Committee for Risk Assessment

RAC

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

Adopted

8 June 2023

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September 2023: latest version, replacing all previous versions

8 June 2023

CLH-O-0000007326-73-01/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical name: **folpet (ISO); *N*-(trichloromethylthio)phthalimide**

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

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

The proposal was submitted by **Austria** and received by RAC on **21 June 2022.**

In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

PROCESS FOR ADOPTION OF THE OPINION

Austria has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **8 August 2022**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **7 October 2022**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Karine Angeli**

Co-Rapporteur, appointed by RAC: **Riitta Leinonen**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was adopted on **8 June 2023** by **consensus**.

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	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	GHS08 GHS07 GHS09 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 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. 1A; H317: C ≥ 0,001% M = 1	
RAC opinion	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 Aquatic Chronic 1 **Modify** Acute Tox. 2 Eye Dam. 1 Skin Sens. 1A	**Retain** H351 H400 **Add** H372 (respiratory tract) H410 **Modify** H330 H318	**Retain** GHS08 GHS09 **Add** GHS05 GHS06 **Modify** Dgr **Remove** GHS07	**Retain** H351 H317 **Add** H372 (respiratory tract) **Modify** H330 H318 H410	**Add** EUH066	**Retain** M = 10 **Add** inhalation: ATE = 0,30 mg/L (dusts and mists) STOT RE 1; H372: C ≥ 5% STOT RE 2; H373: 5% > C ≥ 0,5% Skin Sens. 1A; H317: C ≥ 0,001% M = 10	
Resulting Annex VI entry if agreed by COM	613-045-00-1	folpet (ISO); *N*-(trichloromethylthio)phthalimide	205-088-6	133-07-3	Carc. 2 Acute Tox. 2 STOT RE 1 Eye Dam. 1 Skin Sens. 1A Aquatic Acute 1 Aquatic Chronic 1	H351 H330 H372 (respiratory tract) H318 H317 H400	GHS08 GHS07 GHS09 Wng	H351 H330 H372 (respiratory tract) H318 H317 H410	EUH066	inhalation: ATE = 0,30 mg/L (dusts and mists) STOT RE 1; H372: C ≥ 5%	

						H410				STOT RE 2; H373: 5% > C ≥ 0,5% Skin Sens. 1A; H317: C ≥ 0,001% M = 10 M = 10	

Based on the data submitted during the consultation, the DS modified their proposal for skin corrosion/irritation from classification as Skin Irrit. 2 (H315) to EUH066

GROUNDS FOR ADOPTION OF THE OPINION

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.

