Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

captan (ISO); 1,2,3,6-tetrahydro-*N*-(trichloromethylthio)phthalimide

EC Number: 205-087-0
CAS Number: 133-06-2

CLH-O-0000007361-79-01/F

Adopted

14 September 2023

14 September 2023

CLH-O-0000007361-79-01/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 on **14 September 2023** by **consensus** an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical name: **captan (ISO); 1,2,3,6-tetrahydro-*N*-(trichloromethylthio)phthalimide**

EC Number: **205-087-0**

CAS Number: **133-06-2**

Rapporteur, appointed by RAC: **Brendan Murray**

Co-Rapporteur, appointed by RAC: **Zilvinas Uzomeckas**

Administrative information on the opinion

Austria has submitted on **21 June 2022** 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**.

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 following table provides a summary of the Current Annex VI entry, Dossier submitter proposal, RAC opinion and potential Annex VI entry, if agreed by the Commission.

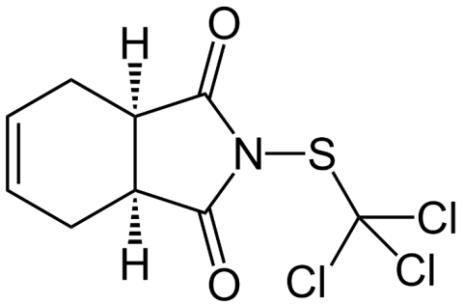
Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	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	Existing	captan (ISO); 1,2,3,6-tetrahydro-*N*-(trichloromethylthio)phthalimide	205-087-0	133-06-2	Carc. 2 Acute Tox. 3 Eye Dam. 1 Skin Sens. 1 Aquatic Acute 1	H351 H331 H318 H317 H400	GHS09 GHS08 GHS05 GHS06 Dgr	H351 H331 H318 H317 H400		M = 10	
Dossier submitters proposal	Existing or TBD	captan (ISO); 1,2,3,6-tetrahydro-*N*-(trichloromethylthio)phthalimide	205-087-0	133-06-2	**Retain** Carc. 2 Eye Dam. 1 Aquatic Acute 1 **Add** STOT RE 1 Aquatic Chronic 1 **Modify** Acute Tox. 2 Skin Sens. 1A	**Retain** H351 H318 H317 H400 **Add** H372 H410 **Modify** H330	**Retain** GHS05 GHS06 GHS08 GHS09 Dgr	**Retain** H351 H318 H317 **Add** H372 **Modify** H330 H410		**Retain** M=10 **Add** inhalation: ATE = 0.22 mg/L (dusts and mists) Skin Sens.: SCL ≥ 0.001% M = 1	
RAC opinion	613-044-00-6 or TBD	captan (ISO); 1,2,3,6-tetrahydro-*N*-(trichloromethylthio)phthalimide	205-087-0	133-06-2	**Retain** Carc. 2 Eye Dam. 1 Aquatic Acute 1 **Add** Repr. 2 STOT RE 1 Aquatic Chronic 1 **Modify** Acute Tox. 2 Skin Sens. 1A	**Retain** H351 H318 H317 H400 **Add** H361f H372 H410 **Modify** H330	**Retain** GHS05 GHS06 GHS08 GHS09 Dgr	**Retain** H351 H318 H317 **Add** H361f H372 **Modify** H330 H410		**Retain** M=10 **Add** inhalation: ATE = 0.22 mg/L (dusts and mists) Skin Sens.: SCL ≥ 0.001% M = 10	
Resulting Annex VI entry if agreed by COM	Existing or TBD	captan (ISO); 1,2,3,6-tetrahydro-*N*-(trichloromethylthio)phthalimide	205-087-0	133-06-2	Carc. 2 Repr. 2 Acute Tox. 2 STOT RE 1 Eye Dam. 1 Skin Sens. 1A Aquatic Acute 1 Aquatic Chronic 1	H351 H361f H330 H372 H318 H317 H400 H410	GHS08 GHS06 GHS05 GHS09 Dgr	H351 H361f H330 H372 H318 H317 H410		inhalation: ATE = 0.22 mg/L (dusts and mists) Skin Sens.: SCL ≥ 0.001% M = 10 M = 10	

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

Captan is a general use pesticide that displays a broad spectrum of antifungal effects, it rapidly penetrates fungal spores and can be used as an effective protectant in a variety of crops. It belongs to the N-sulfinyl phthalimide class of fungicides (or chloroalkylthio group, along with structurally similar molecules such as folpet and captafol). The target sites of captan are numerous and have not been completely identified. It displays multisite local activity, a consequence of its rapid reaction with thiol groups (i.e., sulfhydryl, -SH groups) found on many enzymes and other molecules of biological importance (e.g. the cysteine moiety of glutathione or GSH).



Captan is approved for use in the EU on pome fruit, peaches and nectarines, plums, cherry, tomato, strawberries (field use) and strawberries under greenhouse application. As a preventative fungicide, it is efficacious when applied prior to the establishment of pathogenic fungi. Captan's application for renewal under the approval criteria provided for in EU Reg. 1107/2009 was assessed by the Rapporteur Member State (RMS) Austria and co-RMS Italy. The RMS provided its initial evaluation in the captan RAR which was submitted to EFSA in late 2017. Subsequently EFSA published its peer review in 2020.

Captan has a current entry in Annex VI of the CLP Regulation and thus only a selection of hazard classes is open for assessment in this opinion document. The physical hazard classes are not assessed in the CLH dossier nor are they assessed in this opinion document. The current harmonised classification consists of:

Acute Tox. 3 – H331.
Eye Dam. 1 – H318.
Skin Sens. 1 – H317.
Carc. 2 – H351.
Aquatic Acute 1 – H400.

The RMS proposed to add classification for chronic toxicity (STOT RE 1 – H372) and for aquatic chronic (Aquatic Chronic 1 – H400) with an M factor of 10, and an M factor of 10 to the existing aquatic acute classification, to update classification for skin sensitisation (Skin Sens. 1A – H317) with an SCL of 0.001%, and to retain Eye Dam. 1 – H318 and Carc. 2 – H351. On the basis of new acute inhalation toxicity studies (subsequent to the EFSA peer review), the RMS additionally proposed to modify Acute Tox. (inhalation) from Category 3 to Category 2.

The minimum purity is 910 g/kg. Perchloromethylmercaptan (PCMM), carbon tetrachloride and folpet are considered relevant impurities with maximum contents of 5 g/kg, 0.1 g/kg and 10 g/kg, respectively. The DS considered these impurities not to contribute to the proposed classification. Captan is poorly soluble in water, and it degrades by hydrolysis or reaction with thiol-rich

matrices into two initial degradants through the metabolic cleavage of the nitrogen–sulphur bond, i.e. tetrahydrophthalimide (THPI) and thiophosgene. This generally occurs in the gastrointestinal tract prior to absorption. THPI is relatively stable, while thiophosgene is very reactive and unstable. THPI undergoes a series of metabolic transformations (THPAM, 3-OH-THPI, 3-OHTHPAM, 5-OH-THPI, THPI-Epoxide and 4,5-diOH THPI). THPI and all of its metabolites do not share the same toxicological properties of captan. The hazard profile of captan is associated with the sidechain attached to the isoindole ring nitrogen, the trichlormethylsulfanyl-group (R-$SCCl_3$).

Captan has been extensively studied in a series of guideline and non-guideline toxicokinetic investigations. Captan is extensively and rapidly absorbed from the gastrointestinal tract (> 80%) and is also rapidly metabolized with no evidence for bioaccumulation. There are no apparent sex differences. Urinary excretion predominates (THPI is a major urinary rat metabolite). Rats given captan orally, excreted a third in the faeces and half in the urine within 24 hours. Tissue residues are negligible (highest rates in kidney; < 0.01% of administered radioactive label).

The findings from the toxicokinetic studies help clarify captan's hazard potential, essentially, it is very difficult to achieve acute systemic exposure through the oral route due to its extremely short half-life which is a consequence of its rapid reactivity with thiols. Toxicological effects are restricted to points of initial contact such as mucus membranes and intestinal surfaces due to direct interactions at the site of first contact with the rapid release of thiophosgene and its reactivity towards thiols. The terminal half-life of the parent material in human blood at 37°C is 0.97 seconds (Gordon and Williams, 1999), the corresponding half-life of thiophosgene has been measured at 0.6 seconds (Arndt and Dohn, 2004).

Captan gastrointestinal fate in rat and mouse was compared involving administration of single oral doses of 5 or 250 mg/kg bw [^{14}C]-trichloromethylthio labelled captan to animals, pre-treated by dietary exposure to 5000 ppm captan for 90 or 148 days. Levels of duodenal captan and metabolites were proportionately higher in the mouse at high doses compared to low doses. In the rat the proportions were similar at both doses and similar to those in the low dose mouse. In the high dose groups, markedly higher duodenal levels of captan were found in the mouse compared to the rat.

The toxicokinetic studies help to clarify captan's hazard profile of acute local irritation and adverse effects. There is no evidence for systemic exposure towards the intact parent molecule. The toxicophore, trichlormethylsulfanyl-group, is highly reactive and severely irritating to mucous membranes (respiratory, digestive or ocular). Primary effects are therefore expected to occur at the first site of exposure before entering the systemic compartment. Any observed systemic effects are therefore assumed to arise from primary irritating effects and depletion of essential thiol-rich compounds.

No unique human metabolite is found from the *in vitro* intraspecies comparative metabolism study using rat and human liver microsomes. According to the final EFSA review report, and with regard to the assessment of the endocrine disruption potential for humans as laid out in the ECHA/EFSA guidance (2018), captan does not meet the Endocrine Disruptor (ED) criteria for EATS modalities.

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

Oral route

There is no current Annex VI entry for acute toxicity via the oral route. The DS proposed no classification for captan with respect to acute oral toxicity. There were four acceptable, guideline-compliant (OECD TG 401, 1981-1987), acute oral studies available to the DS (one in mouse and three in rats, table 11 of the CLH report), the acute oral LD_{50} was consistently > 2000 mg/kg bw in all studies. Only study 1 (rat) was noted as performed to GLP standard. Nevertheless, the DS noted all studies were acceptable.

Study 1 (Anonymous, 1991; RAR 3.1.1.1)

The acute oral LD_{50} was found to be > 2000 mg/kg bw for Sprague-Dawley rats (5/sex/dose) for both sexes. Only a single dose was tested (2000 mg/kg bw). No mortalities and no signs of systemic toxicity were recorded during the study. All animals showed expected body weight gain during the study and there were no abnormalities detected at necropsy.

Study 2 (Anonymous, 1984; RAR 3.1.1.2)

The acute oral LD_{50} was found to be > 5000 mg/kg bw for Sprague-Dawley rats (10/sex/dose) in both sexes. Only a single dose was tested (5000 mg/kg bw). A variety of non-specific clinical signs were observed in all animals. Following dose administration, 2 males died on days 1 and 12 and one female died on day 4. Necropsy findings were reported for the two males that died and showed gastrointestinal and liver involvement.

Study 3 (Anonymous, 1982; RAR 3.1.1.3)

Two tests were conducted using different doses and number of Sprague-Dawley rats. Test 1 used 5 animals/sex/dose at levels of 0, 5000, 6500, 8300, 10800 and 14000 mg/kg bw. Test 2 used 10 animals/sex/dose at levels of 0 and 7800/7200 (M/F) mg/kg bw. High mortality was observed at all doses > 6500 mg/kg bw. At 6500 mg/kg bw, 1/5 males and 2/5 females died (day 5) with the remaining males recovering between days 2-5 and females recovering in 4-5 days. No mortality occurred in the 5000 mg/kg bw dose groups in either sex. Clinical findings were not reported with respect to the dose administered. The acute oral LD_{50} was calculated to be 7000 mg/kg bw (males) and 6170 mg/kg bw (females).

Study 4 (Anonymous, 1983; RAR 3.1.1.4)

A fourth acute oral toxicity study was conducted in the mouse. Groups of 5 animals/sex/dose were given a single oral dose of captan at 1500, 1890, 2380 or 3000 mg/kg bw and observed for 14 days. There were no mortalities and just minor clinical signs following the 1500 mg/kg bw in both males and females. At 1890 mg/kg bw, one male died on day 1 and one female died on day 2. At this dose level, clinical signs included reduced activity, reduced frequency of respiration, staggering, tremor and abdominal ache. Higher dose levels produced severe and generalised clinical signs with significant mortality. Macroscopic examination of the abdominal cavity revealed hyperaemia of the gastrointestinal tract. The combined acute oral LD_{50} for both sexes was 2110 mg/kg bw.

Dermal route

There is no current Annex VI entry for acute toxicity via the dermal route. The DS presented three studies in total; one in rabbit (1984) and two in rats (1989, 1991) where it was

demonstrated that captan technical was of low acute dermal toxicity. All studies adhered to or followed closely to OECD TG 402 (1981) but only the two rat studies were noted to be GLP compliant. The DS did not propose classification for acute dermal toxicity.

Study 1 (Anonymous, 1984; RAR 3.2.1.1)

The acute dermal LD_{50} was found to be > 2000 mg/kg bw. A single dose of captan was applied to the closely clipped abdominal skin of male and female Stauffland albino rabbits at a dose of 2000 mg/kg bw (5 animals/sex). The skin was abraded on half of the animals and left intact on the others. There were no mortalities. All animals appeared normal throughout the observation period, except two rabbits which had wet areas around the eyes on days 4 and 5. There were no signs of dermal irritation. No abnormalities were observed at necropsy.

Study 2 (Anonymous, 1989; RAR 3.2.1.2)

The acute dermal LD_{50} was found to be > 2000 mg/kg bw. A group of 5 male and 5 female CD rats were given a single 24-hour dermal application at a dose of 2000 mg/kg bw. The test substance was applied at a concentration of 72.7% w/v in distilled water to the intact, clipped lumbar skin. Animals were observed for 14 days for clinical signs. There were no mortalities and no signs of systemic toxicity or dermal irritation. All animals showed the expected gain in body weight during the study. No abnormalities were observed at necropsy.

Study 3 (Anonymous, 1991; RAR 3.2.1.3)

The acute dermal LD_{50} was found to be > 2000 mg/kg bw. A single captan dose of 2000 mg/kg bw was applied to intact, clipped skin (back and flanks clipped to expose an area of approximately 10% total body surface) of 5 male and 5 female Sprague Dawley rats for 24 hours. There were no mortalities and no signs of systemic toxicity or dermal irritation. All animals showed the expected gain in body weight during the study. No abnormalities were observed at necropsy.

Inhalation route

The current Annex VI entry for acute toxicity via the inhalation route is Acute Tox. 3; H330. The DS presented four rat studies in total where it was demonstrated that captan technical was of moderate acute inhalation toxicity. In all studies, the LC_{50} values were below the upper cut-off value of 5 mg/L for dusts or mists. The DS proposed Acute toxicity Cat. 2; H330: Fatal if inhaled with an ATE of 0.22 mg/L based on the results of study 4 detailed below.

Study 1 (Anonymous, 1995; RAR 3.3.1.1)

The acute (4-hour) inhalation LC_{50} for rats exposed to captan was 1.21 mg/L or 1.05 mg/L in males and females, respectively. Captan technical was administered as a single 4-hour exposure dose (nose only) to CD strain rats. Three groups of five males and five females were each exposed to achieved concentrations of 0.43, 0.82 and 1.36 mg/L. The study was guideline compliant (OECD TG 403, 1981) and performed according to GLP and considered reliable with restrictions by the DS. However, it is noted that a respirable fraction was not obtained (MMAD of 4.95 - 6.51 µm) and consequently the acute inhalation toxicity may have been underestimated.

Study 2 (Anonymous, 1991; RAR 3.3.1.2)

The acute (4-hour) inhalation LC_{50} for rats exposed to captan was 0.90 mg/L and 0.67 mg/L in males and females, respectively. Captan technical was administered as a single four-hour exposure (nose only) to Sprague-Dawley rats. Three groups of five males and five females were each exposed to mean achieved concentrations of 0.23, 0.94 or 4.81 mg/L. The study was guideline compliant (OECD TG 403, 1981) and performed according to GLP and considered acceptable by the DS. All animals exposed to 4.81 mg/L died or were killed in extremis during exposure. There were no deaths of animals exposed at 0.23 mg/L. The particle size distribution confirmed that a sizable respirable fraction was generated at each dose level (MMAD of 1.6 – 1.8

µm). There were seven deaths in the group exposed to 0.94 mg/L. Animals that died or were killed in extremis showed similar abnormalities at necropsy including lungs that appeared haemorrhagic, swollen and fluid filled.

Study 3 (Anonymous, 1985; RAR 3.3.1.3)

The acute (4-hour) inhalation LC_{50} for rats exposed to captan was 0.72 mg/L and 0.87 mg/L in males and females, respectively. Captan technical was administered as a single four-hour exposure (whole body) to Sprague-Dawley rats. Three groups of ten males and ten females were each exposed to mean achieved concentrations of 0.56, 0.71 or 1.39 mg/L. The study was guideline compliant (US EPA, 1982) but was not performed according to GLP though it was considered acceptable by the DS with some noted deviations from OECD guidelines. All males and 8/10 females exposed to 1.39 mg/L died. At 0.71 mg/L, 5/10 males and 5/10 females died within 3 days. One female died on day 1 and 3/10 males died within 2 days at 0.56 mg/L. Animals that died showed common abnormalities including lungs that appeared reddened, mottled, and failed to collapse properly. The MMAD was > 4 µm for low and mid dose concentrations.

Study 4 (Anonymous, 2000; RAR 3.3.1.4)

The acute (4-hour) inhalation LC_{50} for rats exposed to captan was 0.22 mg/L and 0.32 mg/L in males and females, respectively. Captan technical was administered as a single four-hour exposure (nose only) to Sprague-Dawley rats. The study was designed to include a comparison of testing milled test substance (three exposure levels) with non-milled (typical manufactured product) test substance (one exposure level). The non-milled material had an MMAD = 7.4 µm and was therefore unsuitable for regulatory testing according to updated guidelines. The milled material generated a sufficient respirable fraction at all tested doses (MMAD of 2.5 – 3.0 µm). Within the milled substance study, three groups of 5 males and 5 females were each exposed to mean achieved concentrations of 0.072, 0.65 or 2.28 mg/L. The study was guideline compliant (US EPA OPPTS 870.1300, 1998), performed according to GLP and considered acceptable by the DS even though some deviations were noted. The top, mid and low dose exposures to the milled substance resulted in mortalities of 100, 80 and 10%, respectively, within 3 days after exposure. No mortality occurred in the group exposed to the non-milled test substance.

Comments received during consultation

Oral route

There was a single comment only and this was from one Member State Competent Authority (MSCA). They noted that only a combined acute oral LD_{50} of captan in mice for both sexes was reported and suggested separate values for males and females should be calculated especially when sex sensitivity was apparent. The MSCA noted this was especially important because the proposed LD_{50} was close to the cut-off value for Cat. 4 of 2000 mg/kg bw/d and the resulting individual values could have a bearing on the classification proposal. The DS noted that the appropriate detail was not reported in the specific study summary. Access to the original study data by RAC and assessment through simple non-linear regression confirms that males and females both have LD_{50}'s > 2000 mg/kg bw.

Dermal route

There was a single comment only and this was from one MSCA supporting no classification for acute dermal toxicity in line with the proposal by the DS.

Inhalation route

There were four comments in total, two from Industry arguing for the existing classification of Acute Tox. Category 3 (H331) be retained; and two comments from MSCA supporting the DS proposal to modify the existing harmonised classification to Acute Tox. Category 2 (H330). The Industry arguments centred around the most appropriate size distribution of the material as produced and they also made the point of utilising a split-entry approach based upon particle size. The DS disagreed on both counts. The study with milled material was well conducted and satisfied the requirements of the technical guidelines. The split-entry approach was not accepted by the DS.

Additional key elements

Acute oral toxicity study in the mouse (Anonymous, 1983).

***Table**: Summary of lethality findings from acute oral study #4 (Anonymous, 1983)*

Parameter/Dose (mg/kg bw)	Males				Females			
	1500	**1890**	**2380**	**3000**	**1500**	**1890**	**2380**	**3000**
Animals found dead:								
24 h:	0/5	1/5	3/5	5/5	0/5	0/5	1/5	5/5
48 h:	0/5	0/5	0/5		0/5	1/5	1/5	
7 d:	0/5	0/5	1/5		0/5	0/5	1/5	
Total:	**0/5**	**1/5**	**4/5**	**5/5**	**0/5**	**1/5**	**3/5**	**5/5**

Using simple non-linear regression, the dose response curves estimate the following LD_{50} values:

Males: LD_{50} = 2121 mg/kg bw

Females: LD_{50} = 2245 mg/kg bw

Combined: LD_{50} = 2179 mg/kg bw

The combined value above is in line with the DS reported finding of 2110 mg/kg bw.

Captan's acute toxicity assessed in the mouse supports the conclusions from the rat studies. The acute oral median lethal dose is greater than the cut-off limit of 2000 mg/kg bw, the data do not meet the criteria for classification and labelling.

Assessment and comparison with the classification criteria

Oral route

Acute toxicity was assessed in mouse (1 study) and rat (3 studies) following overnight fast and administration by oral gavage. The studies were conducted between 1982 and 1991 and aligned with test guideline OECD TG 401, versions 1981 and 1987. The purity of the tested substance was not reported for any of the three available rat studies (only the mouse study stated a purity for the technical substance of 92.7%).

In order to be classified with acute toxicity category 4, the LD_{50} must fall between the following range: 300 < LD_{50} ≤ 2000 mg/kg bw. All the oral studies consistently reported LD_{50} values > 2000 mg/kg bw. Calculated LD_{50} values were determined by RAC for both sexes independently, from data in the original study report for the mouse (Anonymous, 1983). These values substantiate the proposal of the DS to not classify for acute oral toxicity.

As the acute oral median lethal dose is greater than the upper criterion of 2000 mg/kg bw, the classification criteria are not meet and **RAC concludes that no classification for acute oral toxicity is warranted,** as proposed by the DS.

Dermal route

Two studies in rats and one in the rabbit have demonstrated that captan technical is of low acute dermal toxicity. As the acute dermal median lethal dose is greater than the upper criterion of 2000 mg/kg bw, the classification criteria are not meet and **RAC concludes that no classification for acute dermal toxicity is warranted,** in line with the proposal by the DS.

