-SENS™
KE 3 Monocytic /dendritic cell response</td>
<td>Folpet
Purity:95.8%
U937 cells
CD86 marker
1st run: 1 to 200 µg/mL
2nd run: 1 to 50 µg/mL
-ve control: Lactic acid
+ve control: TNBS</td>
<td>Acceptance criteria fulfilled
Results
<table>
<tr><th></th><th>EC_{150} (µGg/mL)</th><th>CV_{70} (µg/mL)</th><th>S.I. of CD86</th></tr>
<tr><td>Exp 1</td><td>1.3</td><td>13</td><td rowspan="2">> 150% at no cytotoxic concentrations</td></tr>
<tr><td>Exp 2</td><td>1.3</td><td>12</td></tr>
</table>
Positive→ skin sensitiser (with complementary information)</td>
<td>Study report, 2021 confidential attachment Submitted docs.zip</td>
</tr>
<tr>
<td>GLP, GARDskin assay
Similar to OECD TG 442E (2022) (performed before)
KE 3Monocytic /dendritic cell response</td>
<td>Folpet
Purity:95.8%
SenzaCell™ cell line
17 µM
Triplicate
Vehicle: DMSO
+ve control: PPD</td>
<td>Acceptance criteria fulfilled
Results:
Gene expression levels in cell cultures exposed to folpet analysed using the GARD Data Analysis Application (GDAA):
Mean DV (decision value) = 5.41 (> 0)
Positive→ skin sensitiser (with complementary information)</td>
<td>Study report, 2021 confidential attachment Submitted docs.zip</td>
</tr>
<tr>
<td>GLP, GARDskin dose-response
Adapted from OECD TG 442E</td>
<td>Folpet
Purity:99.6%
SenzaCell™ cell line
0.23, 0.47, 0.94, 1.9, 3.8 and 7.5 µM
Duplicate (deviation)
Vehicle: DMSO
+ve control: PPD</td>
<td>Acceptance criteria fulfilled
Results:
<table>
<tr><th></th><th>Concentration µM</th><th>DV</th><th>Classification</th></tr>
<tr><td>Folpet</td><td>7.5</td><td>2.21</td><td>S</td></tr>
<tr><td>Folpet</td><td>3.8</td><td>0.214</td><td>S</td></tr>
<tr><td>Folpet</td><td>1.9</td><td>-0.437</td><td>NS</td></tr>
<tr><td>Folpet</td><td>0.94</td><td>739</td><td>NS</td></tr>
<tr><td>Folpet</td><td>0.47</td><td>-1.011</td><td>NS</td></tr>
<tr><td>Folpet</td><td>0.23</td><td>-1.47</td><td>NS</td></tr>
<tr><td>PPD</td><td>75</td><td>5.48</td><td>S</td></tr>
<tr><td>DMSO</td><td>0.20%</td><td>-1.4</td><td>NS</td></tr>
</table>
S: sensitizer, NS: Non-Sensitizer
→ cDV_0= 3.03 µM (95% CI: 2.18-3.9%)
Estimated LLNA EC3: 0.64% (95% CI: 0.249-1.65%)</td>
<td>Study report, 2023</td>
</tr>
</table>

GLP compliant *in vitro* assays were submitted during the consultation to investigate the potential of folpet to induce certain key events in the skin sensitization AOP. Regarding key

event 1 "covalent interaction with skin proteins", the DPRA assay (OECD TG 442C) was terminated because no suitable assay compatible solvent could be found to dissolve folpet. The KeratinoSens assay (OECD TG 442D) investigating key event 2 "keratinocyte responses", the USens assay (OECD TG 442E) and the GARD assay (similar to OECD TG 442E) both investigating key event 3 "dendritic cell responses" were all positive concurring with the results observed in the guinea pig assays.

In a supplementary GARDskin Dose-Response response assay (adapted from OECD TG 442E), performed to characterise the potency of folpet, six concentrations were tested from 0.23 µM to 7.5 µM) to generate a decision value (DV) for each concentration and then to calculate the cDV_0 corresponding to DV=0 by linear interpolation between DVs.

A cDV_0 of 3.03 µM (95% CI: 2.18-3.9%) was obtained and a corresponding LLNA EC3 of 0.64% (95% CI: 0.249-1.65%) was estimated using a linear regression model on log-transformed values (Gardin, 2021) which corresponds to the potency of a strong skin sensitiser.

Overall, the *in vitro* tests further support that folpet is a skin sensitizer. Regarding potency determination, the GARDskin Dose-Response response assay is not validated, as mentioned by the study author (disclaimer), and not cited in the OECD guideline 497 dedicated to Defined Approaches for Skin Sensitisation. Therefore, the estimated LLNA EC3 value is considered of lower reliability for sub-categorisation than the results from the GPMT studies.

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, TA1537 E.coli WP2 uvrA	0.50, 1.58, 5.00, 15.8, 50.0, 158, 500 µg/plate	Positive (+/- S9) Lower potency under conditions of metabolic activation.	(R-38829) Study 9 (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)	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
- only one dose group (3 µg/ml) met all three experimental conditions - 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) - 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	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
- no HCD data are reported Supplementary information (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	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
analysed/ animal - mitotic index was determined in 500 scored cells (instead of 1000)				
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	Rat	Intraperitoneal injection and oral gavage for 5 days	Inconclusive	(R-545) Study 8 (1982)

Method, guideline, deviations if any	Test system	Relevant information about the study (as applicable)	Observations	Reference
conditions - no information regarding the dose volume used - no information on preparation of the animals 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)		2.5, 5 and 10 mg/kg bw/d (i.p.) 50, 100 and 200 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 -	Male albino mice	Intraperitoneal injection (single dose) 0, 5 and 10 mg/kg bw/d	Negative	R-6073 Study 17 (1971)

Method, guideline, deviations if any	Test system	Relevant information about the study (as applicable)	Observations	Reference
Not reliable (due to major study deviations and IBT as the testing facility, where the study was conducted)				

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)

Method, guideline, deviations if any	Test system	Concentrations/Doses tested	Result	Reference
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 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)	Chinese hamster cells (CHL/IU)	0, (313), 625, 1250, 2500, 5000 µg/mL	Inconclusive* *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)	CHO cells - S9	Phthalic acid (as sodium salt): 0, 10, 20,	Negative	Phillips et al. 1982

Method, guideline, deviations if any	Test system	Concentrations/Doses tested	Result	Reference
supplementary information (reliable with restrictions)		50 mM		
Micronucleus test in vivo (public literature) supplementary information (reliable with restrictions)	Bone marrow of male ICR mice	Phthalic acid: 0, 20, 100, 500, 2500, 12500 µg/kg bw	Negative	Lee & Lee 2007
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	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)

Method, guideline, deviations if any	Test system	Concentrations/Doses tested	Result	Reference
- Reporting of cytotoxicity is limited supplementary information (reliable with restrictions)				
Chrom. Aberration in vitro (public literature) - 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)	CHO cells - S9	Phthalic anhydride: 0, 6, 8, 10 mM	Chromosome aberrations at top dose (precipitation)	Hilliard et al. 1998
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.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

The DS proposed no classification for germ cell mutagenicity based on a weight-of-evidence assessment: while folpet was consistently positive *in vitro*, it is not systemically available itself, the *in vivo* studies carried out with folpet are all negative and folpet's systemic metabolites are consistently negative in Ames test.

Comments received during consultation

One MSCA State and Industry supported the DS proposal.

Assessment and comparison with the classification criteria

The genotoxic potential of folpet has been extensively investigated. A recent *in vitro* data set complying with the current OECD TG is available. The other submitted regulatory studies are more dated but generally performed according to OECD TG in force at the time they were carried out. They are considered reliable unless further specified. Results from the studies performed by Yu *et al*. (2006) are considered as supplementary data. In a literature review on folpet's genotoxic potential (Arce, 2010), results from studies not submitted in the CLH dossier were also reported.

In vitro studies with folpet

Mutagenicity in bacteria cells

In a bacterial gene mutation assay (study 9, 2017), fully compliant with OECD TG 471, folpet induced gene mutation in all the tested strains (*S. typhimurium* TA98, TA100, TA1535, TA1537 and *E.coli* WP2 uvrA) with and without metabolic activation. Positive results were also obtained in previous reverse mutation assays (study 10, 1993a; study 11, 1993b; Yu, 2006 and Arce, 2010).

Mutagenicity mammalian cells

In an *in vitro* mammalian cell gene mutation assay (HPRT-Locus) in Chinese Hamster V79 cells (study 12, 2018a), fully compliant with OECD TG 476, folpet was mutagenic both in the absence and presence of metabolic activation. An older assay (study 13, 1986) with several deviations from OECD TG 476 (2016) is considered as inconclusive. In Arce (2010), two other positive mammalian cell gene mutation assays are reported.

Clastogenicity in mammalian cells

In an *in vitro* chromosome aberration test in human lymphocytes (study 14, 2018b), fully compliant with OECD TG 473, folpet was positive with and without metabolic activation. Two other chromosome aberration tests presenting several deviations from OECD TG 473 (2016) are available. One performed in human lymphocytes (study 15, 1987) is considered as inconclusive while the second one performed in Chinese hamster ovary cells is positive with or without metabolic activation (study 16, 1989).

DNA damage in mammalian cells

In an *in vitro* Comet assay in human peripheral mononuclear cells (Yu, 2006; no validated guideline, considered supplementary data), folpet tested at 0, 0.1, 1.0 and 10 µg/mL induced a significant increased tail length from 1.0 µg/mL.

Overall, folpet exerts genotoxic activity (both mutagenic and clastogenic) in *in vitro* test systems. In all tests, folpet's potency to induce gene mutations or chromosome aberrations was decreased under conditions of metabolic activation. This may be explained by an increased thiol-pool provided by the addition of S9 mix, which reacts and degrades folpet resulting in mitigation of its mutagenic potency *in vitro*.

In vivo studies with folpet

In vivo tests somatic cell - systemic exposure

Mutagenicity

Folpet was tested in a mouse somatic cell mutation assay (spot test, study 4, 1985), similar to OECD TG 484 (1986). 140 C57B1/6 pregnant female mice per group (mated with T-strain male mice) were exposed during gestation day 8-12 to diet containing 0, 100, 1500 or 5000 ppm folpet (equivalent to 0, 17, 300 and 965 mg/kg bw/d). Maternal and consequent foetal and neonatal toxicity occurred at 5000 ppm. On lactation days 12 and 28, folpet did not induce increase in the number of pups with recessive coat spots (RCS) or differentiation spots in any groups, while a significant increase of pups with RCS was observed from dams treated with the positive control (ethylnitrosourea).

Clastogenicity

In a mouse micronucleus test (study 1, 1985) performed according to OECD TG 474 (1983), male and female CD-1 mice were treated with folpet at concentrations of 10, 50 and 250 mg/kg in 0.5% carboxymethyl cellulose. Five animals per sex per group were killed 24 hours after treatment and the bone marrow extracted and prepared. In addition, five males and five females from the vehicle control group and the high dose group were killed at 48 and 72 hours

after treatment. Folpet treatment did not result in any significant increase in the frequency of micronucleated polychromatic erythrocytes (MNPCE), while the positive control chlorambucil showed a significant increase. The study is considered reliable; however, the maximum tolerated dose (MTD) was not reached at the highest dose tested.

In a non-GLP mouse micronucleus test (Yu, 2006; similar to OECD TG 474, see Supplemental information), 5 male and female mice per dose group were treated with folpet at concentrations of 0, 1000, 2000 and 4000 mg/kg bw via oral gavage, once per day for 2 consecutive days. They were sacrificed 6 h after the second administration and the bone marrow was extracted and prepared. Folpet treatment did not result in any significant increase in the frequency of MNPCE, while the positive control cyclophosphamide showed a significant increase.

In a GLP compliant mammalian bone marrow chromosomal aberration test in SD rats (study 2, 1983), similar to OECD TG 475 (1984), folpet was administered by gavage to groups of 12 animals per sex at dose levels of 0, 150, 500, 1500 and 2000 mg/kg bw in 0.5%. Folpet showed no clastogenic effect in bone marrow at 6, 24 or 48 hours after dosing while the positive control cyclophosphamide induced chromosomal aberrations.

None of the three *in vivo* tests investigating *in vivo* clastogenicity on bone marrow, provides direct evidence of bone marrow exposure (polychromatic erythrocytes/normochromatic erythrocytes (PCE/NCE) not affected by treatment in the micronucleus tests in mice, no plasma levels measurement performed and no systemic toxicity observed in any of the three tests). However, indirect evidence that bone marrow exposure occurred in both species is supported by the toxicokinetic studies.

DNA damage

An *in vivo* comet assay (Yu, 2006) in five SD rats per sex per dose, exposed to folpet by diet for 90 days up to 2150 mg/kg bw/day, tail length of peripheral mononuclear cells (PMNC) was not affected by treatment. In the absence of positive control and with respect to the very high tested dose compared to dose range used in the available rat 90 days studies, this test is considered of low reliability.

In vivo tests somatic cell - local exposure duodenum

DNA damage

With respect to folpet's reactivity in the first site of exposure and occurrence of small intestinal tumours in mice carcinogenicity studies, folpet induced effects on mouse duodenum were investigated in three GLP compliant comet assays (study 3, 2004; study 6, 2007; and study 5, 2008) performed similarly to OECD TG 489.

In study 3 (2004), eight CD-1 female mice per group were dosed at 0, 1000 or 2000 mg/kg bw folpet, or the positive control, N-methylN-nitrosourea (MNU) by single gavage administration. Folpet did not induce DNA damage in the duodenum compared to controls at the 2-hour and 6-hour sampling times (measured by tail length, percent tail intensity or tail moment).

In study 6 (2007) four CD-1 mice per sex per group were dosed by gavage at 0, 1000 or 2000 mg/kg bw folpet, or the positive control, MNU. A very slight but statistically significant increase in the median olive tail moment (OTM) was observed only in the high dose females after 3-hour expression time. The increase was driven by one outlier animal and was not reproduced

after 14-hour expression time. According to the study author, the very slight increase in the OTM median was of no biologically significance (3.14 vs 1.73 in controls). Furthermore, the results could have been compromised by a high frequency of ghost cells (hedgehogs) in this study (> 40% in all groups including the vehicle control) hampering an appropriate discrimination between scorable cells and hedgehogs and confounding the scoring. A follow-up study (study 5, 2008), where female animals were treated with a single dose of 2000 mg/kg bw and sampled at 3 hours was clearly negative. The OTM and the frequency of ghost cells (< 1%) for both vehicle and folpet animals were clearly lower than those of study 6 (2007), supporting that scoring in study 6 (2007) was biased by the high frequency of hedgehogs. In conclusion, folpet was also negative in these comet assays.

In a micronucleus test in duodenum of CD-1 mice, mentioned in Arce (2010) and considered acceptable by US EPA, folpet treatment up to 2000 mg/kg bw/d for 5 days did not induce micronuclei or apoptotic cells in the duodenal crypts. However, this test was not submitted in the CLH dossier.

In vivo tests germ cell

Two dominant lethal tests in rats and one in mice are available. The first one (study 7, 1980) is considered reliable with no compromising deviation from the current OECD TG 478 and the second one (study 8, 1982) as supplementary data due to major deviation (e.g. no positive control). The dominant lethal tests in mice (study 17, 1971) is considered as not reliable due to major shortcomings.

In the dominant lethal test (study 7, 1980), folpet in 1% carboxymethyl cellulose (CMC) was given by gavage to groups of 20 Osborne-Mendel male rats at dose levels of 50, 100 and 200 mg/kg bw/d for five days. Folpet was negative with total number of implants, corpora lutea, live implants, early deaths, and late deaths comparable to control. Significant expected responses were obtained in the positive control group (triethylenemelamine).

In study 8 (1982), folpet was administrated to 15 Osborne-Mendel male rats per group either orally (gavage) up to 200 mg/kg bw/d or by intraperitoneal injection up to 10 mg/kg bw/d for five days. Folpet treatment induced mix results and is considered as inconclusive.

The test in mice (study 17, 1971), intraperitoneal administration up to 10 mg/kg bw is considered negative.

In another test in ICR/SIM mice mentioned in Arce (2010) and considered acceptable by US EPA, folpet did not induce dominant lethal effects when administrated via diet up to 5000 ppm. However, this later test was not submitted in the CLH dossier.

In a spermatogonial chromosome aberration test (Yu, 2006 supplementary data), no significant differences in the aberration frequency were observed in male mice treated with folpet up to 4000 mg/kg bw via oral gavage.

Overall, the weight of evidence indicates that folpet is not genotoxic in *in vivo* mammalian germ cell mutagenicity tests.

Genotoxicity of systemically available metabolites

The *in vitro* genotoxicity tests on phthalimide and phthalic acid are of lower quality than the most current studies for folpet however, the *in vitro* data on both metabolites coming from the open literature do not indicate genotoxic potential.

Comparison with the criteria

- Category 1A

No human data are available.

- Category 1B

Folpet is negative in the available heritable germ cell mutagenicity tests in mammals (dominant lethal tests).

Folpet is also negative in the available *in vivo* somatic cell mutagenicity tests in mammals exploring mutagenicity (spot test in mice) and clastogenicity (micronucleus test in mice and chromosome aberration in rats). Furthermore, toxicokinetic studies indicate that folpet is not systemically available itself and therefore unlikely to interact with the genetic material of germ cells.

The published data on its systemically available metabolites do not raised concern on their genotoxic potential.

Consequently, RAC considers that the criteria for categories 1A or 1B are not met.

- Category 2

While, folpet consistently exerts genotoxic activity (both mutagenic and clastogenic) in *in vitro* systems, the *in vivo* follow-up studies in mammals are consistently negative.

In addition, *in vivo* comet assays in the mice duodenum do not indicate genotoxic potential at the first site of exposure.

Consequently, RAC considers that the criteria for Category 2 are not met.

In accordance with the criteria laid down in the CLP Regulation RAC agrees with the DS **that classification for germ cell mutagenicity is not warranted.**

Supplemental information - In depth analyses by RAC

Evidence of bone marrow exposure in *in vivo* tests investigated clastogenicity in bone marrow.

