# Committee for Risk Assessment

# RAC

## Opinion

proposing harmonised classification and labelling
at EU level of

## silthiofam (ISO); *N*-allyl-4,5-dimethyl-2-(trimethylsilyl)thiophene-3-carboxamide

## EC Number: -
## CAS Number: 175217-20-6

CLH-O-0000001412-86-245/F

## Adopted

## 30 November 2018

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CLH-O-0000001412-86-245/F

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

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

**Chemical name:** **silthiofam (ISO); *N*-allyl-4,5-dimethyl-2-(trimethylsilyl)thiophene-3-carboxamide**

**EC Number:** **-**

**CAS Number:** **175217-20-6**

The proposal was submitted by **Ireland** and received by RAC on **22 February 2018.**

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

# PROCESS FOR ADOPTION OF THE OPINION

**Ireland** has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **23 April 2018**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **22 June 2018**.

## ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Nathalie Printemps**

Co-Rapporteur, appointed by RAC: **Michael Neumann**

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

The RAC opinion on the proposed harmonised classification and labelling was adopted on **30 November 2018** by **consensus**.

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

| | Index No | International Chemical Identification | 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 | No current Annex VI entry | | | | | | | | | | |
| Dossier submitters proposal | TBD | silthiofam (ISO); *N*-allyl-4,5-dimethyl-2-(trimethylsilyl)thiophene-3-carboxamide | N/A | 175217-20-6 | Repr. 2<br>STOT RE 2<br>Aquatic chronic 2 | H361d<br>H373<br>H411 | GHS08<br>GHS09<br>Wng | H361d<br>H373<br>H411 | - | - | - |
| RAC opinion | TBD | silthiofam (ISO); *N*-allyl-4,5-dimethyl-2-(trimethylsilyl)thiophene-3-carboxamide | N/A | 175217-20-6 | STOT RE 2<br>Aquatic Chronic 2 | H373<br>H411 | GHS08<br>GHS09<br>Wng | H373<br>H411 | - | - | - |
| Resulting Annex VI entry if agreed by COM | TBD | silthiofam (ISO); *N*-allyl-4,5-dimethyl-2-(trimethylsilyl)thiophene-3-carboxamide | N/A | 175217-20-6 | STOT RE 2<br>Aquatic Chronic 2 | H373<br>H411 | GHS08<br>GHS09<br>Wng | H373<br>H411 | - | - | - |

# GROUNDS FOR ADOPTION OF THE OPINION

## RAC general *comment*

Silthiofam is a pesticide active substance approved as a selective fungicide under regulation (EC) No 1107/2009. There is no existing entry in Annex VI of the CLP regulation for silthiofam. Therefore, the proposal of the dossier submitter (DS) addressed all physical, human health and environmental endpoints.

## RAC evaluation of physical hazards

### Summary of the Dossier Submitter's proposal

The DS did not propose classification for physical hazards. The data on physico-chemical properties did not indicate any concerns and therefore silthiofam does not meet the criteria for classification.

### Comments received during public consultation

No comments were received.

### Assessment and comparison with the classification criteria

Tests conducted according to EEC A.10/A.16 showed that silthiofam is not flammable or auto-flammable. In addition, the structural formula of silthiofam does not contain any of the chemical groups characteristic of an oxidising solid. Test method EEC A.14 showed that silthiofam is not explosive. Therefore, RAC agrees with the DS that **classification is not required for physical hazards**.

# HUMAN HEALTH HAZARD EVALUATION

## RAC evaluation of acute toxicity

### Summary of the Dossier Submitter's proposal

#### *Oral route*

No classification is proposed based on the absence of mortality at 5 000 mg/kg bw observed in an acute oral toxicity study in rats.

#### *Dermal route*

No deaths occurred in an acute rat dermal toxicity study. The acute dermal $LD_{50}$ was greater than 5 000 mg/kg bw in the study. On this basis, no classification was proposed by the DS.

***Inhalation route***

The $LC_{50}$ observed in rats exposed to a single dose of silthiofam (dust) was greater than 2.8 mg/L which was the highest technically attainable concentration. As no deaths occurred at this dose, no classification was proposed by the DS.

## Comments received during public consultation

No specific comments were received.

## Assessment and comparison with the classification criteria

***Acute toxicity: oral***

Silthiofam was tested in an OECD TG 401 study (GLP compliant) in the rats at 5 000 mg/kg bw. No deaths occurred and the $LD_{50}$ was thus greater than 5 000 mg/kg bw in both sexes. In this study, signs of non-specific toxicity were observed in 3 out of 10 animals (e.g. decreased activity, excessive salivation, decreased faecal volume, red stains on the snout). Based on the criteria in the CLP regulation, RAC agrees with the DS that no classification is warranted.