Inhalation route

The available studies for acute inhalation toxicity are summarised below.

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

Study	TG / GLP	Comments	LC_{50} (mg/L)	C&L
Study 1 (Anonymous, 1995; RAR 3.3.1.1) Nose only 4h exposure	TG 403 (1981) / GLP	Test atmosphere → suspension of test substance in air using Wright dust feed generator. Dose: 0.43 0.82 1.36 mg/L MMAD: 4.95 5.59 6.51 µm Deaths: 1 (0) 2 (1) 3 (3) F (M) • Dose response but not dependant on smaller (< 4 µm) particle size. • Acute clinical signs noted, necropsy detailed for lung, little other info. • Purity 98.1%.	1.21 (M) 1.05 (F)	Cat. 4
Study 2 (Anonymous, 1991; RAR 3.3.1.2) Nose only 4h exposure	TG 403 (1981) / GLP	Test atmosphere → suspension of test substance in air using Wright dust feed generator. Dose: 0.23 0.94 4.81 mg/L MMAD: 1.6 1.8 1.8 µm Deaths: 0 (0) 4 (3) 5 (5) F (M) • Dose response, deaths within 4h. • Acute clinical signs noted, necropsy details lung, other organs involved, liver, GIT. • No details about micronizing the test substance, just the generation of the dry powder aerosol. • Purity not reported.	0.90 (M) 0.67 (F)	Cat. 3
Study 3 (Anonymous, 1985; RAR 3.3.1.3) Whole body 4h exposure 10 rats/dose	US EPA (1982) / No GLP	Test atmosphere → suspension of test substance in air using modified NBS dust generator. Dose: 0.56 0.71 1.39 mg/L MMAD: 5.7 5-5.8 2.95 µm Deaths: 1 (3) 5 (5) 8 (10) F (M) • Dose response, deaths within 3 days. • Acute clinical signs noted, necropsy details lung, reddening, oedema. • Purity not reported.	0.72 (M) 0.87 (F)	Cat. 3
Study 4 (Anonymous, 2000; RAR 3.3.1.4) Nose only 4h exposure	OPPTS 870.1300 (1998) / GLP	Test atmosphere → suspension of test substance in air using Wright dust feed generator. Test substance milled (3 concentrations tested) | non-milled (1 concentration tested). Dose: 0.072 0.648 2.28 | 0.668 mg/L MMAD: 2.55 2.61 2.98 | 7.4 µm Deaths: 0 (1) 4 (4) 5 (5) | 0 (0) F (M) • Dose response, apparent dependence on smaller (< 4 µm) particle size? • Acute clinical signs noted, necropsy limited → lung, reddened, oedema. • Purity 91.66%.	Milled: 0.22 (M) 0.32 (F)	Cat. 2

GIT: gastrointestinal track

Lethality is primarily associated with the achieved dose. How differences in particle size may factor into the lethality is far less certain and has not been sufficiently investigated. The clinical effects and macroscopic findings described were typical of those expected from exposure to irritant particles. These findings were evident in all groups in all studies (reduced respiratory rate, exaggerated or laboured breathing, rales, increased lung weight, hepatisation of lungs, swollen lungs and oedema), except for the lowest dose with the milled test material from study 4 (Anonymous, 2000).

The lowest ATE may be derived from a well conducted study where the test material adheres to test guidelines regarding an aerosol containing a primary proportion of respirable particles (MMAD 1-4 µm). For captan, the milled test material in study 4 (Anonymous, 2000) complies with these requirements as does study 2 (Anonymous, 1991). The criteria outlined in OECD TG 403 are quite specific and require rodents to be exposed to an aerosol comprised of mostly respirable particles to ensure hazard driven testing irrespective of the form of the technical material available to market. Under section 1.2.3.2 of the CLP guidance (ECHA, 2017), "*the assumption is made that the testing conditions of valid animal assays reflect the hazards to man and these data must be used for classification*". However, some margin for the pragmatic evaluation of effects of particulate materials is allowed for when considering "*any limitations due to the fact that the specific form of the tested substance or mixture does not or not perfectly represent that to which human exposure may occur during intended, known, or reasonably expected use*". Within the CLP guidance there is also a brief reference to the paper by Pauluhn (2008) which tries to address these regulatory challenges through consideration of the EU split-entry concept. However, RAC considers that while there are some indications that toxicity could be particle size dependent, overall, there is insufficient testing on this point to support such a concept. One of the main tenets for split-entry consideration (according to Pauluhn, 2008), is that toxicity is confined only to the gas exchange region of the lungs. Study 2 (Anonymous, 1991) necropsy results indicate other organ systems are also affected, noting both liver and GIT involvement. Study 3 (Anonymous, 1985), though not ideal, also shows that a low LC_{50} value can be attained with particle sizes of 5-5.8 µm, thus illustrating that toxicity is not only confined to a size distribution ≤ 4 but also associated with larger inhaled particles penetrating beyond the larynx, i.e., particle sizes that in theory are confined to the thoracic region. On the basis that the CLP guidance stresses the importance of hazard driven testing (see also section 3.1.2.3.2 dealing with "*Special considerations concerning aerosols (dusts and mists)*"), RAC concurs with the DS that there is sufficient data from the well conducted study 4 (Anonymous, 2000) and its milled material to derive a lowest value ATE for inhalation (0.22 mg/L), such that a classification proposal of Acute Tox. 2 (H330) is supported. In addition, the individual LC_{50} values for both males and females in study 4 (Anonymous, 2000) support category 2.

Hazard criteria:

Cat. 3: 0.5 < ATE ≤ 1.0 mg/L (dusts and mists).

Cat. 2: 0.05 < ATE ≤ 0.5 mg/L (dusts and mists).

Therefore, in accordance with the criteria laid down in the CLP Regulation, **RAC concludes that classification for Acute Toxicity Category 2; H330 is warranted, as proposed by the DS's proposal.**

An **ATE of 0.22 mg/L** is also proposed based on the lowest LC_{50} in males from study 4 (Anonymous, 2000).

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. Clinical signs were mainly observed at lethal dose levels in acute oral and inhalation studies (> 1000 mg/kg bw, > 0.072 mg/L), these included: reduced activity, ataxia, convulsions, coma, sedation and reduced reflexes, as well as more general signs of toxicity. The symptoms were transient in all studies, except in two (acute oral in mouse, (Anonymous, 1983) and acute inhalation in rat (Anonymous, 2000)). The DS acknowledged that transient organ effects such as respiratory tract irritation could also lead to a STOT SE classification, but clinical signs occurred in most studies at dose levels where mortality was observed. Based on the available data for captan, acute respiratory irritation is likely potentially justifying a STOT SE Category 3 classification, however, the classification as acute inhalation toxicant is based on the same underlying irritation effect on the respiratory tract which results in oedema and subsequent mortality. The classification proposal for inhalation toxicity in category 2 is more protective than a STOT SE 3 classification and is recognised by the DS to cover the potential to cause respiratory tract irritation.

Comments received during consultation

There were two comments from MSCAs and one from Industry.

Both MSCAs agreed with a no classification for STOT SE. One MSCA commented that the respiratory tract can and should be viewed as a specific target organ in contrast to what the DS had stated. Both MSCAs agreed the acute effects were most likely linked to the mortality and were already covered by the classification for acute inhalation toxicity.

Industry commented that there was no specific target organ for captan. Hence, any STOT classification *per se* miscommunicates the hazard associated with captan exposure.

Assessment and comparison with the classification criteria

No human data are available. The acute inhalation toxicity studies indicate respiratory irritant effects confirmed by clinical signs (dyspnoea, irregular respiratory rate, laboured breathing) and pathology (increased lung weight, swollen lungs and oedema) relevant for STOT SE 3 (H335). However, these effects were observed in the presence of lethality in most studies and captan has a proposed classification for acute inhalation toxicity category 2 (due to mortality by oedema caused by irritation) which takes precedence over STOT SE. Captan's hazard potential for acute exposure, as explained by the DS, is sufficiently described and communicated by the (newly) proposed more severe acute classification. **RAC concludes that no classification is warranted for STOT SE, as proposed by the DS .**

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

In a primary dermal irritation study (Anonymous, 1991; R-6293), performed according to OECD TG 404 (1981) guidelines and GLP compliant, a group of three male, young adult New Zealand White rabbits were given a single 4-hour application of 0.5 g of captan technical (moistened with distilled water) to clipped skin of the flank under a semi-occlusive dressing. The DS noted that

none of the animals showed a mean score of ≥ 2.3 for erythema or oedema and concluded that the data from this study did not meet the criteria for classification. Further reference by the DS to the acute dermal toxicity studies and a repeated dose dermal toxicity study also showed no support for dermal irritant effects. In contrast to folpet (a close structural analogue), only slight effects were observed in a repeated dose dermal toxicity study.

Comments received during consultation

There were two comments, one from a MSCA and one from Industry. The MSCA agreed with no classification for dermal irritation. The Industry comment also agreed no classification and noted that there were novel and recently published data, (two *in vitro* studies submitted during the consultation), indicating that captan does not induce irritation in models with human-like epithelia. They referred to Kluxen *et al.* (2022) and two study reports (20273734 and 20273736).

Additional key elements

New data provided following the consultation.

The publication by Kluxen et al. (2022) summarises the results from the two *in vitro* captan reports investigating skin corrosion and irritation.

The authors investigated how New Approach Method (NAM) studies may predict certain known acute endpoints (e.g., eye and skin irritation and skin sensitisation), for the contact fungicides captan and folpet. They postulated that this approach could provide more human relevant toxicology hazard data with the advantage of contributing to a reduction in animal testing. The main conclusion of this publication is that the alternative *in vitro* tests are suitable for regulatory testing of chemicals and that the available animal test data for both folpet and captan may be overly protective.

A total of 9 new studies were conducted to specifically investigate captan's inhalation, eye, and skin effects. This list also included a GARD skin sensitisation assay and rat EpiAirway acute airway toxicity assays (the latter with single and repeated exposure). All studies were GLP compliant (except the EpiAirway studies), and followed OECD test guidelines (except the GARD skin and EpiAirway studies for which there are currently no formal guideline tests available).

The two skin corrosion/irritation tests generate data where skin corrosion or irritation are expressed in terms of the remaining cell viability (relative to the negative controls). The two tests in the Kluxen et al. (2022) paper are identical to the original study reports supplied during the consultation phase of the CLH dossier.

The results from both studies indicate that captan is not corrosive and not irritating under the respective testing conditions as outlined by the appropriate OECD guidelines.

The criteria for a positive corrosivity test according to OECD TG 431 are:

- Corrosive: mean remaining cell viability < 50% after 3 minutes, or
- Corrosive: mean remaining cell viability ≥ 50% after 3 minutes and < 15% after 1 hour, or
- Non-corrosive: ≥ 50% after 3 minutes and ≥ 15% after 1 hour.

The criteria for a positive irritant test according to OECD TG 439 are:

- Irritation: mean remaining cell viability < 50% after 15 ± 0.5 minutes, or
- No irritation: > 50% after 15 ± 0.5 minutes.

***Table**: Summary of the two in vitro skin corrosion/irritancy tests.*

Study	TG / GLP	Comments	Result	Reference
Study 1 Captan 94.6%	TG 431 (2019) / GLP	Skin corrosion Reconstructed human epidermis (EpiDerm EPI-200). (+) Control (8M KOH): 1-hour mean relative tissue viability 11%. Test: 3 min mean relative tissue viability 101%. Test: 1-hour mean relative tissue viability 89%.	Not corrosive	Anonymous, 2021 Report 20273734
Study 2 Captan 94.6%	TG 439 (2021) / GLP	Skin irritation Reconstructed human epidermis (EpiSkin Small Model). (+) Control (5% SDS): 15 ± 0.5 min mean relative tissue viability 4.3%. Test: 15 ± 0.5 min mean relative tissue viability 94%.	Not irritating	Anonymous, 2021 Report 20273736

Assessment and comparison with the classification criteria

A well conducted rabbit acute irritation study with a single 4-hour dermal exposure showed no skin irritation relevant for classification. Only very slight erythema was noted at all treated skin sites 1 and 24 hours after patch removal and in one animal at the 48-hour time point. All treated skin sites appeared normal 72 hours after treatment.

Mean scores: erythema: 0.33, 0.67, 0.33; oedema: 0, 0, 0.

As none of the animals showed a mean score of ≥ 2.3 for erythema or oedema, the data do not meet the criteria for classification and captan is considered to be non-irritant to the skin.

Supplementary *in vitro* studies also indicate that captan does not induce irritation in OECD guideline and GLP-compliant tests using human-like epithelia models. **RAC concludes that no classification is warranted for skin corrosion/irritation, as proposed by the DS.**

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

The DS proposed to retain the current classification of Eye Damage Cat. 1; H318: causes serious eye damage. The DS quoted two primary eye irritation studies (Anonymous, 1991; Anonymous, 1982) where severe, non-reversible, ocular irritation was observed following instillation of powdered captan (the substance is poorly water soluble).

***Table**: Summary table of animal eye irritation studies.*

Study	TG / GLP	Comments	Reversibility	C&L
Study 1 (Anonymous, 1991; R-6294) 1 female, no rinsing of eye.	OECD TG 405 (1987) / GLP	82 mg of captan technical administered → ocular effects were evaluated 1- and 5-hours following administration. Effects: diffuse corneal opacity, iridial inflammation and severe conjunctival irritation. Dulling of the normal lustre of the corneal surface and haemorrhage of the nictitating and conjunctival membranes. 5-hous post exposure: killed for humane reasons.	Persistent effects to study termination.	Eye Damage Cat. 1
Study 2 (Anonymous, 1982; R-6334) 6 females, no rinsing of eyes. 3 females, exposure for 30s followed by rinsing of eyes for 1min.	Similar to TG 405 (2012) / GLP	100 mg of captan technical administered. (1) Effects, no rinsing: diffuse corneal opacity, iridial inflammation and severe conjunctival irritation. • roughened cornea in 3 animals to end of the study. • corneal pannus in 3 animals to study termination. • conjunctival bleeding was common in most animals from 24- to 96-hours, persisting in 1 case, to day 21. • conjunctival necrosis in one animal to 10-days. • cornea blistered in 1 animal to 17 days. (2) Effects, rinsed eyes: slight to no effects, reversible by day 7.	Persistent effects to study termination.	Eye Damage Cat. 1

Comments received during consultation

There were two comments, one from a MCSA and one from Industry. The MSCA agreed with retaining the existing classification for Eye Dam. 1. The Industry comment also agreed with the existing classification but noted that there were new, relevant *in vitro* data available (two *in vitro* studies submitted during the consultation; report 20273725, a BCOP test and report 20273728, an EpiOcular test), indicating captan does induce eye irritation at a lower level that supports a category 2 classification. They also referred to Kluxen *et al.* (2022) where this data is also summarised.

The DS responded that *in vitro* effects should not be used to overrule positive *in vivo* data unless the *in vivo* data is unreliable. Such is not the case here. The DS pointed out some of the limitations of the *in vitro* tests (these *in vitro* assays have corneal opacity as an endpoint, the BCOP also tests corneal permeability which reflects a loss in corneal barrier function and cell-to-cell membrane junctions of the corneal epithelium), specifically with regard to accessing those effects that involve other tissues of the eye or active involvement of a living eyeball over a longer period of time such as conjunctival bleeding, roughed cornea and pannus formation in the cornea. Such effects are outside the applicability domain of the two *in vitro* assays supplied during the consultation.

Additional key elements

Two GLP compliant *in vitro* assays, the BCOP (OECD TG 437, 2020) and EpiOcular assay (OECD TG 492, 2019) were submitted during the consultation and provide conflicting results.

Table: Summary of the two in vitro eye corrosion/irritancy tests.

Study	TG / GLP	Comments	Result	Reference
Study 1 Captan 94.6%	TG 437 (2020) / GLP	BCOP assay: 1st Experiment - absence of moisture: IVIS (0.6) ≤ 3 → no classification. 2nd Experiment – in the presence of moisture: IVIS (5.7) > 3 ≤ 55 → inconclusive.	Negative (1st exp.) and no prediction can be made (2nd exp.)	Anonymous, 2021 Report 20273725
Study 2 Captan 94.6%	TG 492 (2019) / GLP	EpiOcular assay: (+) Control (CH_3COOCH_3): 6 ± 0.25 hour mean relative tissue viability 19%. Test: 6 ± 0.25 hour mean relative tissue viability 3.7% → potentially irritant or corrosive.	Irritating / corrosive?	Anonymous, 2021 Report 20273728

The BCOP assay (OECD TG 437) is overall inconclusive. The first experiment (test item tested dry) did not indicate any irritation potential. In the second experiment, there was an increased *In Vitro* Irritancy Score (IVIS) relative to the negative control (5.7), this value however falls within the 'no prediction can be made' zone (3 < IVIS ≤ 55), thus classification is not possible based on this result.

In the EpiOcular assay (OECD TG 492), using reconstructed human cornea-like epithelium (RhCE), no prediction can be made since the viability is lower than 60%. It is noted that the viability is 3.7%, substantially less than that of the positive control (19%), indicating a potentially irritant or corrosive substance.

Assessment and comparison with the classification criteria

Classification as an eye irritant category 1 is required when a mean Draize score at or above 3 (corneal opacity) or > 1.5 (iritis) is observed from gradings at 24, 48 and 72 hours following installation of the test substance in at least 4 out of 6 (2 out of 3) animals and/or effects in one animal on the cornea, iris or conjunctiva that are not expected to reverse or have not fully reversed within the typical observation period of 21 days.

The individual eye irritation scores in study 2 (Anonymous, 1982) from the 6 animals in the unrinsed group clearly meet the criteria for a category 2 classification (table below) at a minimum. However, the persistence of ocular effects (corneal opacity, conjunctival redness and discharge, in some cases more severe than the mean scores for the combined 24, 48 and 72 hour timepoints, along with roughened corneas and conjunctival bleeding) in 2 – 4 animals to study termination at day 21 supports a category 1 classification.

***Table**: Mean values for ocular lesions 24 - 72 hours after instillation*

Animals	Corneal opacity	Iridial lesions	Conjunctival	
			Redness	Discharge
1. F20	**2.3***	1	**3***	**3***
2. F22	**2.3***	1	**3***	**3***
3. F53	0.3	1	3	2
4. F54	**2.3***	1	3	**1***
5. F55	0.7	0.3	3	2.7
6. F56	**2.7***	1	3	3
CLP Criteria: Eye Irrit. (Cat. 2)	≥ 1	≥ 1	≥ 2	≥ 2
CLP Criteria: Eye Dam. (Cat. 1)	≥ 3	> 1.5	NA	NA

*: did not resolve by day 21, Na: not applicable

In animals where the eyes were rinsed, no corneal opacity was observed during the 21-day observation and other effects were greatly diminished and fully reversible within 7 days.

One animal was tested in study 01 (Anonymous, 1991), but had to be terminated 5-hours post instillation for humane purposes. There is not a great deal of information available from the CLH report or the DAR on this study but suffice to say that the study author felt it necessary to terminate the study early and not to proceed with treating further animals. It was noted that there was severe irritation of the conjunctiva 5-hours post instillation and this included haemorrhaging of the nictitating and conjunctival membrane. The study was reported by the RMS to be acceptable and conform to OECD TG 405 (1987) and be GLP compliant.

Two GLP compliant *in vitro* assays, the BCOP assay (OECD TG 437, 2020) and the EpiOcular assay (OECD TG 492, 2019) were submitted during the consultation. The results from these studies did not align with each other, one indicating a potential irritation / corrosion effect, the other inconclusive. These two studies do not affect or change the overall assessment of captan for eye damage and irritation.

Captan fulfils the CLP classification criteria for serious eye damage. Based on conclusive data, **RAC concludes that classification as Eye Dam. 1; H318, is warranted, as proposed by the DS**.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

The DS described a single Magnusson and Kligman skin sensitisation assay (Anonymous, 1991), the results of which supported a proposal of skin sensitisation Cat. 1A, H317: May cause an allergic skin reaction. The current harmonised classification is Skin Sens. 1 with no sub-categorisation. In addition, because the test results were indicative of high potency and an extreme skin sensitiser, an SCL of 0.001% was also proposed.

In the main study, a 0.1% w/v concentration of test substance in arachis oil was used for the intradermal induction injections and a 50% w/w concentration of the test substance in arachis oil was used for the topical induction applications. Control animals were treated in a similar way but with vehicle alone. For the 24-hour challenge exposure, all test and control animals were given a topical application of a 10% w/w concentration of the test substance in arachis oil on one flank and vehicle alone on the other flank.

The study was considered robust and acceptable, adhered to OECD TG 406 (1981 & 1992) and was performed under GLP. No adverse reactions were noted at the vehicle control sites in test animals or at any sites of control animals at the 24 and 48-hour observations. Captan produced a 100% sensitisation rate in the guinea pigs and was considered an extreme sensitiser.

Comments received during consultation.

There were three comments, two from MSCAs and one from Industry. The MSCAs agreed with the DS proposal to upgrade the classification to Skin Sens. 1A; H317, with an SCL of 0.001%. The Industry comment seemed to agree with the existing classification but disagreed with the potency assessment. Industry submitted new *in vitro* data (GARDskin assay (Theorin, 2021); KeratinoSens assay (Gijsbrechts (2021); U-Sens assay (Gijsbrechts (2021) and a GARDskin *in silico* modelling report designed to estimate potential sensitising potency) during the consultation indicating captan to be at most a moderate sensitiser without the need to specify an SCL.

Industry also referred to Kluxen *et al.* (2022) where this data is also summarised.

The DS replied indicating the basis of the classification proposal is the well performed, OECD guideline and GLP compliant guinea pig maximisation test, the results of which according to CLP guidance justify captan being identified as an extreme sensitiser. In their view, the new *in vitro* data were not appropriate to allow potency considerations and they justified their decision based on guidance in OECD TG 497 "Guideline on Defined Approaches for Skin Sensitisation" with specific reference to methods used to predict potency.

Additional key elements

Several GLP-compliant, *in vitro* assays (GARDskin assay (Theorin, 2021); KeratinoSens assay (Gijsbrechts (2021); U-Sens assay (Gijsbrechts (2021) and a GARDskin *in silico* modelling report designed to estimate potential sensitising potency) were submitted during the consultation.