None of the three available tests provide direct evidence of bone marrow exposure (PCE/NCE was not affected by treatment in the micronucleus tests in mice, no plasma levels measurement was performed, and no systemic toxicity was observed in any of the three tests). However, indirect evidence that bone marrow exposure occurred comes from the toxicokinetic data carried out with radiolabelled folpet. While the parent molecule is not likely to reach the systemic compartment, a bioavailability of its metabolites (phthalimide part) higher than 80% is estimated based on radioactivity excreted via urine within 24 h single and repeated administration of 10 mg/kg bw in rats (study 1, 1991). In another toxicokinetic study, after oral administration of 75 mg/kg bw of ^{14}C-folpet (6.3 µg/mL blood in males, and 5.4 µg/mL in females) radioactivity in blood reached a peak at 45 minutes post-dose (0.045% of dose/mL) (study 3, 1974). In a comparative TK/mechanistic study in rats and mice similar excretion patterns were observed after a pulse dose of 50 and 5000 ppm ^{14}C-folpet in the diet; in mice the highest level of radioactivity in blood (0.043-0.047% of the dose) was reached after 6 hours (study 4, 1991).

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 were not processed histopathologically	Folpet Purity 93%, Treatment of 0, 1000, 5000 and 12000 ppm for 112-113 weeks CD-1 mouse 80/sex/treatment group Control= 104/sex	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: 10%), stat. sign. Mucosal hyperplasia in duodenum (68% males, 53% females), stat. sign. extramedullary haematopoiesis of the spleen (34% males, 35% females), stat.	(R-6036) Study 1 (1982)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
		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 survival to 104 weeks <u>1000 ppm (123 mg/kg bw/d)</u> histopathological findings of non-neoplastic and neoplastic lesions in GI tract	(R-3650) Study 2 (1985)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
		(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 <u>1350 ppm (119.9 mg/kg bw/d)</u> 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 <u>3200 ppm (161.8 mg/kg bw/d)</u> 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. <u>200 ppm (9.9 mg/kg bw/d)</u> 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).

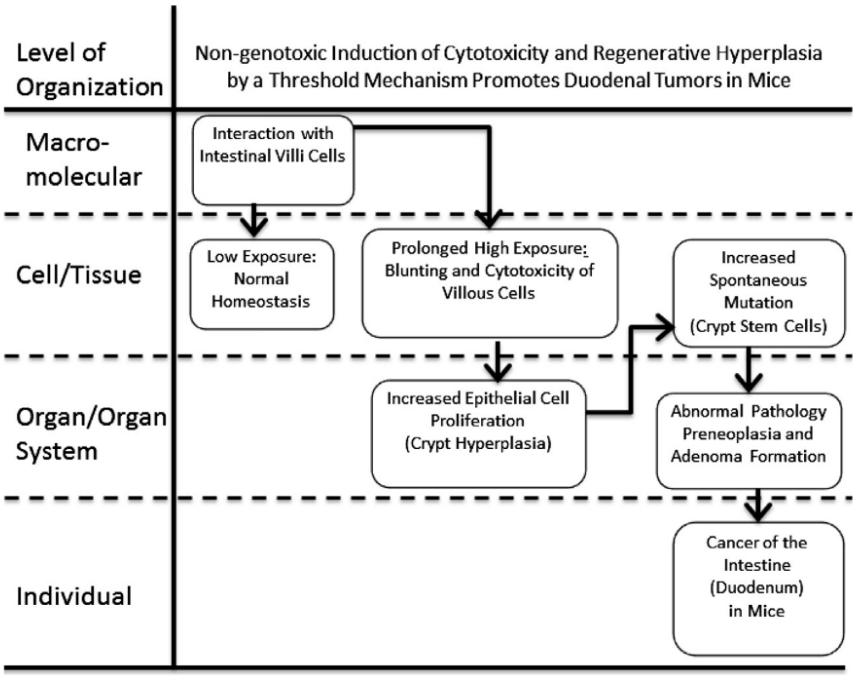


Figure 4: From Becker *et al.* 2015 "Depiction of the AOP of induction of cytotoxicity and regenerative hyperplasia by oral CrVI leading to duodenal tumours in mice." Becker et al. 2015 further states a similarity to effects observed for folpet and captan.

[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.

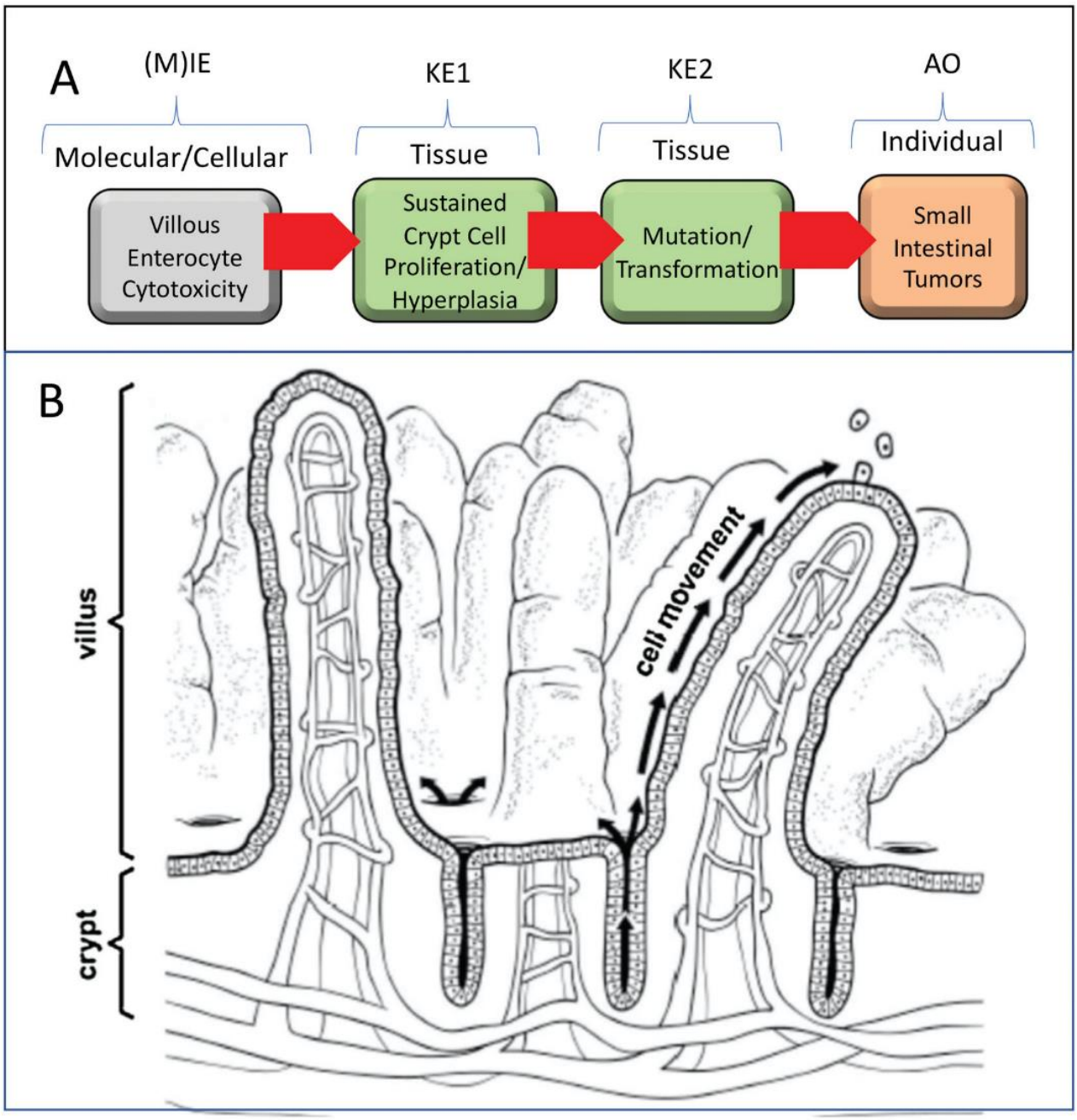


Figure 5: From Bhat *et al.* 2020 "Proposed AOP for cytotoxicity-mediated SI cancer in mice. (A) AOP diagram. See text for discussion regarding (M)IE. Future evolution of this AOP may better define the (M)IE associated with villous cytotoxicity. (B) Structure of intestinal mucosa. Villi are finger- or 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.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

The DS proposed to retain the existing classification of folpet as Carc. 2; H351. While carcinogenicity after oral administration has been demonstrated in a single species (mouse) and in a single target tissue (duodenum) in three independent studies, the underlying MoA has been identified and is initiated by local irritation at the first site of contact. Thereby,

repeated irritating doses of folpet are needed for tumour formation, limiting the strength of evidence.

Comments received during consultation

One MSCA considered that a classification as a carcinogen in Category 1B would be warranted, since there is no evidence that the proposed MoA is not relevant for humans and the exposure scenario was the main argument to limit folpet classification to Category 2, instead of 1B, while the CLP criteria are based on the presence of a hazard.

The DS answered that regarding the specific threshold MoA (cytotoxicity and regenerative cell proliferation by continuous irritation) and the clear established threshold for tumour development in mice gastrointestinal tract, Category 2 is proposed.

Industry disagreed considering that folpet's inherent hazard property is acute irritation and not carcinogenicity and is appropriately classified as an irritant (eye irritation, acute inhalation toxicity) which appropriately communicates its inherent hazard property. The reversibility of the irritating effects in mice duodenum, the species-specificity (mice) of the gastrointestinal tumours, the absence of exposure scenario for humans that results in life-long, or even short-term, irritating concentrations of folpet via the diet represent additional lines of evidence that support a non-classification of folpet (refer to the RCOM for further details).

The DS acknowledged that the adverse outcome pathways (AOP) developed by Bhat *et al*. (2020) concluded that the key events (KEs) become quantitatively implausible in humans after accounting for background levels of human exposure. Nevertheless, the authors also concluded that the KEs are qualitatively plausible in humans. Classification is hazard based, therefore considerations about exposure and risk are not relevant.

Assessment and comparison with the classification criteria

In the absence of human data, the carcinogenic potential is evaluated based on animal studies performed by oral route in mice and rats. No inhalation studies are available.

Oral carcinogenicity studies in mice

Three GLP compliant carcinogenic studies (study 1, 1982; study 2, 1985 and study 3, 1994, similar to OECD TG 451) are available.

In study 1 (1982), folpet was administered in the diet to groups of 80 male and female Swiss CD-1 mice at levels of 1000, 5000 and 12000 ppm (equivalent to 93/96, 502/515 and 1282/1284 mg/kg bw/d in males/females, respectively) for 112-113 weeks. While survival was not affected by treatment body weight was significantly reduced from the mid dose level (17% and 16% less than controls in high dose males and females respectively). Non-neoplastic effects, elsewhere than in the gastrointestinal tract, consisted mainly in hair loss around the eyes and skin irritation as well as slight anaemia in the high dose animals. There were dose-related increases in mucosal hyperplasia from the low dose in the duodenum and at the high dose in the jejunum and ileum.
There were significant and dose-related increases in intestinal tumours, particularly adenocarcinomas, predominantly in the duodenum but also present in the jejunum at the high dose. There was a dose-related increase in incidence of stomach squamous papilloma in males.

Table: *Incidences (%) of lesions in the gastrointestinal tract in study 1 (1982).*

Study 1 (1982)		**Sex**	**ppm (M/F mg/kg bw/d)**			
CD-1 mice n = 80/sex/group, Control = 104/sex			0	1000 (93/96)	5000 (502/515)	12000 (1282/1284)
Stomach	Papilloma	M	1	1	6	8
		F	1	5	6	1
Duodenum	Hyperplasia	M	3	33**	43**	68**
		F	*10*	*40***	*41***	*53***
	Adenoma	M	1	2	3	14**
		F	0	2	4	18**
	Adenocarcinoma	M	0	2	10**	48**
		F	0	0	7*	40*
Jejunum	Hyperplasia	M	1	0	3	40**
		F	0	2	5	37**
	Adenoma	M	0	0	2	2
		F	0	0	0	3
	Adenocarcinoma	M	0	2	0	11**
		F	0	0	0	4
Ileum	Hyperplasia	M	0	0	0	10**
		F	1	0	0	5
Chi-squared/Yates * $p < 0.05$; ** $p < 0.01$						

In study 2 (1985), folpet was administered in the diet to groups of 52 male and female B6C3F1 mice at dose levels of 0, 1000, 5000 and 10000 ppm reduced to of 0, 1000, 3500 and 7000 ppm due to deterioration (equivalent to 123/141, 564/608 and 1264/1300 mg/kg bw/d in males/females, respectively) for 104 weeks. There was a reduction in survival in the mid and high dose animals. However, the survival rate exceeded 66% in all groups. Body weight was significantly reduced from the mid dose level (18% and 23% less than controls in high dose males and females respectively, at termination).

Skin irritation was observed in the high dose animals in the early weeks of the study before the downward adjustment.

There were dose related increases in the incidence and severity of hyperkeratosis of the skin, oesophagus and forestomach in all treated groups. In the intestine atypical hyperplasia and proliferation of mucosal glands were observed from the low dose. There was a significant (by trend analysis) increase of adenomas and carcinomas in the duodenum of all treated groups and carcinomas in the jejunum of high dose animals. Papillomas and squamous cell carcinomas in the stomach were observed in all treated groups being statically significant (by trend analysis) only in females. There was also an increase (trend analysis) in the incidence of malignant lymphomas in treated females, while in males a dose-related decrease was observed. The incidences were 30.7, 36.5 and 50% in low, mid and high dose females respectively vs 30.7% in controls. While no historical control data (HCD) from the laboratory are available, contemporaneous historical controls from NTP studies in B6C3F1 mice provide a mean incidence (standard deviation) [range]

of 12% (7.2%) [2-32%] for males and 25.1% (10%) [8-62%] for females (Haseman, 1984). Another publication mentionned a mean incidence [range] of 23.9% [4.3-52%] for females based on 29 carcinogenicity studies between 1988 and 1998 (Eiben, 2001). Malignant lymphoma is a common spontaneous systemic neoplasm in B6C3F1 mice especially in females. Furthermore, at the high dose level the MTD is exceeded as evidenced by a 23% decreases body weight in females. Overall, the increased incidence of malignant lymphoma in high dose females is considered rather linked to the high spontaneous background of this type of tumour in B6C3F1 than reflecting folpet's carcinogenicity potential.

Table: *Number of animals with neoplastic lesions in study 2 (1985)*

Study 2 (1985)		**Sex**	**ppm (M/F mg/kg bw/d)**			
B6C3F1 mice, n = 52/sex/group			0	1000 (123/141)	3500 (564/608)	7000 (1264/1300)
Stomach	Papilloma	M	0	2	3	2
		F	2	1	5	7**
Duodenum	Tubular adenoma	M	0	0	0	1
		F	1	0	3	1
	Papillotubular adenoma	M	0	1	0	1
		F	1	0	2	0
	Carcinoma	M	0	3	17	24***
		F	0	1	5	18***
Jejunum	Carcinoma	M	0	0	0	1
		F	0	0	0	1
Multiple organs	Malignant lymphoma	M	13	11	12	9
		F	16	16	19	26**
Peto's test for trend ** p < 0.01; *** p < 0.001						

In study 3 (1994), folpet was administered in the diet to groups of 52 male and female CD-1 mice at concentrations of 150, 450 and 1350 ppm (equivalent to 16/16, 47/51 and 151/154 mg/kg bw/d in males/females, respectively) for 2 years. This study was performed to establish a threshold for intestine tumour development in CD-1 mice and to supplement the two previous mouse carcinogenicity studies, consequently the MTD was not reached.
There was a greater incidence of keratoacanthosis in the non-glandular stomach in high dose females occasionally associated with acute inflammation and ulceration. Villous hyperplasia of the duodenal mucosa was seen in three high dose females and one mid dose male. Hyperplasia of the duodenal lamina propria was seen in two high dose males. One high dose male was found to have hyperplasia in the jejunum and ileum.
A duodenal adenoma was found in one high dose female and benign papillomas were noted in the forestomach in one high dose male, three high dose and one mid dose females.

Table 14: *Number of animals with neoplastic lesions in study 3*

Study 3 (1994)		**Sex**	**ppm (M/F mg/kg bw/d)**			
CD-1 mice n = 52/sex/ group Control = 100/sex			0	150 (16/16)	450 (47/51)	1350 (151/154)
Stomach	Papilloma	M	0	0	0	1
		F	0	0	1	3*

Duodenum	Benign B-adenoma	M	0	0	0	0
		F	0	0	0	1
Fischer's exact test * $p < 0.05$						

Oral carcinogenicity studies in rats

Three GLP compliant studies in rats (diet) are available: study 4 (1985, similar to OECD TG 453 including 1-year time point), study 5 (1985 similar to OECD TG 451) and study 6 (1989, chronic toxicity similar to OECD TG 452).

In study 4 (1985), folpet was administered in the diet to groups of 50 male and 52 female Crl:CD(SD)BR rats at concentrations of 200, 800 and 3200 ppm (equivalent to 10, 40 and 162 mg/kg bw/d) for 2 years. Survival and body weight were not affected by treatment (body weight gain for the high dose animals was slightly decreased (5%) after one year).
In high dose males and females, increased incidence of hyperkeratosis/acanthosis with erosion/ulceration in the forestomach occasionally associated to inflammation were observed at the terminal kill and in unscheduled deaths. At the 1-year interim kill a very slight increase in incidence of these lesions was noted.
No gastric or intestinal neoplastic lesion were noted. The thyroid C-cell adenoma and the interstitial (Leydig cell) tumours seen in males were not considered to be treatment related based on a lack of a dose-response relationship and/or being within the biological variability of this strain and HCD range.