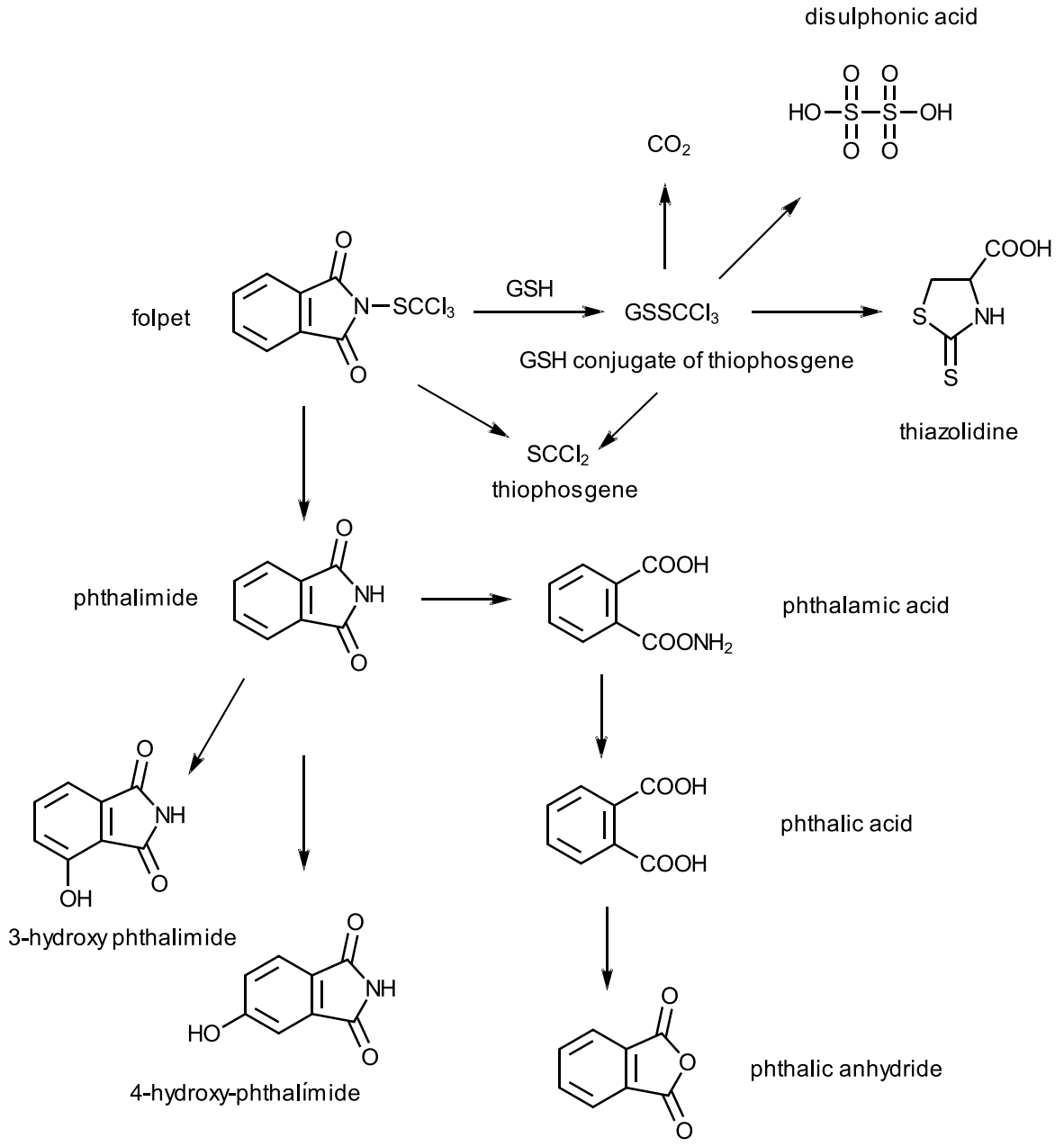

***Figure**: Generalised metabolic pathway for folpet in rodents following oral administration*

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 toxophore 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.

HUMAN HEALTH HAZARD EVALUATION

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	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

- 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)					
GLP, OECD TG 403 (1981) Nose-only exposure	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 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 LC50 for males is 0.30 mg/L and (converted to 100% purity)
- In study 3 (nose-only exposure), the LC50 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).

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 (see Background document) 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.**

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 (see summary table in the Background document).

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.**

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	Individual 24-72 h means 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 per day from 24-72 hours - Unclear if fluorescein staining was used	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	Individual 24-72 h means for animals without eye washing Cornea opacity: **2.67** (pannus present from day 10 onwards), 0, 0, 0, **2.33** (pannus present from day 13 onwards), **0.67** (pannus present from day 7 onwards) corneal opacity **not reversible within 13 days (max follow-up)** Iritis: 0, 0, 0, 0, 0, **0.33 not reversible within 13 days (max follow-up)** Conjunctiva – redness: **3,** 3, 3, 2.67**, 3, 3 not reversible within 13 days (max follow-up)** Conjunctiva – chemosis: **3,** 3.3, 3, 2, **3.3, 3 not reversible within 13 days (max follow-up**) Individual 24-72 h means for animals with eye washing 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)	(R-1737) Study 2, 1979
GLP, EPA August 1978 Deviations from OECD TG	NZW rabbits, 9 males	Folpet technical, no purity reported	0.1 g, eyes were not washed in	Individual 24-72 h means for animals without eye washing Cornea opacity: 0, 0, 0, 0, 0, 2.67 (pannus present	(R-7091) Study 3, 1982

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			the 3-day post exposure period In 3 animals eyes were washed 30 s after exposure	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	
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 Background document).

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).**

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 in the Background document).

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**.**

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. (See Background Document).

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%

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 Background Document), 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.**

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.**

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. (See Background document)

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

			No evidence of a specific sensitive period	
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 13^{th} 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 13^{th} 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.**

ENVIRONMENTAL HAZARD EVALUATION

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 Background Document)
- 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.

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ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in 'RAC boxes'.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and RAC (excluding confidential information).

Appendix

Carcinogenicity

Mode of action

The aetiology of the small intestinal tumours has been thoroughly investigated in mechanistic studies in CD-1 mice (study 7 to 12) and published studies. A MoA driven by cytotoxicity with subsequent regenerative proliferation which if sustained, increases the probability of spontaneous mutation leading finally to tumours, has been proposed.