***Table**: Summary of the in vitro sensitising tests*

Study	TG / GLP	Comments	Result	Reference
Study 1 Captan 94.6%	TG 442D (2018) / GLP	KeratinoSens: 1st Experiment: Imax = 5.8-fold 2nd Experiment: Imax = 11.9-fold C < 1000 µM, viability > 70%.	Positive (> 1.5-fold induction)	Gijsbrechts, 2021 Report 20273730
Study 2 Captan 94.6%	TG 442E (2018) / GLP	U-Sens (U937 cell line activation test): 1st Experiment: SI >> 150% 2nd Experiment: SI >> 150% Rel. cell viability > 70%.	Positive	Gijsbrechts, 2021 Report 20273732
Study 3 Captan 94.6%	Non-guideline / GLP	GARDskin: Captan: mean DV = 5.6 Positive Ctrl: mean DV = 9.3 Negative Ctrl: mean DV = -1.2 Rel. cell viability ≥ 90%.	Positive	Theorin, 2021 Report 1063-2002

1. 1. KeratinoSens (reporter gene assay, without a functional skin barrier): captan is positive, it activates the antioxidant / electrophile responsive element (ARE)-dependent pathway in keratinocytes.
2. 2. U-Sens: captan is positive, it increases CD86 cell surface marker expression, a key event in the activation of dendritic cells as part of the skin sensitisation adverse outcome pathway (AOP).
3. 3. GARDskin assay: captan shows a gene expression pattern that leads to a decision value (DV) > 0 and is thus considered positive and a category 1 sensitiser.
4. 4. Results from the GARDskin assay were used in an in silico potency prediction model to estimate LLNA EC3 and human NOEL values. The report itself notes that in order to arrive at more accurate estimates of a compound's potency a GARDskin dose-response assay is required, this was not available. The crude estimate from this report indicates a sensitiser of moderate potency, however, there is little weight that can be placed on the result of this paper due to the large deficiencies noted with the approach.

In conclusion, the results from the skin sensitisation assays concur with the predictions observed in the guinea pig assay.

Assessment and comparison with the classification criteria

Classification into sub-categories is required when data are sufficient. The animal test results (100% of animals responding as positive at an intradermal induction dose of 0.1%) clearly exceed the criteria for sufficient evidence to place captan in sub-category 1A.

> Guinea pig maximisation test (section 3.4.2.2.3.2 of CLP Regulation):
>
> Sub-category 1A
> (i) ≥ 30 % responding at ≤ 0.1 % intradermal induction dose or,
>
> (ii) ≥ 60 % responding at > 0.1 % to ≤ 1 % intradermal induction dose.

According to section 3.4.2.2.5 of the CLP guidance, captan identifies as an extreme sensitiser (where ≥ 60 % responding at ≤ 0.1 % intradermal induction dose). The recommendation for setting an SCL for an extreme sensitiser is set at 0.001 % (w/v).

The results from the *in vitro* skin sensitization assays concur with the predictions observed in the guinea pig assay. These particular studies do not allow for the prediction of potency.

RAC concludes that classification as Skin Sens. 1A; H317 with an SCL of 0.001% is warranted, as proposed by the DS.

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

Summary of the Dossier Submitter's proposal

The DS reported that captan was tested in a range of studies, (GLP compliant, with varying levels of accordance to OECD TG) covering a range of species including the rat, mouse and dog in oral, dermal and inhalation studies lasting from 4 weeks to 2 years.

Eight studies in total were considered under STOT RE:

1. Dog, 28-day oral capsule study (1987).
2. Dog, 1-year oral capsule study (1988).

3. Rabbit, 21-day dermal toxicity study (1987).
4. Rat, 90-day inhalation toxicity study (1989).
5. Rat, 2-year carcinogenicity, oral dietary study (1982).
6. Rat, 2-year plus, carcinogenicity, oral dietary study (1983).
7. Mouse, 2-year carcinogenicity, oral dietary study (1981).
8. Mouse, 2-year carcinogenicity, oral dietary study (1983).

The effects of captan following repeated dosing by the oral or dermal route were mainly on body weight, food consumption and irritation. Irritation was evident in the skin in the rabbit dermal study. In the mouse, irritation in the duodenum resulting in mucosal hyperplasia and subsequent tumour formation occurred in both males and females and this hazard is dealt with under carcinogenicity.

The only study with severe effects within the general guidance values for STOT RE category 1 is the 90-day inhalation toxicity study in rat (Anonymous, 1989; R-5603). This is considered the key study for the proposal of STOT RE 1 by the DS. This is a complex situation because the effects of inhalation exposure might be also indicative of an irritant effect.

Exposure of rats for 90 days to captan aerosols at atmospheric concentrations of 0.13, 0.60, 5.06 and 12.98 µg/L resulted in five treatment-related mortalities in males exposed to the highest concentration. Treatment-related effects were confined to the respiratory tract including the lungs and larynx. Mortalities were not observed earlier than week 5. Following a 4-week recovery period at the end of the 13-week inhalation exposure study (designed for the control and top dose groups only), the lung (site of damage resulting in death) and nasal passage effects had resolved, but the laryngeal effects (squamous metaplasia and squamous hyperplasia) were still present in the top dose group (low and mid dose groups were not examined).

Effects in the larynx (squamous hyperplasia) and nasal cavity (degeneration/atrophy olfactory epithelium) were present at ≥ 0.13 µg/L. Folpet, which has the same toxicophore, shows similar morphological effects in its 28-day inhalation study investigating effects on the respiratory tract. However, one key difference is that folpet does not result in mortality following repeated dose inhalation.

Comments received during consultation

Three MSCAs agreed with the DS' proposal.

However, Industry disagreed, they considered acute irritation as the main toxicological hazard responsible for the effects noted in the 90-day rat inhalation study. Histopathological findings were noted throughout the respiratory tract. The DS considered whether the resulting effects were an adaptive change (e.g. in the larynx) or if they led to sufficiently adverse (e.g. irreversible) changes. Often degeneration of the original epithelial cells with subsequent regeneration hyperplasia and squamous metaplasia occurs; this was observed in the available toxicology package for captan, so in principle they could be considered as an adaptive response arising from the inhalation of irritants. However, the DS pointed out the severity of the findings from the 90-day inhalation study. Mortalities occurred and in the same study, changes in the larynx were still apparent in male and female rats exposed to 12.98 µg/L after the 4-week recovery period. Squamous hyperplasia of the epithelium on the arytenoid projections was still present but with reduced severity and incidence. Squamous metaplasia in the ventral part of the larynx was also still evident. The DS considered this a case for repeated dose toxicity and proposed STOT RE 1 – H372 (respiratory tract).

Industry’s main argument centred around the effects in the repeated exposure study being the result of multiple, subsequent acute irritation events noting captan's mode of action (MoA) was well established as acute *in situ* membrane reactivity, cytotoxicity and irritation. Thus, they

considered highly unlikely that the effects observed in the repeated exposure studies were the result of toxicity other than repeated acute contact irritation. Industry also submitted a new *in vitro* study; modelling rat tissue using the rat EpiAirway assay (Anonymous, 2021; study 787149) demonstrating that captan induced histopathological changes after only 1 day of treatment, i.e., a single exposure resulted in a higher incidence and severity of degenerative type changes (squamous differentiation or intercellular separation), in the respiratory tract compared to controls. This demonstrates that histopathological changes occur after single exposure events and are thus of acute aetiology. A comparison of the effective dose in the 90-day inhalation study with concentrations inducing lethality in acute studies (while not so easy because of different times of exposure as well as the number of times of exposure) showed that these were very close, thus further supporting the non-relevance of classification for repeated exposure because the effects were ultimately due to acute local toxicity with subsequent treatments exacerbating the toxicity.

The DS countered these arguments noting:

1. Similar MoAs can lead to classification for different hazard classes.
2. Microscopic findings in the EpiAirway assay were considered to be of limited reliability, because only two samples per group were assessed (non GLP). Other evidence brought into question the reliability of the positive control.
3. The DS assumed that no steady-state condition was achieved for repeated inhalation exposure (due to captan's rapid degradation), thereby resulting in exceptions to Haber's rule and the DS objecting to the applicability of Haber's rule for extrapolating between captan's acute and repeated-dose inhalation toxicity dose levels.

The DS' response was clear: in the 90-day inhalation study mortalities occurred (presumed due to bronchial damage in the lungs) in addition, squamous hyperplasia and squamous metaplasia in the larynx persisted following a 4-week recovery period, therefore classification as STOT RE 1 was appropriate.

Additional key elements

Captan: Evaluation of upper airway cytotoxicity in vitro using the MatTek EpiAirway rat test system (Anonymous, 2021; study 787149)

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 was submitted during the consultation.

Rat tissues were exposed to 8 dilutions of the test item for either a single 24-hour period (single exposure test), or for 3 continuous 24-hour periods (with rinsing in between; repeated exposure test). The following analytical measurements were carried out in this study:

1. TEER as a measure of the integrity of tight junctions between cells.
2. LDH release/leakage into the culture medium as a measure of cellular damage.
3. Histopathology following treatment.

The assay appeared to be well conducted. The DS questioned the reliability of the study based on results with the positive control (formaldehyde at 14.7 mg/mL, giving a low value for mean LDH release) and the low numbers of rat samples tested (2 per test group).

The positive control indicated an increase in the release of LDH (7.6%) relative to controls (2-4.5%) and this was in line with results for captan at 100 µg/mL (6.7%) though lower than treatment with captan at 250 µg/mL (30.8%) following 24 hours incubation. Formaldehyde appeared to perform adequately in the TEER assay where it resulted in a substantial reduction in

tissue integrity on par with captan concentrations of 250-400 µg/mL following 24 hours incubation.

Summary of the results

Pre-dose TEER for all tissues was high (> 590 Ω/cm^2) indicating good tissue barrier integrity. Pre dose LDH release was varied between tissues with incidences of apparently elevated LDH spread throughout treatment groups.

Treatment with concentrations of ≥ 100 µg/mL resulted in clear evidence of toxicity by TEER and LDH release assay results in the Rat EpiAirway model. These effects were clearly induced by 24h exposure and slightly further elevated over time.

Treatment	Single Exposure (Mean LDH Release %)	Repeat Exposure (Mean Cumulative LDH Release (%)		
	24 h	24 h	48 h	72 h
Vehicle Control (Mineral Oil)	2.02	3.42	5.44	5.61
Vehicle Control (Water)	4.48	N/A		
ALI (Untreated)	1.05	0.18	0.18	0.72
Positive Control (Formaldehyde 14.7mg/mL)	7.58	N/A		
Captan (600 µg/mL)	45.09	44.82	47.40	47.4
Captan (400 µg/mL)	38.47	40.33	41.40	41.54
Captan (250 µg/mL)	30.78	29.51	42.54	45.27
Captan (100 µg/mL)	6.70	5.92	7.63	9.26
Captan (60 µg/mL)	3.18	3.94	5.26	5.58
Captan (40 µg/mL)	2.45	1.89	1.89	1.99
Captan (10 µg/mL)	1.59	2.92	4.24	4.63
Captan (6 µg/mL)	2.09	2.51	2.51	3.26

TEER (Ω x cm^2)								
Treatment	Single Exposure		Repeat Exposure					
	24 h		24 h		48 h		72 h	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Vehicle Control (Mineral Oil)	639	100	342	66	517	112	629	137
Vehicle Control (Water)	682	287	N/A					
ALI (Untreated)	656	71	436	109	363	119	566	186
Positive Control (Formaldehyde 14.7mg/mL)	67	11	N/A					
Captan (600 µg/mL)	36	11	47	6	38	6	22	10
Captan (400 µg/mL)	55	9	56	13	35	9	21	3
Captan (250 µg/mL)	59	11	74	17	42	8	30	7
Captan (100 µg/mL)	365	469	600	347	708	450	611	407
Captan (60 µg/mL)	796	477	327	115	519	193	880	333
Captan (40 µg/mL)	726	229	609	182	676	225	632	125
Captan (10 µg/mL)	528	74	598	142	603	52	590	86
Captan (6 µg/mL)	474	87	684	142	569	181	698	158

Regarding the histopathological evaluation, only 2 samples per treatment were investigated but the results indicated clear cytotoxicity. A single exposure to captan over a 24-hour period was associated with a higher incidence and severity of degenerative type changes, compared to controls, in samples exposed to ≥ 100 µg/mL. Individual instances of increased squamous differentiation or intercellular separation were detected in tissues treated with 60 or 10 µg/mL captan, respectively when compared to control tissues. Repeated exposure over a 72-hour period was associated with a higher incidence and severity of degenerative type changes (decreased (i.e. absent) squamous differentiation and increased epithelial loss) in samples exposed to ≥ 250 µg/mL. In contrast, the formaldehyde positive control samples exhibited minimal to moderate intercellular separation and minimal necrosis of cells (similar to negative controls). In general,

captan treated samples did not show intercellular separation and all treatment groups had minimal cells necrosis similar to the negative controls.

The study indicates that captan is cytotoxic in this test system with histopathological changes occurring after both single exposures and multiple exposures. Histopathological effects (decreased squamous differentiation and increased epithelial loss) seemed more pronounced with repeated treatments. These results do not distinguish between acute mediated toxicity and repeated dose toxicity but show both occur. The study supports the cytotoxic MoA at the local point of contact.

The study is considered supplementary, limitations arise because of the low number of tissue samples analysed and the performance of the positive control in the LDH release assay, in addition the exposure time is 24 hours instead of 6 hours as in the rat 90-day inhalation study which makes comparison difficult, however, it may be concluded that the results support the proposed captan MoA.

Further details: captan: 90-day inhalation toxicity study in the rat (RAR/Annex I to the CLH Report, section 3.12.1.4).

This key study is the only repeat dose study with significant, serious toxicological effects at dose levels below the general guidance values for STOT RE that also support a potential category 1 classification.

The RMS and DS considered the study acceptable. It is a 13-week, nose only study. Test atmospheres were generated by the suspension of captan particulate in air using a Wright dust feed generator. Control and top dose groups had 20 animals per sex (incorporating a satellite group of 10 animals for recovery), other groups were composed of 10 animals/sex/dose. At the end of the exposure period, the 10 animals from the control and top dose satellite groups were sacrificed after a 4-week recovery period. The MMADs for each exposure group were 0.95; 1.22; 1.57 and 1.60 µm from low to top dose respectively.

Exposure of rats for 90 days to captan aerosols at atmospheric concentrations of 0.13, 0.60. 5.06 and 12.98 µg/L resulted in 5 treatment-related mortalities in males exposed to the top concentration. Treatment-related effects were confined to the respiratory tract.

Four male rats exposed to 12.98 µg/L were found dead over weeks 5 to 13 and another male from the same dose group was killed *in extremis* in week 11 (totalling 5 deaths during weeks 5-13). The incidence was 5/20 animals (25%) in total from this dose group. Respiratory noise was present towards the end of the study, primarily in those animals in the higher dose groups. There were no treatment-related clinical signs evident in any group. Haematology, clinical chemistry, urinalysis and organ weights were all unremarkable across all dose groups.

The pathology findings for the 5 deceased animals noted that all five males showed either marked or moderate necrosis of the epithelium lining the bronchi and larger bronchioles, and one male had a small focus of sub-epithelial cellular necrosis in a bronchus. Other changes affecting the airways of males were peribronchial inflammation, bronchitis hyperplasia of bronchiolar epithelium and an inflammatory proteinaceous exudate within the lumen of bronchioles. Changes in alveoli were alveolitis, alveolar macrophage infiltration, alveolar epithelialisation and hyaline membrane formation.

In the satellite groups (control and 12.98 µg/L dose groups), following a four-week recovery period without exposure to captan, all treatment-related lung changes seen at termination of the main treatment groups had resolved. However, changes in the larynx were still apparent in all exposed male and female rats. Squamous hyperplasia and metaplasia of the epithelium on the laryngeal arytenoid projections was still present in both males and females but the severity and incidence were reduced.

The DS proposed STOT RE 1 due to significant toxic effects (mortality) occurring at concentrations below the guidance value of 0.02 mg/L 6h/d for 90-day inhalation toxicity studies in rats exposed to dust/mist/fume, according to the CLP Regulation.

Assessment and comparison with the classification criteria

In the absence of human data, the evaluation of STOT RE was based on 8 repeated-dose toxicity studies across several species and routes of exposure. These studies were GLP compliant though not always guideline compliant and that may simply be due to the date when first initiated (i.e. pre-dating OECD or USEPA guideline acceptance).

A summary of the studies presented in the CLH report by the DS may be seen in the table below. This table summarises the approximate equivalent guidance values for the different animal studies and assesses their ability to support STOT RE classification or not to support classification.

***Table**: Approximate adjusted general guidance values for STOT RE 1 and 2 in the relevant repeated dose animal studies. Further detail may be found in table 49 of the CLH report.*

Study	Species	Comments	Cat. 1	Cat. 2
Study 1 (Anonymous, 1987) 28-day, oral, capsule Captan ?%	Dog	Relevant dose for considering STOT RE: 30; 100 mg/kg bw/d **Effects:** none / insufficient for classification	≤ 30 mg/kg bw/d	≤ 300 mg/kg bw/d
Study 2 (Anonymous, 1988) 1-year, oral, capsule Captan 90.4%	Dog	Relevant dose for considering STOT RE: 12.5 mg/kg bw/d **Effects:** none / insufficient for classification	≤ 2.5 mg/kg bw/d	≤ 25 mg/kg bw/d
Study 3 (Anonymous, 1987) 21-day, dermal Captan ?%	Rabbit	Relevant dose for considering STOT RE: 12.5; 110 mg/kg bw/d **Effects:** none / insufficient for classification	≤ 80 mg/kg bw/d	≤ 800 mg/kg bw/d
Study 4 (Anonymous, 1989) 90-day, inhalation Captan 88.7%	Rat	Relevant dose for considering STOT RE: **0.013 mg/L** (top dose) **Effects:** lethality / necrosis of the bronchiolar epithelium / squamous hyperplasia and metaplasia of the epithelium on the laryngeal arytenoid projections, persistent even after a 4-week recovery period.	**≤ 0.02 mg/L**	≤ 0.2 mg/L
Study 5 (Anonymous, 1982) 2-year, carcinogenicity Captan 89%	Rat	Relevant dose for considering STOT RE: No dose relevant. All higher than GV. **Effects:** none / insufficient for classification	≤ 1.25 mg/kg bw/d	≤ 12.5 mg/kg bw/d

Study 6 (Anonymous, 1983) 2-year, carcinogenicity Captan ?%	Rat	130 week study. Relevant dose for considering STOT RE: 5 mg/kg bw/d **Effects:** none / insufficient for classification	≤ 1.25 mg/kg bw/d	≤ 12.5 mg/kg bw/d
Study 7 (Anonymous, 1981) 2-year, carcinogenicity Captan 90.7%	Mouse	113 week study. Relevant dose for considering STOT RE: No dose relevant. All higher than GV. **Effects:** none / insufficient for classification	≤ 1.25 mg/kg bw/d	≤ 12.5 mg/kg bw/d
Study 8 (Anonymous, 1983) 2-year, carcinogenicity Captan 89%	Mouse	94 week study. Relevant dose for considering STOT RE: No dose relevant. All higher than GV. **Effects:** none / insufficient for classification	≤ 1.25 mg/kg bw/d	≤ 12.5 mg/kg bw/d

?: unknown; GV: guidance values

It can be seen from the above table that only the 90-day rat inhalation study supports classification with STOT RE. The rabbit teratology studies were also assessed by RAC, the dosing period in these studies was generally from GD6-GD28, constituting a 22-day dosing period in total (in some older studies only 12 days). The guidance values for STOT RE 1 and STOT RE 2 would then be ≤ 40 and ≤ 400 mg/kg bw/d respectively. Maternal toxicity was evident and generally comprised of a reduction in faecal output, highly variable and decreased body weight and body weight gain and substantial reductions in food consumption. All effects appeared to be reversible upon cessation of exposure. The rat developmental toxicity study also showed similar effects which were also shown to be reversible upon cessation of treatment.

The 90-day rat (nose-only) inhalation study resulted in 5 treatment-related mortalities in males exposed to the top concentration. Treatment-related effects were confined to the respiratory tract, and the death incidence was 5/20 animals (25%). According to the pathological report, the main factor identified as contributing to death in these males was considered to be marked or moderate necrosis of the bronchial/bronchiolar epithelium of the lung. In the satellite groups (control and 12.98 µg/L dose groups, 10 animals per sex), following a four-week recovery period, all treatment-related lung changes seen at termination of the main treatment groups had resolved. However, the changes in the larynx were still apparent in the exposed male and female rats. Squamous hyperplasia and metaplasia of the epithelium on the laryngeal arytenoid projections was still present in both males and females but the severity and incidence were reduced.

These changes to the respiratory tract were also evident in the 5.06 µg/L group, i.e. sub-epithelial cellular necrosis in the lung (6/10 males, 2/10 female), ulceration (3/10 females) and vacuolar degeneration of squamous epithelium in the larynx (3/10 males, 4/10 females), and degeneration (atrophy) of olfactory epithelium in the nasal cavity (1/10 males).

Effects on the duodenal mucosa in the rat and mouse carcinogenicity studies occurred at dose levels above the guidance values for STOT RE (all categories).

In addition, a non GLP, non-guideline *in vitro* rat EpiAirway assay indicated that captan was cytotoxic in the test system with histopathological changes occurring after single and multiple exposures. Some histopathological effects were also more pronounced with repeated treatments (24, 48 and 72-hours). Several limitations were identified in the study but overall RAC considers

it acceptable and to provide supplementary information supporting the captan MoA, i.e. cytotoxicity at the local point of contact.

Though the CLP guidance indicates that one approach to distinguish between acute and repeated dose toxicity is by way of dose comparison (see section 3.9.2.5.1), it must be pointed out that this is in respect of severe toxicological effects due to corrosivity. Captan acts by way of cytotoxicity and is presumed to result from the chemical reactivity with free sulfhydryl groups on extracellular and transmembrane macromolecules. It is not classified as corrosive though it is recognised to be highly irritating, especially to mucous membranes. It is acknowledged that the only study to support classification is the 90-day rat inhalation study where the effects were very severe, particularly in the top dose group. These effects occurred after a prolonged period of time (weeks 5-13) with some effects, though minor, indicative of tissue change in the larynx that did not resolve following a 4-week recovery period. RAC also notes that effects evident in the intermediate dose group (5.06 µg/L) may also support classification.