In study 5 (1985), folpet was administered in the diet to groups of 60 male and female Fischer F344 rats at dose levels of 0, 500, 1000 and 2000 ppm (equivalent to 28/37, 58/67 and 108/133 mg/kg bw/d in males/females respectively) for 2 years. Survival was not affected by treatment. The mean body weight in treated males was slightly lower (less than 3%) than in controls.
Non-neoplastic treatment-related microscopic findings were mainly seen in the gastro-intestinal tract with hyperkeratosis in the forestomach from the mid dose (affecting all high dose animals) and in the oesophagus only in the high dose animals.
Increase incidence of benign fibro-epithelial tumours in the mammary gland and thyroid C-cell adenoma in females and malignant lymphoma in the highest treatment groups were not considered to be treatment related based on a lack of a dose response and/or being within the biological variability of this strain and NTP historical control range (Haseman, 1984; Haseman, 1985).

In study 6 (1989), folpet (91.1%) was administered in the diet to groups of 20 male and female Fischer F344 rats at dose levels of 0, 250, 1500 or 5000 ppm (equivalent to 0, 12/16, 83/104 or 296/359 mg/kg bw/d in males/females, respectively). There was no evidence of carcinogenicity in this study. Survival was not affected by treatment. Mean body weight gain was significantly decreased in both sexes at 5000 ppm.
In both sexes, histopathological finding consisted in the increased incidence and severity of diffuse hyperkeratosis of the forestomach from the mid dose and of the oesophagus at the high dose.

RAC notes that the doses tested in rats carcinogenicity studies were lower than in mice studies.

While irritation of the forestomach (hyperkeratosis/acanthosis in all animals) was observed at high dose levels in studies 4 and 5, the reported marginal body weight changes do not indicate that the MTD was reached in any of the two studies.

Overall, folpet consistently induces glandular tumours (adenomas and adenocarcinomas) in the duodenum and jejunum of both CD-1 and B6C3F1 mice. There was also a marginal increase of papillomas in the forestomach in both strains.

While some uncertainties remain in respect to the lower doses tested, the available carcinogenicity studies do not provide evidence that folpet is carcinogenic in rats.

The aetiology of the small intestinal tumours has been thoroughly investigated in mechanistic studies supporting a MoA driven by cytotoxicity with subsequent regenerative proliferation which if sustained, increases the probability of spontaneous mutation leading finally to tumours. Recently, an AOP on mouse small intestinal tumours mediated by the initiating event "sustained enterocyte cytotoxicity" has been published (Bhat *et al.*, 2020). Folpet, its sibling captan and hexavalent chromium have been used as stressors to provide the empirical support of this AOP (See Appendix 1 for RAC's analysis and further details).

Comparison with the criteria

- Category 1A

No epidemiological studies in humans investigated folpet's carcinogenic potential are available.

- Category 1B

RAC considers that the experimental studies provide sufficient evidence of carcinogenicity according to CLP criteria since folpet induces benign and malignant neoplasms in the gastrointestinal tracts in three independent well-conducted studies in mice. These tumours occurred in both sexes with a clear dose-response relationship.

- Category 2

However, RAC has also taken into consideration several factors that may decrease the level of concern for human carcinogenicity.

- Tumours were limited to one tissue (small intestine).
- There is sufficient evidence that folpet is not mutagenic *in vivo*. Especially, no DNA damage in duodenal was noted in two independent comet assays in mice.
- Based on a weight of evidence analysis, RAC considers that the proposed MoA driven by enterocyte cytotoxicity with subsequent regenerative proliferation is sufficiently substantiated in mice.
- While this MoA is considered qualitatively relevant for human, RAC acknowledges that a clear threshold for tumour-development in mice is established and sustained irritating concentrations are necessary to trigger the downstream key events.

RAC considers that the above mentioned elements support Category 2 classification.

Based on a weight of evidence analysis and in accordance with the criteria laid down in the CLP Regulation, RAC agrees with the DS to **classify folpet as carcinogen in category 2; H351, i.e. retaining the current classification.**

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,	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-	(R-4347) Study 1 (1986)

<table>
<tr>
<td>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
- 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>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>
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</td>
<td>Folpet batch SX-1388 (technical grade), purity 89.5%</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)</td>
<td>(R-6134)
Study 2 (1985)</td>
</tr>
</table>

<table>
<tr>
<td>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><u>800 ppm</u>
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>
<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			

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Developmental toxicity OECD 414 (2001) GLP Oral (gavage) Rat, Rj Han:SD 24 mated females/group 20 litters/group used for foetal examination	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: 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). 100 mg/kg bw/day: ↓ 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). 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	(000101015) Study 4 (2007)
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	Folpet batch 631729 (technical grade), purity 91.1% 0, 150, 550 or 2000 mg/kg bw/day on gestation days 6-15	*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	(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**
the day prior to scheduled kill) GLP Oral (gavage) Rat, CD(SD) 22 mated females/group	Vehicle: CMC+ acetic acid	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 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)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Preliminary developmental toxicity Non-guideline Non-GLP Oral (gavage) Rat, CD(SD) 6 mated females/group supplementary information (reliable with restrictions)	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 (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 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	(R-14258) Study 9 (2002)
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	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	(R-18200) Study 11 (2006)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
25 mated females/group		*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). 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)	Folpet batch SX-1338 (technical grade), purity 89.5%	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.	(R-6183) Study 13 (1985)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Rabbit, New Zealand White (DIa Hra:NZW) 20 mated females/group	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 Vehicle: CMC + Tween 80	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.	
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 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)	Folpet technical grade Purity: not reported Vehicle: corn oil	Limited data reported. Small groups of animals used. 10 pregnant rats dosed with 100 mg/kg bw/day gestation days 6-15 4 pregnant rats dosed with 500 mg/kg bw/day gestation days 8-10	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 foetal abnormalities. Internal structure appeared normal. Well-defined skeletal development generally observed.	Kennedy, Fancher & Calandra. (1968) Toxicology and Applied Pharmacology, 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	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*	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
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)			1/65 foetuses abnormal with microphthalmia. Study not appropriate to derive NOAEL	
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	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.

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
(due to very limited reporting- only the abstract of the 8th annual meeting is available).				Abstract only
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	Folpet technical grade purity 87% Gavage vehicle corn oil:acetone 9:1 SC vehicle:	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**
CD-1 mice 7-8/group Supplementary information (Only one dose level/route, severe maternal toxicity when administered by inhalation route, age of mice unknown) (reliable with restrictions)	DMSO 100 mg/kg/day oral & SC gestation days 6-15 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	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
not reported, lack of reporting for foetal effects, e.g. weight, sex, … , no negative 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	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, ↓

		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%)	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 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%	Yes: Incomplete ossification in the pelvis, pubis and/or ischium- not stat. significant Assumed to occur secondary to maternal toxicity

		days 6-20); ↓ food consumption (11% days 6-13, 15% days 13-20)	
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)	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.

		[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)] *	Assumed to occur secondary to maternal toxicity (No hydrocephaly)
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),	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

		few (13/25) or pale (5/25) faeces [body weight loss (-0.03kg cf. +0.03kg controls days 6-10); ↓ body weight gain (69% days 6-29)]*	Assumed to occur secondary to maternal toxicity
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). 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.	**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
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.) 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	Folpet batch 631933 (technical grade), purity 91% 0, 250, 1500 or 5000 ppm Vehicle: laboratory animal diet	*Parental toxicity* <u>5000 ppm (250 mg/kg bw/day)</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) 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]	Study 1 (1986)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
		250 ppm (12.5 mg/kg bw/day) 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* 5000 ppm (250 mg/kg bw/day) F1: ↓ body weight gain from day 7 (27% days 7-21) F2: ↓ body weight gain from day 7 (12% days 7-21) 1500 ppm (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. 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	Folpet batch SX-1388 (technical grade), purity 89.5% 0, 200, 800 or 3600 ppm Vehicle: laboratory animal diet	*Parental toxicity* 3600 ppm F0: ↓ slight body weight gain males (5.6%, days 1-155) F1(b): ↓ slight body weight gain males (5.7%, days 1-155) 800 ppm No effects NOEL 800 ppm *Reproductive toxicity* No effects at any dose level NOAEL 3600 ppm *Offspring toxicity* 3600 ppm 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%) 800 ppm No effects NOAEL 800 ppm *Test item intake in mg/kg bw/day*	(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>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><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

Type of study/data	Relevant information about the study (as applicable)	Observations	Reference
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) LOAEL of 10 mg/kg bw/d	Allingham (2016) Study 18 (2016)

9.10.11 Conclusion on classification and labelling for reproductive toxicity

Folpet is proposed to be not classified for reproductive toxicity.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Sexual function and fertility

No classification of folpet for sexual function and fertility has been proposed by the DS based on the absence of effects on reproduction in two two-generation studies up to 5000 ppm (equivalent to 370 and 435.6 mg/kg bw/d in males and females, respectively), dose level inducing reduced body weight and food consumption in parental animals and reduced pup growth.

Developmental toxicity

No classification of folpet for developmental toxicity has been proposed by the DS, the developmental effects observed in rats (delayed ossification as well as reduced foetal weigh) being assumed to be secondary to high maternal toxicity. In the rabbit the increased post implantation loss and late resorptions, reduced foetal weight, reduced skeletal ossification, increased incidences of extra ribs and vertebra observed in several studies and lens

malformations (observed in a single study) are also assumed to be secondary to high maternal toxicity and consequent foetal immaturity.

Regarding, the increased incidences of hydrocephalus observed in one study in the presence of severe maternal toxicity, a following pulse dose study failed to identify a specific sensitive period. Furthermore, HCD showed a peak in hydrocephalus at the time the study was conducted.

Lactation

No classification of folpet for effects on or via lactation has been proposed by the DS since no adverse effect of folpet due to transfer of the chemical in the milk or on the quality of the milk was indicated in the multigeneration studies.

Comments received during consultation

One MSCA and IND supported no classification for reproductive toxicity.

The DS pointed out for RAC discussion that folpet's metabolite phthalimide has a structure similar to thalidomide which is a known teratogenic substance in the rabbit. In a developmental study (study 15, 2006), the metabolite phthalimide was tested clearly below the MTD, therefore effects at higher dose, capturing maternal toxicity, cannot be excluded.

Assessment and comparison with the classification criteria

Sexual function and fertility

Two GLP compliant two-generation reproductive toxicity studies in rat exposed by diet are available (study 1, 1986 and study 2, 1985, similar to OECD TG 416 (1983)). Although sensitive parameters (e.g. sperm parameters, age at puberty onset, ovarian follicles counts) required in the current version of OECD TG 416 (2001) were not investigated, RAC agrees with the DS that the deviations are unlikely to alter the conclusions reached. Study 1 (1986) is given more weight since the highest dose induced systemic toxicity which was not the case in study 2 (1985).

A non-GLP three-generation dietary study, study 3 (1967) in rat up to 1000 ppm, is not considered reliable.

Folpet has also been tested in an exhaustive battery of GLP compliant *in vitro* and *in vivo* endocrine assays under the Endocrine Disruptor Screening Program (EDSP) Tier 1 of US EPA, corresponding to level 2 and 3 of the OECD Conceptual Framework for Testing and Assessment of Endocrine Disrupters (as revised in 2012).

In study 1 (1986) folpet was administered in the diet to groups of 25 male and female CD(SD) rats at concentrations of 250, 1500 and 5000 ppm (equivalent to 19/23, 112/133 and 370/436 mg/kg bw/d in F0 males/females respectively) during a 14-week premating period, pairing, gestation and lactation until weaning of the F1 litters. Twenty five males and females were selected from the F1 litters to form the next generation and were exposed to the same treatment regimen as F0 (equivalent to 25/28, 150/168 and 520/565 mg/kg bw/d in F1 males/females respectively).

Food consumption and mean body weight were significantly lower than the controls in high dose animals of both generations throughout the study (F0 males -8.5% at the end of the

premating period; F0 females -5%, -7% and -4% at the end of the premating period, gestation and lactation respectively; F1 males -14% at the end of the premating period and F1 females -8%, -9% and -5% at the end of the premating period, gestation and lactation respectively).

Histopathological examination revealed increases in the incidence of diffuse hyperkeratosis in the forestomach from the mid dose groups of both sexes in both generations in line with the findings of the repeated toxicity studies.

Absolute testes weight was statistically significantly reduced (-8%) in the high dose group in the F1 generation.

In both generations, there was no test substance-related effects on oestrous cycle, gestation length, mating, fertility and gestation indices, litter size or sex ratio.

Neo-natal viability index was not affected by treatment. Lactation index (i.e. pup survival post culling) was slightly decreased in the high dose group in both generations but did not reach statistically significance. Significantly lower mean pup weights were recorded in high dose offspring from post-natal day (PND) 7 (-8%) in F1 pups and on PND21 (-9.5%) in the F2 pups.

In the study 2 (1985), folpet was administered in the diet to groups of 30 male and female CD(SD) rats at concentrations of 0, 200, 800 and 3600 ppm (equivalent to 15/88, 60/73 and 263/314 mg/kg bw/d in F0 males/females respectively) during a 62-day premating period, pairing, gestation and lactation for two successive litters (F1a and F1b). Thirty males and females were randomly selected from the F1b litters to form the next generation and were exposed to the same treatment regimen as F0 during a 12-week premating period, pairing, gestation and lactation for two successive litters (F2a and F2b) through to weaning of the F2b litters (equivalent to 22/23, 91/95 and 421/436 mg/kg bw/d in F1b males/females, respectively).

Mean body weights at termination were slightly decreased in high dose F0 (-5%) and significantly decreased in F1b males (-9%). There were no other systemic effects nor histopathological findings (GIT was not analysed).

There was no effect on any reproductive parameters in any generations.

Lactation indices in high dose groups were similar or higher than their respective controls in all generations (F1a, F1b, F2a and F2b).

Mean pup weights in the high dose group were significantly reduced in all litters in both generations (10 to 20% less); in the F1a and F1b litters, weights were reduced on PND21 and from PND14 in the F2a and F2b litters.

The results of the GLP compliant endocrine assays conducted according to OPPTS guidelines are summarised in the following table.

Table: *Summary table of endocrine assays*

Assay	Results
Level 2 assays (*in vitro* assays providing data about selected endocrine mechanism(s) / pathways(s))	
Study 25 (2012), OPPTS 890. 1250 Oestrogen Receptor Binding Assay	Negative: folpet does not interact with the rat oestrogen receptor.
Study 26 (2012), OPPTS 890. 1300 Oestrogen Receptor Transcriptional Activation Assay	Negative: folpet is not an agonist to hERα in the HeLa-9003 model.
Study 23 (2012), OPPTS 890.1150 Androgen Receptor Binding Assay (Rat Prostate)	Negative: folpet does not interact with the androgen receptor.

Study 24 (2012), OPPTS 890. 1200 Human Recombinant Aromatase Assay	Equivocal: average aromatase activity between 50 and 75%
Study 27 (2012), OPPTS 890.1550 Steroidogenesis Assay	Negative in H295R cell line
Level 3 assays (*in vivo* assays providing data about selected endocrine mechanism(s) / pathway(s))	
Study 22 (2012), OPPTS 890.1600 Uterotrophic Assay	Negative: folpet did not show agonist or antagonist estrogenic activity in ovariectomized SD rats (tested doses: 0, 313 or 1000 mg/kg bw/d for 3 days)
Study 19 (2012), OPPTS 890. 1400 Hershberger Assay	Negative: folpet did not exhibit agonist or antagonist androgenic activity in castrated male SD rats (tested doses: 0, 100, 250 or 800 mg/kg bw/d for 10 days)
Level 4 assays (*in vivo* assays providing data on adverse effects on endocrine relevant endpoints)	
Study 20 (2012), OPPTS 890.1500 Pubertal Assay in Female Rats	Negative: folpet does not adversely pubertal development in female rats (tested doses: 0, 400 or 800 mg/kg bw/d for 21/22 after weaning)
Study 21 (2012), OPPTS 890.1450 Pubertal Assay in Male Rats	Negative: folpet does not adversely pubertal development in male rats (tested doses: 0, 200, 400 or 800 mg/kg bw/d for 31/32 days after weaning)

Levels as stated in the Conceptual Framework for Testing and Assessment of Endocrine Disrupters (as revised in 2012).

Overall, the battery of *in vitro* and *in vivo* endocrine assays does not provide evidence that folpet could interact with the oestrogen, androgen or thyroid pathways in mammals.

Based on a weight of evidence analysis according to the EFSA/ECHA ED guidance (2018), EFSA peer review concluded in 2022 that folpet does not meet the ED criteria for human health https://www.efsa.europa.eu/en/topics/topic/pesticides#peer-review (EFSA report, conclusion not available yet).

In the repeated dose toxicity studies in rats and mice, folpet did not induce adverse effect on the reproductive organs. In the dog, decreased testis weight and testicular degeneration observed at high dose levels (exceeding the TG recommended limit dose) in a 90-day study (study 7, 1985) and in a 52-week studies (study 8, 1988) are considered to be a consequence of the overt toxicity (poor general conditions and marked decrease of bodyweight) rather than a direct effect of folpet on the testis.

Comparison with the criteria

RAC acknowledges that the two-generation studies have some limitations (omissions in the study design compared to the current test guideline requirements of OECD TG 416). Nevertheless, they do not indicate effects on sexual function or fertility up to dose levels inducing systemic toxicity (5000 ppm). There is no convincing evidence that folpet impacts reproductive organs in repeated dose toxicity studies or interferes with the oestrogen or androgen pathways based on an exhaustive battery of endocrine assays.

Therefore, in accordance with the criteria laid down in the CLP Regulation, **RAC concurs with the DS that no classification for sexual function and fertility is warranted.**

Development

Studies in rats

Two GLP compliant prenatal developmental toxicity studies (PNDTS) in the rat, study 4 (2007) and study 5 (2003) performed according to OECD TG 414 (2001) are available.

Two older studies (non-GLP, with quality assurance inspections) but with design similar to OECD TG 414 (2001) for study 7 (1983) and OECD TG 414 (1981) for study 6 (1987) are also considered reliable in the absence of major deviations.
In the four studies, folpet in CMC-based vehicle was administrated by gavage to pregnant Sprague-Dawley rats (at least 20 animals/group) on gestation days (GD) 6-20 (study 4), GD6-19 (study 5, 2003 and study 7, 1983) or on GD6-15 (study 6, 1987). The dose selection relied on dedicated preliminary studies.
The same dose regimen (i.e. 0, 20, 100 or 800 mg/kg bw/day) was applied in studies 4 and 5.