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.

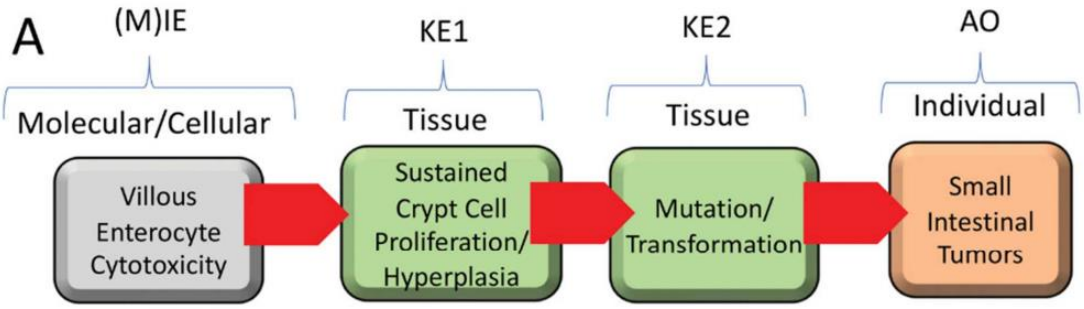

Figure: From Bhat et al. 2020 "Proposed AOP for cytotoxicity-mediated SI cancer in mice". AOP diagram.

RAC has analysed the available data with folpet supporting the proposed pathway.

Key events

- Initiating event "villous enterocyte cytotoxicity"

As demonstrated in the toxicokinetic studies by oral route, folpet is rapidly degraded to phthalimide and thiophosgene in the gastrointestinal tract. Breakdown occurs by either hydrolysis (readily under neutral and alkaline conditions) or reaction with GHS and other thiols (proteins).

The interaction of folpet and thiophosgene with thiol groups, leads to cytotoxicity in the duodenum as any other mucous membranes.

In the mechanistic study 10 (1997), folpet dietary treatment with 5000 ppm for 28 days induced duodenal cytotoxicity in male and female CD-1 mice reflected by reduced villi height and villi fusion (villous blunting), associated with increased numbers of inflammatory cells in the lamina propria. Such changes were not observed after single dose of 5000 ppm in diet or 900 mg/kg bw/d via gavage (study 11, 2004).

Chappell *et al*. (2019) investigated the gene expression profile of duodena from female B6C3F1 mice exposed at dose level of to 6000 or 16000 ppm in the diet for 28 days. Folpet (similarly to captan and Cr(VI)) upregulated hypoxia inducible factor 1 (HIF-1) and activator protein 1 (AP1) signalling pathways, downstream biomarkers of cytotoxicity.

Essentially of the initiating event

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. In study 9 (1995), protein and non-protein thiol concentrations in the duodenum S9 fraction increased after 28-day exposure of 5000 ppm by diet, this increase was reversible after a 28-day recovery period.

RAC considers that there is strong evidence that the initial KE is triggered by folpet.

- Key event 1 "sustained crypt cell proliferation/hyperplasia"

The 28-day mechanistic studies 7 to 10 in CD-1 mice provide consistent evidence that folpet dietary exposure with concentrations (5000 ppm) that result in small intestinal tumours in carcinogenetic studies, induce hyperplasia of the crypts observed at histopathological examination and measured as an increase in labelling indices for either bromodeoxyuridine (BrdU) or proliferating cell nuclear antigen (PCNA).

Thompson *et al*. (2017) also showed duodenal hyperplasia (crypts epithelium and hyperplasia and villous enterocyte hypertrophy) in B6C3F1 mice exposed to folpet (6000 ppm by diet) for 28 days.

In the available carcinogenicity studies, increased incidence of duodenal hyperplasia was observed in both strains from 1000 ppm in the diet.

Essentially of the key event 1

Reversibility or attenuation of the duodenal hyperplasia have been demonstrated in the recovery groups included in the 28-day repeated toxicity studies in both CD-1 mice (study 9, 1995 and study 12, 2011) and B6C3F1 mice (Thompson, 2017).

RAC considers that there is strong evidence that folpet induces crypt cell proliferation/hyperplasia.

- Key event 2 "mutation and transformation"

While it is broadly accepted that chronic cell proliferation/hyperplasia increases the probability of spontaneous mutations to occur, specific data to address this key event are lacking.

- Adverse outcome "small intestinal tumours"

There is strong evidence that folpet induces small intestinal tumours in mice based on positive carcinogenicity studies in both sexes of two different strains.