Assessment of adverse effects for classification

Captan does not induce specific indications of potential neurotoxicity, neurological signs or neuropathological lesions in toxicity studies at relevant dose levels.

There are no toxicological alerts from subchronic and chronic mammalian studies that suggest an immunologic MoA.

Exposure of rats for 90 days to Captan aerosols at atmospheric concentrations of 0.13, 0.60, 5.06 and 12.98 µg/L resulted in five treatment-related mortalities in males exposed to the highest concentration, and numerous histopathological effects both in the top dose and intermediate dose groups triggering classification for STOT RE 1; H372 (respiratory tract) occurring at concentrations below the guidance value of 0.02 mg/L 6h/day (dust/mist/fume).

No specific concentration limits for STOT RE were proposed by the DS. Since the effects on the respiratory tract occurred at a concentration just below the guidance value for a 90-day inhalation exposure study, RAC does not propose to apply an SCL.

In conclusion, **RAC concludes that classification as STOT RE; H372 (respiratory tract) is warranted, as proposed by the DS.**

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

The DS reported that captan was tested in a range of GLP and OECD guideline compliant *in vitro* and *in vivo* genotoxicity assays (the table below contains reliable with or without limitation studies), details were supplied in tables 29 and 30 of the CLH report.

In vitro assays included:

- 3 × *in vitro* Ames test (reverse mutation assay with *Salmonella typhimurium* and *Escherichia coli*), all positive ± S9 mix.
- 3 × *in vitro* mammalian cell gene mutation tests, all positive in the absence of S9 mix (negative in the presence of S9 mix).
- 2 × *in vitro* mammalian chromosome aberration tests, positive in the absence of S9 mix (negative in the presence of S9 mix in one study).

In vivo assays included:

- 1 × mouse micronucleus test in bone marrow: negative.
- 1 x somatic cell gene mutation assay in pregnant C57B1/6J mice: negative.
- 2 x mammalian bone marrow clastogenicity studies: 1 negative, 1 positive.
- 1 x transgenic mouse mutation assay: negative.
- Set of duodenal clastogenicity studies: negative.
- 1 × rat unscheduled DNA Synthesis (UDS) test in hepatocytes: negative.
- 2 x dominant lethal assays: 1 negative, 1 positive.
- 1 x mammalian spermatogonial chromosome aberration study; positive.

***Table**: RAC Summary of reliable* genotoxicity tests with captan*

Study	Result	Methods and acceptability	Reference
***In vitro* studies:**			
Bacterial mutagenicity Captan 96.5%	Positive ± S9	GLP, OECD TG 471 (1997), acceptable* *Salmonella* Strains: TA1535, 100, 1537, 98 Other: *E. coli* WP2 uvrA strain S9 ↓ potency to induce gene mutations	RAR 3.8.1.1 Anonymous, 2017
Bacterial mutagenicity Captan 92.4%	Positive	Non GLP, non-guideline Ames test, limited* *Salmonella* Strain: TA100 only Positive without glutathione or cysteine else negative. No metabolic activation	RAR 3.8.1.2 Anonymous, 1985
Bacterial mutagenicity Captan 93%	Positive (strain TA1535) ± S9	Non GLP, non-guideline Ames test, limited* *Salmonella* Strain: Response only in one strain - TA1535	RAR 3.8.1.3 Carere, 1978
Clastogenicity Captan 96.4%	Positive in absence of S-9 mix	GLP, OECD TG 473 (2016), acceptable* Human lymphocytes	RAR 3.8.1.4 Anonymous, 2018
Clastogenicity Captan 99.9%	Positive in absence of S-9 mix	Non GLP, non-guideline, limited* Chinese hamster V79 cell line Not tested with S9 mix	RAR 3.8.1.5 Tezuka, 1980
Mammalian cell mutagenicity Captan 96.5%	Positive in absence of S-9 mix	GLP, OECD TG 476 (2016), acceptable* HPRT locus. Chinese hamster V79 cell line	RAR 3.8.1.7 Anonymous, 2017
Mammalian cell mutagenicity Captan 92%	Positive in absence of S-9 mix	Non GLP, non-guideline, acceptable* L5178Y mouse lymphoma thymidine kinase locus assay	RAR 3.8.1.8 Anonymous, 1986
Mammalian cell mutagenicity	Positive in absence of S-9 mix	Non GLP, non-guideline, acceptable* CHO/HGPRT system	RAR 3.8.1.9 O'Neill, 1981

Study	Result	Methods and acceptability	Reference
***In vivo* studies:**			
Micronucleus Captan 94%	Negative up to limit dose (1000 mg/kg)	Non GLP, OECD TG 474, acceptable* CD1 Mouse bone marrow Positive controls → significant increase in the number of MNPCEs Exposure of the target tissue is unknown Captan is rapidly degraded	RAR 3.8.2.1 Anonymous, 1985
Somatic cell gene mutation assay Captan 92.2%	Negative	Non GLP, non-guideline, acceptable* No treatment related increase in the frequency of recessive somatic mutant spots in mice	RAR 3.8.2.2 Anonymous, 1981
Mammalian bone marrow clastogenicity Captan ?%	Negative	Non GLP, non-guideline, acceptable* No evidence of damage to rat chromosomal structure	RAR 3.8.2.3 Anonymous, 1979
Mammalian bone marrow clastogenicity Captan 96.5%	Positive	Non GLP, non-guideline, limited* (1) ↑ MN of PCE in mouse bone marrow (2) ↑ chromosome aberrations in bone marrow cells. See also RAR 3.8.2.13	RAR 3.8.2.4 Feng and Lin, 1987[#]
Transgenic Mice (Muta Mouse) Mutation Assay Captan 95%	**Negative**	GLP, OECD TG 488, acceptable* Did not induce gene mutations in the liver or duodenum of transgenic mice	RAR 3.8.2.6 Anonymous, 2016
Duodenal clastogenicity studies Captan 94 - 99%	**Negative**	Non GLP, non-guideline, acceptable* No increase in aberrant nuclei in mouse crypt cells with or without glutathione depletion. **Key study**	RAR 3.8.2.7 Chidiac and Goldberg, 1986
UDS Captan 91.2%	Negative	GLP, OECD TG 486 compliant, acceptable*. Male rat (Alpk:APfSD) hepatocytes	RAR 3.8.2.8 Anonymous, 1990
Dominant lethal assay Captan >98%	Negative	Non GLP, non-guideline, acceptable but limited* Male C3H mice were treated orally	RAR 3.8.2.10 Tezuka, 1978
Dominant lethal assay Captan ?%	Positive[##]	Non GLP, pre-guideline, limited* (1) statistical ↑ in mean early deaths/pregnancy, rats and mice, orally dosed, mating weeks 1, 2, positive dose response (for i.p., mice, top dose, weeks 4, 5) (2) No treatment effect on mean total implantations/pregnancy in rats or mice (3) No antifertility effects	RAR 3.8.2.11: tables B.3.8.2.11-1 and B.3.8.2.11-2 Collins, 1972[##]
Mammalian spermatogonial chromosome aberration Captan 96.5%	Positive	Non GLP, non-guideline, limited* (1) ↑ chromosomal aberration in mouse spermatogonia (2) ↑ chromosomal aberration in mouse primary spermatocytes (3) ↑ Sperm-head abnormalities	RAR 3.8.2.13 Feng and Lin, 1987[#]

* This listing differs from that in the CLH report. It only considers studies that were determined by the RMS with respect to the DAR/RAR and the evaluation by the RAC to be acceptable from a regulatory context. Some of the studies presented in the CLH report have been omitted from this table because they were assessed to be unreliable and therefore of no value to the hazard

assessment process. Studies described as limited means they still have some data of value but deficiencies with regards to adherence to guidelines, their data pack as a whole, or quality of reporting, limit their reliance or weighting in the assessment process. These are however, still regarded in a weight of evidence approach.

The publication by Feng and Lin (1987) though accepted and designated as limited by the RMS in the RAR (section 3.8.2.13), has been considered unreliable in the review by Arce et al., (2010) who noted mathematical errors were present that made data interpretation difficult. In addition, industry has concluded this study not to be reliable because of the protocol, the source, and the purity of the test material.

Collins (1972) considered positive. Unstable chromosome aberrations in sperm will lead to foetal deaths after fertilisation at around the time of implantation in the uterus wall. According to Adler *et al*. (2012), these can be scored as early dead foetuses in the uterus wall of the females at mid-pregnancy. This is also in line with comments by Yauk *et al.* (2015).

In addition to the *in vitro* and *in vivo* studies listed in the table above, the DS also supplied details of supporting studies investigating both the stability of captan in human blood and the genotoxicity of its main metabolite THPI, which is systemically available.

Degradation of captan in human blood was time-dependent and exceptionally rapid, with a calculated half-life of 0.97 seconds. It is stable in normal saline for 60.4 seconds at 37°C demonstrating that blood components (thiols) are required for degradation (RAR 3.8.2.15). Any systemically available captan will degrade rapidly in whole human blood, to degradants also seen in rat studies.

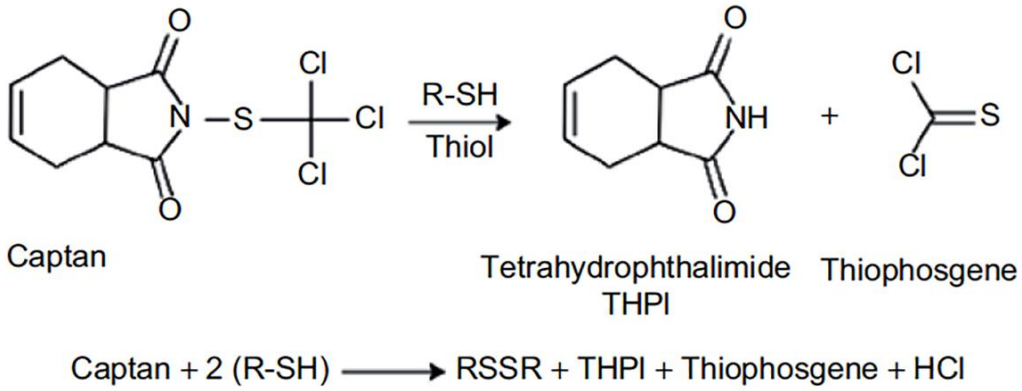


Figure: Simplified chemical reaction of captan with thiols and its degradation products.

THPI was tested in three *in vitro* genotoxicity assays: (i) reverse mutation in bacteria, OECD TG 471 and GLP compliant; (ii) chromosome aberration test in CHO cells, OECD TG 473 and GLP compliant; and a mammalian cell gene mutation test in L5178Y TK+/- mouse lymphoma cells, OECD TG 476 and GLP compliant (RAR 3.8.2.16; 3.8.2.17; 3.8.2.18, respectively). THPI was not mutagenic in any of these test systems. In addition, THPI did not induce micronuclei or apoptotic bodies in mouse duodenal crypt cells following gavage dosing from 250 to 1500 mg/kg bw (Chidiac and Goldberg, 1986).

In vitro results

Captan induces gene mutation in microbial systems, and the mutation frequency is greatly diminished or eliminated by the addition of thiol containing components. *In vitro* mammalian test systems for both gene mutation and chromosomal damage show positive results in the absence of S9 and little or no activity when S9 is present.

In vivo results

Generally, there has been little to no genotoxicity reported from *in vivo* assays on somatic cells when captan is administered by gavage or in the diet. A gene mutation assay with transgenic male mice (Muta™ Mouse) was also conducted to assess the potential of captan administered in the diet, to induce gene mutation (reporter gene: lacZ) in the liver and duodenum. There was no evidence of gene mutation in either tissue. The most relevant results (considered a key study), regarding the effects seen in rodent carcinogenicity studies are from a series of nuclear aberration assays in mouse duodenum reported by Chidiac and Goldberg (1986). Captan does not induce clastogenic changes in the carcinogenic target tissue and is not regarded as an *in vivo* genotoxicant by the DS.

Positive results for genotoxicity in cytogenetic assays in mouse bone marrow (both micronuclei and chromosomal aberrations) and in germ cells (chromosomal aberrations mouse spermatogonia and spermatocytes) have been reported in a single paper where technical captan was administered by gavage (Feng and Lin, 1987). There were a number of deficiencies associated with the reporting and publication of this study but a clear explanation for the positive results is lacking. There were also positive results in one of the dominant lethal assay studies (Collins, 1972), which were considered acceptable but in a limited capacity by the DS and RMS for the RAR.

The DS further noted some mechanistic results and an extensive review article on both folpet and captan. Purification of liver, jejunum and duodenum samples from captan-treated mice did not demonstrate any clear evidence for a reaction of captan or its breakdown products with DNA. The review by Arce *et al.* (2010) concluded that both folpet and captan have *in vitro* mutagenic activity but are not genotoxic *in vivo* due to their rapid degradation in the presence of thiol-rich matrices typically found *in vivo*.

Comments received during consultation

There were three comments, two from MSCAs and one from Industry. All agreed with the DS proposal that no classification for mutagenicity is required.

Assessment and comparison with the classification criteria

Captan's *in vitro* mutagenicity is ascribed to thiophosgene which *in vivo* is also rapidly neutralized by thiol-containing molecules.

Captan genotoxic potential has been extensively investigated but many of the *in vivo* studies are old and of limited reliability. The publication by Feng and Lin (1987) indicated positive effects for micronuclei of polychromatic erythrocyte (PCE) in mouse bone marrow and chromosomal aberrations in bone marrow and germ cells. Though accepted as limited by the RMS in the RAR (section 3.8.2.13), it has been considered unreliable in the review by Arce *et al.* (2010). Other *in vivo* tests were generally negative though there was one, acceptable, though limited positive dominant lethal assay study (Collins, 1972).

Regarding *in vivo* genotoxicity assays in germ cells, the data is rather limited and again is derived from old studies, pre guideline and none GLP compliant. Of the 4 dominant lethal assays (DLAs) reported, 2 are considered unreliable and therefore unsuitable for assessment of hazard (see table below). That leaves 3 studies, 2 DLAs and a chromosome aberration assessment. These had mixed results, 2 positive, 1 negative.

***Table**: Results of in vivo genotoxicity assays in germ cells with captan*

Study	TG / GLP	Comments	Reliability	Result	Ref.
Study 1 Dominant lethal assay in the mouse Captan > 98%	No / Not GLP	Male germ cells. Oral route Oral: 0, 200, 600 mg/kg bw	RMS: Limited DS: Limited RAC: Limited	Negative	Tezuka *et al.*, 1978
Study 2 Dominant lethal assay in mice and rats Captan ?%	No / Not GLP	Male germ cells Oral gavage and i.p. Oral: 0, 50 to 200 mg/kg IP: 0, 2.5 to 10 mg/kg …(for five days)	RMS: Limited DS: Limited RAC: Limited	**Positive**	Collins, 1972
Study 3 Dominant lethal assay in the mouse Captan ?%	No / Not GLP	Male germ cells Oral gavage and i.p. Oral: 0, 500, 800 mg/kg (once) Oral: 0, 25, 50 mg/kg (for five days) IP: 0, 9 to 30 mg/kg …(once)	RMS: NA DS: NA RAC: NA	Negative	Epstein *et al.*, 1972
Study 4 Chromosomal aberrations Captan 96.5%	No / Not GLP	Mouse spermatogonia and spermatocytes Oral gavage Oral: 0, 10 to 1000 mg/kg bw (for 5 days).	RMS: Limited DS: Limited RAC: Limited	**Positive**	Feng and Lin, 1987
Study 5 Dominant lethal assay in the mouse Captan 89.8%	No /Not GLP	Male germ cells Oral (diet) Oral: 0, 500, 3000, 7000 ppm Test house → unfaithful reporting of data	RMS: NA DS: NA RAC: NA	Negative	Salaman and Smith, 1977

NA = not acceptable

Overall, the weight of evidence indicates that captan does not produce mutagenic or clastogenic effects following oral ingestion *in vivo* especially when taking into account the results on the target tissue, the duodenum. If allowed access to DNA, as in *in vitro* test systems, captan and/or thiophosgene have the ability to induce mutagenic effects in prokaryotic and eukaryotic cells. The presence of an increased thiol-pool provided by the addition of S9 mix, or thiol-containing small molecules and macromolecules in blood and in the whole animal neutralise captan (and thiophosgene) before it can induce DNA damage.

Captan is metabolised very rapidly by whole human blood to THPI, a metabolite which is not genotoxic. As half-life in human blood was determined to be < 1 second, it is plausible that captan is thought to have too short a half-life to be systemically available and thus unlikely to reach germ cells *in vivo*. On the basis of the available evidence, it is assumed captan will not induce heritable mutations in the germ cells of humans. **RAC concludes that no classification as germ cell mutagenicity is warranted, as proposed by the DS.**

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

The DS proposed to keep the existing classification as Carc. 2; H351. All study details were summarised in table 32 in the CLH report. Carcinogenicity attributable to captan oral administration has been demonstrated in a single species (mouse), both sexes and in a single target tissue (duodenum) in 2 independent studies. The underlying MoA has been described in detail by Bhat *et al.* (2020) and is initiated by local irritation leading to villous enterocyte toxicity along with sustained crypt cell proliferation and hyperplasia eventually leading to (non-genotoxic) mutations and transformation in the duodenum.

Four rodent carcinogenicity studies conducted to GLP and similar to current OECD guidelines were available to the DS where animals were fed captan in the diet:

- Studies in rats:
    - Study 1, (Anonymous, 1982; R-9282); 2-year duration; 0, 25, 100 or 250 mg/kg bw/d. There were no treatment-related clinical signs. Survival rate was not affected by treatment. At the top dose bodyweight decreased by approximately 20% for both males and females. Only small effects on mean food consumption observed. Relative liver weight changes were statistically significantly increased for animals in the top dose group at the 12-month, 18-month and terminal timepoints (males: +14%, +47%, +21%; females: +24%, +17%, +27%).
    - Study 2, (Anonymous, 1983; R-3608); 130-week duration; 0, 5, 24 or 98 mg/kg bw/d. There were no treatment-related clinical signs. Survival rate was not affected by treatment. At the top dose bodyweight decreased from 8-10% for males and females. Liver weight (relative to bw) increased in males by about 15%.

There were no treatment-related effects on haematological or clinical chemistry parameters. There were no treatment related increases in tumour incidence in either study. It is noted that the dosing levels employed in the rat studies are much lower than those employed in the mice studies.

The DS proposed to retain Carc. 2; H351 on the basis of results from the two mouse studies.

- Studies in CD-1 mice:
    - Study 3, (Anonymous, 1981; R-8292); 113 weeks duration, captan purity 90.7%, 80 animals per sex and dose; 0, 599/634, 1030/1080 and 1890/1880 (M/F) mg/kg bw/d. There were minor treatment-related clinical signs. Survival rate was affected in the high dose group. At the top dose bodyweight decreased by approximately 20% for both males and females. Food consumption was also reduced across all treatment groups.
    - Study 4, (Anonymous, 1983; R-7995); 94 weeks duration, captan purity 89%, 100 animals per sex and dose; 0, 15/18, 61/70, 123/142 and 925/1043 (M/F) mg/kg bw/d. The incidence of mortality for the top dose males (35%) was higher than for control males (15%). Body weight decreases were minor as were treatment related clinical signs.

Neoplastic findings

Evidence for treatment-related carcinogenicity is only provided by the mice studies. On the basis of the investigations into the mutagenicity of captan, it is presumed that a non-genotoxic mechanism of carcinogenicity operates in the context of the mouse tumours.

Study 3 (Anonymous, 1981; R-8292)

There was a clear increased incidence of duodenal adenomas and adenocarcinomas in both males and females at all dose levels. The number of thymic lymphosarcomas was also slightly (but not clearly dose-related), increased ($p < 0.09$) in high-dose females (4/26) compared to the control (0/30).

Study 4 (Anonymous, 1983; R-7995)

The DS noted that duodenal sections were re-evaluated in 1994. It was concluded from this later re-evaluation that there was an increase in benign and malignant duodenal neoplasia in females at 1043 and 142 mg/kg bw/d and a positive response was also noted by the DS in males at 925 mg/kg bw/d. There was an increase in duodenal adenocarcinoma in both sexes at 925-1043 mg/kg bw/d. Although adenomas were present in control males and females, there were no adenocarcinomas in the control mice.

Other data

The DS also presented some epidemiological data relating to humans exposed to captan from two unpublished reports (Wagner Palshaw, 1980; 1987). No duodenal cancers were observed in the 134 workers included in these studies. The DS considered this (limited) data to support the conclusion that captan is not a human carcinogen.

Mechanistic evidence was also summarised by the DS in the CLH report supporting a non-genotoxic mechanism of carcinogenicity in the mouse, associated with captan irritant nature. Studies showed no evidence of captan covalent binding with DNA and there was a lack of nuclear aberrations in duodenal crypt cells. This is further supported by the fact there is a threshold for duodenal tumours in mice: there is a clear NOAEL for tumours at 60.9 mg/kg bw/d for males (study 4, Anonymous, 1983; R-7995).

In summary, the DS proposed a mechanism for duodenal tumour induction in the mouse that is evident at high doses; captan degradation in the duodenum results in the formation of the irritant thiophosgene (captan is stable in the stomach but readily breaks down to thiophosgene in the higher pH of the small intestine), leading to villus cell damage and irritation and as a result there is enhanced cell replication. This in turn leads to crypt cell hyperplasia, adenoma and ultimately carcinoma. It is well understood that sustained hyperplasia can progress to adenoma and carcinoma if prolonged. Evidence suggests the oncogenic effect manifested in the mouse duodenum results from a non-genotoxic mechanism for which a NOAEL has been established. Recent data evaluating an AOP for mouse small intestinal tumours (Bhat *et al*., 2020) supports this interpretation.

Background of the proposed adverse outcome pathway

Intestinal tumours are rare in rodent bioassays and historically lack a clear aetiology. In addition, the physiology and metabolic capacity of the small intestine is not well studied in relation to human health. As a result, the default regulatory position has been to assume that the MoA is mutagenicity. However, in recent years following the results of Cr(VI) investigations in rodents that illustrated Cr(VI) caused duodenal tumours in mice via a non-mutagenic MoA, regulatory authorities have come to accept a clear precedent for a cytotoxicity-mediated MoA for small intestinal tumours. Captan and folpet are hypothesised to induce duodenal tumours in mice

through a similar MoA of sustained damage to the small intestinal villi resulting in chronic intestinal epithelial hyperplasia.