In study 4 (2007), at 800 mg/kg bw/day, there was marked maternal toxicity evidenced by 2 deaths, clinical signs (dyspnoea and loud breathing) and a 25% decrease of the net body weight gain (GD 6-21). Some maternal toxicity (decreased net body weight gain of 20%) was also observed at 100 mg/kg bw/day. Post-implantation loss was slightly increased in treated groups (3.6, 9.8, 6.3 and 7.8 in controls, low, mid and high dose, respectively). In the absence of dose-response relationship, this effect is considered unlikely to be related to treatment. No adverse effects on foetal viability and growth or on teratogenic potential were demonstrated in this study.

In study 5 (2003), maternal toxicity was restricted to the high dose females evidenced by a 21% decrease of the net body weight gain (GD 6-19) and increased salivation after dosing. Consistently with study 4 (2007), there were no significant substance-related developmental effects.

In study 7 (1983), folpet was administrated at 0, 10, 60 or 360 mg/kg bw/day. At the high dose, clear maternal toxicity was evidenced by clinical signs (dyspnoea, salivation, decreased motor activity, soft/liquid faeces) and decreased net body weight gain (27% less on GD6-19). At the mid dose, a decreased net body weight gain (15%) was also noted. There was no effect on foetal viability, on mean foetal weight or on malformations incidences. In the high dose group, a slight non-statistically significant increased incidence of incomplete ossification in the pelvis, pubis and/or ischium (3 litters affected versus 1 in controls) was observed.

In study 6 (1985), higher dose levels were investigated (0, 150, 550 and 2000 mg/kg bw/day) but exposure only covered the period of organogenesis (GD6-15). At the highest dose level, marked maternal toxicity was evidenced by 1/22 death, clinical signs, decreased net body weight gain of 50% and decreased food consumption during the whole period of dosing. Some maternal toxicity (decreased net body weight gain of 14% and decreased food consumption during the first few days of dosing) was also observed at the mid dose level.
There was no effect on foetal viability. Foetal weight was reduced in the mid (4%) and high dose groups (7%) reaching statistical significance only at the high dose. There was a significant increase of small foetuses (< 3 g) from the mid dose level (33 and 75 in mid and high doses respectively vs 20 in control).
A number of skeletal variations characteristic of transient developmental retardation with delayed ossification were observed in the mid and high dose groups. These included: dose related and statistically significant reduced ossification of cranial bones, sternebrae, pubes, metacarpals and metatarsals. Foetuses in the low dose group were not significantly affected, except for the interparietal bone when analysed on a per litter basis (calculated as the sum of individual litter frequencies/number of litters x 100). In the low dose groups the incidence of angulated ribs was within the historical control range (from the same laboratory 15 studies in SD rats conducted between 1984 and 1987). Incidence of reduced ossification of the interparietal bone was within the historical control for the low and mid dose groups.

Table: *Selected foetal findings in study 6 (1985) in rats*

Anomaly	Incidence No (%)	Dose Level of folpet (mg/kg bw/day)				HCD from 15 studies conducted 1984-1987
		0	150	550	2000	
No. of litters examined		22	22	22	20	
Reduced ossification interparietal bone	Foetus	23 (13.45)	29 (18.13)	22 (15.94)	49 (36.57)***	3-51 (1.8-31.7%)
	Litter[$]	12 (13.23)	13 (18.73)*	12 (18.56)*	19 (38.53)***	2-17 (1.7-33.0%)
	Litter[#]	12 (54.5)	13 (59.1)	12 (54.5)	19 (95.0)	
Angulated ribs	Foetus	0 (0.00)	5 (3.13)	4 (2.90)	6 (4.48)*	0-5 (0-3.1%)
	Litter[$]	0 (0.00)	3 (2.84)**	3 (6.49)***	5 (6.51)***	0-3 (0-3.3%)
	Litter[#]	0 (0.00)	3 (13.6)	3 (13.6)	5 (25.0)	

* p < 0.05; ** p < 0.01; *** p < 0.001

[$] Incidence mean % calculated as the sum of individual litter frequencies/number of litters x 100
[#] Incidence mean % calculated as number of litters with affected foetuses/total number litters examined x 100 (not statistically analysed)

The multigeneration studies in rats did not show any effect on foetal development. There were no effects on birth, viability or lactation indices.

Birth weight was not affected by treatment. However, in study 1 (1986) decreased pup weight was noted in high dose pups, in the presence of maternal toxicity (decreased body weights during gestation and lactation). In study 2 (1985) decreased pup weight was also noted in high dose pups not earlier than PND21 for the F1 generation and PND14 for the F2 generation, in the absence of significant effects on maternal body weights.

In a publication (Kennedy, 1968) of limited reliability (poor reporting, low number of animals, tested dose levels and exposure period), teratogenicity of the three fungicides folpet, captan and difolatan, their metabolites phthalimide and tetrahydrophthalimide (THPI) was investigated in rats and rabbits in comparison with thalidomide. The authors concluded that 100 mg/kg bw/d administered of folpet on GD6-15 to SD pregnant rats reduced maternal body weight but did not induce developmental effects while at exposure to 500 mg/kg bw/d administered on GD6-10, the foetus showed a marked retardation of development.

Table*: Summary of maternal and developmental toxicity from the PNDTS in rats*

Dose level of folpet (mg/kg bw/day) References	Maternal toxicity	Developmental toxicity		
		Death	Structural abnormality	Altered growth
2000 Study 6, 1985	1/22 death, clinical signs, ↓ 50% net bw gain (GD 6-15) ↓ food consumption	No	No malformation ↓ skeletal ossification ↑ litter incidence of angulated ribs	↓ 7% foetal weight ↑ number of small foetuses
800 Study 4, 2007	2/24 deaths dyspnoea ↓ 25% net bw gain (GD 6-21)	No	No	No

800 Study 5, 2003	↑ salivation post dosing ↓ 21% net bw gain (GD6-20)	No	No	No
550 Study 6, 1985	clinical signs, ↓ 14% net bw gain (GD6-15)	No	No malformation ↓ skeletal ossification ↑ litter incidence of angulated ribs	↓ 4% foetal weight ↑ number of small foetuses
360 Study 7, 1983	clinical signs, ↓ 27% net bw gain (GD6-20)	No	No malformation ↓ ossification in the pelvis, pubis and/or ischium (slight non-stat.)	No
150 Study 6, 1985	No	No	↑ litter incidence of angulated ribs and ↓ reduced ossification of the interparietal bone (low incidence, within HCD)	No
100 Study 4, 2007	↓ 20% net bw gain (GD6-21)	No	No	No
100 Study 5, 2003	No	No	No	No
60 Study 7, 1983	↓ 15% net bw gain (GD6-20)	No	No	No
20 Study 4, 2007	No	No	No	No
20 Study 5, 2003	No	No	No	No
10 Study 7, 1983	No	No	No	No

Assessment

RAC has analysed in a weight of evidence approach, the effects from the available data in rats, relevant for development classification.

From a consistent and reliable set dataset, there is no evidence that folpet induces significant toxic effect in the rat offspring i.e. embryo/foetal lethality or structural malformations up to a dose twice higher than the recommended limit dose in OECD TG 414.

Across studies, foetal toxicity as indicated by decreased foetal weight and/or delayed ossification was observed concurrently with moderate to marked maternal toxicity which could at least partly contribute to the observed developmental findings. However, RAC notes that significant increased incidence (% as calculated by the study author) of reduced ossification of the interparietal bone and angulated ribs were observed in study 6 (1985) in the low dose group without evidence of maternal toxicity. Reduced ossification of the interparietal bone is categorised as a variation by the DevTox Project (devtox.org). RAC notes that the incidence on a litter basis at the low and mid dose levels are close or equal to that in the concurrent control group and well within the range of HCD. Angulated ribs is not referenced in the current harmonized nomenclature of DevTox Project and may be considered as synonym of "wavy rib" (Stazy, 1991) but also to "bent ribs". Distinction between wavy ribs categorized as a variation by DevTox and bent ribs categorized in grey zone is based on appearance rather than on aetiology and causation. Several studies have showed that wavy ribs are reversible within a few days or weeks after birth in rodents (Kast, 1994; Soleki, 2013). Low information is available on the postnatal fate of "bent" ribs probably due to variation in terminology. However, Kast (1994) suggested that bent, undulated, nodulated, bulbous, flexible, kinky, distorted, or misshapen ribs as well as mineralization defects are synonyms for waved or ''wavy'' ribs and emphasized the transient nature of wavy ribs finding and its association with delays in ossification. RAC considers that while a treatment related effect cannot be excluded

in study 6 (1985), the low dose group incidence is within the HCD range and the dose response relationship is not clear.

Regarding postnatal development, in study 2 (1985), decreased pup weight was noted in high dose pups not earlier than PND21 for the F1 generation and PND14 for the F2 generation, in the absence of significant effects on maternal body weights. As the pups gradually start to consume food from around PND14, the effects on bodyweight seem related to a direct exposure from the diet.

Overall, there is slight evidence on toxicity on development. In view of the nature and the low severity of the developmental findings (slight depressed foetal weight and/or retarded ossification and wavy ribs) observed concomitantly with marked maternal toxicity, RAC considers that the four PNDTS performed in rats do not raise concern regarding toxicity for development.

Studies in rabbits

Study performed with folpet

Three GLP compliant studies in the rabbits (study 11, 2006; study 12, 1984; study 13, 1985) are available and considered reliable.

Study 11 (2006) fully complies with OECD TG 414 (2001) with a treatment period from day 6 to day 28 of gestation and is considered as the key study. Study 12 (1984) design is similar albeit a lower number of dams with litters at term than recommended in the current test guideline. Study 13 (1985) is a follow-up of study 12 (1984) using a pulse dosing regimen.

A non-GLP (with report on quality assurance inspections), study 14 (1985) is compliant with OECD TG 414 (1981) with a treatment period from GD 6 to 19, it is also considered reliable albeit a lower number of dams with litters at term than recommended in the current test guideline. In this study, folpet in CMC-based vehicle was administrated by gavage to pregnant New Zealand White rabbits.

In study 11 (2006), 25 mated female rabbits received folpet via gavage at doses of 0, 10, 30, or 60 mg/kg bw/day. Maternal toxicity was observed in all treated groups. There were highly increased incidence of thin build and few or pale faeces in high dose dams and similar pattern of effects albeit less prominent at lower dose levels. Reduced food consumption was about 20%, 35% and 50% less during the dosing period in low, mid and high dose groups respectively and was less marked at the end of the treatment. Adjusted body weight change was significantly reduced in high dose dams. One animal in each treated groups aborted. At the high dose level, there was a statistically significant increase in the mean number of late resorptions and consequently a statistically significant increase in mean post-implantation loss (12.3% vs. 4.6% in controls). Mean foetal body weight at the high dose level was statistically significant decreased (18% less). A 7% non-statistically significant decrease was also observed at the mid dose level.

From the mid dose level, there was an increased incidence of skeletal abnormalities (i.e. thoracolumbar supernumerary ribs associated with 20 thoracolumbar vertebrae and incompletely ossified/unossified epiphyses, astragalus, metacarpals/phalanges) as well as increased incidence of atelectatic lungs. These effects were not statistically significant (on foetus and litter basis) but showed a clear dose response relationship and could reflect a developmental delay.

At the high dose group (60 mg/kg bw/d), there was an increased incidence of foetuses (8 foetuses in 2 litters) with lens alterations (small, misshapen, opacity). According to the study author, there were no previous records of this finding in the laboratory HCD and although this represents an unusual and severe malformation, only an unequivocal relationship to treatment is established in view of the low incidence.
From individual data, both dams (no. 89 and 99) with foetuses showing lens malformations lost weight during gestation phase and had late resorptions. Mean foetal weights in the litters of dams No. 89 and 99 were 24.9 and 24.1 g, respectively, both lower than the mean foetal weight of this group (32.8 g) and the mean foetal weight of the concurrent control group (40.2 g). The majority of these foetuses (6/8) also showed delayed ossification.

According to the study author these findings in conjunction indicate a slight developmental delay in the high dose group, probably associated with the observed significant decrease in mean foetal weight and the significantly reduced mean maternal bodyweight gain.

Table: *Selected foetal findings in study 11 (2006) in rabbits*

Observations	Dose level folpet (mg/kg bw/d)			
	0	**10**	**30**	**60**
Major malformations				
Number of foetuses (litters) examined	227 (25)	177 (22)	184 (22)	191 (22)
Number of foetuses (litters) affected	3 (2)	3 (1)	1 (1)	11 (5)
Mean % affected per litter	8	4.5	4.5	22.7
Eye – Lens malformation (litter)	-	-	-	8 (2)
Skeletal abnormalities				
Number with 12/13 or 13/13 ribs, foetus (litter)	127 (22)	108 (22)	134 (20)	144 (22)
20 thoracolumbar vertebrae, foetus (litter)	65 (15)	47 (17)	91 (19)	103 (21)
Incomplete ossification/unossified epiphyses, foetus (litter)	5 (5)	8 (5)	12 (8)	43 (12)
Incomplete ossification/unossified astragalus, foetus (litter)	2 (1)	1 (1)	3 (3)	10 (4)
Incomplete ossification/unossified metacarpals/phalanges, foetus (litter)	11 (7)	11 (7)	36 (15)	47 (14)
Visceral abnormalities				
Lungs atelectatic, foetus (litter)	3 (2)	2 (2)	12 (5)	17 (5)

In study 12 (1984), 20 inseminated female rabbits received folpet via gavage at doses of 0, 10, 20, or 60 mg/kg bw/day. One, 4, 4 and 6 females were found to be not pregnant in the control, low, mid and high dose groups, respectively. At the high dose, maternal toxicity resulted in 1/20 death (gastric ulceration), reduced food consumption throughout treatment period (about 50%) and reduced net body weight gain. Maternal toxicity was also evidenced by decreased food consumption (about 20% less during the dosing period) at the mid dose level.

In the high dose group, a total of 4 foetuses (3 live and 1 dead) from 3 litters were found to be hydrocephalic (the dead foetus was in the litter of the female found dead on GD27). These foetuses were reported to have correlated anomalies of the skull (domed head, holes in parietals, irregularly shaped fontanelle). The study 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.

A review of HCD from the laboratory between 1980 and 1991 indicates that there was a higher prevalence of hydrocephaly at the time study 12 and 13 were conducted (1984-1985) compared to the previous and subsequent periods. However, the incidence (4 in 3 litters) at the high dose level still slightly exceeds the HCD range from 1982-1986 when the dead foetus is taken into account.

Table: *Incidence of hydrocephaly in live foetuses in study 12 (1984) in rabbits*

Observations	**Dose level folpet (mg/kg bw/day)**				**HCD range** from 38 studies 1982-1986
	0	**10**	**20**	**60**	
Number of foetuses (litters) examined	96 (16)	73 (14)	115 (16)	64 (11)	
Hydrocephaly Foetus Litter	0 0	0 0	1 (0.9) 1 (6.2)	3 (4.8)** 2 (18.2)	0-3 (2.7) 0-2 (11.8)

** $p \leq 0.01$
HCD are related to live foetuses. In high dose folpet study 1 dead foetus from the dam found dead also had hydrocephaly.

Study 13 (1985) was undertaken in the same laboratory as study 12 (1984) to investigate the possible association of hydrocephaly with a specific window of development using a pulse dosing regimen. Twenty inseminated female rabbits received via gavage 60 mg/kg bw/d of folpet for 3 day periods (GD7-9, GD10-12, GD13-15 or GD16-18). A concurrent control group of 20 inseminated females was included. Administration of folpet significantly decreased the mean daily food consumption during the dosing periods more severely and persistently in groups exposed later in the gestation period. Internal hydrocephalus was observed in one foetus of the GD10-12 group and in another foetus of the GD16-18 group. The incidences of hydrocephaly in this study are consistent with the HCD. No particular cluster around a particular window of gestation was demonstrated.

Table: *Incidence of hydrocephaly in study 13 (1985) in rabbits*

Observations	**Control**	**Dose level folpet 60 mg/kg bw/day**			
		GD7-9	**GD10-12**	**GD13-15**	**GD16-18**
Number of foetuses (litters) examined	111 (18)	76 (14)	113 (15)	108 (15)	82 (14)
Hydrocephaly Foetus Litter	0 0	0 0	1 (0.9) 1 (6.7)	0 0	1 (1.2) 1 (7.1)

In study 14 (1985), folpet was administered by oral gavage to 14 mated female rabbits at dose levels of 0, 10, 40 and 160 mg/kg bw/d on gestation days 7 to 19.

Marked maternal toxicity was observed in the high dose group as indicated by clinical signs (soft faeces, few or no faeces) decreased food consumption (more than 50% during dosing period) and decreased body weight gain. In high dose females, post implantation loss was significantly higher (21.8% vs 14.4% in controls).

The proportion of foetuses defined as small (< 30.0 g) was significantly higher in the high dose group and the mean foetal weight was slightly lower (< 10%) but did not gain significance.

No increase of malformation was observed. From the mid dose level, evidence of delayed ossification (fewer than 16 caudal vertebrae centra ossified, reduced ossification of long bone epiphyses and sternebrae 1-4) and a dose-related significant increase of supernumerary ribs and vertebrae when analysed on a per litter basis (calculated as the sum of individual litter frequencies/number of litters x 100 by the study author) were reported.