***Acute toxicity: dermal***

The $LD_{50}$ of silthiofam in rats was greater than 5 000 mg/kg bw in an OECD TG 402 study (GLP compliant) in both sexes. No classification is warranted based on the CLP criteria.

***Acute toxicity: inhalation***

In an OECD TG 403 study, rats were exposed nose-only to 2.8 mg/L/4h of silthiofam (dust). No mortality was observed in this group. The clinical signs from this study are described under the section on STOT SE (below). As 2.8 mg/L was the highest attainable concentration, there is no evidence that the $LC_{50}$ in rats is below the 5 mg/L cut-off for classification for acute toxicity by inhalation. Therefore, RAC agrees with the DS that classification of silthiofam for acute toxicity following inhalation exposure is not warranted.

In conclusion, RAC agreed that **no classification is warranted for acute toxicity via the oral, dermal and inhalation routes**.

# RAC evaluation of specific target organ toxicity – single exposure (STOT SE)

## Summary of the Dossier Submitter's proposal

The DS concluded that silthiofam was of low acute toxicity and that there is no basis for classification for STOT SE in category 1 or 2. Moreover, no evidence or indication of transient respiratory tract irritation or narcosis was observed in the available studies. Therefore, no classification was proposed by the DS.

## Comments received during public consultation

No specific comments were received.

### Assessment and comparison with the classification criteria

There was no relevant data on humans in the dossier. RAC agreed with the DS that that the data available from studies involving single exposure to silthiofam provided no basis for classification for STOT SE in category 1 or 2.

From the acute inhalation toxicity study, clinical signs suggestive of respiratory irritation such as laboured respiration and red ocular discharge and test material on the nose as well as hair stained with urine and faeces were observed in one or more rats during and immediately after exposure (exact number of animals not reported), at the highest attainable concentration tested (2.8 mg/L). These clinical signs were reversible by day 1 and no abnormalities were observed at necropsy. As the substance is a solid, the mechanical effect of solid particles may have contributed to the irritation observed. No lower concentrations were tested in the study. The substance was without irritant effect in the eyes or the skin of rabbits. No gross pathological findings in the lung were observed at necropsy. Therefore, RAC agrees with the DS' proposal not to classify silthiofam as STOT SE 3 for respiratory irritation.

Overall, RAC agrees with the DS that **classification for STOT SE is not warranted** for silthiofam.

## RAC evaluation of skin corrosion/irritation

### Summary of the Dossier Submitter's proposal

In an OECD TG 404 study, silthiofam (ground and moistened with saline) was applied to the skin of 6 rabbits. Exposure was for 4 hours under a semi-occlusive dressing. As no irritation was observed, the DS proposed no classification for skin irritation/corrosion.

### Comments received during public consultation

No specific comments were received.

### Assessment and comparison with the classification criteria

In the absence of any signs of irritation in an OECD TG and GLP compliant study, RAC agrees with the proposal of the DS **not to classify silthiofam for skin irritation/corrosion**.

## RAC evaluation of serious eye damage/irritation

### Summary of the Dossier Submitter's proposal

In an OECD TG 405 study (GLP compliant), solid silthiofam was instilled in the eyes of six rabbits. Slight conjunctival irritation was observed after one hour which was resolved by 48 h (mean 24-72 h score: max. 0.7). On this basis, no classification was proposed by the DS.

### Comments received during public consultation

No specific comments were received.

### Assessment and comparison with the classification criteria

RAC agrees with the DS proposal **not to classify silthiofam for eye damage/irritation** based on the absence of irritation observed in an OECD TG 405 study (which was GLP compliant).

## RAC evaluation of respiratory sensitisation

### Summary of the Dossier Submitter's proposal

No classification was proposed by the DS due to lack of data.

### Comments received during public consultation

No specific comments were received.

### Assessment and comparison with the classification criteria

RAC agrees with the DS that silthiofam **should not be classified as respiratory sensitiser due to lack of data.**

## RAC evaluation of skin sensitisation

### Summary of the Dossier Submitter's proposal

Silthiofam was tested in a guinea-pig maximisation test (GPMT), performed according to OECD 406 (GLP compliant). As no dermal response to challenge was observed in treated and control animals, no classification was proposed by the DS.

### Comments received during public consultation

No comments were received.

### Assessment and comparison with the classification criteria

RAC agrees with the DS that silthiofam **should not be classified as a skin sensitiser** based on the negative GPMT.

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

### Summary of the Dossier Submitter's proposal

The evaluation of STOT RE was based on nine repeated-dose toxicity studies. The studies consisted of three oral studies in dogs (28-day range-finding, 90-day and one year), two oral studies in mice (28-day and 60-day), two oral studies in rats (28-day, 90-day with a pilot reproductive toxicity phase) and one rat 21-day dermal toxicity study. In addition, a rabbit range-finding developmental toxicity study was considered relevant for this endpoint.