There is no clear evidence for a specific initiating event for small intestinal tumours at the molecular level. The AoP described in Bhat *et al*. (2020)suggests that captan, upon entering the duodenum, reacts with sulfhydryl-containing cell surface molecules resulting in cytotoxicity of the villous epithelium and this is proposed to be the molecular initiating event (MIE). Over time, to compensate for the rate of enterocyte loss, the regenerative capacity of the gut epithelium declines, particularly at higher compound concentrations.

The first Key Event (KE) is sustained regenerative crypt proliferation/hyperplasia. Due to the extensive repair capacity of the gut, this would likely require exposure times in the order of 6-12 months to create more opportunities for the second KE, spontaneous mutation/transformation, ultimately leading to small intestine tumours.

The small intestine is the primary site of nutrient absorption and the large surface area created by the villi projections facilitate this. The villi are lined by mature differentiated enterocytes supplied by stem cells that reside at the base of the crypts or glands of Lieberkühn. The intestinal mucosa is amongst the most proliferative tissue in the body with cells moving from the villi crypts to the tip of the enterocytes (along the crypt villus axis) in approximately 2-3 days in rodents and 3-6 days in humans. A continuous supply of daughter cells emerging from the intestinal crypts move towards the villi where they become committed to non-dividing enterocytes and which in turn are sloughed off the upper tips of the villi by passing intestinal content. The prevailing theory of intestinal cancer is that tumours arise from mutations that occur within the crypt stem cells. These cells remain at the base of the crypt and so have time to acquire multiple mutations leading to transformation. The crypts reside below the intestinal surface and are filled with mucous that is continuously secreted by goblet cells. In general, the intestinal structure is similar in rodents and humans.

Captan or folpet are not readily metabolised in the acidic pH of the stomach. However, at the higher pH in the intestinal lumen, they are readily hydrolysed by reaction with cellular/tissue thiols and/or DNA to form thiophosgene and further depletion of cellular glutathione (GSH), which results in cytotoxicity in the small intestine. Chemicals that deplete GSH in intestinal villi, very likely accelerate enterocyte sloughing, resulting in increased regenerative proliferation. Higher duodenal captan concentrations occur in exposed mice compared to rats and this is considered by the US EPA to potentially explain the sensitivity of mice to these agents.

Thiophosgene can readily react with thiols on cellular macromolecules including protein and DNA. The most likely route of toxicity of these agents is nonspecific, protein binding, and alteration of the cellular redox balance. Importantly villous enterocytes are committed, nonproliferating cells that slough into the intestinal lumen with 24-48h of reaching the villi. Therefore, the likelihood of intestinal tumours arising from DNA damage to differentiated enterocytes is not thought to be feasible. In the normal small intestine, cell proliferation is limited to the crypt compartment with stem/progenitor cells residing at the base of the crypt. The prevailing view on the theory of intestinal carcinoma is that mutations occur and accumulate in the stem/progenitor cells in the crypt. Sustained crypt cell proliferation/ hyperplasia leads to mutation and transformation and these in turn lead to small intestinal tumours in mice.

Captan and folpet do not induce small intestinal tumours in rats. Rats do exhibit signs of crypt hyperplasia and villous damage, but to a much lesser extent than mice. In rats, the transit time from the stomach to the duodenum is 4-times slower than in mice thus possibly facilitating greater susceptibility to these agents in the mouse gut. Human relevance cannot be reasonably excluded on the basis of a lack of fundamental qualitative interspecies differences. Instead the weight of evidence is sufficient to establish a MoA in mice with qualitatively plausible key events in humans (and rats). With regard to species differences, it seems likely that in this case the key

events become quantitatively unlikely in humans. Exposure levels used in animal carcinogenicity studies are always higher than expected in humans, to compensate for the shorter study duration, so that levels may never be achieved to result in small intestinal tumours. Even so this does not negate the potential hazard associated with duodenal exposure to captan.

Comments received during consultation

One MSCA considered that classification as Carc. 1B was warranted since there is no evidence that the proposed captan MoA is not relevant for humans and importantly, reproducible tumours observed in one species would be sufficient for classification as Carc. 1B. However, as pointed out by the DS, the consideration of a Cat. 2 rather than Cat. 1B classification was not based on risk arguments even though all parties agreed that levels of exposure needed for irritation to result in cancer are not reached in humans. The DS pointed out that the MoA for captan was established as non-genotoxic, but irritation-driven, with reversibility of early and mid-key events. In addition, the DS clearly outlined CLP guidance that stated if a secondary mechanism of action with the implication of a practical threshold above a certain dose level (e.g. chronic stimulation of cell proliferation) was evident then this could lead to a downgrading of a Cat. 1 to Cat. 2 classification. The effect appears to be species-specific to mice, but the highest dose tested in rats (250 mg/kg bw/d, study 1, Anonymous, 1982) just exceeds by two-fold the mouse LOAEL of 123 mg/kg bw/d (study 4, Anonymous, 1983). There are no gastrointestinal tumours in the other species, even if gastrointestinal irritation is observed and this leads to a further easing of concern.

A second MSCA agreed that classification for carcinogenicity based on findings in the duodenum in mice studies was warranted. This MSCA also noted effects in the lungs (squamous hyperplasia and metaplasia) from the 90-day inhalation study in rats which it understood to be irreversible in nature. These effects could lend further support to the evaluation of captan carcinogenicity. The DS disagreed and considered the responses to be indicative of an adaptive response to an irritant effect rather than as a set of pre-neoplastic lesions.

Industry disagreed with captan carcinogenicity classification, they considered captan's inherent hazard property was acute irritation and not carcinogenicity. This is more appropriately classified as an irritant (eye irritation, acute inhalation toxicity) which aptly communicates the hazard in this case. The reversibility of the irritating effects in mice duodenum, the species-specificity (mice) of the gastrointestinal tumours, the absence of an exposure scenario for humans that results in life-long, or even short-term, irritating concentrations of captan via the diet represent additional lines of evidence that support a non-classification.

The DS acknowledged that the AOP developed by Bhat *et al.* (2020) concluded that, the KEs become quantitatively implausible in humans, especially after accounting for background levels of human exposure. Nevertheless, the authors also concluded that the KEs are qualitatively plausible in humans. The DS reiterated its proposal to retain Carc. 2 classification.

Assessment and comparison with the classification criteria

Summary

Overall, the available carcinogenicity studies do not provide evidence that captan is carcinogenic in rats. However, captan consistently induces tumours (adenomas and adenocarcinomas) in the duodenum/small intestine of CD-1 mice. There was an increased incidence of malignant and/or benign neoplasm of intestinal (duodenal and jejunum/ileum combined) and duodenal crypt cells alone in females at 142 mg/kg bw/d, and both sexes at 925/1043 mg/kg bw/d (M/F) from the study 4 (Anonymous, 1983) and this corroborates the results in study 3 (Anonymous, 1981).

***Table**: Oral carcinogenicity study in the mouse (Anonymous, 1981): summary of incidence of duodenal neoplasms.*

	Dose M/F (mg/kg bw/day)			
	Control	599 / 634	1030 / 1080	1890 / 1880
Females				
Number examined[1]	72	78	76	76
Number duodenal neoplasms	2	24***	19***	29***
Adenocarcinoma[2]	0	17***	14***	20***
Adenoma[2]	2	10*	8	12*
Undifferentiated sarcoma	0	0	0	0
Males				
Number examined[1]	74	73	72	75
Number duodenal neoplasms	2	20***	21***	39***
Adenocarcinoma[2]	1	10***	14***	30***
Adenoma[2]	1	11*	7	11*
Undifferentiated sarcoma	0	1	0	0

[1] Excluding severely autolysed and missing tissues.
[2] Tabulated by total number of neoplasms; some had multiple (i.e. both benign and malignant) neoplasms.
Significantly different from the control * $p < 0.05$, *** $p < 0.001$.

***Table**: Second oral carcinogenicity study in the mouse (Anonymous, 1983): initial summary of incidence of all intestinal neoplasms. Tumours of the small intestine in yellow.*

Tissue	**Lesion**	**Dose (ppm)**				
		0	**100**	**400**	**800**	**6,000**
Males						
Stomach	Number examined	97	95	98	92	97
	adenoma/polyp(s)	0	2	0	1	2
	carcinoma (primary)	0	1[1]	0	0	0
	squamous cell carcinoma (primary)	0	0	0	0	0
Duodenum	Number examined	84	79	90	89	85
	adenoma/polyp(s)	0	3	0	0	3
	carcinoma (primary)	0	0[3]	0	0	1
Jejunum/ileum	Number examined	81	78	77	74	79
	adenoma/polyp(s)	0	1	1	0	0
	carcinoma (primary)	0	0	0	0	0
Cecum and colon	Number examined	75	87	87	77	72
	adenoma/polyp(s)	0	0	0	0	0
	carcinoma	0	0	0	0	0
	leiomyosarcoma	0	0	0	0	0
Females						
Stomach:	Number examined	97	96	99	96	97
	adenoma/polyp(s)	0	0	1	1[2]	2
	carcinoma (primary)	0	0	0	0	0
	squamous cell carcinoma (primary)	0	0	0	0	1
Duodenum	Number examined	85	83	89	82	93
	adenoma/polyp(s)	0	0	1[4]	1[2]	3[4,5]
	carcinoma (primary)	0	0	0	0	1
Jejunum/ileum	Number examined	85	81	78	83	87
	adenoma/polyp(s)	0	1	2[4]	2	2[4]
	carcinoma (primary)	0	0	0	0	1
Cecum and colon	Number examined	85	85	85	84	87
	adenoma/polyp(s)	0	0	0	0	1
	carcinoma	1	0	0	0	0
	leiomyosarcoma	0	0	0	1	0

[1] One animal metastatic to duodenum.
[2] One animal with polyps in two organs (stomach and duodenum).
[3] One animal metastatic from stomach (for primary tumour see stomach).
[4] One animal with polyps in two organs (duodenum and jejunum/ileum).
[5] One animal had two duodenal lesions (one hyperplasia and one polyp).

***Table**: Second oral carcinogenicity study in the mouse (Anonymous, 1983): Updated assessment of duodenal sections (1994); summary of incidence of neoplasms (yellow).*

	Dose M/F (mg/kg bw/day)				
	0	15/18	61/70	123/142	925/1043
Males					
Number examined	91	83	93	87	84
Number missing	9	17	7	13	16
Adenocarcinoma (malignant)	0	0	0	0	2
Adenoma (benign)	2	3	0	1	4
Focal mucosal hyperplasia	4	2	7	6	12
Adenoma with atypia (benign)	0	0	0	0	0
Lymphoid proliferation	9	8	10	12	23
Amyloidosis	60	61	75	54	49
Females					
Number examined	85	82	83	81	91
Number missing	15	18	17	19	9
Adenocarcinoma (malignant)	0	0	0	0	1
Adenoma (benign)	3	1	1	7	3
Focal mucosal hyperplasia	11	9	8	13	20
Adenoma with atypia (benign)	0	0	0	0	3
Lymphoid proliferation	10	13	14	18	18
Amyloidosis	52	51	47	45	55

The aetiology of the small intestinal tumours has been investigated in several mechanistic studies supporting a MoA driven by cytotoxicity with subsequent regenerative proliferation which if sustained, finally leads to increases in spontaneous mutation followed by 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, captan and hexavalent chromium have been used as stressors to provide the empirical support of this AOP. This AOP is considered relevant for human.

Relevance of the rat data

It is not entirely understood why tumours of the duodenum are not observed in the rat. Based on studies with Cr(VI) (Cullen *et al.*, 2016), rats exhibited some signs of small intestinal lesions (crypt hyperplasia and villous damage). It is speculated that the AoP for duodenal tumours in mice might also be relevant to rats receiving sufficiently high concentrations of a chemical stressor to induce severe chronic cytotoxicity and regenerative hyperplasia (Bhat *et al.*, 2020).

RAC notes that the dose levels in the rat studies were lower than those in the mouse studies (table below). Also, there is some evidence for differences in toxicokinetic such as transit time from the stomach to the duodenum which is about 4-times slower for the rat than in mice (Kirman *et al.* 2012), thus possibly facilitating greater susceptibility to captan by way of greater target tissue exposure in the mouse gut. Higher duodenal concentrations of captan were detected in mice compared to rats exposed to 250 mg/kg bw radiolabelled captan (Wong and Chang, 1985).

***Table**: Dosing levels in the rodent carcinogenicity studies*

Study	TG / GLP	Comments	Ref.
Study 1 (Anonymous, 1982) CR CD rat Acceptable	Non Guideline / GLP yes	Captan purity 89% Dose: 0, 25, 100, 250 mg/kg bw/d, 104 weeks	RAR/Annex I to CLH report 3.9.1.1
Study 2 (Anonymous, 1983) SPF (Cpb:WU; Wistar random) rat Acceptable	Non Guideline / GLP yes	Captan purity ?% Dose: 0, 5, 24, 98 mg/kg bw/d, 130 weeks	RAR/Annex I to CLH report 3.9.1.2
Study 3 (Anonymous, 1981) CD1 mouse Acceptable	Non Guideline / GLP yes	Captan purity 90.7% Dose: 0, 599/634, 1030/1080, 1890/1880 mg/kg bw/d (M/F), 113 weeks	RAR/Annex I to CLH report 3.9.1.3
Study 4 (Anonymous, 1983) CD1 mouse Acceptable	Non Guideline / GLP yes	Captan purity 89% Dose: 0, 15/18, 61/70, 123/143, 925/1043 mg/kg bw/d (M/F), 94 weeks	RAR/Annex I to CLH report 3.9.1.4

? = unknown

RAC also notes that there is no comparative data for captan with respect to the small intestinal lesions that were extensively investigated in the mouse but no such undertaking was performed in the rat. No studies were presented in the CLH report to indicate if similar histopathological lesions were present in the rat duodenum.

Comparison with the criteria

Category 1A

No definitive epidemiologic data in humans investigating captan's carcinogenic potential were available. The epidemiology data reported by the DS was only concerned with mortality within a cohort of captan workers (two unpublished reports; Wagner Palshaw, 1980 and 1987, RAR section 3.9.2.1). No duodenal cancers were observed in the 134 workers included in these studies.

Category 1B

RAC considers that the mouse studies provide sufficient evidence of carcinogenicity according to CLP criteria since captan induces benign and malignant neoplasms in the gastrointestinal tracts in two independent well-conducted studies. These tumours occurred in both sexes, but a clear dose-response relationship was not always evident and could just be an indicator of the dose spacings employed in both studies.

Clearly there is concern with carcinogenicity of the small intestine and duodenum in particular in the mouse, with both sexes affected, that can be replicated across studies. This in itself fulfils the criteria for classification in Cat. 1B, however there is notable uncertainty introduced as the evidence in the second mouse study is not as robust as in the first study, which could simply be

due to the dosing regimen employed. In addition, the lack of tumours in the rat studies could suggests the overall evidence is not sufficient to support a Cat. 1B classification. However, in the rat studies, dosing may also be an important factor to consider, since higher doses may have resulted in neoplastic effects.

Category 2

RAC has 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 captan is not mutagenic *in vivo*. No DNA damage was evident in duodenal investigations in mice.
- Based on a weight of evidence approach, involving mechanistic data and agreement with the KEs of the proposed AOP, RAC considers that the proposed MoA, i.e., neoplastic responses driven by enterocyte cytotoxicity with subsequent regenerative proliferation, is biologically plausible in mice.
- While this MoA is considered qualitatively relevant for humans, 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.

Conclusion

Carcinogenicity attributable to oral administration of captan has been demonstrated in a single species (mouse) and in a single target tissue (small intestine/duodenum) in two independent studies. RAC considers that the weight of evidence (table below) is sufficient to establish that a MoA driven by cytotoxicity underlays the occurrence of small intestinal tumours in mice. While this MoA is considered qualitatively relevant for human, RAC acknowledges that a clear threshold for tumour-development in mice is established which reduces the level of concern for human carcinogenicity.

***Table**: Compilation of factors taken into consideration for the assessment of carcinogenicity*

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**
Rat: CD	No increase in tumour incidence	n/a	n/a	n/a	n/a	n/a	Oral diet	n/a
Rat: Wistar	No increase in tumour incidence	n/a	n/a	n/a	n/a	n/a	Oral diet	n/a
Mouse: CD-1 Study 1	Duodenal adenoma and adenocarcinoma Rare < 1% in CD-1 mouse	No, reduced tumours in male lung and liver	Yes	Yes	Both	Pronounced effect on body weight all doses, high dose exceeds guideline maximum	Oral diet	Non genotoxic mechanism potentially relevant to humans
Mouse: CD-1 Study 2	Duodenal adenoma and adenocarcinoma	No, but examination confined to GI tract, lungs and abnormal tissues	Yes	n/a	Both	No	Oral diet	Non genotoxic mechanism potentially relevant to humans

Based on a weight of evidence analysis and in accordance with the criteria laid down in the CLP Regulation, **RAC concluded that retaining the classification as Carcinogen Category 2; H351 is warrented, as proposed by DS.**

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Sexual function and fertility

Two rodent generation studies are available where animals were fed captan in the diet:

- Studies in COBS CD rats:
    - Study 1, (Anonymous, 1982; 3.10.1.1), 3-generation reproduction study, pre-guideline, non-GLP; 0, 25, 100 and 250 and 500 mg/kg bw/d.

      No treatment-related effects were seen in the general behaviour or appearance of the treated parental rats or pups. Survival rate was not affected by treatment. No consistent treatment-related effects on male and female fertility or length of gestation were observed.

      No treatment-related or statistically significant differences were noted in male and female fertility, mean numbers of nonviable foetuses, early and late resorptions and post implantation loss per dam or foetal sex distribution in any of the captan treated groups compared to the control. A very slight reduction in the mean number of viable foetuses per dam and a corresponding slight decrease in total implantations was determined at 250 and 500 mg/kg bw/d.

      Parental group mean body weights were statistically significantly decreased by approximately 13-28% for both males and females in the two top dose groups across all generations. Moderate effects on mean food consumption was observed at ≥ 250 mg/kg bw/d (-12 to -22 % of controls).

      Substantial reductions in pup body weights were dose related and consistent across generations on PND0 and PND21, weight reduction maintained into adulthood. There was no investigation into effects on sexual maturation.

    - Study #02, (Anonymous, 1982, 3.10.1.2), 1-generation reproduction study, pre-guideline, GLP compliant; 0, 6, 12.5, 25 mg/kg bw/d. There were no treatment-related clinical signs. No adverse effects noted.

The DS found no adverse effects on sexual function and fertility in the rat to warrant classification as a reproductive toxicant affecting sexual function and fertility. The DS also summarised the results from a number of endocrine assays. Both *in vitro* (binding and transcriptional activation assays) and *in vivo* assays (uterotrophic and Hershberger) did not support endocrine activity via oestrogenic (ER) or androgenic (AR) modalities while captan gave equivocal results in a Human Recombinant Aromatase Assay.

***Table**: Summary table of a number of endocrine assays potentially relevant for toxicity affecting sexual function and fertility*

Study	TG / GLP	Comments	Ref.
Study 14 (Anonymous, 2012) Androgen Receptor Binding Assay (Rat Prostate)	OPPTS 890.1150 / GLP yes	Negative: Captan does not interact with the androgen receptor.	RAR 3.10.3.4
Study 10 (Anonymous, 2012) Hershberger Assay	OPPTS 890.1400 / GLP yes	Negative: captan does not exhibit agonist or antagonist activity in castrated male rats.	RAR 3.10.3.8
Study 16 (Anonymous, 2012) Human Recombinant Aromatase Assay	OPPTS 890.1200 / GLP yes	Equivocal: The mean aromatase activity was 60.3% of control at 10-5 M, compared to 0.74% for the positive control.	RAR 3.10.3.6
Study 13 (Anonymous, 2012) Oestrogen Receptor Binding Assay	OPPTS 890.1250 / GLP yes	Negative: captan does not interact with the rat oestrogen receptor.	RAR 3.10.3.3
Study 15 (Anonymous, 2012) Oestrogen Receptor Transcriptional Activation Assay	OPPTS 890.1300 / GLP yes	Negative: captan is not an agonist to hERα in the HeLa-9003 model.	RAR 3.10.3.5
Study 17 (Anonymous, 2012) Steroidogenesis Assay	OPPTS 890.1550 / GLP yes	Ambiguous results: captan effects were inconsistent across three runs. There was a decrease in oestradiol in one run; a decrease in testosterone in another run and no effects in a third run (albeit at a high dose that was three orders of magnitude lower). A valid third confirmatory run was missing.	RAR 3.10.3.11
Study 9 (Anonymous, 2012) Uterotrophic Assay	OPPTS 890.1600 / GLP yes	Negative: captan did not affect uterine weight (i.e. show oestrogenic activity) in ovariectomized rats.	RAR 3.10.3.7
Study 11 (Anonymous, 2012) Pubertal Assay in Male Rats	OPPTS 890.1500 / GLP yes	Noteworthy adverse effects: sndrogen-dependent tissue weights (ventral prostate, LABC) and serum testosterone were significantly decreased at doses at or above MTD.	RAR 3.10.3.9
Study 12 (Anonymous, 2012) Pubertal Assay in Female Rats	OPPTS 890.1450 / GLP yes	Noteworthy adverse effects: Delay in vaginal opening, day of first oestrus, decreased number of corpora lutea, and decreased pituitary gland, ovarian, and uterine weights at MTD.	RAR 3.10.3.10

MTD: maximum tolerated dose

RAC notes the adverse effects in the two pubertal assays which are further discussed in the assessment and comparison with the classification criteria as additional evidence for reproductive toxicity effects.

Developmental toxicity

The DS did not propose to classify captan for developmental toxicity. The DS described one rat developmental study and four rabbit developmental studies with captan administered via gavage and one rabbit study with the principal metabolite THPI.

The developmental effects observed in rats (consisting of an increase in minor skeletal variations as well as a minor reduction in foetal weights) did not support classification.