Table: *Selected foetal findings in study 14 (1985) in rabbits*

Anomaly	Incidence No. (%)	Dose Level of folpet (mg/kg bw/day)				HCD from 8 studies 1985-1989
		0	10	40	160	
No. Foetuses (litters) examined		123 (14)	120 (14)	114 (14)	94(12)	
Fewer than 16 caudal vertebral centra ossified	Foetus Litter$ Litter#	0 (0.00) 0 (0.00) 0 (0.0)	1 (0.88) 1 (0.89) 1 (7.1)	2 (1.79) 2 (2.22)*** 2 (14.3)	5 (5.68)* 5 (5.37)*** 5 (41.7)	0 – 3.03 0 – 3.19
Reduced / irregular ossification among sternebrae 1-4	Foetus Litter$ Litter#	1 (0.81) 1 (0.89) 1 (7.1)	1 (0.83) 1 (1.02) 1 (7.1)	8 (7.02)* 5 (7.00)*** 5 (35.7)	9 (9.57)** 5 (9.20)*** 5 (41.7)	0 – 0.81 0 – 0.89
Reduced ossification of long bone epiphyses	Foetus Litter$ Litter#	26 (21.4) 10 (19.40) 10 (71.4)	19 (15.83) 7 (14.54)* 7 (50.0)	28 (24.56) 10 (24.51) 10 (71.4)	40 (42.55)** 10(38.04)*** 10 (83.3)	16.70 – 37.40 17.20 – 34.40
13 thoracic vertebrae& 13 pairs of thoracic ribs	Foetus Litter$ Litter#	1 (0.81) 1 (1.79) 1 (7.1)	0 (0.00) 0 (0.00) 0 (0.00)	3 (2.63) 2 (4.29)* 2 (14.3)	5 (5.32) 3 (5.36)*** 3 (25.0)	0 – 0.81 0 – 1.79
13 (lumbar) rib present bilaterally	Foetus Litter$ Litter#	64 (52.03) 13 (49.70) 13 (92.8)	57 (47.50) 12 (47.42) 12 (85.7)	67 (58.77) 14 (59.48)** 14 (100.0)	79 (84.04)*** 12 (83.58)*** 12 (100.0)	17.16 – 69.10 20.06 – 67.90
13 (lumbar) rib present unilaterally	Foetus Litter$ Litter#	11 (8.94) 8 (7.72) 8 (57.1)	14 (11.67) 7 (11.92) 7 (50.0)	8 (7.02) 6 (5.68) 6 (42.9)	5 (5.32) 3 (5.28) 3 (25.0)	

* p < 0.05; ** p < 0.01; *** p < 0.001
$ Incidence mean % calculated as the sum of individual litter frequencies/number of litters x 100
# Incidence mean % calculated as umber of litters with affected foetuses/total number litters examined x 100 (not statistically analysed)

From the open literature, three publications (Fabro, 1966; Kennedy, 1968; McLaughlin, 1969) explored folpet developmental toxicity in rabbits. While considered of limited reliability (poor reporting, number of animal tested, only single or two dose levels tested), none of these studies indicated teratogenicity potential of folpet up to 150 mg/kg bw/d. In the publication of Kennedy (1968), folpet at dose level up to 75 mg/kg bw/d was administered on GD6-18 to NZW and Dutch Belted (DB) rabbits. At 75 mg/kg bw/d folpet induced maternal mortality, reduced maternal and foetal body weight and increased post-implantation loss. Maternal tolerance was higher and foetal resorption was not evident in the DB rabbits.

Table: *Summary of maternal and developmental toxicity from the PNDTS in rabbits*

Dose level of folpet (mg/kg bw/day) References	Maternal toxicity	Developmental toxicity		
		Death	Structural abnormality	Altered growth
160 Study 14 (1985)	↓ food consumption (> 50% during dosing period) Clinical signs (↓ faeces)	↑ post-implantation loss	No malformation ↓ skeletal ossification ↑ extra ribs and vertebrae	↓ non stat. foetal body weight (7%)

60 Study 11 (2006)	↓ food consumption (about 50% during dosing period Clinical signs (↓ faeces) thin physique	↑ late resorption ↑ post-implantation	↑ lens malformations ↓ non-stat skeletal ossification ↑ non stat. extra ribs and vertebrae	↓ foetal weight (18%)
60 Study 12 (1984)	1 death (gastric ulceration) ↓ food consumption (about 50% during dosing period	No	Hydrocephaly stat. at foetal incidence within HCD (live foetuses)	No
60 Study 13 (1985) pulse study	↓ food consumption (about 35% during dosing period)	No	1 hydrocephaly in group GD10-12 and in group GD16-18 within HCD No evidence of a specific sensitive period	No
40 Study 14 (1985)	Slight clinical signs (soft faeces white mucous excrement)	No	No malformation ↓ skeletal ossification ↑ extra ribs and vertebrae	No
30 Study 11 (2006)	↓ food consumption (about 35% during dosing period)	No	↓ non stat. skeletal ossification ↑ non stat. extra ribs and vertebrae	↓ non stat. foetal weight (7%)
20 Study 12 (1984)	↓ food consumption (about 20% during dosing period)	No	1 hydrocephaly, within HCD	No
10 Study 11 (2006)	↓ food consumption (about 20% during dosing period)	No	No	No
10 Study 12 (1984)	No	No	No	No
10 Study 14 (1985)	No	No	No	No

Studies with phthalimide

Based on toxicokinetic data, there is no evidence for systemic exposure towards folpet which rapidly degrades in the presence of thiol-containing components. Liberation of thiophosgene by hydrolysis or by the mean of reactions with thiol compounds yields phthalimide (metabolite systemically available) which is further metabolised. The metabolite phthalimide (and its downstream metabolites) may therefore reach the developing offspring following maternal administration of folpet.

A GLP compliant study (study 15, 2006) was performed according to OECD TG 414 (2001) in the same laboratory as folpet's study 11 (2006). In this study, phthalimide was administrated to 25 female rabbits by gavage at dosages of 0, 5, 15 or 30 mg/kg bw/d from days 6 to 28 after mating. The dose levels were chosen as molar equivalent doses of folpet tested in study 11 (2006). There were no indicators of any maternal or developmental toxicity up to the 30 mg/kg bw/d (equivalent to 60 mg/kg bw/d of folpet). However, the chosen dose regimen does not allow to thoroughly investigate the potential developmental toxicity of phthalimide in the absence of maternal toxicity at the high level which represents a deviation from the OECD TG 414.

In the publication of Kennedy (1968), phthalimide administrated to 9 female DB rabbits by gavage at dosage of 75 mg/kg bw/d on GD6-16 did not induce post-implantation losses or malformations. Thalidomide at the same dose level (75 mg/kg bw/d) exerted its well-known teratogen action in both WNZ and DB strains.

In the publication of Fabro (1966), phthalimide administrated to 3 WNZ and 3 Chinchilla female rabbits by gavage at 150 mg/kg bw/d on GD7-12 did not induce teratogenic or embryotoxic effect.

Studies with captan and its metabolite THPI

The structurally analogue captan and its metabolite THPI were also investigated (study 16, 2006; study 17, 2006) and found not to be developmental toxicants in rabbits.

These two studies were carried out concurrently with study 11 (2006, folpet) and study 15 (2006, phthalimide) in the same facility, using the NZW from the same colony and according to the same design (OECD TG 414, 2001) providing a directly comparable set of data.

Dedicated studies to specific sensitivity of rabbits

Folpet may impact the gut microflora via antibiotic activity.

This is of particular concern in caecotrophs such as rabbits (i.e. species relying on orally take up partly digested material from the anus to inoculate their gastrointestinal system and assure nutrient supply). Rabbit is a species particularly susceptible to gastrointestinal disturbances, which may in part be mediated through changes in the gut microflora. Oral administration of antibiotics to rabbits may induce microflora imbalance (caecal dysbiosis) leading to gastrointestinal symptomology known as "gastrointestinal stasis" or "antibiotic toxicity" identified in studies undertaken to characterize teratology of antibiotics as pointed out in the ICH S5 (R3) guideline on reproductive toxicology (2020).

In GLP compliant minimum inhibitory concentration (MIC) assays (Anonymous 2005a; 2005b), folpet (2, 10, 20, 50,100, 200, 500, 1000 and 2000 µg/mL in DMSO) and phthalimide (1, 2, 5, 10, 20, 50, 100, 200, 500 and 1000 µg/mL in DMSO) were tested against isolates of two anaerobic bacterial organisms of the genus *Bacteroides sp*. and *Enterococcus faecalis*, and one yeast, *Candida albicans*, identified as representatives of selected rabbit gut flora species.

Folpet showed marked antimicrobial activity towards all 3 species (MIC of 5, 50 and 200 µg/mL for *Candida albicans, Bacteroides sp.* and *Enterococcus faecalis*, respectively), while phtalimide demonstrated no antimicrobial activity.

In the GLP compliant mechanistic study 18 (2016), 10 male and female rabbits were treated for 9 days orally (gavage) with folpet at 0, 10, 30 and 90 mg/kg bw/d. Clear dose dependent effects evocating gastrointestinal stasis (decreased body weight and food consumption, up to 62% in high dose females), decreased daily faeces weight, -65% in high dosed females), were demonstrated consistently with effects observed in PNDTS in rabbits. However, no treatment related effects on bacterial flora (fresh faecal samples before and after treatment and from caecum at termination) or on mean faecal concentration of *Clostridium difficile* toxin were demonstrated.

Assessment

RAC has analysed in a weight of evidence approach, the effects from the available data set in rabbits, relevant for development classification.

From a large and reliable set dataset, there is no evidence that folpet induces developmental effects in the absence of maternal toxicity in rabbits while increased post implantation losses, increased incidence of structural abnormalities and decreased foetal weights were observed in several studies at dose levels inducing severe maternal toxicity evidenced by a drastic fall in food consumption during the dosing period and decreased faeces.

RAC acknowledges that the *in vitro* data (MIC assay) showed that folpet may potentially affect the rabbit gastrointestinal tract microflora. However, the *in vivo* mechanistic study failed to demonstrate gut bacteria flora changes after 9 days of treatment. Based on these inconclusive data, no definitive conclusion can be drawn on the mechanism underlying the increased susceptibility of rabbit dams compared to rat dams.

RAC agrees with the DS that the increase of in post-implantation losses and the decreased foetal weight observed at the high dose levels in study 11 (60 mg/kg bw/day, 2006) and study 14 (160 mg/kg bw/day, 1985) can be assumed to be secondary to the high maternal toxicity as indicated by clinical signs decreased faeces and decreased food consumption of 50% or more during the entire dosing period. Several studies on effects of caloric restriction alone during pregnancy in rabbit have shown that 50% undernutrition from GD6 to GD18 result in embryo foetal mortality and decreased foetal weights (Matsuzawa, 1981; Cappon, 2005; Matsuoka, 2006; Lopez-Tello, 2019).

While severe maternal toxicity may also explain the delayed ossification (Cappon, 2005; Lopez-Tello, 2019) and increased incidence of lumbar supernumerary ribs (Chernoff, 2004) observed in studies 11 (2006) and 14 (1985), a direct link between maternal toxicity and increased incidence of structural malformations has not been established in the light of current knowledge.

Regarding the increased incidence of lens malformation observed in 8 foetuses of 2 litters of the high dose group in study 11 (2006), RAC notes that these lesions occurred in severely underweight and developmentally immature foetuses. Though, a direct causal link between lens alterations and foetal immaturity has not been substantiated. However, in respect to the low incidence observed in study 11 (2006) and the absence of such finding in the 3 other PNDTS studies, RAC considers that a specific substance-related effect is questionable.

Regarding the increased incidence of hydrocephaly observed in the high dose group of study 12 (1984), RAC considers that a specific substance-related effect seems unlikely based on the following considerations:

- The incidence of hydrocephaly in the high dose group (3 foetuses in 2 litters) is within the HCD range of the laboratory (0 to 3 foetuses in 2 litters) when considering the live foetuses which is the usual practice in teratogenicity studies. A review of HCD from the laboratory between 1980 and 1991, also supports a higher prevalence of hydrocephaly at the time study 12 and 13 were conducted (1984-1985) compared to the previous and subsequent periods.
- The investigative study 13 (1985) with pulse dosing failed to identify a particular sensitive window of exposure during gestation which would be expected for a chemically induced malformation.
- No hydrocephaly was observed in study 11 (2006, more recent study involving higher number of animals tested at the same dose levels) nor in study 14 (1985), where higher dose levels were tested.
- No hydrocephaly was observed in the PNDTS (study 15, 2006) performed with phthalimide (systemically available metabolite of folpet) up to 30 mg/kg bw/d molar equivalent doses of folpet 60 mg/kg bw/d tested in study 12 (1984).
- While of limited reliability, studies in rabbits from the open literature do not report hydrocephaly or other teratogenic effects of folpet or its metabolite phthalimide.

- No hydrocephaly was observed in the PNDTS performed with the structural analogue captan (study 16, 2006) or its metabolites THPI (study 17, 2006).

Overall, RAC agrees with the DS that the development effects (slight increased post implantation losses, decreased foetal weight, delayed ossification and increased number of 13th ribs) observed in rabbit studies are most likely secondary consequences of excessive maternal toxicity than specific chemically induced developmental effects. Although some remaining uncertainty on its aetiology, RAC considers that the slight increase in lens malformations observed in immature foetuses at the high dose level in one study, seems unlikely to be substance related since it was not observed in any of the three other PNDTS.

Studies in other species

In a non-GLP study from the published literature (Robens, 1970), which is not considered reliable due to severe shortcomings (e.g. 1-3 litters evaluated for many dose levels due to high maternal mortality), the teratogenic effects of derivatives of phthalimide, including folpet, were tested in groups of 2-8 pregnant golden hamsters. Folpet was given orally as a single doses of 400 to 1000 mg/kg bw on GD7 or GD8, or as a daily dose of 200 to 500 mg/kg bw/d on GD6-10. No malformations were reported in the groups receiving repeated doses of folpet. Some malformed foetuses were observed in the groups treated with a single dose of folpet but these doses induced maternal lethality. Neither maternal toxicity nor teratogenic effects were observed in groups of hamsters treated up to 1000 mg/kg bw of phthalimide on GD7 or GD8 (single dosing).

In another non-GLP study from the open literature of limited reliability (Courtney, 1983), pregnant mice were exposed to folpet by oral gavage or by subcutaneous injection at a dose of 100 mg/kg bw/d on GD6-15 or by inhalation 624 mg/m^3, 4 h/day on GD6-13. There was approximately 10% maternal mortality on the inhalation route only. No foetal toxicity was observed after any of three routes of exposure.

The teratogenic potential of folpet has also been evaluated in the non-human primates (Vondruska, 1971, considered of poor reliability). Folpet was administered orally in a solution of cream of coconut to pregnant rhesus monkeys and stump-tailed macaques (4 to 6 animals per group) during the period of foetal limb development (GD21-34). No evidence of teratogenicity was observed up to 75 mg/kg bw/d in any of the two species. Despite the study's limitations, the study design was validated to some extent using thalidomide as a positive control.

Assessment

While of limited reliability, RAC notes that the studies from the open literature performed in other species do not raise concern about folpet developmental toxicity.

The DS pointed out for RAC discussion that folpet's metabolite phthalimide has a structure similar to thalidomide which is a known teratogenic substance in the rabbit and that the highest dose tested in the developmental study (study 15, 2006) was clearly below the MTD, therefore effects at higher dose, capturing maternal toxicity, cannot be excluded based on this study.

Folpet and thalidomide share the same phthalimide core. The other part in folpet is the TCM group while glutarimide ring is present in thalidomide. It is noteworthy that the teratogenic activity of thalidomide is not only related to the phthalimide group but critically also to the glutarimide structure (absent in folpet) as shown by a review of the structure–activity relationship of over 50 structural analogues (Smith and Mitchell, 2018).

RAC agrees with the DS that the dose tested in study 15 (2006) was too low to adequately investigate the developmental toxicity potential of phthalimide. Nevertheless, no teratogenic potential was demonstrated up to 30 mg/kg bw/day. Furthermore, while of limited reliability (old studies with poor reporting, low number of animals, limited exposure duration), publications from open literature are consistently negative in rabbits (up to 100 mg/kg bw/d in rabbits (Kennedy, 1966; Febro, 1966), and up to 1000 mg/kg bw (single dose) in Hamster (Robens, 1970). In all these publications, thalidomide was also tested and was teratogenic.

From ECHA dissemination site (2023): Toxicity for reproduction of phthalimide was investigated in a combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (1999). Groups of 12 animals per sex and dose were dosed by gavage with 0, 250, 500, or 1000 mg/kg bw/d of phthalimide. While this study is not dedicated to investigate structural abnormality, no pups were found with any malformation and body weight at birth was not affected up to 1000 mg/kg bw/d.

In addition, folpet's PNDTS in rats (up to 2000 mg/kg bw/d corresponding to a molar equivalent dose of 1333 mg/kg bw/d) provide some indirect evidence that phthalimide is not teratogenic in rats.

Overall, RAC acknowledges that some uncertainties remain due to the low dose level tested in the available GLP compliant PNDTS in rabbits and the poor reliability of the supplementary data. However, none of these data provide evidence for embryonic/foetal lethality or teratogenicity of phthalimide. Therefore, phthalimide is not considered as toxic for the development based on inconclusive dataset.

Comparison with the criteria

There are no epidemiological data available that could support classification of folpet in Category 1A.

From animal studies, there is no clear evidence of an adverse effect on development in the absence of other toxic effects that could support classification of folpet in Category 1B.

There is some evidence on toxicity on development in the prenatal developmental toxicity studies in rats at high dose levels. However, in view of the nature and the low severity of the developmental findings (depressed foetal weight and/or retarded ossification, wavy ribs) only observed concomitantly with marked maternal toxicity, RAC considers that the criteria for classification in Category 2 are not fulfilled.

RAC also considers that development effects (slight increased post implantation loss, decreased foetal weight, developmental delay e.g. delayed ossification and increased number of 13th ribs) observed in the prenatal developmental toxicity studies are most likely secondary consequences of the severe maternal toxicity rather than specific chemically induced developmental effects. Although some remaining uncertainty on its aetiology, RAC considers that the slight increase in lens malformations in underweight foetuses observed at the high dose level in one study, unlikely to be substance-related since not reproduced in any of the other three reliable PNDTS.

RAC acknowledges that while some uncertainties remain due to the low dose level tested in the available GLP compliant PNDTS in rabbits and the poor reliability of the supplemental data, none of these data provide evidence for embryonic/foetal lethality or teratogenicity of phthalimide (systemically available metabolite of folpet).

Therefore, in accordance with the criteria laid down in the CLP Regulation **RAC concurs with the DS that no classification for development is warranted.**

Lactation

In the two-generation studies summarized in the chapter on adverse effects on sexual function and fertility, offspring animals were exposed during lactation. There were no effects on pup survival, litter size of the pups at birth, nor indication of impaired nursing behaviour or decreased pup viability during lactation.