<u>Biological plausibility of the key event relationships (KER)</u>

It is broadly accepted that turnover of normal villi occurs by migration of epithelial cells from the base of the intestinal crypts, and that stem cells serve as the source of these new epithelial cells. Hyperplasia in intestinal crypts is a well-documented response to villous cytotoxicity. Prolonged hyperplasia in the stem cells increases the probability of cloning a transformed cell ultimately leading to an increased incidence of duodenal adenomas and adenocarcinomas.

Sustained cytotoxicity associated with regenerative hyperplasia is a well-documented pathway for tumour promotion.

Therefore, the biological plausibility of KER1 (villous enterocyte cytotoxicity leading to sustained crypt cell proliferation), KER2 (sustained crypt cell proliferation/ hyperplasia leading to mutation and transformation) and KER3 (mutations and transformation leading to small intestinal tumours) is considered strong.

<u>Empirical support of the key event relationships (KER)</u>

Folpet's data set support the KERs. In particular, the different studies demonstrate dose and temporal concordance of the KER, establishing a strong causal association.
A clear threshold can be established with tumours occurring only after a prolonged exposure to cytotoxic doses.
Consistency of the KERS among two strains of mice tested with folpet is demonstrated. However, small intestine tumours do not occur in rats at irritating doses (hyperkeratosis in the forestomach).
In addition to the different dose regimen used in rat, several hypotheses have been put forward to explain the species differences (i.e. greater folpet intake of mice when compared to rat, greater

reliance on glutathione for the detoxification of mouse or anatomical differences of the gut between the two species).
Overall, the evidence provided by the empirical support is considered strong.

Analogy

Captan, which is structurally related to folpet and share thiophosgene as major metabolite, also induces small intestine tumours in mice.

Alternative mode of action: genotoxicity.

While, folpet consistently exerts genotoxic activity (both mutagenic and clastogenic) in *in vitro* systems, it is negative in *in vivo* follow-up studies.
Especially, folpet did not induce DNA damage in the duodenum of CD-1 mice after a single administration of 2000 mg/kg bw in two independent comet assays (study 3, 2004 and study 5, 2008). 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.
Therefore, RAC considers that early genotoxic events are unlikely to be involved in the cancerous process leading to small intestine tumours after exposure to folpet.

Human relevance of the proposed mode of action according to WHO/IPCS human relevance framework a MoA

The weight of evidence is considered sufficient to establish a MoA driven by cytotoxicity with subsequent regenerative proliferation which if sustained, increases the probability of spontaneous mutation leading ultimately to small intestine tumours in mice.
The different KEs are plausible in human, and the human relevance of this MoA cannot be excluded based on qualitative interspecies differences.
There is no data on toxicokinetic/dynamic difference between human and mice. *In vitro* comparative metabolism in rat and human microsomes did not reveal any relevant differences.
However, the different KEs become less plausible when background levels of human exposure (no exposure scenario resulting in sustained irritating concentrations of folpet via the diet is expected) are considered.

Conclusion:
RAC considers that the weight of evidence is sufficient to establish that a MoA driven by cytotoxicity underlies the occurrence of small intestinal tumours in mice.
While this MoA is considered qualitatively relevant for humans, RAC acknowledges that a clear threshold for tumour-development in mice is established which decreases the level of concern for human carcinogenicity.

Factors to be taken into consideration in the hazard assessment

Species and strain	Tumour type and background incidence	Multi-site responses	Progression of lesions to malignancy	Reduced tumour latency	Responses in single or both sexes	Confounding effect by excessive toxicity?	Route of exposure	MoA and relevance to humans
Rats SD	Intestinal tumours do not occur at the low doses used	-	-	-	-	-	Oral diet	-
Rats Fisher	Intestinal tumours do	-	-	-	-	-	Oral diet	-

Species and strain	Tumour type and background incidence	Multi-site responses	Progression of lesions to malignancy	Reduced tumour latency	Responses in single or both sexes	Confounding effect by excessive toxicity?	Route of exposure	MoA and relevance to humans
F344	not occur in rats at the low doses used							
Mice CD-1	Duodenal adenoma and adeno-carcinoma. Rare CD-1 mouse	No	Yes	No information	Both	Tumours	Oral diet	Non-genotoxic mechanism
Mice B6C3F1	Duodenal adenoma and adeno-carcinoma Rare CD-1 B6C3F1	No	Yes	No information	Both	High dose exceeds MTD in females However tumours also observed at lower dose levels	Oral diet	Non-genotoxic mechanism