In the rabbit, increased post implantation loss and late resorptions were a feature, along with reduced foetal weight, and several minor variations including reduced skeletal ossification, and increased incidences of extra ribs and vertebra. The rabbit was more prone to maternal toxicity (marked in the rabbit at ≥ 100 mg/kg bw/d) with significant decreased body weight and food consumption during the dosing period. The DS assumed the foetal toxicity to be secondary to high maternal toxicity. There was no consistent evidence of a specific malformation or set of malformations; the diversity of malformations as well as their individual low numerical incidence in the presence of severe maternal toxicity made it difficult to assign a treatment-related effect on the developing foetus. Any malformations observed were considered to be spontaneous in origin. The DS concluded there was no evidence of teratogenicity in either the rat or rabbit.

One prenatal developmental toxicity study with THPI was conducted in the rabbit. THPI has a structure similar to thalidomide which is a known teratogenic substance in the rabbit. The dose rates used were precisely comparable with those of captan used by Anonymous (2006; 3.10.1.7). The DS concluded that no effects with regard to maternal toxicity or developmental toxicity was observed.

The DS also reviewed a position paper (Anonymous, 2018; Captan: A Review of the Potential to Induce Developmental Toxicity) which provided comments on the animal study included in the CLH dossier as well as investigating published studies in the literature searching for information on captan potential to induce developmental effects. A number of points were articulated this review:

1. The very short half-life of captan indicates that the developing offspring *in utero* will not be exposed to captan. The principal metabolite THPI may reach the developing offspring following maternal administration of captan.
2. A series of directly comparable rabbit developmental toxicity studies, conducted in 2005, provide clear evidence that neither captan and its metabolite THPI, nor the structurally related chemical folpet and its metabolite, phthalimide, induce malformation in the rabbit. There is also a lack of evidence from the published literature for the structurally similar chemical folpet, and its metabolite phthalimde.
3. No evidence from published studies that either captan or THPI induce malformations in the rabbit.
4. No evidence from published studies that either captan or THPI induce malformations in the rat.
5. A review of the data available in the published literature confirms that neither captan nor THPI induce malformations in the mouse or primate.

The DS concluded that captan is not a developmental toxicant in several mammalian species including the rat and rabbit. Consequently, they did not propose a classification as reproductive toxicant.

Lactation

No classification of captan for effects on or via lactation was proposed by the DS. There was no indication of impaired nursing behaviour or decreased pup viability during lactation from the data presented in the two rat generation studies. There were no indications of any direct, adverse effects on the offspring due to transfer of the active substance via the milk or to the quality of the milk.

Comments received during consultation

There were three comments, two from MSCAs and one from industry. All agreed with the DS proposal, no classification for reproduction. The DS in one response indicated that captan's metabolite THPI has a structure similar to thalidomide which is a known teratogenic substance in the rabbit. In a developmental study (Anonymous, 2006; RAR 3.10.3.2), the metabolite THPI was tested clearly below the MTD, therefore the potential for effects at a higher dose, capturing maternal toxicity, has not been addressed.

Assessment and comparison with the classification criteria

Sexual function and fertility

Captan has been evaluated for adverse effects on sexual function and fertility in one key, rat, 3-generation study (Anonymous, 1982; 3.10.1.1). A second 1-generation study (Anonymous, 1982; 3.10.1.2) was also performed but at much lower dose levels, this second study did not have any impact regarding adverse effects on either the parent animals or their offspring.

The key study (commenced prior to GLP and OECD test guidelines) was acceptable but there were notable omissions in the data including no evaluation of oestrus cyclicity, no evaluation of sperm, no calculation of pre-coital interval, no evaluation of pup physical development or sexual maturation, no parental or pup organ weights, parental or pup histopathology. Overall, the key study is considered to be acceptable but limited for a robust assessment of sexual function and fertility.

Of particular note is that adverse effects on parental animals (including maternal toxicity) were observed at 250 and 500 mg/kg bw/d in the form of significantly reduced body weight and food consumption metrics (see below).

There was no indication of an adverse effect of Captan on sexual function that was sufficient to warrant a proposal for classification. There were offspring effects (reduced bw) that were biologically significant and may be considered in the context of developmental toxicity. However, the presence of moderate maternal toxicity may negate these offspring effects. Other effects as seen in the recent (2012) pubertal development and thyroid function studies with both male and female rats may indicate a potential for adverse effects on fertility.

***Table**: Summary table of animal studies on sexual function and fertility.*

Study	TG / GLP	Comments	Ref.
Study 1 (Anonymous, 1982) COBS CD strain rats.	Substantial deviations from TG 416 (2001) / non-GLP	3-gen rat dietary study → captan purity 89%. Dose: 0, 25, 100, 250, 500 mg/kg bw/d • consistent ↓ pup weight (PND0, 21) across generations. • consistent ↓ pup weight (PND0, 21) dose related. • puberty endpoints not investigated. Parental tox: • ↓ bw, top dose: males 17-28%; females 12 – 24% • substantial ↓ mean food consumption at ≥ 250 mg/kg bw/d (-12 to -22 % of controls)	RAR 3.10.1.1
Study 2 (Anonymous, 1982) COBS CD strain rats.	Non-guideline but similar to OECD TG 415 (1983) / GLP yes	1-gen rat dietary study → captan purity 89% Dose: 0, 6, 12.5, 25 mg/kg bw/d • no significant adverse effects at any dose level.	RAR 3.10.1.2

Slight to moderate decreases in parental food consumption (g food/rat/day) relative to controls were of particular note in males and females of treatment groups dosed ≥ 250 mg/kg bw/d, in all three generations:

F0: Males; 15-19% reduction in food consumption.
Females; 15-24% reduction.

F1: Males; 14-22% reduction.
Females; 12-20% reduction.

F2: Males; 13-18% reduction.
Females; 4-19% reduction.

Throughout all generations, a statistically significant, dose-dependent decrease in male and female parental body weights was also observed in the 250 and 500 mg/kg bw/d treatment groups and together with the effects on food consumption led to moderate maternal toxicity and dosing.

Dose-related, statistically significant reductions in pup body weights were seen in all litters in the 250 and 500 mg/kg bw/day groups throughout lactation to weaning. The weight reductions were sustained in these pups giving rise to the subsequent F1 and F2 parental populations. Foetal observations at caesarean section in the F2c teratology cohort showed a small statistically significant ($p < 0.05$) reduction in foetal bw at the highest dose (500 mg/kg bw/d). There was no data recorded from this study to investigate if pubertal attainment was affected in any way. There was no data on anogenital distance. The consistent and dose related reduction in mean pup body weight and subsequent sustained reduction of body weight relative to controls in the emerging parental generations (F1 and F2, table below) is consistent with moderate growth retardation and therefore developmental delay which may also be considered for classification under developmental toxicity. The influence of maternal toxicity on these effects is difficult to

ascertain but the recent studies on pubertal development indicate adverse effects in the absence of maternal toxicity.

***Table**: 3-generation reproduction study in the rat: mean pup and parental % body weight changes relative to controls PND1 to weaning to adult hood (for F1, F2, F0 included for completeness).*

Generation	Controls	25 mg/kg bw/d	100 mg/kg bw/d	250 mg/kg bw/d	500 mg/kg bw/d
PND 1, Sexes combined[1]					
F1 pups	7.3 g	-6%	-9%	-11%	-18%
F2 pups	7.1 g	-6%	-6%	-12%	-14%
F3 pups	6.9 g	-1%	-4%	-7%	-14%
PND 21, Males					
F1 pups	46.0 g	-2%	-8%	-30%	-36%
F2 pups	49.0 g	-2%	-13%	-27%	-38%
F3 pups	49.5 g	-3%	-8%	-19%	-36%
PND 21, Females					
F1 pups	44.4 g	-2%	-8%	-19%	-37%
F2 pups	46.4	-3%	-13%	-26%	-36%
F3 pups	47.7 g	-4%	-8%	-19%	-36%
Parental, Males, after 32-36 weeks of treatment					
F0	653 g	-5%	-8%	-13%	-18%
F1	614 g	+2%	-6%	-16%	-27%
F2	614 g	-5%	-8%	-19%	-24%
Parental, Females, after 32-36 weeks of treatment					
F0	337 g	-2%	-4%	-8%	-12%
F1	346 g	-3%	-7%	-14%	-22%
F2	344 g	-4%	-4%	-13%	-18%

1. individual data for males and females not summarised in the original report.

Additional evidence for reproductive toxicity effects

The EFSA peer review of captan (2020) concluded that captan did not meet the criteria for endocrine disruption for both humans and non-target organisms through EATS modalities, but noteworthy adverse effects are presented below.

Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats **(Anonymous, 2012, RAR 3.10.1.9)**

Administration of captan from PND23 (through PND53 or 54) to male rats at dose levels of 100 or 200 mg/kg bw/d were not considered to exceed the MTD by the RMS (due to a lack of adverse clinical observations, or signs of systemic toxicity), in their evaluation of the RAR. The study was performed according to USEPA OPPTS 890.1500 guidelines and was GLP compliant.

This study is useful in removing the maternal toxicity factor from the assessment of any adverse effects that were observed. Captan (94.4%) was administered to 16 animals per dose group. All male rats administered the vehicle control (1% CMC) or 100 mg/kg bw/d captan survived to scheduled euthanasia with no evidence of moribundity and clinical signs, when observed, were minor (100 mg/kg bw/d: one rat with rales, a second animal with alopecia of the dorsal area on study days 31 and 32). Two out of 16 animals administered 200 mg/kg bw/d were euthanized prior to scheduled termination (on PND40 and on PND49). These two animals were observed with abnormal breathing (gasping). No signs of dosing error were observed at necropsy: gastrointestinal dilatation was observed in both animals. According to the study author, the mortality of these two rats may have been a result of oral gavage-related reflux that results in serious respiratory effects and mortality rather than to any 'systemic' toxicity of captan (no further detail was presented). This conclusion was also accepted and agreed by the RMS in the RAR. There is no further evidence to suggest otherwise. The same effect appears to have occurred in the female rat study. The final body weights of male rats administered 100 or 200 mg/kg bw/d were decreased of 9 to 13% relative to the mean control body weight.

Body weight at preputial separation was slightly reduced (-8 to -13%) but there was little effect on day of attainment of preputial separation. Effects were seen in some androgen-dependant tissues; ventral prostate (VP) weight was significantly decreased in rats administered 100 or 200 mg/kg bw/d compared to the vehicle control group (-15 to -20%). The levitor ani-bulbocavernosus (LABC) muscle weight was also significantly decreased in animals administered 200 mg/kg bw/d (-14.5%). Histopathology in several tissues was uneventful. In addition, administration of 200 mg/kg bw/d significantly decreased serum thyroxine (T4, -17.7%) and serum testosterone concentrations (-23 and -42% for the 100 and 200 mg/kg bw/d dose groups, respectively) compared to the vehicle control group. There was no change in serum TSH concentration.

***Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Female Rats* (Anonymous, 2012; RAR 3.10.3.10)**

Administration of captan (94.9%) from PND22 (through PND42 or 43), to female rats at dose levels of 300 or 600 mg/kg bw/d (16 animals per dose) were not considered to exceed the MTD by the RMS in their evaluation. The study was performed according to USEPA OPPTS 890.1450 guidelines and was GLP compliant. This study is also useful in removing the maternal toxicity factor from the assessment of any adverse effects since juvenile rats were dosed naively from PND22 onwards, i.e., there was no previous exposure via any route prior to the commencement of the study.

All female rats administered 0 or 300 mg/kg bw/d captan survived to scheduled euthanasia with no evidence of moribundity. Clinical signs noted in two rats were minor and resolved quickly. Two out of 16 animals administered 600 mg/kg bw/d were euthanized prior to scheduled termination due to moribundity (abnormal breathing (gasping), on PND26 and the other on PND38). No signs of dosing error were observed at necropsy: gastrointestinal dilatation was observed in both of these animals. Small intestinal dilatation appears to be a dose related effect; it was observed in one animal administered 300 mg/kg bw/d and 12 animals administered 600 mg/kg bw/d. The RMS stated these findings were a result of gavage-related reflux. This may be symptomatic of a local effect rather than a systemic one. The final body weights of female rats administered 300 or 600 mg/kg bw/d were decreased of -9.4% and -9.8%, respectively, relative to the mean control body weight, there was no evidence of a dose related decrease.

Administration of captan to female rats was not associated with histopathologic changes in the uterus, left ovary, or left kidney. In the ovary, the number of corpora lutea in animals administered 300 or 600 mg/kg bw/d was significantly decreased compared to vehicle controls.

In addition, thyroid gland follicular cell height was statistically increased and colloid area decreased in animals administered 600 mg/kg bw/d.

In summary, a statistically significant delay in vaginal opening (+4 to + 5.3 days, with no effect on body weight at time of VO), day of first oestrus (+ 2.5 and 5.2 days), decreased number of corpora lutea (-29 to -30%), and decreased pituitary gland (-10.5 to -19%), ovarian (-18 to -23%), and uterine weights (blotted; -12 to -34%) were observed in rats administered 300 or 600 mg/kg bw/d and indicate effects on pubertal/thyroid development and therefore may be considered as a potential adverse effect on fertility.

In addition, the serum concentration of T4 was significantly decreased below the normal range in female rats administered 300 (-27.4%) or 600 (-41.7%) mg/kg bw/d, compared to the control group. There were no significant changes in serum TSH concentrations. A steroidogenesis assay using the H295R human adrenocortical carcinoma cell line was inconclusive.

Conclusion

The effects observed in the male and female pubertal assays are not secondary to adverse parental toxicity as juvenile rats were naively exposed from PND22 onwards. The difficulty with putting these results into perspective here is that there is a lack of a modern multigeneration study with a concurrent pubertal assessment to substantiate these findings. However, the design of these juvenile rat pubertal and thyroid development studies is such that maternal toxicity is removed as a factor in the consideration of the adverse effects. As standalone studies they indicate noteworthy adverse effects on fertility, particularly in females and provide support for captan reproductive toxicity.

Assessment of sexual function and fertility for classification

According to the CLP Regulation (section 3.7.1.3 of Annex I), *any effect of substances that has the potential to interfere with sexual function and fertility has to be regarded for a classification for reproductive toxicity. This includes, but is not limited to, alterations to the female and male reproductive system, adverse effects on onset of puberty, gamete production and transport, reproductive cycle normality, sexual behaviour, fertility, parturition, pregnancy outcomes, premature reproductive senescence, or modifications in other functions that are dependent on the integrity of the reproductive systems*.

Category 1A or 1B is not supported, as there is no human data available and the 3-generation rat study is not robust in terms of data for important parameters that were never investigated. Category 2 is supported on the basis of the results from the juvenile rat pubertal and thyroid development studies, where the adverse effects noted include:

1. Significant delay in vaginal opening (+4 – to + 5.3 days, with no effect on bw at the time of VO) for the mid and top dose groups.
2. Significantly decreased serum T4 in males (-17.7%) and females (-41.7%) in the top dose group, clear dose response in females.
3. Significantly decreased serum testosterone concentrations in males (-23 and -42%) for the mid and top dose groups.
4. Reduction in the weight of androgen-dependant tissues in males; VP -20% and LABC -14.5% in the top dose group.
5. Significantly decreased number of corpora lutea (-29 to -30%) for the mid and top dose groups.
6. Significantly decreased ovarian (-18 to -23%), and uterine weights (blotted; -12 to -34%) at the top dose group.

Overall, **RAC concludes that a classification as Repr. 2; H361f is warranted**.

Adverse effects on development

The developmental toxicity of captan (and its metabolite THPI) was investigated in six prenatal developmental toxicity studies, one in rats (Anonymous, 1987) and five in rabbits (Anonymous, 1991; 1987; 1981; 2006a and 2006b). One of the rabbit studies investigated the teratology of THPI and found no evidence for adverse effects (table below).

***Table**: Summary table of key effects in animal studies on developmental toxicity*

Study	TG / GLP	Comments	Ref.
Study 1 (Anonymous, 1987) CR CD rat Acceptable	OECD TG 414 (2001) / GLP yes	Captan teratology study → purity 91% Dose: 0, 18, 90, 450 mg/kg bw/d • Dosing period GD6-GD15 • No treatment-related foetal malformations were observed, only an increase in variations • No impact on classification Maternal tox: • ↓ bw over the dosing period in the top dose dams (< 10%) • ↓ Day 7-9 food consumption at top dose (-46.6%)	RAR 3.10.1.3
Study 2 (Anonymous, 1991) NZW rabbit Excessive maternal toxicity Limited	USEPA Guideline 83-3 / GLP yes	Captan teratology study → purity 91.2% Dose: 0, 10, 30, 100 mg/kg bw/d • Dosing period GD7-GD19 • ↑ in early/late intra-uterine deaths (11-12% vs 3.1-3.5% in controls) • ↑ in total malformations, non-specific, difficult to associate with treatment. 5 litters affected in the top dose group vs 1 litter in control group (table 3.10.1.5-5, RAR) • ↓ mean foetal bw (-17%) Maternal tox: • ↓ bw gain over the dosing period dramatic in the top dose does (a loss of 159 g) relative to controls (a gain of 238 g) • ↓ Day 7-19 food consumption at top dose (-65%) • Top dose: 1 x doe sacrificed.	RAR 3.10.1.4
Study 3 (Anonymous, 1987) NZW rabbit Limited	USEPA Guideline 83-3 / GLP yes	Captan teratology study → purity 91%. Dose: 0, 10, 40, 160 mg/kg bw/d • Dosing period GD7-GD19 • 2/16 top dose animals sacrificed, aborting • ↑ post implantation loss, including one total resorption (19.6% vs 5.8%) • no remarkable malformations observed at necropsy	RAR 3.10.1.5

		Maternal tox: • ↓ bw over the dosing period minor in the top dose does • ↓ Day 7-19 food consumption drastic at top dose (-53 to -83 %)	
Study 4 (Anonymous, 1981) NZW rabbit Limited Animals from different sources	Non Guideline / GLP yes	Captan teratology study → purity 89%. Dose: 0, 6, 12, 25, 60 mg/kg bw/d • Dosing period GD6-GD28 • no treatment-related effect on litter size and post implantation loss • no malformations observed at necropsy Maternal tox: • ↓ bw gain over the dosing period (-23%) in the top dose dams • ↑ non-pregnant does at top dose (6/15 vs 1/15 in controls)	RAR 3.10.1.6
Study 5 (Anonymous, 2006a) NZW rabbit Acceptable	OECD TG 414 (1981) / GLP yes	Captan teratology study → purity 95% Dose: 0, 10, 20, 45 mg/kg bw/d • Dosing period GD6-GD28 • ↑ post implantation loss (15.3% vs 4.9%) • ↓ foetal bw (-12 to -14%) in the top dose group. • Top dose group 4/180 foetuses (2/23 litters) had absent kidney and ureter vs 0 in control group • no other treatment related malformations observed at necropsy Maternal tox: • Little effect on bw • ↓ bw gain in top dose group (-77%)	RAR 3.10.1.7
Study 6 (Anonymous, 2006b) NZW rabbit Acceptable	OECD TG 414 (1981) / GLP yes	THPI teratology study → purity 98.4% Dose: 0, 5, 10, 22.5 mg/kg bw/d • Dosing period GD6-GD28 • Prenatal development unaffected Maternal tox: • no treatment-related deaths or any other indicators of maternal toxicity	RAR 3.10.3.2

NZW: New Zealand White

In addition, the rat 3-generation study shows a clear effect at 250 and 500 mg/kg bw/d on pup body weight at birth and to weaning through to each generation. These effects however are noted at dose levels that clearly exceed the MTD for the parental animals where significant decreases in food consumption and body weight were reported.

In the rat prenatal developmental toxicity study (study 1, Anonymous, 1987; 3.10.1.3), captan did not induce foetal malformations at the highest dose level administered, 450 mg/kg bw/d. The rabbit developmental studies clearly illustrate the susceptibility of the rabbit to captan with dramatic reductions in food consumption and highly variable body weight changes. Some foetal malformations were seen in some of the rabbit studies but there was no indication of a specific malformation or set of malformations, and the diversity of malformations as well as their

individual low numerical incidence in the presence of severe maternal toxicity introduces substantial uncertainty in assigning a treatment-related effect of captan on the developing foetus.

One prenatal developmental toxicity study with THPI was conducted in the rabbit (study 6, Anonymous, 2006b, 3.10.3.2). THPI has a structure similar to thalidomide which is a known teratogenic substance in the rabbit. The dose rates used were precisely comparable with those of captan in study 5 (Anonymous, 2006a, 3.10.1.7). Single malformations were recorded at 5 and 10 mg/kg bw/d. These were considered to be spontaneous in origin and were not related to the administration of THPI.

Maternal toxicity was marked in the rabbit at ≥ 100 mg/kg bw/d. Effects above this dose level included reductions in food consumption > 65%, clinical signs and weight loss during pregnancy.

Assessment of adverse effects on development for classification

There are no epidemiological data available that could support captan classification into Category 1A.

The animal studies provide no clear or robust evidence of an adverse effect on development in the absence of other toxic effects that could support classification into Category 1B.

From the available toxicity dataset, there is no evidence that captan induces developmental effects in the absence of maternal toxicity in either rats or rabbits. Increased post implantation losses, increased incidences of malformations and structural abnormalities and decreased foetal weights were observed in several studies at dose levels inducing moderate to high maternal toxicity as evidenced by a drastic decrease in food consumption along with reduced body weights during the dosing period and reduced faecal output. At 100 and 160 mg/kg bw/d, resorption and possibly abortion are likely attributable to maternal toxicity. At lower dose levels, the effects on the foetus were confined to slightly reduced body weight and an increase in skeletal variations causing no permanent structural impairment.

A position paper (Anonymous, 2018) was submitted to investigate captan potential to induce developmental effects in mammalian species with respect to potential reproductive classification. Several points were noted by the DS including:

- Captan very short half-life in blood indicates little to no exposure of the developing offspring in utero.
- Rabbit developmental toxicity studies provide evidence that neither captan nor its metabolite THPI induce malformation in the rabbit.
- No evidence is available from other rabbit developmental toxicity studies, including those in the published literature, of malformations induced by captan or THPI.
- Similarly, no evidence of malformations induced by captan or THPI is available from rat studies.
- No evidence from mouse or primate studies in the published literature.

Overall, RAC agrees with the DS that the development effects observed in the rat and rabbit studies are most likely secondary consequences of moderate maternal toxicity rather than specific, captan induced developmental effects. **RAC concludes that no classification for developmental toxicity is warranted, as proposed by the DS.**

Assessment of effects on or via lactation

For classification for effects on or via lactation, the CLP criteria require:

i. Human evidence indicating a hazard to babies during the lactation period…and/or…

ii. Results of one or two generation studies in animals which provide clear evidence of adverse effects in the offspring due to transfer in the milk or adverse effect on the quality of the milk…and/or…

iii. ADME studies indicating the substance is present in potentially toxic levels in breast milk.