In study 1 (1986), pup body weight gain at the highest dose level (5000 ppm) was reduced from PND7 in both F1 and F2 pups, dams of this group showed reduced body weight gain and food consumption during gestation and lactation.

In study 2 (1985), reduced body weight and body weight gain could be observed at the high dose level (3600 ppm). Reduced pup body weight was evident not earlier than PND21 for the F1 pups and PND14 for the F1 and F2 generations respectively and therefore considered more related to direct food consumption.

No specific data on transfer in the milk or on the quality of the milk is available from the two studies.

In a mouse somatic cell mutation assay (spot test, study 4, 1985), described in the mutagenicity part, decreased pup survival was observed at the high dose level, in the presence of overt maternal toxicity (high lethality).

Therefore, in accordance with the criteria laid down in the CLP Regulation, RAC concurs with the DS that **no additional labelling of folpet for "adverse effects on or via lactation" is warranted.**

Supplemental information - In depth analyses by RAC

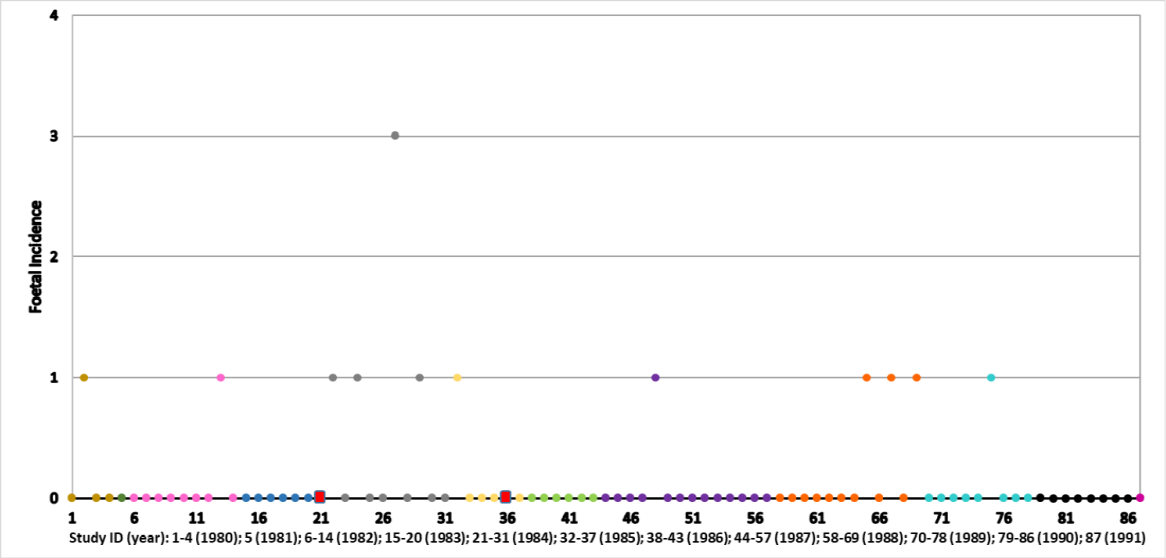
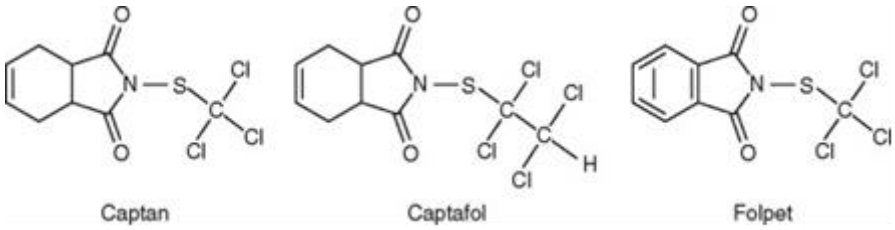
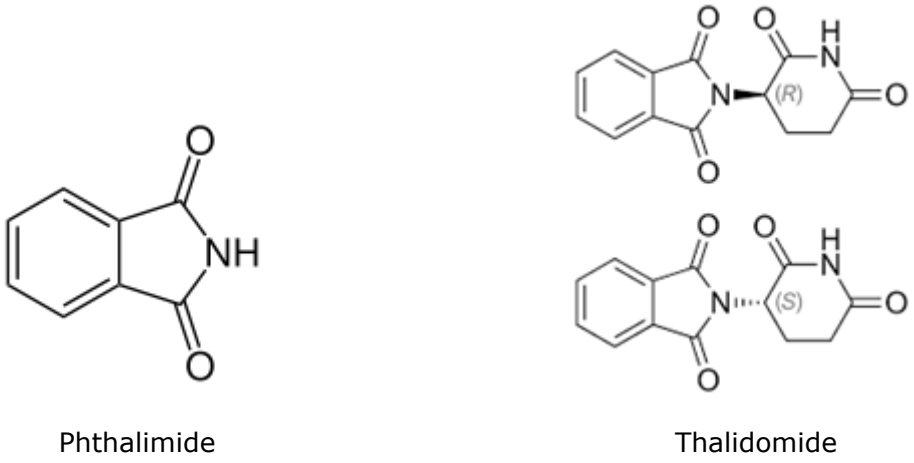


Figure: Foetal incidence of hydrocephaly, marked or extreme dilation of the lateral brain ventricles in control animals from studies conducted 1980 - 1991 with study 12 (1984) and 13 (1985) shown in red.

Chemical structures





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	**Assessment**

and CLP guidance[4]	
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: *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).*	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 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*	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

[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

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 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.*	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
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	Acute toxicity classifications describe folpet's hazard properties appropriately.

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.	• 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.

RAC evaluation of specific target organ toxicity – single exposure (STOT SE)
Summary of the Dossier Submitter's proposal

The DS did not propose classification for STOT SE either due to its proposed acute classifications (eye, skin, inhalation) or because the effects were not sufficiently adverse after single exposure (gastrointestinal tract).

Comments received during consultation

Industry (IND) disagreed with the conclusion on classification for STOT RE in the CLH report and mentioned that if a STOT classification should be considered for folpet's effects on the respiratory system, then classification as STOT SE 3 (H335) would be the appropriate one. However, this would result in a "double classification" since folpet is already classified for acute inhalation toxicity.

The DS answered that since effects relevant for STOT SE 3 (H335) classification occurred at doses which caused mortalities, no classification as STOT SE 3 is warranted.

Assessment and comparison with the classification criteria

No relevant human data are available. In addition to the acute toxicity studies, two other studies are relevant for the purpose of STOT SE classification i.e. one mechanistic study (study 11, 1997) investigating intestinal irritation in CD-1 mice after 24-hour exposure to folpet and a second one (study 1, 2015) investigating toxicity in Wistar rats after 24-hour dietary with 13-days recovery.

In the mechanistic study in mice (study 11, 1997), in a first trial folpet was administered to groups of 3 female mice by oral gavage at 900 mg/kg bw (followed by untreated diet) or in the diet at 200 or 5000 ppm over a 24-hour period. Animals were terminated after 24 hours. Marked irritation in the proximal region of the duodenum was observed in animals treated with 900 mg/kg bw by gavage and in animals exposed to 5000 ppm (845 mg/kg bw) via the diet. In the second trial, with optimized histopathological procedure (eight step serial sections of the duodenum), folpet was administrated to groups of 15 female mice by oral gavage at 900 mg/kg bw (followed by untreated diet) or in the diet at 50, 200, 500 or 5000 ppm over a 24-hour period. Five animals in each group were killed after 1, 3 or 7 days, respectively. No duodenal irritation was observed in any treated females. A bolus dose of folpet at 900 mg/kg bw caused minimal erosion in the stomachs of two of the five mice.

The GLP compliant 24-hour feeding study in rats (5 animals/sex/dose dosed with 0, 313, 1250 or 5000 ppm sacrificed on day 1 or 14) showed no irritation or histopathological findings in the gastrointestinal tract nor systemic effects up to 5000 ppm (equivalent to 393.22 mg/kg bw in males and 386.9 mg/kg bw in females, study 1, 2015).

From the available data addressing acute exposure, there is no evidence for a primary target organ. All mucous membranes were affected by folpet exposure. The severity on the mice gastrointestinal tract is minimal after single exposure of 900 mg/kg bw.

The acute inhalation toxicity studies clearly indicate respiratory irritant effects in terms of clinical signs (dyspnoea, irregular respiratory rate, laboured breathing) and pathology (increased lung weight, swollen lungs and oedema) relevant for STOT SE 3 (H335). While, respiratory irritation may also occur at non-lethal concentrations, there are no studies to support this assumption. Indeed in the available studies, irritant effects were observed from the lowest tested concentrations in the presence of lethality (table below in supplementary information) and folpet is already proposed to be classified for acute inhalation toxicity

Category 2 (due to mortality by oedema caused by irritation), which takes precedence over STOT SE. Folpet's hazard profile for acute exposure is considered sufficiently described and communicated by the proposed acute classifications.

Therefore, in accordance with the criteria laid down in the CLP Regulation**,** RAC supports the DS proposal of **no classification for STOT SE.**

Supplemental information - In depth analyses by RAC

The available studies for acute inhalation toxicity are summarized in the table below.

Table: *Summary table of effects in acute inhalation toxicity studies*

Acute inhalation studies	Dose levels, duration of exposure	Effects	Lethality	Value LC_{50}
GLP, Acute Inhalation – Rat US EPA OPP 81.3. Study 1, 1988	0, 0.21, 0.53, 0.95, 1.49 mg/L for 4 hours	Abnormal respiratory sounds; laboured breathing; gasping all doses Compound-related lesions present in the lung and trachea at all exposure levels. Lung: fibrinous oedema and haemorrhage. Chronic interstitial pneumonia in animals surviving longer. Acute tracheitis	From 0.21 mg/L (1/5 M and 1/5 F)	M: 0.34 mg/L F: 1 mg/L
AEPA, Proposed Guidelines for Registering Pesticides in the U.S., Part II, August 22, 1978 in rats Study 2, 1979 Supplementary information	0.64, 0.65, 0.67, 2.68, 3.61 mg/L for 4 hours	Gasping, lacrimation, nasal discharge dyspnoea and salivation at all doses Gross pathology: fluid in trachea and bronchi from 2.68 mg/L haemorrhagic lungs at 3.61 mg/L	From 0.64 mg/L (1/5 M and 1/5 F)	M: 1.38 mg/L F: 1.30 mg/L
GLP, OECD TG 403 (1981) in rats Study 3, 1991	0.14, 0.36, 1.06, 4.35 mg/L for 4 hours	Laboured respiration Various lung changes haemorrhage, abnormal redness, dark foci and swelling from 0.14 mg/L. Haemorrhagic lungs at higher concentrations.	From 0.14 mg/L (1/5 M)	M: 0.39 mg/L F: 0.43 mg/L
GLP, EPA Guideline No. 83-1 (equivalent to OECD TG 403 (1981)) in rats Study 4, 1993	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	Rales, gasping, irregular respiration, deep respiration. For decedents dark lungs, caseous material in the trachea and firm lungs Slightly high lung weights in animals killed after 14 days of observation	From 0.79 mg/L 1/5 F and 3.57 mg/L 2/5 M	M: > 4.35 mg/L F: 1.08 mg/L
GLP, EPA Guideline No. 83-1 (equivalent to OECD TG 403 (1981)) in rats Study 5, 1993	2.14 mg/L for 4 hours	Irregular respiratory movements during exposure period Dark mandibular lymph nodes no other macroscopic findings	No death	M: > 2.14 mg/L F: > 2.14 mg/L

Supplementary information: MMDA: 14.3 µm				
GLP, OECD TG 403 (1981) in rats Study 6, 1993	0, 0.8, 1.6, 1.99 mg/L for 4 hours	Changes in respiratory rates and pattern, gasping, rales, vocalisation from 1.6 mg/L. Macroscopic changes attributed to treatment only seen in decedents: fluid in the trachea, dark lungs, incomplete collapse of the lungs and increased lung weights.	From 1.6 mg/L (3/5 M and 1/5 F)	M: 1.54 mg/L F: 2.89 mg/L

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.	Folpet Purity 93.5% 1000, 3000, 5000, 12000	NOAEL = 5000 ppm (585 mg/kg bw/d) 12000 ppm (585 mg/kg bw/d)	(R-6116) Study 1 (1979)

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)	ppm 21 d 5/sex/group SD rats	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%)	
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	Folpet Purity 89.5% 20, 60, 180,	Due to small number of animals no NOAEL can be set <u>540 mg/kg bw/d</u>	(R-6135) Study 4 (1983)

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)	540 mg/kg/day Capsule 2/sex/group Beagle dog	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%)	
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 - 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	Folpet Purity ≥ 88.6% 0, 2000, 4000, 8000 ppm 20/sex/group F344 rats	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%) 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	(R-1800) Study 5 (1982)

<table>
<tr>
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<td>4000 ppm (270 mg/kg bw/d
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
2000 ppm (136 mg/kg bw/d)
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 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</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
10000 ppm (614 mg/kg bw/d)
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>
</table>
</td>
<td>(R-6118)
Study 6 (1981)</td>
</tr>
</table>

<table>
<tr>
<td>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></td>
<td><table>
<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></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><td></td><td></td></tr>
<tr><td></td><td>males</td><td>females</td></tr>
<tr><td>Slight atrophy</td><td>0</td><td>1</td></tr>
<tr><td>Moderate/marked atrophy</td><td>4</td><td>1</td></tr>
<tr><td>with depletion of lymphocytes</td><td>4</td><td>2</td></tr>
<tr><td>Fibrosis</td><td>4</td><td>1</td></tr>
<tr><td>Bone marrow</td><td></td><td></td></tr>
<tr><td>Slight depletion</td><td>0</td><td>3</td></tr>
<tr><td>Moderate depletion</td><td>1</td><td>1</td></tr>
<tr><td>Marked depletion</td><td>3</td><td>0</td></tr>
<tr><td>Replacement by watery fat</td><td>3</td><td>4</td></tr>
<tr><td>Replacement by connective tissue</td><td>1</td><td>0</td></tr>
</table>
Testis
<table>
<tr><td>Slight testicular degeneration</td><td>0</td></tr>
<tr><td>Moderate testicular degeneration</td><td>0</td></tr>
<tr><td>Marked testicular degeneration</td><td>4</td></tr>
<tr><td>Cryptochid</td><td>0</td></tr>
<tr><td>Partial aspermia</td><td>0</td></tr>
<tr><td>No spermatogenesis</td><td>1</td></tr>
</table></td>
<td>(R-3654)
Study 7 (1985)</td>
</tr>
</table>

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<tr>
<td></td>
<td></td>
<td>
1800 mg/kg bw/d

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
<table>
<tr><td>Slight testicular degeneration</td><td>2</td></tr>
<tr><td>Moderate testicular degeneration</td><td>2</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>1</td></tr>
<tr><td>No spermatogenesis</td><td>1</td></tr>
</table>

790 mg/kg bw/d

bw ↓ (please refer to table 3.12.2.15 in 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.

<table>
<tr><td>Slight testicular degeneration</td><td>1</td></tr>
</table>
</td>
<td></td>
</tr>
</table>

		<table><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></table> Testis	
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	Folpet Purity 82.7-91.3% 325, 650, 1300 mg/kg bw/d 5/sex/group Beagle dogs	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 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	(R-4663) Study 8 (1988)

		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), 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	(R-6035) Study 9 (1986)

		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

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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.

RAC evaluation of specific target organ toxicity – repeated exposure (STOT RE)

Summary of the Dossier Submitter's proposal

The DS proposed a classification STOT RE 1 based on effects in the respiratory tract after repeated inhalation exposure.

Comments received during consultation

Two MSCAs agreed with the DS's proposal.

However, IND disagreed, considering that histopathological findings in the larynx observed in the 28-day rat study by inhalation have an acute aetiology (i.e. well established *in situ* membrane reactivity of folpet). The following arguments were put forward: folpet is already classified for acute inhalation toxicity, modelling rat tissue using the rat EpiAirway assay demonstrates that acute folpet exposure induces histopathological changes, folpet is cytotoxic upon direct contact in various *in vitro* assays, the comparison with concentrations inducing lethality in acute studies further supports the non-relevance of classification for repeated exposure as well (refer to RCOM for more details).

The DS answered that similar modes of action can lead to classification for different hazard classes and STOT RE is justified based on histopathological effects in the larynx but also findings in the nasal turbinates, trachea and lung, the persistence of those effects being indirectly supported in a 90-day inhalation with the sibling captan including a recovery period. Furthermore, the DS considered the EpiAirway assay of poor reliability and questioned the applicability of Haber's rule for extrapolating folpet's acute and repeated dose toxicity by inhalation due to folpet rapid degradation.

A non GLP *in vitro* assay using MatTekEpiAirway™ rat test system to evaluate the upper airway cytotoxicity was submitted during the consultation and is further discussed below.

Assessment and comparison with the classification criteria

In the absence of human data, the evaluation of STOT RE endpoint was based on twenty repeated-dose toxicity studies that are generally GLP compliant (the oldest non GLP ones had statement on quality control) and performed according to OECD TG in force at the time they were carried out:

Oral route:

- Four studies in dogs (capsule): one 28-day study (study 4, 1983, range finding, supplementary), one 90 day-day study (study 7, 1985, similar to OECD TG 409) and two 1-year studies (study 8, 1988 and study 9, 1986 similar to OECD TG 452);
- Five studies in mice (diet): two 28-day study (study 2, 1978 and study 3, 1981, non GLP, supplementary), and three carcinogenic studies (study 1, 1982; study 2, 1985 and study 3, 1994, similar to OECD TG 451);
- Nine studies in rats (diet): one 21-day range-finding study (study 1, 1979, non-GLP, supplementary), two 90-day studies (study 6, 1981 and study 5, 1982, non-GLP, similar to OECD TG 408), a 13-week neurotoxicity study (study 12, 1982, similar to OECD TG 424), two 2-generation reproductive toxicity studies (study 2, 1985 and study 1, 1986 similar to OECD TG 416), one chronic toxicity (study 6, 1989, similar to OECD TG 452), two carcinogenic studies (study 4, 1985, similar to OECD TG 453 including 1-year time point; study 5, 1985 similar to OECD TG 451).