There is no human evidence to indicate a hazard to babies.

In the rat multi-generation and single-generation studies offspring were exposed during lactation and also in the diet in increasing amounts from around PND14 onwards. At 250 mg/kg bw/d a variable small reduction in survival was noted through lactation day (LD) 4 of the F1a, F2a and F3a litters, reaching statistical significance for the F2a (96-95% LD1-4) and F3a litters (97% LD4). Variable treatment-related reductions in pup survival were also observed on LD4 at 500 mg/kg bw/d in the litters of all three generations (85-93%). There was no indication that treatment affected dam nursing behaviour affecting the weight gain development of the pups. Pup body weight was significantly impacted from LD1 to weaning and beyond.

No information was available on the quantity or quality of the milk produced by the dams, nor was the rat milk analysed for the presence of captan or metabolites. Absorption, metabolism, distribution and excretion studies indicate captan is quickly eliminated and rapidly decomposed such that there is no evidence to indicate any substantial systemic absorption can occur. Consequently. it is unlikely to be present in breast milk. The available data is inconclusive with respect to linking the effects in the post-natal rat pups to lactation. The retarded body weight development during the lactation period is not entirely clear, it may be a secondary effect of significant maternal toxicity. Transfer of the active substance into milk has not been demonstrated. RAC agrees with the DS that **no classification 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

Captan (ISO) is a PPP active substance used on many crops to prevent disease infection and establishment and has a current entry in Annex VI of the Regulation (EC) 1272/2008 (CLP) as Aquatic Acute 1 (H400) with an M-factor of 10.

The DS considered that captan should be considered as rapidly degradable and not expected to have a bioaccumulation potential.

For acute aquatic toxicity, the DS considered fish as the most sensitive trophic level. However, they noted that no reliable fish acute toxicity test with the active substance (captan) is available, as no appropriate exposure throughout the test duration was maintained in the available test. Therefore, the DS proposed classification as Aquatic Acute 1 with an M-factor of 10, based on

the 96h LC_{50} value of 0.0147 mg/L for *Oncorhynchus mykiss* based on mean measured concentrations of captan using an 83% WP formulation.

For chronic aquatic toxicity, the DS considered that the similarity between captan and folpet regarding mechanistic properties, endpoint specific MoA, and empiric properties was sufficient for read-across. Observed differences in the structure of captan and folpet (cyclic compound) are not considered to have an impact on the toxicity of the active substance considering the MoA. Therefore, available chronic toxicity data of folpet might be used for aquatic chronic classification of captan as well. Hence, the DS proposed to classify captan as Aquatic Chronic 1 with an M-factor of 1 based on the NOEC value of 0.00881 mg folpet/L and folpet being not rapidly degradable, from a valid and reliable fish early life stage study conducted with folpet.

Degradation

Hydrolysis

Two studies were available for captan. According to the first hydrolysis study (US EPA 161-1, study 1, 1989a), captan was found to be hydrolytically instable, with DT_{50} values at 25°C of 18.4h (pH 5), 4.8h (pH 7) and 7.8 min (pH 9).

The second hydrolysis study (US EPA 161-1, study 2, 1989b) with captan and its metabolite Tetrahydrophthalimido-thiocarbonate (THPC), indicated similar DT_{50} values at 25°C of 11.7h (pH5), 4.6h (pH7) and 8.1 min (pH9) for captan. DT_{50} values of 15.1h (pH 5), 8.1h (pH 7) and 12 min (pH 9) at 25°C for the metabolite THPC were determined. No further information was provided in the CLH report regarding the metabolite THPC.

One hydrolysis study on the further metabolite 4,5-cyclohexene-1,2-dicarboximide (THPI) and one hydrolysis study on further metabolite 3-cyclohexene-1-carboxylic acid-6-(aminocarbonyl) (THPAM), both according to OECD TG 111, have been provided in CLH report. DT_{50} values for metabolite THPI at 25°C were stable (pH 4), 152 d (pH 7), and 3.2 d (pH 9). DT_{50} values for metabolite THPAM at 25°C were: 4 d (pH 4), 360 d (pH 7) and stable (pH 9).

Photolysis

One study for each of captan, the metabolite THPI, and the metabolite THPAM are available on photochemical degradation. Studies on both metabolites according to SETAC, OECD guidance document (97)21 indicates that both metabolites are stable at pH 7 and 22°C. Photolysis of captan was investigated in sterile aqueous buffer solution at pH 5 according to the US EPA 161-2 guideline. The DT_{50} value of 9.9h was derived in the irradiated solution, and 10.1h in the dark control. As results indicate that the chemical hydrolysis is the principal route of degradation and that photolysis is a very minor route of degradation, no photolytic degradation products were determined.

Ready Biodegradability

Based on a ready biodegradability test (OECD TG 301B) indicating 13% degradation after 28 days, captan is considered as not readily biodegradable.

Simulation studies

One study (OECD TG 309) on aerobic mineralisation of captan in surface water under aerobic conditions in the dark at 20 ± 3°C and at pH 8-8.1 for 60 days indicated that captan underwent rapid primary degradation and was not detectable after 1 h of incubation (DT_{50} < 1h).

One water/sediment study (following the SETAC guideline) indicated that in two aerobic natural water/sediments systems under laboratory conditions in the dark at 20°C and up to 90 days, captan degraded very rapidly with a DT_{50} < 1 day to metabolites including THPI. The

mineralisation of 49-53% CO_2 were determined as AR after 90 days application. THPI was further degraded to THPAM and 4-cyclohexene-1,2-dicarboxylic acid (THPAI).

Whole system DT_{50} values for THPI of 4.7 days and for THPAM of 13.6 days were obtained. No further information on THPAI in the CLH report have been provided regarding the degradation pathway.

Overall, following the results summarised above, the DS considered that captan is rapidly degradable in the aquatic environment due to rapid primary degradation (DT_{50} < 16 days) and the degradation products (THPI and THPAM) do not meet the criteria for classification as hazardous to the aquatic environment.

Bioaccumulation

Two studies on the fish bioaccumulation were provided for captan. Based on the fish bioaccumulation study (Anonymous, 1988a) with *Lepomis macrochirus*, a BCF (whole fish) of 250 was estimated. Based on the other fish bioaccumulation study (Anonymous, 1988b), also with with *L. macrochirus*, a BCF (whole fish) of 134 was estimated. Depuration after 14 days was greater than 89% and 94%, respectively. While both studies were conducted with only one test concentration instead of two and have some further deficiencies (lack of information on the lipid content of the fish and the lack of detailed information on the environmental test conditions (temperature, pH, dissolved oxygen) and information on residues and metabolites), the studies were considered as valid and reliable by the DS, giving a highest $BCF_{steady\text{-}state}$ of 250.

The measured log P_{ow} of captan was determined to be between 2.50 and 2.52. The estimated log P_{ow} was 2.85 by using US EPA software KOWWIN (EPIWEB 4.1).

The experimentally determined log P_{ow} for metabolites THPAM of -0.40 and THPI of 0.22 were provided as well. For the metabolite THPAI, a log P_{ow} of 0.77 was obtained by using US EPA software KOWWIN (EPIWEB 4.1).

Overall, based on the results summarised above, the DS concluded that captan has a low potential for bioaccumulation.

Aquatic Toxicity

The aquatic toxicology test results from available acute and chronic studies of captan and its formulations (80 WDG and 83% WP) are summarised in the following table and sections. The DS indicated that information on the composition of the formulations is provided in the confidential annex to the CLH report. The formulations mainly consist of the active substance (~80%) and the co-formulants used are not considered to significantly increase the toxicity of the formulation.

Regarding aquatic toxicity of the degradation products, the DS provided aquatic acute toxicity studies for metabolites THPI and THPAM and considered that degradation products do not meet the criteria for classification as hazardous to the aquatic environment. The provided information indicated that aquatic acute toxicity of the degradation products (THPI and THPAM) for fish were 96h LC_{50} > 120 mg/L, for invertebrates – 48h EC_{50} > 120 – 220 mg/L, and for algae 72h E_rC_{50} 41 – > 180 mg/L.

<u>Acute Aquatic toxicity of captan and formulations</u>

Regarding aquatic toxicity of the parent substance, the DS provided available aquatic acute toxicity studies and considered that most sensitive trophic level was fish.

***Table**: Short-term aquatic toxicity data for captan*

Test method	Test organism	Short-term result (endpoint)	Reference / Test item
Fish			
OECD TG 203 (1992)	*Salmo trutta*	4h LC_{50} = 0.0511 mg/L (mm) 24h LC_{50} = 0.0246 mg/L (mm) 96h LC_{50} = 0.0913 mg/L (im)	Anonymous (2016a) captan 95.6%
OECD TG 203 (1984)	*Oncorhynchus mykiss*	96h LC_{50} >0.036 mg/L (mm)	Anonymous (1993a) 83% WP formulation
OECD TG 203 (1992)	*Oncorhynchus mykiss*	96h LC_{50} = 0.0147 mg/L (mm)	Anonymous (1995) 83% WP formulation
Aquatic invertebrates			
OECD TG 202 (1984)	*Daphnia magna*	48h EC_{50} = 0.289 mg/L (mm)	Anonymous (1996) merpan 80 WDG
Algae/other aquatic plants			
OECD TG 201 (2011)/EC C.3 (2009)	*Raphidocelis subcapitata*	72h E_rC_{50} = 0.66 mg/L (mm) 72h E_yC_{50} = 0.432 mg/L (mm)	Anonymous (2016c) captan 95.8%
OECD TG 201 (2011)/EC C.3 (2009)	*Raphidocelis subcapitata*	72h E_rC_{50} = 0.78 mg/L (mm) 72h E_yC_{50} = 0.44 mg/L (mm)	Anonymous (2016c) captan 80 WDG
OECD TG 201 (1984)	*Raphidocelis subcapitata*	72h E_rC_{50} = 0.316 mg/L (mm) 72h E_yC_{50} = 0.154 mg/L (mm)	Anonymous (1994) 83% WP formulation

mm – mean measured; im – initial measure; nom – nominal concentrations.

The acute fish toxicity study (OECD TG 203) with brown trout (*S. trutta*) and conducted with captan, was not considered valid and reliable by the DS as no appropriate exposure throughout the test duration was maintained. Nevertheless, a 96h LC_{50} of 0.0913 mg/L was calculated, based on initial measured concentrations. In addition, for classification purposes 4h and 24h LC_{50}s, based on geometric mean measured concentrations, were derived. However, the reliability of the endpoint is low, considering that no confidence interval could be calculated due to the steep dose response. The 24h LC_{50} of 0.025 mg a.s./L was determined based on predicted mean measured concentrations. The DS considered this endpoint as additional information.

Acute toxicity studies with the rainbow trout (*O. mykiss*) conducted with an 83% WP formulation were considered valid and reliable by the DS. The studies were conducted under flow-through test conditions and the endpoints of 96h LC_{50} >0.036 mg/L and 96h LC_{50} of 0.0147 mg a.s./L, were expressed as mean measured concentrations.

An acute toxicity test with *Daphnia magna* conducted with the formulation Merpan 80 WDG was considered reliable by the DS although there were also some uncertainties. The study was conducted under semi-static test conditions and an endpoint was derived based on the available data. Analytical measurements were only conducted in three of the nine test concentrations. The 48h EC_{50} of 0.289 mg a.s./L based on mean measured concentrations was calculated. The endpoint was determined considering predicted mean measured concentrations based on the available analytical measurements.

The DS did not provide acute toxicity tests with *D. magna* conducted with the active substance captan. It is noted in the CLH report that such studies were assessed to be not reliable because of the rapid degradation of captan in the test medium, and exposure throughout the study duration could not be maintained.

Aquatic toxicity studies with the green algae *Raphidocelis subcapitata* were conducted with the active substance captan and the formulations captan 80 WDG and 83% WP. All studies were conducted under static test conditions and no appropriate exposure could be maintained throughout the study duration of 72/96 hours. Due to the lack of analytical verification of the test substance, it was agreed to express the endpoint based on mean measured concentrations. As a pragmatic approach, LOQ/2 was used to calculate the mean measured concentrations for the test concentrations below the LOQ. The lowest endpoint for algae was the 72h E_rC_{50} of 0.316 mg a.s./L, derived from a study with the 83% WP formulation. For the active substance captan, the lowest endpoint was 72h E_rC_{50} of 0.660 mg a.s./L, based on mean measured concentrations.

Overall, based on the results summarised above, the DS proposed to classify captan as Aquatic Acute 1 based on the 72h LC_{50} of 0.0147 mg/L (mean measured) for *O. mykiss* conducted with an 83% WP formulation. The 24h LC_{50} of 0.0246 mg/L (mean measured) for *S. trutta* conducted with active substance captan was proposed as supportive information. As these acute toxicity value falls within the $0.01 < L(E)C_{50} \leq 0.1$ mg/L range, the acute M-factor proposed by the DS was 10.

Aquatic Chronic toxicity of captan and formulations

***Table**: Long-term aquatic toxicity data for captan*

Test method	Test organism	Long-term result (endpoint)	Reference / Test item/ Purity
Fish			
OECD TG 210 (2013)/US EPA OPPTS 850.1400	*Oncorhynchus mykiss*	95d NOEC = 0.2 mg/L (nom)*	Anonymous (2016) captan 80 WDG
Aquatic invertebrates			
OECD TG 211 (2012)	*Daphnia magna*	28d NOEC = 0.4 mg/L (nom)*	Anonymous (2017) captan
Algae / other aquatic plants			
OECD TG 201 (2011)/EC C.3 (2009)	*Raphidocelis subcapitata*	72h NOE_rC = 0.217 mg/L (mm)	Anonymous (2016c) captan
OECD TG 201 (2011)/EC C.3 (2009)	*Raphidocelis subcapitata*	72h NOE_rC = 0.293 mg/L (mm)	Anonymous (2016d) captan 80 WDG
OECD TG 201 (1984)	*Raphidocelis subcapitata*	72h NOE_rC = 0.077 mg/L (mm)	Anonymous (1994) 83% WP formulation

mm – mean measured; im – initial measure; nom – nominal concentrations; * - Not considered reliable by the Dosser Submitter.

The chronic fish study (OECD TG 210) with Rainbow trout (*O. mykiss*) conducted with a captan 80% WDG formulation and the chronic toxicity test with *D. magna* conducted with the active substance captan were not considered valid and reliable by the DS. In both cases, the measured concentrations do not remain within 80-120 % of the nominal concentrations, therefore the effect concentrations cannot be based on nominal or initial measured concentrations.

Aquatic toxicity studies with the green algae *R. subcapitata* were conducted with the active substance captan, as well as the formulations captan 80 WDG and 83% WP. All studies were conducted under static test conditions and hence no appropriate exposure could be maintained throughout the study duration of 72/96 hours. Due to the lack of analytical verification of the test substance, it was agreed to express the endpoint based on mean measured concentrations. As a pragmatic approach, it was agreed to use the LOQ/2 to calculate the mean measured

concentrations for the test concentrations below the LOQ. The lowest endpoint for algae was the 72h NOE_rC of 0.077 mg a.s./L, derived from a study with the 83% WP formulation. For the active substance captan, the lowest endpoint was 72h NOE_rC of 0.217 mg a.s./L, based on mean measured concentrations.

Overall, the DS considered that no reliable chronic toxicity data with the most sensitive species are available and noted that:

- no chronic classification is foreseen considering the rapidly degradation of the active substance in the water-sediment system and the low potential for bioaccumulation (BCF ≤ 500, log K_{ow} ≤ 4)

Nevertheless, the DS considered that the study used for chronic classification of another active substance folpet might also be considered for captan for which no appropriate chronic data with fish are available. Thus, the DS provided the read-across assessment conducted using structural information, available fate and toxicity data, and supporting information generated using the OECD QSAR Toolbox (ver. 4.5.) to demonstrate the similarity between captan and folpet. The DS consequently proposed to classify captan as Aquatic Chronic 1 (M = 1) based on the NOEC of 0.00881 mg folpet/L obtained in valid and reliable fish early life stage study, also considering folpet as rapidly degradable.

Comments received during the consultation

Four Member States (MS) and one National Authority (NA) commented on the environmental part of DS's proposal. All of them agreed or did not express objection on the proposed classification as Aquatic Acute 1 (M = 10). However, they disagreed with the DS or had comments regarding:

Degradation and/or degradation products

One MS disagreed with the DS's conclusion on rapidly degradability. The MS indicated that captan is not readily biodegradable according to the ready biodegradation screening study and the results of the simulation studies would not change this conclusion. Although the DT_{50} in the whole water sediment system for captan and its metabolites THPI and THPAM is below 16 days, this refers only to primary degradation, not ultimate degradation. Mineralisation in the water/sediment study is only 49-53% on day 90 after application. This means that a DT_{50} considering mineralisation is about 90 days. Therefore, captan is not ultimately degraded with a half-life of below 16 days. The rapid hydrolysis is only primary degradation and there is no mineralisation of above 70% within 28 days. Therefore, captan should be considered as not rapidly degradable.

The NA also noted that degradation studies do not demonstrate that > 70% of the substance is ultimately degraded within 28 days under environmentally relevant conditions. In addition, the NA indicated that surface water and water-sediment simulation studies were conducted at around pH 8 which favours the hydrolysis of THPI, whereas the hydrolysis DT_{50} for THPI are considerably greater (≥ 16 days) under more acidic or neutral environmental conditions at pH 4 and 7 than at pH 9.

In addition to the degradation products, the NA noted that for degradation products THPI and THPAM, no information on the chronic toxicity to fish and aquatic invertebrates is available. The NA also indicated that for THCY and THPAI (other identified major metabolites) no aquatic toxicity data is available at all. The metabolite THPC formed rapidly and degraded rapidly so was considered only a transient degradant.

In answer to the comments related to degradation and/or degradation products, the DS replied that captan is degraded in water/sediment with DT_{50} < 16 days and its degradation products THPI and THPAM do not fulfil the criteria for classification as hazardous to the aquatic environment. Therefore, according to the CLP regulation captan is classified as rapidly degradable. The metabolite THPAI does not exceed the trigger values (2 x > 5 %, > 10 %, max at end of study) in water or in sediment separately and was therefore not included in the residue definition and not considered further in the assessment. The metabolite THCY was not detected in an aerobic water/sediment study and was only detected under anaerobic conditions in soil and water/sediment studies and in systems with (assumed) low oxygen concentrations. Under aerobic conditions THCY quickly degrades in soil. Therefore, the metabolite THPAI and THCY was not included in the residue definition and not considered further in PPP assessment, resulting in no aquatic toxicity studies being submitted by the applicant.

Although the DS agreed that there are no chronic toxicity data with the degradation products THPAM and THPI they noted that submission of chronic toxicity data is not triggered and is also not required for the renewal of the active substance captan.

Formulations

The NA asked to confirm whether it is appropriate to use these formulation studies because formulation studies have been used as key studies and the information on the composition of the formulations is in a confidential annex. The DS confirmed that studies conducted with formulations were also used for the risk assessment and therefore are considered acceptable by the DS to be used for classification purposes. The formulations mainly consist of the active substance (~ 80%) and the co-formulants used are not considered to significantly increase the toxicity of the formulation.

Read across

The NA pointed out that the CLH proposal for folpet, which has been used as part of the read-across in this CLH proposal for captan, has been subject to public consultation and is currently awaiting RAC discussion. The consultation comments and RAC opinion should ideally be finalised before a conclusion on read-across is accepted as there could be an impact on the rapid degradability of folpet which could further impact the rapid degradability conclusion for captan.

Aquatic toxicity studies

The NA noted that in two acute toxicity studies with *S. trutta* (Anonymous, 2002b; 2016a) analysis of test/stock solutions at 0h indicated correct dosing with measured concentrations within 80-120% of the nominal. Rapid primary degradation of captan is expected over acute exposure periods and available data for the degradants indicate that these are much less toxic, with endpoints not meeting the classification criteria for hazardous to the aquatic environment. This suggests that captan is driving the toxicity observed in the studies with the parent substance. In the absence of more reliable data, acute toxicity endpoints for captan based on nominal or initial measured concentrations could be relevant for classification, despite the lack of chemical analysis over the whole exposure duration. Test validity criteria were met in both of the acute *S. trutta* studies. The DS indicated that the CLP guidance is clear that when measured concentrations do not remain within 80-120 % of the nominal concentrations, the effect concentrations cannot be based on nominal or initial measured concentrations and should be expressed as mean measured concentrations. The DS pointed out that especially for rapidly degradable substances the endpoints may differ greatly depending on whether they are expressed in terms of nominal/initial measured or mean measured concentrations. The use of endpoints expressed in terms of nominal or initial measured concentrations might be acceptable in the risk assessment but not for classification.

Regarding chronic toxicity studies, the MS indicated that in contrast to the DS statement that no reliable chronic toxicity data for the most sensitive species are available, many chronic fish toxicity test data are available for captan and provide reference to the RIVM report (2008), which is largely based on Draft Assessment Report (DAR, 2005) for captan: https://www.rivm.nl/bibliotheek/rapporten/601716004.pdf. The MS pointed out that some of studies cited in the RVIM report, such as a Fish Full Life Cycle Toxicity with Fathead minnow (*Pimephales promelas*) (Hermanutz *et al*., 1973) and/or chronic development study with *Cancer magister* (Caldwell *et al*., 1978), could provide relevant data for the classification of captan.

In addition, the MS referred to the Fish Short-term Reproduction Assay (FSTRA, OECD TG 229, 2012) and Amphibian Metamorphosis Assay (AMA, OECD TG 231, 2013), which are available in the US EPA Endocrine Disruptor Screening Program and can be found at the website: https://www.epa.gov/endocrine-disruption/status-endocrine-disruptor-screening-program-tier-1-screening-results-and-data.

The MS also asked the DS to explain why the lowest value 72h NOE_rC of 0.077 mg a.s./L for algae *R. subcapitata* from the study conducted with 83% WP formulation (Anonymous, 1994) have not been considered for classification.