Dermal route: one 28-day study in rats (study 10, 1988, similar OECD TG 410)

Inhalation: one 28-day study in rats (study 11, 2008, OECD TG 412)

The folpet's toxicological profile after repeated exposure is in line of its MoA as irritant at the first site of exposure. Accordingly, the identified target organs are the gastro-intestinal tract and the respiration tract via oral and inhalation routes respectively. No other target organ due to systemic toxicity is identified. Folpet did not reveal neurotoxic potential in a 90-day neurotoxicity study (study 12, 1982) in rats.

Gastrointestinal tract

By oral route, studies show an early decrease in food consumption with corresponding decreased body weight. In diet studies, low palatability is suspected to be involved in food reduction because of a distinct chemical smell of folpet. However, decreased body weight observed in rodents studies but also in dog dosed by capsules is most likely be a secondary effect of digestive mucous membrane irritation.

In the oral studies in rodents, mucous membrane irritation of the upper gastrointestinal tract is consistently observed across studies in the form of hyperkeratosis and acanthosis in the forestomach and ulceration/erosion at high dose levels.

In the mice, irritation also induces hyperplasia of the duodenal and jejunal mucosa and subsequent tumour formation in long term studies which is covered by the classification for carcinogenicity.

In dog studies histological examination showed no evidence of abnormality in the gastrointestinal tract. However, gastrointestinal irritation is supported by clinical signs with vomiting and diarrhoea increasing in a dose dependent manner.

In all studies, histopathological findings in the gastrointestinal tract were observed at dose levels above the guidance value of 100 mg/kg bw/d for 90-day exposure or the adjusted standard guidance values of 25 and 12.5 mg/kg bw/d for 1 and 2 years exposure, respectively.

Respiratory tract

In a GLP compliant 28-day inhalation toxicity study (study 11, 2008) similar to OECD TG 412, Sprague-Dawley rats (5 per dose/sex) were exposed to 0, 5, 25 and 100 µg/L (measured concentrations) 6 hours/day, 5 days/week. The average mass median aerodynamic diameter ranged from 1.9 to 2.1 µm with an average geometric standard deviation of 1.87 to 2.03 µm. In the high-exposure group, one male was sacrificed on day 13 due to pulmonary oedema, and mean body weights were 7.9% and 9.2% less for males and females, respectively compared to the control group. Laboured breathing in few animals at 100 µg/L was noted during the exposure periods.

Histopathological findings in the larynx

In all treated animal, moderate squamous/squamoid metaplasia, as well as minimal to moderate hyperplasia of the squamous epithelium with dose-related increase in incidence and severity were present. Minimal to slight mucosal fibrosis also occurred at all treatment levels in both sexes and mixed inflammatory cells were present within the mucosa.

Histopathological findings in the nasal turbinates

Degeneration/atrophy of the olfactory epithelium was present in one male in the 25 µg/L exposure group and in all high exposure animals accompanied by erosion/ulceration of the olfactory epithelium (1 male and 3 females).

One male at 25 µg/L showed squamous/squamoid metaplasia in the respiratory epithelium. Other changes in the respiratory epithelium of the nasal turbinates occurred primarily at 100 µg/L.

Histopathological findings in the trachea

Mixed inflammatory cells were present within the mucosa in all groups, but were increased in severity in both sexes in the treatment groups. Slight squamous/squamoid metaplasia occurred in 4 males and 1 female in the high exposure group.

Histopathological findings in the lungs

Inflammatory changes observed in the lungs were mainly observed in the 100 µg/L exposure group with minimal to slight subacute to chronic peribronchiolar inflammation (4/5 males and all females). While perivascular mixed inflammatory cells were observed in all groups, they were slightly increased in severity in the high exposure group. Pulmonary inflammation was more extensive in the decedent and included subacute to su-bchronic interstitial inflammation.

A non GLP *in vitro* rat EpiAirway assay measuring transepithelial electrical resistance (TEER), lactate dehydrogenase (LDH) release into the culture media and histopathological findings in a 3D cell model of the rat mucociliary airway epithelium is available. This assay indicates that folpet is cytotoxic in this test system with histopathological changes occurring after single

exposure (1-day exposure). Effects seem more pronounced with repeated treatments (3-day exposure). However, there is no validated guideline and the identified limitations compromise the reliability of the test.

Comparison with the criteria

In the 28-day inhalation toxicity study, effects in the larynx squamous/squamoid hyperplasia and metaplasia, mucosal fibrosis and inflammation occurred from 0.005 mg/L which is below the adjusted guidance value for 28-day exposure for STOT RE 1 of 0.06 mg/L.

While laryngeal effects may be considered as adaptive response to inhalation of irritants as folpet is, quite severe effects are observed from the lowest concertation level i.e. moderate squamous metaplasia in all animals, slight to moderate squamous hyperplasia in three out of five males and one female and minimal to slight mucosal fibrosis already observed in five animals. At higher concentrations, the effects were increased in incidence and severity. Therefore, RAC considers laryngeal changes as adverse from the low exposure.

Furthermore, other effects in the respiratory tract were observed below the adjusted guidance values i.e., atrophy of the olfactory epithelium (1/5 male), squamous/squamoid metaplasia in the respiratory epithelium of the nasal cavity (1/5 male) and increased tracheal inflammation. At the highest concentration, effects in nasal cavity, trachea and lungs progressed in incidence and/or severity and one death occurred in one out five males.

In the absence of a recovery group, the reversibility of the histopathological findings in the respiratory tract cannot be estimated. An indirect line of evidence of irreversibility is provided by the results obtained in a 90-day inhalation toxicity study carried out with the sibling captan, where squamous hyperplasia and metaplasia in the larynx persisted in animals after a 4-week recovery period in animals exposed to 0.013 µg/L.

Regarding atrophy of the olfactory epithelium, RAC points out that this effect is not considered to be reversible. If exposure is stopped, the olfactory epithelium may be substituted with respiratory epithelium, but the functions of the olfactory epithelium will never return.

With respect to folpet's MoA as irritant at the first site of exposure, according to the CLP guidance, 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), classification as specific target organ toxicant (repeated exposure) would be warranted even if the substance is also classified as acutely toxic and/or corrosive.

No histopathology of the respiratory system was performed and no low concentrations were tested in any of the available acute inhalation studies hampering a direct comparison of the effects after acute and repeated exposure. However considering the lowest tested concentration in the acute inhalation toxicity studies i.e. 0.14 mg/L for 4-hour treatment (equivalent 0.09 mg/L for 6 hours) where animals showed clinical signs and one out of five males died, a factor of 18 is obtained between the lowest concentration causing effects in the larynx in the 28-day study and the lowest concentration causing clinical signs in the acute inhalation toxicity study considering a 6-hour treatment.

Based on the above-mentioned elements and in accordance with the criteria laid down in the CLP Regulation, RAC considers that **classification for STOT RE 1 (H372: Causes damage to the respiratory tract) is warranted.**

No specific concentration limits (SCLs) for STOT RE has been proposed by the DS. Since the effects on larynx occurred at a concentration of more than one magnitude below the adjusted guidance value for 28-day inhalation exposure (dust/mist/fume) of 0.06 mg/L for Category 1, the following SCL should apply.

STOT RE 1; H372: C ≥ 5%

STOT RE 2; H371: 5% > C ≥ 0.5%

Supplemental information - In depth analyses by RAC

Table: *Histopathological findings in the larynx*

	Males				**Females**			
Dose levels (mg/L)	0	0.005	0.025	0.1	0	0.005	0.025	0.1
Number of animals	5	5	5	5	5	5	4*	5
Epithelium, squamous/squamoid metaplasia								
Minimal	0	0	0	0	0	0	0	0
Slight	0	0	0	0	0	0	0	1
Moderate	0	5	5	5	0	5	4	4
Total incidence	0	5	5	5	0	5	4	5
Averaged weighted incidence	0.0	5.0	5.0	5.0	0.0	5.0	4.0	4.7
Epithelium, metaplastic keratinization								
Total incidence	0	5	5	5	0	5	4	5
Epithelium, squamous, hyperplasia								
Minimal	0	0	0	0	0	1	1	0
Slight	0	2	2	1	0	0	2	1
Moderate	0	1	2	4	0	0	1	4
Total incidence	0	3	4	5	0	1	4	5
Averaged weighted incidence**	0.0	2.3	3.3	4.7	0.0	0.3	2.7	4.7
Mucosa fibrosis								
Minimal	0	1	1	1	0	1	0	1
Slight	0	2	2	4	0	1	4	4
Moderate	0	0	0	0	0	0	0	0
Total incidence	0	3	3	5	0	2	4	5
Averaged weighted incidence	0.0	1.7	1.7	3.0	0.0	1.0	2.7	3.0
Mucosa, mixed inflammatory cell								
Minimal	1	0	0	0	1	0	0	0
Slight	4	1	3	3	4	1	2	1
Moderate	0	4	2	2	0	4	2	4
Total incidence	5	5	5	5	5	5	4	5
Averaged weighted incidence	3.0	4.7	4.0	4.0	3.0	4.7	3.3	4.7

*The slide not prepared properly for reading

** Weight 1, 2, 3 for minimal, slight and moderate respectively

Table: *Histopathological findings in the nasal turbinates*

	Males				Females			
Dose level [mg/L]	0	0.005	0.025	0.1	0	0.005	0.025	0.1
Number of animals	5	5	5	5	5	5	5	5
Respiratory epithelium, squamous/squamoid metaplasia								
Total incidence	0	0	1	4	0	0	0	5
Olfactory epithelium, degeneration/atrophy								
Total incidence	0	0	1	5	0	0	0	5
Olfactory epithelium, ulcer/erosion								
Total incidence	0	0	0	1	0	0	0	3
Respiratory epithelium, thinning/atrophy								
Total incidence	0	0	0	1	0	0	0	1
Lumen: inflammatory cells/debris								
Total incidence	0	0	0	4	0	0	0	4
Mucosa (respiratory/olfactory), acute/subacute inflammation								
Total incidence	0	0	0	3	0	0	0	4

Table: *Histopathological findings in the trachea and in the lungs*

	Males				Females			
Dose level [mg/L]	0	0.005	0.025	0.1	0	0.005	0.025	0.1
Number of animals	5	5	5	5	5	5	5	5
Trachea Epithelium squamous/squamoid metaplasia								
Total incidence	0	0	0	4	0	0	0	1
Trachea Mucosa, mixed inflammatory cells								
Minimal	3	2	2	0	4	2	2	1
Slight	0	1	2	3	1	1	0	2
Moderate	0	1	0	1	0	1	1	1
Total incidence	3	4	4	4	5	4	3	4
Averaged weighted incidence	1.0	2.3	2.0	3.0	2.0	2.3	1.7	2.7
Lung Peribronchiolar subacute/chronic inflammation								
Total incidence	0	0	0	4	0	0	0	5
Lung Perivascular mixed inflammatory cells								
Minimal	3	3	3	3	4	5	5	4
Slight	0	0	0	1	0	0	0	1
Moderate	0	0	0	0	0	0	0	0
Total incidence	3	3	3	4	4	5	5	5
Averaged weighted incidence	1.0	1.0	1.0	1.7	1.3	1.7	1.7	2.0

Table: *In vitro assay using MatTekEpiAirwayTM rat test system*

Method, guideline, deviations	Dose levels duration of exposure, species, strain, sex, no/group	Results	Reference
No OECD TG guideline available **Non-GLP** **Supplementary**	Folpet Purity: 95.8% MatTekEpiairway™ (3D) cell model of the rat mucociliary airway epithelium 1st trial: 0, 0.6, 6, 10, 40, 60, 100, 400 and 600 µg/mL 24 hours 2nd trial: 0, 0.6, 6, 10, 40, 60, 100, 400 and 600 µg/mL 72 hours Vehicle: mineral oil 3 controls: untreated, vehicle, water +ve control: formaldehyde	1st trial ≥ 100 µg/mL: increase LDH release, decrease TEER and histopathological findings. 2nd trial ≥ 100 µg/mL: increase cumulative LDH release, decrease TEER. Increased magnitude with treatment duration (72-hour vs 24-hour measurements) Histopathological findings No statistical analysis. Poor repeatability (1st run and 24-hour time point 2nd run) shift to a squamous phenotype of the test system over time	Study report, 2021 confidential attachment Submitted docs.zip

The rat EpiAirway assay is a 3D cell model of the rat mucociliary airway epithelium (MatTek Corp., Ashland, MA). Two different trials were performed: a single 24-hour period (single exposure), or three continuous 24-hour periods (rinsing every 24 hours with phosphate-buffered saline, repeated exposure). In both trials eight concentrations were tested from 0.6 µg/mL to 600 µg/mL. Pre-dosing and after each 24-hour exposure period transepithelial electrical resistance (TEER) and lactate dehydrogenase (LDH) release into the culture media were measured. Histopathology was performed following the single (first trial 24, hour) or final exposure period (second trial, 72 hours).

In the first trial (single exposure), LDH release was increased in a dose-related manner from 100 µg/mL; TEER was decreased at 600 µg/mL and degenerative changes (squamous differentiation, epithelial thinning cell necrosis) were observed from 100 µg/mL with marked changes at the two highest concentrations. In the second trial (repeated exposure). Cumulative LDH release was increased from 100 µg/mL; TEER was decreased from 400 µg/mL. Degenerative changes (squamous differentiation, epithelial thinning and cell necrosis) were noted in all groups including untreated and vehicle samples indicated a shift to a squamous phenotype over time in the culture, compromising the assessment. However, the two-highest concentrations showed more severe degenerative changes with epithelial loss compared with the vehicle treated controls. Overall, repeating the treatment seem to increase the severity of the findings.

However this assay presents several limitations:

- No statistical analysis of the data;
- Different values of TEER and LDH in the first trial vs 24-hour measurement in the second trial (supposed to be similar) compromising the repeatability of the test;
- Shift to a squamous phenotype over time in untreated and control-treated samples limiting the sensitivity of the test;
- Only two samples per condition for histopathological analysis.

This assay indicates that folpet is cytotoxic on the rat mucociliary airway epithelium with histopathological changes occurring after single exposure. Effects seem more pronounced with repeated treatments. However, the identified limitations compromise its reliability.

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 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 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-cyanobenzoic acid and benzamide are considered major degradation products under the conditions of this study.	Anonymous, 1994
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 %	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	Anonymous, 1998

Method	Results	Remarks	Reference
	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)	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.	
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, 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, 1988b
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).	Legacy study broadly in line with OECD 111	Anonymous, 1992b

Method	Results	Remarks	Reference
	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)		
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: [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, 2016g

Method	Results	Remarks	Reference
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 (*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, 1999
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.		Anonymous, 2007c

Method	Results	Remarks	Reference
	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)		
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 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, 1991a
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 %	Legacy study broadly in line with OECD 307	Anonymous, 2001a

Method	Results	Remarks	Reference
	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).		
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 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, 2015b
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	Study performed with folpet metabolite phthalamic acid	Anonymous, 2015e

Method	Results	Remarks	Reference
	acid radiopurity 95.3 % GLP: Yes Study considered valid		
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
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).		Anonymous, 1991d

Method	Results	Remarks	Reference
	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).		
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 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, 2000a

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.

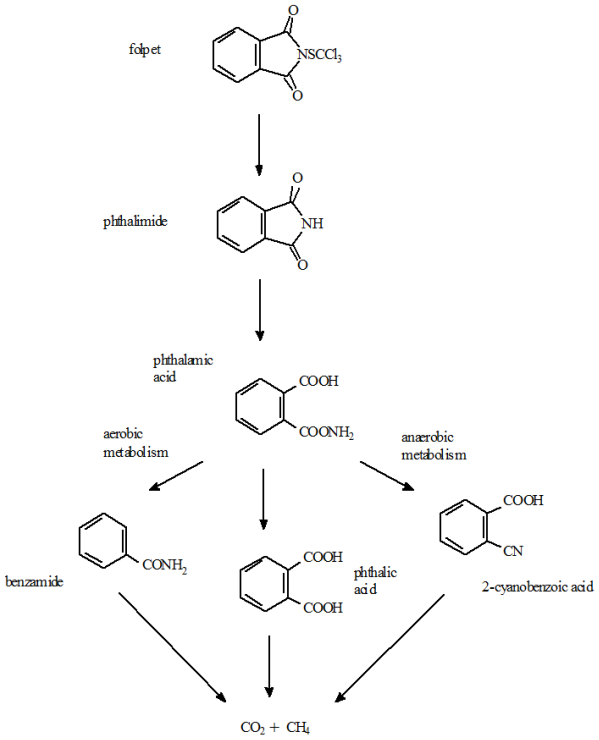

Figure 6: Proposed route of degradation of phenyl labelled folpet in aquatic systems

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 <u>phthalamic acid</u> 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 <u>phthalic acid</u> 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 <u>2-cyanobenzoic acid</u> 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 <u>benzamide</u> 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 <u>folpet</u> (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	Anonymous, 2015b
			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	
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) $f_T = Q_{10}^{\wedge}[(T_{act} - T_{ref})/10]$ 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 <u>phthalimide</u> (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 (%)	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 <u>phthalamic acid</u> (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			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 <u>phthalic acid</u> (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)
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	2-cyanobenzoic acid Batch #: BGBB6262V	pH 2.4 log P_{OW} = 0.89 (20°C)	Acceptable GLP: Yes	Anonymous (2015)

Method	Test substance	Results	Remarks	Reference
(HPLC)	Purity: 98.6%			
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)

Method	Species	Test material	Test condition	Exposure time	Results [1] [mg a.s./L]	Reference
		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*	Phthalimide	Static	72 h	E_rC_{50} > 46 mm	Anonymous (2015)

Method	Species	Test material	Test condition	Exposure time	Results [1] [mg a.s./L]	Reference
	Green algae	Batch #: SZBD221XV Purity: 99.9%			E_yC_{50} > 46 $_{mm}$	
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 $0.001 < NOEC \leq 0.01$ 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

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

Folpet (ISO) is a biocide and a fungicide. The substance is classified as Aquatic Acute 1 with an M-factor of 10 in Annex VI of the CLP Regulation. The Dossier Submitter (DS) proposed to maintain the classification based on the 96-hour LC_{50} of 0.015 mg/L for fish which warrants an M-factor of 10 ($0.01 < LC_{50} \leq 0.1$ mg/L). In addition, the DS proposed to add Aquatic Chronic 1, with an M-factor of 1 ($0.001 < NOEC \leq 0.01$ mg/L for rapidly degradable substances), based on a 33-day NOEC of 0.00881 mg/L for fish.