In answer to these points raised by the MS, the DS stated that these studies were not submitted by the applicant or were not considered relevant during the renewal of the active substance captan and were thus not evaluated by the DS. All data submitted by the applicant during the renewal of the active substance captan were included in the CLH report.

The study by Hermanutz *et al*. (1973) was not submitted by the applicant and was also not mentioned in the literature search provided for the renewal of the active substance. Hence, the study was not evaluated by the RMS. This is also the case for the chronic developmental study with *C. magister* (Caldwell *et al*., 1978).

Nevertheless, the DS agreed that the fish short-term reproduction assay might be useful to consider for aquatic chronic classification and noted that a NOEC of 0.01 µg a.s./L (a DS typing mistake as it should be 0.01 mg a.s./L) can be determined. Regarding the Amphibian Metamorphosis Assay, the DS noted that this study might be considered as additional information. However, as most of the endpoints are endocrine disruption-specific endpoints, the relevance of the study is questionable. Still, the DS pointed out that considering fresh weight and development stage, a NOEC of 0.0073 mg a.s./L could be determined.

Regarding the last point raised by the MS, the DS explained that the results from the study with algae *R. subcapitata* conducted with 83% WP formulation (Anonymous, 1994) might be considered as additional information. Nevertheless, considering that fish is the most sensitive trophic level, the DS was of the opinion that the use of the algae study would result in a less conservative classification. Still, the NOEC of 0.077 mg a.s./L might be considered as additional information.

Additional key elements

Additional aquatic toxicity studies

RAC was made aware of additional relevant data during the consultation and has assessed the additional toxicity data provided during the consultation. This data came from the following source reports:

- RIVM (2008) Environmental risk limits for captan
- US EPA Endocrine Disruptor Screening Program (US EPA EDSP)

It should be noted that only relevant chronic toxicity data considered reliable in the source report (Klimisch scores 1/2, or other scoring method) are discussed below.

***Table**: Additional chronic toxicity data on fish and crustacea from the RIVM report on Environmental risk limits for captan (RIVM, 2008) and US EPA Endocrine Disruptor Screening Program (US EPA EDSP)*

Test method	Test organism	Long-term result (endpoint)	Reference/Test item/Klimisch score	Source
Fish				
Fish early life stage (ELS) test/OECD TG 210	*Pimephales promelas*	30d NOEC (growth) = 0.017 mg/L 30d NOEC (mortality) = 0.017 mg/L	EC, 2005 (DAR), RVMI report, Hermanutz *et al*. (1973)/captan 88.4%/2	RIVM (2008)
Fish Short-term Reproduction Assay (FSTRA)/OECD TG 229/EPA Guideline 890.1350	*Pimephales promelas*	21d NOEC ≥ 0.01 mg/L	US EPA EDSP, 2012, EPA MRID Number 48669501/captan 94.9%/Requirement Satisfied	US EPA EDSP
Fish early life stage (ELS) test/OECD TG 210/EPA Guideline 850.1500	*Pimephales promelas*	45w NOEC (growth) = 0.0168 mg/L 45w NOEC (mortality) = 0.04 mg/L	US EPA EDSP, EPA MRID Number 00057846/captan 88.4% / -	RIVM (2008) and US EPA EDSP
Fish early life stage (ELS) test/OECD TG 210	*Pimephales promelas*	45w NOEC (growth) = 0.017 mg/L 45w NOEC (mortality) = 0.04 mg/L	EC, 2005 (DAR), RVMI report, Hermanutz *et al*., 1973/captan 88.4%/2	RIVM (2008) and US EPA EDSP
Crustacea				
Chronic developmental study	*Cancer magister 1st stage zoae*	69d NOEC (molting) = 0.0031 mg/L 36d NOEC (mortality) ≥ 0.51 mg/L 80d NOEC (mortality) ≥ 0.29 mg/L 75d NOEC (mortality) ≥ 0.34 mg/L	EC, 2005 (DAR), RVMI report, Caldwell *et al*. (1978)/captan 92.8% /1	RIVM (2008)
Other aquatic organisms				
Amphibian Metamorphosis Assay (AMA)/OECD 231/EPA Guideline 890.1100	*African Clawed Frog (Xenopus laevis)*	96h L(E)C_{50} = 0.0774 mg/L 21d NOEC > 0.0072 mg/L	US EPA EDSP, 2013, EPA MRID Number 49136701/captan 93%/Requirement Satisfied	US EPA EDSP

Information from the RIVM report "Environmental risk limits for captan" (2008)

There are no robust summaries for the studies provided, so RAC was not able to evaluate acceptability and reliability of these studies. RAC is also not in a position to clarify why studies

available in the Draft Assessment Report (DAR, 2005) was not considered in the Draft Renewal Assessment Report (DRAR, 2012). As RAC do not have access to the full RAR or DRAR of captan, evaluation of these studies can only rely on available information in the RVIM report.

Overall, the chronic development toxicity with *C. magister* study indicates a 69d NOEC of 0.0031 mg/L for molting. Although given the reliability of this study is stated as 1 in the source report, RAC concedes that is not standard test and not standard species used in classification process. Without robust summary of the study, RAC cannot evaluate and confirm that obtained NOEC is acceptable and reliable from classification perspective according to CLP criteria.

Overall, fish early life stage test with *P. promelas* is a common test which are frequently used in classification process. Although Klimisch score of the study is stated as 2, without further information RAC is not in the position to assess reliability and acceptability of the obtained 30 d-NOEC (growth) of 0.017 mg/L. As such, this study can only be used as supporting information.

Information from the US EPA Endocrine Disruptor Screening Program (US EPA EDSP)

It should be noted that for both studies of relevance for CLP, only "Requirement Satisfied" was indicated, no Klimisch scores have been assigned.

The 21 day short-term reproduction assay (MRID 48669501) of captan with fathead minnow (*P. promelas*) was conducted under flow-through conditions. Mean-measured concentrations were < 0.000014 (<LOQ; controls), 0.00011, 0.00099, and 0.010 mg a.s./L. The test system was maintained at 24 to 26°C and a pH of 7.0 to 7.7. Overall survival in the negative (clean water) and solvent controls was 88 and 96%, respectively. Overall survival was 100% in all captan treatment groups and was not significantly different from the negative control. Significant increases in male body weight at the high treatment level and in male body length at the mid and high treatment levels were observed relative to the negative control. There were no treatment-related effects on secondary sex characteristics or clinical signs. In the negative and solvent controls, spawning occurred at least every 4 days in three out of four replicates in the negative control and two out of four replicates in the solvent control. Fecundity and fertilization success was not significantly different in any treatment group relative to the negative control. Plasma vitellogenin (VTG) was significantly increased in males at the mid and high treatment levels relative to the negative control. However, the increase was not dose related. No EC_{10} or NOEC was obtained.

Overall, no reliable EC_{10} or NOEC can be derived from the available information. However, RAC considers that significant increases in male body weight at the high treatment level and in male body length at the mid and high treatment levels suggest a 21d NOEC of ≥ 0.01 mg/L.

The 21 day assay (MRID 49136701) of captan on Amphibian Metamorphosis (AMA) of African clawed frog (*Xenopus laevis*) was conducted under flow-through conditions. Mean-measured concentrations were < 0.0000091 (<LOQ; controls), 0.000064, 0.00077, and 0.0073 mg a.i./L. Reviewer-calculated time-weighted average (TWA) measured concentrations were < 0.0000091 (<LOQ; controls), 0.000060, 0.00074, and 0.0072 mg a.i./L. The test system was maintained at 21 to 23°C and a pH of 6.9 to 7.8. There were no significant differences between the negative control and solvent control. Throughout the 21-day exposure period, mortality did not exceed 3% in any treatment group. There were no significant effects of treatment on body weight or snout-vent length. As indicated in the study after 96 hours of exposure, the LC_{50}/EC_{50} values (based on sublethal effects) were 0.0774 mg/L. No EC_{10} or NOEC was obtained.

Overall, the AMA study result is related to endocrine disruptors specific endpoints and usually are not commonly used for classification purposes. Nevertheless, in the past RAC has classified based on ED endpoints. From the available information, no reliable EC_{10} or NOEC can be derived. At the highest treatment level no significant effects was observed. Therefore, RAC considers that a 21d

NOEC of >0.0072 mg/L could be obtained. However, this value might only be used as supportive information in the classification process.

Information from the RIVM report "Environmental risk limits for captan" (2008) and US EPA Endocrine Disruptor Screening Program (US EPA EDSP)

Fish early life stage (ELS) study for 45 weeks by Hermanutz *et al*. (1973) are presented in both sources. The only difference is the rounded NOEC (growth) value.

Groups of 9 day old Fathead minnow (*P. promelas*) embryos (25 embryos/aquaria; 2 aquaria/dose group) were exposed to captan (88.4% purity) at nominal concentrations of 0 (negative control), 12.5-15.6, 25-31.3, 50-62.5, 100-125, or 200-250 µg/L under flow-through conditions for 45 weeks, with the surfactant (Triton X-100) added at 6.7x10-6% v/v. Concentrations were measured daily and test results are based on mean measured concentrations. For the chronic exposure of Fathead minnow, survival of the parental fish was decreased in the mean-measured 63.5 µg/L treatment group (↓98%). The parental fish length and the F_1 fish survival and length were also decreased in the mean-measured 39.5 µg/L treatment group. At the medium and medium-high concentrations (16.8 and 39.5 µg/L), the mean number of spawnings/female were decreased by 82 and 96%, respectively, compared to the control, and the mean number of eggs spawned/female were decreased, by 77 and 98%, respectively. The mean number of eggs/spawning was also decreased at the medium-high concentration by 42%, compared to the control. Percent hatchability of embryos from unexposed parents incubated at 63.5 µg/L captan was similar to that of control embryos, but all larvae died 5-8 days after hatching. There was 100% mortality of 1 day old larvae from unexposed parents incubated at 63.5 µg/L captan within 24 hours. Survival of 3 day old hatchlings in the medium-high concentration group was decreased by approximately 50%. In conclusion, a NOEC of 16.8 µg/L was derived.

Overall, the fish early life stage test with *P. promelas* is a common test which is frequently used for classification. RAC concludes that based on the available short summary the study (US EPA EDSP, EPA MRID Number 00057846) in the US EPA EDSP, the study seems to be relevant and reliable. Therefore, RAC concludes that the 45w NOEC (growth) of 0.0168 mg/L can be used for classification.

Assessment and comparison with the classification criteria

Degradation

RAC agrees that based on a ready biodegradability test result of 13% after 28 days in a test following OECD TG 301B, captan is considered as not readily biodegradable.

The water/sediment study under laboratory conditions in the dark at 20°C and up to 90 days, derived a DT_{50} < 1 day for captan in water and whole system indicates that captan undergoes rapid primary degradation. However, mineralisation was only around 50% AR determined as CO_2 on day 90 after application (< 10% CO_2 after 30-day).

Aerobic mineralisation of captan in surface water study under aerobic conditions in the dark at 20 ± 3°C at pH 8.0-8.1 for 60 days, gave a DT_{50} < 1 day and indicates that captan undergoes rapid primary degradation. However, mineralisation at maximum amounts of 3.4% AR were detected.

The two hydrolysis studies with captan obtained DT_{50} values of:

- 18.4 hours at pH5, 4,8 hours at pH7 and 7.8 min at pH9 (25°C); and
- 11.7 hours at pH5, 4.6 hours at pH7 and 8.1 min at pH9 (25°C)

indicate that the longest half-life t½ of captan determined within the pH range 4-9 is significantly shorter than 16 days. However, in the first study (study 1, 1989a), at pH 7 and pH 9 two unknown metabolites (> 10% AR) increased during the study and reached their maximum occurrence at the end of the study. A further information specifies that one unknow metabolite was THPC and the other could not be identified, although it was thought to be thiocarbonic acid or a corresponding salt.

Hydrolysis is the main route of degradation of captan under aqueous conditions. Captan is rapidly hydrolysed to several pH-dependent hydrolysis products, although no CO_2 evolution was determined. Based on aerobic mineralisation and water/sediment studies total mineralisation (of the degradation products) occurs at time points > 16 days.

Therefore, although captan quickly hydrolysed with a half-life < 1 day, it is only an initial transformation process and there is no scientific evidence to demonstrate that captan degraded to a level > 70 % within a 28-day period.

Available acute toxicity data formed hydrolysis products (metabolites THPI and THPAM) do not fulfil the criteria for classification as hazardous for the aquatic environment. Nevertheless, there were no chronic toxicity data available on these degradation products. In addition, no aquatic toxicity data were available for metabolite THPC.

Consequently, RAC considers that, despite rapid hydrolysis and further indications of rapid primary degradation, captan is not ultimately degraded to above 70 % within 28 days (equivalent to a half-life below 16 days) and it cannot be demonstrated that all degradation products do not fulfil the criteria for classification as hazardous to the aquatic environment. Therefore, RAC disagrees with the DS and considers captan as not rapidly degradable according to the CLP criteria.

Aquatic Bioaccumulation

The reliable $BCF_{steady\text{-}state}$ of 250 L/kg is well below the CLP trigger value of ≥ 500. The measured and estimated log Pow of captan 2.50 and 2.85, respectively, are also well below the CLP trigger value of ≥ 4.

Therefore, RAC agrees with DS that captan has a low potential for bioaccumulation according to the CLP criteria.

Aquatic Toxicity

RAC acknowledges that formulations (80 WDG and 83% WP) used in acute and chronic toxicity testing mainly consist of active substance captan (>80%). Although the are no aquatic toxicity data with used co-formulants, RAC note that co-formulants are not classified as toxic to the aquatic environment and unlikely to significantly increase the toxicity of the formulation. RAC points out that the Draft Assessment Report (DAR, 2005) and Draft Renewal Assessment Report (DRAR, 2012) themselves were not available to RAC. Therefore, RAC's evaluation is based on the available information in the CLH report including provided Annex's and information obtained during the consultation. Based on the available data the most acutely and chronically sensitive trophic group seems to be fish. However, RAC acknowledges that there are some data gaps and uncertainty regarding acute and chronic toxicity data.

<u>Read-across between captan and folpet</u>

A summary of the methodology of the read across assessment between captan and folpet has been provide in the CLH report, and full QSAR (Read – Across) report was attached as an Annex.

The assessment has been conducted by using the OECD QSAR Toolbox (ver. 4.5.) and indicated that, based on the profiling data, it could be shown that captan and folpet are similar considering

the US EPA Chemical Categories (Imides), the general mechanistic properties (protein binding, ionisation, and hydrolysis half-life) and the endpoint specific properties like acute aquatic toxicity MoA (base surface narcotics or aquatic toxicity classification). There are similarities in organic functional groups, chemical elements, and groups of elements. The structural similarity (based on PubChem features) of folpet does not reach the target structural similarity of > 90% but was shown to be > 70%.

Overall, the similarity between captan and folpet regarding mechanistic properties, endpoint specific properties (MoA), and empirical properties iss considered by RAC to be sufficient, even though the structural similarity is only 70-80% compared to captan. The observed differences in the structure of captan and folpet (cyclic compound) was not considered to have an impact on the toxicity of the active substance considering the MoA as a base surface narcotic.

Folpet (CAS 133-07-3, EC 205-088-6) had a harmonised classification as Aquatic Acute 1 (M = 10). RAC's opinion on the classification of folpet concluded that folpet is not rapidly degradable, has a low bioaccumulation potential and proposes classification as Aquatic Acute 1 with an M-factor of 10, based on 96-hour LC_{50} of 0.015 mg/L for *O. mykiss* and Aquatic Chronic 1 with an M-factor of 10 based on 33-day NOEC of 0.00881 mg/L for *P. promelas*.

Overall, RAC is of opinion that use of read – across assessment between captan and folpet is principally acceptable.

Acute Aquatic toxicity

RAC concludes that reliable acute toxicity data are available for fish, invertebrates, and algae. Regarding aquatic acute toxicity to fish, RAC notes that when measured concentrations do not remain within 80-120 % of the nominal concentrations, the effect concentrations cannot be based on nominal concentrations and should be expressed as mean measured concentrations. Therefore, RAC considers that the 24h LC_{50} value of 0.025 mg a.s./L for *S. trutta* determined based on predicted mean measured concentrations based on the degradation observed in one test concentration could be used as only supportive information.

Overall, RAC agrees with the DS that the lowest endpoint value is the 96h LC_{50} of 0.0147 mg/L (mean measured) for *O. mykiss* conducted with an 83% WP formulation. As this value is < 0.1 mg/L and captan is not rapidly degradable, classification as Aquatic Acute 1 (H400), M = 10 ($0.01 < L(E)C_{50} \leq 0.1$) is warranted.

Chronic Aquatic toxicity

Including the additionally provided chronic toxicity data, RAC considers that there are reliable and valid chronic toxicity data only for fish and algae. Hence, reliable and valid chronic toxicity endpoints obtained are:

- 45-week NOEC of 0.0168 mg/L for fish *P. promelas* derived from additionally provided study with captan (US EPA, 00057846/Hermanutz et al., 1973); and
- 72h NOE_rC of 0.077 mg a.s./L for algae *R. subcapitata* derived from a study with the 83% WP formulation (Anonymous, 1994).

RAC considers the chronic toxicity studies (OECD TG 210) with *O. mykiss* (Anonymous, 2016) and (OECD TG 211) with *D. Magna* (Anonymous, 2017) as not appropriate for classification purpose. The measured concentrations do not remain within 80-120 % of the nominal concentrations and therefore the effect concentrations cannot be based on nominal or initial measured concentrations. Neither should endpoint values be determined and expressed relative to the arithmetic mean concentration for flow-through tests and expressed relative to the geometric mean of the measured concentrations for semi-static tests, as indicated in OECD TG 210.

Overall, RAC considers that adequate chronic toxicity data are not available for all trophic levels as data for invertebrates are missing. In addition, there are no reliable chronic toxicity data on the most sensitive species under acute toxicity testing. Hence, according to CLP criteria classification shall be assessed according to the criteria given in Table 4.1.0(b)(i) and if for the other trophic level adequate acute toxicity data are available according to the criteria given in Table 4.1.0(b)(iii) and should be based on the most stringent outcome:

- Based on the 45-week NOEC of 0.0168 mg/L for fish (*P. promelas*) and 72h NOE_rC of 0.077 mg a.s./L for algae (*R. subcapitata*) using captan, classification as Aquatic Chronic 1 with an M-factor of **1** (0.01 < NOEC ≤ 0.1 mg/L), Table 4.1.0(b)(i) would be warranted.
- As chronic toxicity data for invertebrates are not available, based on adequate acute toxicity data (*D. magna* 48-h EC_{50} of 0.289 mg/L) captan would warrant classification as Aquatic Chronic 1 with an M-factor of **1** (0,1 < L(E)C50 ≤ 1), (Table 4.1.0(b)(iii).
- However, based on the most acutely sensitive species *O. mykiss* (96-h LC_{50} of 0.0147 mg/L) for which no reliable chronic data is available captan would warrant classification as Aquatic Chronic 1 with M factor of **10** (0,01 < L(E)C50 ≤ 0,1), (Table 4.1.0(b)(iii).

RAC notes that the additional chronic toxicity endpoints of 21d NOEC of ≥ 0.01 mg/L for *P. promelas* (FSTRA, US EPA EDSP, 2012, 48669501), 21d NOEC of >0.0072 mg/L for *X. laevis* (AMA, US EPA EDSP, 2013, 49136701), and 69d NOEC (molting) of 0.0031 mg/L for C*. magister* indicate the toxicity of same order of magnitude (resulting in an M-factor of 10). However, these data can be used only as supportive information.

RAC acknowledges that read-across assessment between captan and folpet would also lead to classification as Aquatic Chronic 1 with M-factor of **10** based on a 33d NOEC of 0.00881 mg/L for *P. promelas* (folpet being not rapidly degradable). However, RAC notes that due to the availability of long-term toxicity data for *P. promelas* using captan, a read-across to folpet is not required to classify captan. It is included here to indicate that the outcome is the same when using the approach proposed by the DS.

Overall, RAC disagrees with the DS and concludes that captan warrants classification as Aquatic Chronic 1 with an M-factor of **10,** based on most stringent outcome and supported by additional studies and read-across assessment with folpet.

Conclusion on classification

Overall, captan is considered as not rapidly degradable and does not fulfil the criteria for bioaccumulation.

Based on the available and reliable information, **RAC concludes that below classification is warranted** (in disagreement from the DS proposal):

Aquatic Acute 1 (H400), **M = 10**

Aquatic Chronic 1 (H410), M = 10

Additional references

Gordon, E and Williams M, 1999. The stability of captan and folpet in whole blood. Horizon Laboratories. Laboratory Report No. 10238. (Company file R-11143/TMN-0392A).

Arndt T and Dohn D, 2004. Measurement of the half-life of thiophosgene in human blood. PTRL West, Inc. Laboratory Report No. 1146W-1 (Company file: R-17121).

Kluxen FM, Roper CS, Jensen SM, and Koenig CM, 2022. Characterizing local acute irritation properties of Captan and Folpet with new approach methods, Applied In Vitro Toxicology, 8(9): 83-101

Adler ID, Pacchierotti F, Russo A, 2012. The Measurement of Induced Genetic Change in Mammalian Germ Cells. In: Parry, J., Parry, E. (eds) Genetic Toxicology. Methods in Molecular Biology, vol 817. Springer, New York, NY.

Yauk et al., (2015). Approaches for identifying germ cell mutagens: Report of the 2013 IWGT workshop on germ cell assays. Mutat Res Genet Toxicol Environ Mutagen.;783:36-54. doi: 10.1016/j.mrgentox.2015.01.008.

Cullen JM, Ward JM, Thompson CM, 2016. Re-evaluation and classification of duodenal lesions in B6C3F1 mice and F344 rats from 4 studies of hexavalent chromium in drinking water. Toxicol Pathol. 44:279–289.

Kirman CR, Hays SM, Aylward LL, Suh M, Harris MA, Thompson CM, Haws LC, Proctor DM, 2012. Physiologically based pharmacokinetic model for rats and mice orally exposed to chromium. Chem Biol Interact. 200:45–64.

Wong ZA, Chang HM. 1985. The comparative metabolism of captan in the rat and mouse. Richmond (CA): Chevron Chemical Company. Unpublished study No. SOCAL 1992, S-2163

EFSA, 2020. Conclusion on the peer review of the pesticide risk assessment of the active substance captan. EFSA Journal 2020;18(9):6230

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 and additional information (if applicable).

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