Degradation

In degradation studies radiolabels have been incorporated either in the aromatic ring, in the carbonyl ring, or in the TCM side-chain.

There were two hydrolysis studies (OECD TG 111, GLP) available. In Anon. (1988b), the hydrolysis of [carbonyl-^{14}C]-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. Phthalimide, phthalic acid, and phthalamic acid were identified as degradation products. Phthalic acid was considered stable to hydrolysis at all pH values.

In the other hydrolysis study (Anon. 1992b), [trichloromethyl-^{14}C]-folpet was recovered as 47 and 52% AR at 1 h at pH 5 and pH 7, respectively, but was not found at pH 9. At 24 hours, levels of folpet were 14.9% AR and 1.1% AR at pH 5 and pH 7, respectively. Two unknown compounds were detected in solution. Unknown 1 was detected after one hour at pH 7 (17.3% AR) and pH 9 (14.5% AR) and after 24 hours at pH7 (8.8% AR) and pH 9 (36.0% AR). Unknown 2 was noted at high levels (25% AR at pH 5, 52% AR at pH 9) after

1 hour but not detected at pH 7. Based on the limited information available from this study, significant formation of degradation products deriving from the TCM moiety of folpet in other aquatic systems could not be excluded. It was postulated that Unknown 1 was the primary degradant, probably trichloromethylsulfenic acid salt, which on changes of pH and exposure time degrades to the volatile trichloromethylmercaptan (Unknown 2) which in turn may degrade to thiophosgene, carbon oxysulphide and ultimately CO_2. However, the validity of the study is uncertain because the mass balance of the two sampling points was partly far below 90% AR, although the DS highlights that the study is the only study available with trichloromethyl-labelled folpet in aquatic systems.

The direct photochemical degradation study (Anon. 1989b), broadly in line with OECD TG 316 (GLP), was considered valid and relevant. The recovery of [U-phenyl-14C]-folpet after 8 hrs of irradiation (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. Based on this study, the overall impact of irradiation on the dissipation of folpet in water, was considered negligible.

Two OECD TG 301B ready biodegradability tests (GLP) were considered valid and relevant. In Anon. (1994), cumulative CO_2 production was equivalent to 35% and 46% (mean = 41%) of the CO_2 over the 29-day period. The nominal test concentration was 10 mg C/L of folpet technical. Degradation was slow but progressive throughout and a degradation plateau was not attained. The study result showed that the substance was not readily biodegradable. In the other OECD TG 301B ready biodegradability test (Anon., 1998), mean cumulative $^{14}CO_2$ production by mixtures containing in total 1 mg/L (CLH report indicates 10 mg/L as a typo) of [U-phenyl-^{14}C]-folpet 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 within the 10d window. The DS noted that the results of these tests were conflicting but could not specify the reason. However, they concluded that folpet was readily biodegradable.

Two aerobic surface water simulation studies (OECD TG 309, GLP) were considered valid and relevant. In Anon. (2016g), [U-phenyl-14C]-folpet applied at either 10.5 or 100.8 µg/L disappeared completely within one hour of incubation. Major metabolites were phthalimide, phthalic acid and phthalamic acid. Mineralisation was low until day 7 for both test concentrations. Afterwards, the $^{14}CO_2$ formation increased resulting in maximum fractions at test end of 68.2% AR and 22.9% AR for low and high concentrations, respectively. The formation of organic volatiles for both concentrations was negligible. In the other surface water simulation study (Anon. 2015j), [U-phenyl-14C]-folpet was found to degrade rapidly (DT_{50} of approx. 0.3 hrs). The metabolites phthalimide, phthalamic acid, and phthalic acid were formed in significant amounts. Mineralisation was significant for both dose levels, reaching a maximum value of 53.6% AR on day 21 at the 10 µg/L dose level and 20.8% AR on day 28 at 100 µg/L dose level.

Two OECD TG 308 water/sediment studies (Anon. 1999, Anon. 2007c, GLP) were considered valid and relevant. [U-phenyl-^{14}C]-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). 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 to 12.5% AR at 100 days in Anon. 1999. In Anon. (2007c), the maximum was reached at 30 (19.5% AR) and 62 days (18.8% AR) in Row Pond and Emperor Lake systems, respectively. At 99 day the unextracted residue in those sediments reached 13.2% AR and 16.5% AR, respectively. The unextracted residues were mainly associated with humin, humic acid, and fulvic acid.

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

According to the information provided in the CLH Report, the degradation products phthalimidine, phthalic acid, phthalamic acid, benzamide, and 2-cyanobenzoic acid do not meet the criteria for classification as hazardous to the aquatic environment for short-term hazard.

Based on the above the DS considered folpet to be rapidly degradable.

Bioaccumulation

The bioaccumulation of [^{14}C]-folpet in *Lepomis macrochirus* was investigated in a flow-through system at a nominal concentration of 11 µg/L (Anon. 1989a, GLP). The steady state bioconcentration factor (BCF) after 28 days in whole fish was 56. Despite deficiencies e.g., lack of information on the lipid content and growth-dilution, the DS considered the study valid and reliable.

In a shake flask study (Anon. 1987c) performed according to the USEPA Test Method CG-1400, a log Pow of 3.107 was measured for folpet.

The DS concluded that the information available indicate a low concern on the bioaccumulation of folpet.

Aquatic toxicity

Acute Aquatic Toxicity

***Table**: Relevant information on acute aquatic toxicity*

Method	Test material	Species	Result mg a.s./L	Reference
Fish				
US EPA 72-1 Flow-through	Folpet 90.3%	*Oncorhynchus mykiss*	96 h LC_{50} = 0.015 mm (35-130% of nom.)	Anonymous (1988a)
US EPA 72-1 Flow-through	Folpet 90.3%	*Lepomis macrochirus*	96 h LC_{50} = 0.047 mm (25-40% of nom.)	Anonymous (1988b)
US EPA 72-1 Flow-through	Folpet 90.3%	*Cyprinodon variegatus*	96 h LC_{50} = 0.0655 mm (2.3-11.7% of nom.)	Anonymous (1989a)
Invertebrates				
US EPA 72-2 Flow-through	Folpet 90.3%	*Daphnia magna*	48 h EC_{50} = 0.02 mm (4.7-10% of nom.)	Anonymous (1988)
FIFRA 72-3 Flow-through	Folpet 90.3%	*Americamysis bahia*	96 h LC_{50} = 0.16 mm (2.3-11.7% of nom.)	Anonymous (1989c)

Algae				
OECD TG 201 Static	Folpet 96.6%	*Raphidocelis subcapitata*	72 h ErC50 > 0.161 mm (*	Anonymous (2016e)

mm - mean measured, (* - Measured concentrations only at 0 and 4 hours. After 4 hours, the measured concentrations were below the LOQ for all test concentrations, except for the highest (23% of nominal). The DS used LOQ/2 (0.035 mg/L) for 4, 24, 48 and 72 hours to calculate the geometric mean measured concentrations.

There were reliable acute toxicity data available for the three trophic levels. The lowest acute toxicity value was a 96-hour LC_{50} of 0.015 mg/L for *Oncorhynchus mykiss* based on mean measured concentrations.

Chronic Aquatic Toxicity

***Table**: Relevant information on chronic aquatic toxicity*

Method	Test material	Species	Result mg a.s./L	Reference
Fish				
ASTM (1983) Flow-through ELS	Folpet 90.3%	*Pimephales promelas*	35 d EC_{10} = 0.0234 NOEC = 0.011 mm (10-13% of nom.) hatchability, fry survival	Anonymous (1989)
ASTM (1983) Flow-through ELS	Folpet 93.2%	*Pimephales promelas*	33 d NOEC = 0.00881 mm (17-20% of nom.) growth	Anonymous (1995)
Algae				
OECD TG 201 Static	Folpet 96.6%	Raphidocelis subcapitata	72 h EC_{10} = 0.083 72 h NOE_rC = 0.058 mm (*	Anonymous (2016e)

mm - mean measured, (* - Measured concentrations only at 0 and 4 hours. After 4 hours, the measured concentrations were below the LOQ for all test concentrations, except for the highest (23% of nominal). The DS used LOQ/2 (0.035 mg/L) for 4, 24, 48 and 72 hours to calculate the geometric mean measured concentrations.

There were reliable chronic toxicity data available for fish and algae. No data was available for invertebrates. The lowest chronic toxicity value was a 33-day NOEC of 0.00881 mg/L for *Pimephales promelas* based on mean measured concentrations. No EC_{10} value for growth could be determined in the study.

Comments received during consultation

Three Member States and one company agreed with the proposed classification.

Comments were given on conflicting results in the two ready biodegradability studies. Anon. (1998) concluded that folpet was readily biodegradable in contrary to Anon. (1994) for reasons unknown. It was pointed out that in Anon. (1994) the concentration of folpet used was more than 30 times higher than the aqueous solubility limit. Anon. (1998) was

conducted with a lower concentration of folpet. In the test report of Anon. (1998) it was considered that the biodegradation in Anon. (1994) may have been influenced by its rate of dissolution in the test medium. The DS agreed that as the test concentration in Anon. (1998) was set to the water solubility of folpet, results are considered more reliable even if the folpet test concentration was clearly below the test concentration recommended in OECD TG 301. In addition, rapid degradation/dissipation in other aquatic systems (OECDs TG 308, 309 and 111) show that folpet should be considered readily biodegradable.

A company also commented on the difference between the two ready biodegradability studies. They supported the conclusion that the low dose radio-labelled study (Anon. 1998) is more reliable than the non-labelled study (Anon., 1994). They also agreed that the other studies (OECD TGs 308, 309, and 111) support the ready biodegradability conclusion.

A National Authority (NA) also commented on the ready biodegradability tests. They questioned the validity of Anon. (1998) study due to the test concentration being below the OECD TG 301 test conditions and asked for confirmation from the DS. The DS answered that according to their expert, folpet is considered readily biodegradable and rapidly degradable in the water/sediment system. They specified that the test concentration in Anon. (1998) was 1 mg folpet/L (radiolabelled + unlabelled folpet). Folpet tested comprised 10% radiolabelled and 90% unlabelled folpet, so 0.1 mg labelled and 0.9 mg unlabelled folpet per litre.

The NA also pointed out that chronic toxicity data is not available for degradants to consider if they meet aquatic chronic hazard criterion. They concluded that in case folpet would be considered not rapidly degradable, the surrogate system should be considered for the most sensitive fish species and invertebrate data.

An MS informed that there were more data on the aquatic toxicity of folpet available than those included in the CLH Report. The data found in Status of Endocrine Disruptor Screening Program Tier 1 Screening Results and Data Evaluation Records/US EPA (US EPA EDSP) included fish reproduction tests and an amphibian metamorphosis assay (AMA OECD TG 231). The DS informed that two fish short-term reproduction assays and an amphibian metamorphosis assay were submitted for the ED assessment. They agreed that these fish assays should have been included in the CLH report but noted that the relevant endpoint values determined from these studies (NOEC = 0.0086 mg a.s./L (male VTG) (Anonymous, 2012) and 0.00627 mg a.s./L (Anonymous, 2021) do not have an impact on the proposed classification. The NOEC = 0.0096 mg/L (developmental stage and weight) from the AMA test would not change the classification proposal either.

Assessment and comparison with the classification criteria

Degradation

RAC disagrees with the DS and is of the opinion that folpet is not rapidly degradable based on the decision scheme in the CLP guidance, page 498.

- folpet was not readily biodegradable as Anon. (1998) is considered not reliable (see the next Chapter)
- folpet did not ultimately degrade in the surface water simulation tests
  - At test end the $^{14}CO_2$ formation increased resulting in maximum fractions of 68.2% AR and 22.9% AR for low and high concentrations, respectively.

- folpet was rapidly hydrolysed but it cannot be excluded that the hydrolysis products fulfil the criteria for classification as hazardous to the aquatic environment
  - the detected degradation products differ depending on the position of the ^{14}C-radiolabel ([carbonyl-^{14}C]-folpet and [trichloromethyl-^{14}C]-folpet)
  - there is no chronic data available for the degradation products formed in the carbonyl-^{14}C study
  - the degradation products formed in the trichloromethyl-^{14}C study are not confirmed
- folpet rapidly degraded in the two water/sediment studies but RAC concludes that it cannot be excluded that the degradation products fulfil the criteria for classification as hazardous to the aquatic environment.
  - there is no chronic data available on the degradation products
  - the fate of the thio(trichloromethyl) side chain of folpet in aquatic systems is largely unknown.

RAC therefore considers that folpet should be considered as not rapidly degradable for the purpose of classification.

Bioaccumulation

RAC agrees with the DS's conclusion to consider folpet as having a low potential for bioaccumulation based on the 28-day fish bioconcentration factor of 56 which is below the classification cut-off of 500. The log Pow of 3.107 is also below the classification cut-off of 4.

Aquatic toxicity

There are acute folpet toxicity data available for the three trophic levels. RAC agrees with the DS to consider the 96-hour LC_{50} of 0.015 mg/L for *Oncorhynchus mykiss* the lowest effect value which warrants classification as Aquatic Acute 1 (CLP Annex I Table 4.1.0 (a)) with an M-factor of 10 ($0.01 < LC_{50} \leq 0.1$ mg/L).

There were chronic toxicity data available for fish and algae. RAC took note of the additional long-term studies mentioned in the consultation. Although the effects endpoints are for ED effects, they are potentially relevant, and RAC assessed them for classification and labelling. RAC was not able to assess the lowest NOEC of 0.00627 mg/L due to a lack of information and the other NOEC of 0.0086 mg/L appears to be based on male vitellogenin production, which by itself is not admissible for hazard assessment. RAC agrees with the DS that the remaining additional endpoint value (NOEC = 0.0096 mg/L (developmental stage and weight) from the AMA test) does not alter the classification outcome as it is in the same range as the value for *P. promelas*, albeit higher. RAC agrees with the DS to consider the 33-day NOEC of 0.00881 mg/L for *P. promelas* as the lowest chronic effect value. As RAC considers folpet as not rapidly degradable, classification to Aquatic Chronic 1 category (CLP Annex I Table 4.1.0 (b) (ii)) with and M-factor of 10 is warranted ($0.001 < NOEC \leq 0.01$ mg/L). The surrogate system based on the 48-hour EC_{50} of 0.02 mg/L for *Daphnia magna* results in the same classification outcome (CLP Annex I Table 4.1.0 (b) (iii)).

Consequently, RAC disagrees with the DS and concludes that folpet should be classified as:

Aquatic Acute 1, M=10 and

Aquatic Chronic 1, M=10.

Supplemental information - In depth analyses by RAC

Ready biodegradability

The two available ready biodegradation tests were performed following OECD TG 301B. In Anon. (1994), the biodegradation after 28 days was 41% CO_2 and in Anon. (1998) 73% AR $^{14}CO_2$. Inoculum in both tests was activated sludge from a local domestic STTP. Test concentrations were different. In Anon. (1994), 27.5 mg/L folpet technical (10 mg/L C/L) was used and in Anon. (1998) 1 mg/L (0.1 mg/L [U-phenyl-14C]-folpet + 0.9 mg/L folpet) was used. Cumulative CO_2 production was 21.2 mg and 25.7 mg in Anon. 1994 and 49.8 and 49.2 mg in Anon. 1998.

A general condition applying to the OECD TG 301B method is a test concentration 10-20 mg/DOC/L. This condition is not fulfilled in Anon. (1998). Hence, the reliability of the test for assessing ready biodegradability is uncertain.

The validity criteria for OECD TG 301B states that the total CO_2 evolution in the inoculum blank at the end of the test should not normally exceed 40 mg/L medium. In Anon. (1998), this value was exceeded. The CLH Report mentioned 10 mg/L of total folpet. However, in the answer to the consultation comment, the DS specified that the test concentration was 1 mg folpet/L (radiolabelled + unlabelled folpet). Folpet tested in Anonymous (1998) comprised 10% radiolabelled and 90% unlabelled folpet, so 0.1 mg labelled and 0.9 mg unlabelled folpet per litre.

Based on the activated sludge respiration inhibition test (OECD TG 209, GLP) presented in Volume 3 – B.9 of the draft RAR (2018/03), a 3-hour EC_{50} > 320 mg/L, EC_{20} of 23.23 mg/L and a NOEC of 10 mg/L as nominal concentrations calculated for folpet technical (96.1%) no inhibitory effect in Anon. (1994) was foreseen. OECD TG 301 Annex II states that "if inhibition due to toxicity is to be avoided, it is suggested that the test substance concentrations used in ready biodegradability testing should be less than 1/10 of the EC_{50} values (or less than EC_{20} values) obtained in toxicity testing. Compounds with an EC_{50} value greater than 300 mg/L are not likely to have toxic effects in ready biodegradability testing."

The water solubility of folpet is 0.80 mg/L at 25 °C. The OECD TG 301B test is suitable for poorly soluble substances. There is no information available in the CLH Report or the Annexes if magnetic stirrers or special attention as instructed in Annex III of the OECD TG 301B has been used in either test. However, the DS provided data from the study reports indicating in Anon. (1994) test incubations samples have neither been stirred or kept agitated, whereas in Anonymous (1998) test incubation samples have been kept agitated by means of a magnetic stirrer.

Based on the above and mainly related to the test substance concentration RAC considers Anon. (1998) not reliable for assessing ready biodegradability.

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			

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14 ANNEXES

Annex I: Human Health

Annex II: Ecotoxicology I (AS)

Annex III: Environmental Fate & Behaviour

Confidential Annex