The liver was identified as the main target organ in dogs, rats and mice. Elevated liver weight and serum enzyme markers for liver toxicity were observed in all three species. However, liver histopathology was observed only in rats and mice. No liver histopathological findings were observed in dogs up to significantly toxic doses.

Liver findings in rats and mice occurred at doses in excess of the guidance values for classification as STOT RE 2. As the liver effects observed in dogs occurred without histopathological correlates and were consistent with severe general systemic toxicity rather than on a specific target organ, no classification was proposed by the DS for the liver.

***Table**: Selected findings in repeated-dose toxicity studies in dogs exposed to silthiofam*

| | **28-day** (0, 10, 50, 150, 350/250 mg/kg bw/d) *(STOT RE 2 ≤ 300 mg/kg bw/d)* | **90-day** (0, 1, 10, 50, 75*/125 mg/kg bw/d) *(STOT RE 2 ≤ 100 mg/kg bw/d)* | **1-year** (0, 1, 5, 20, 80 mg/kg bw/d) *(STOT RE 2 ≤ 25 mg/kg bw/d)* |
|---|---|---|---|
| Mortality | 350/250 mg/kg bw/d: 1/2 (f) (d24) + 2/2 (m) (d12, d18) | 75/125 mg/kg bw/d: 1/5 (f)(d50) | - |
| Body weight (bw) and food consumption (fc) | At 350/250 mg/kg bw/d: ↓ fc, bw loss | 75/125 mg/kg bw/d: ↓ fc, bw loss | At 20 mg/kg bw/d: ↓ bw gain (f)<br>At 80 mg/kg bw/d: bw loss |
| Biochemistry | ≥150 mg/kg bw/d: ↑ APPT (m), ↑ γGT, ↑ ALP (m/f)<br><br>50 mg/kg bw/d: ↑ ALP (f) | 75/125 mg/kg bw/d: ↑ ALP (m/f), ↑ γGT (f), ↑ APPT (m/f)<br><br>At 50 mg/kg: ↑ APPT (m/f) ↑ ALP (f) | 80 mg/kg bw/d: ↑ ALP, γGT, potassium, phosphorus<br><br>20 mg/kg bw/d: ↑ potassium, phosphorous; ↑ ALP (f) |
| Liver relative weight | ≥150 mg/kg bw/day: ↑ weight in m/f (15 %/26 %) | ≥ 75/125 mg/kg bw/d: ↑ weight in m/f (29 %/19 %) | 80 mg/kg bw/d: ↑ weight (m/f)<br>≤ 20 mg/kg bw/d: no effects |
| Liver necropsy | Not investigated | No effects | No effects |

APPT: activated partial prothrombin time; ALP: alkaline phosphatase; γGT : gamma glutamyltransferase; *top dose was reduced to 75 mg/kg bw/d in females after 7 weeks; m: males, f: females

However, the DS proposed to classify silthiofam as STOT RE 2 for lethality observed in the rabbit range-finding developmental toxicity study. In this range-finding study, rabbits were dosed with 0, 5, 15, 50, 100 and 150 mg/kg, from days 7 to 19 of pregnancy. Four of six and 5/6 females died in the 100 and 150 mg/kg/day dose groups, respectively. Deaths occurred between gestation days 13-16 and 15-22 in these respective groups. All deaths except for one intubation error in the 100 mg/kg group were considered treatment-related. A clear steep dose response relationship was observed as no toxic effects were observed at the top dose of 60 mg/kg bw/d in the main study. Mortality occurring at ≥ 100 mg/kg bw/d fall within the guidance values for classification in category 2 (28-day study ≤ 300 mg/kg bw/d).

## Comments received during public consultation

One Member State (MS) supported the classification as STOT RE 2 based on mortality observed in dogs and rabbits at relevant dose levels and in rats at higher dose levels. Moreover, the MS further asked for the potential relevance of the mechanism of action (MoA) in fungi (e.g. inhibition of ATP export from the mitochondrial matrix to the cytosol) with regard to humans because this MoA may add support to the relevance of mortality seen in animals to humans. In addition,

according to the MS, effects observed in the liver also fulfilled the criteria for STOT RE 2 (no further explanation provided). The DS agreed that mortality in dogs may also be relevant for STOT RE. No further information on the relevance of the MoA in fungi to humans was included by the DS.

A second MS supported the DS's proposal to classify silthiofam for STOT RE based on the mortality observed in pregnant rabbits. Nevertheless, the MS proposed to use the actual time of death. At 100 mg/kg bw/d, the death of dams, observed within 7-9 days, were within the range of the guidance values for STOT RE 1 classification (for 7 days, Cat. 1 ≤ 130 mg/kg bw/d), and the death observed at day 10 was only just above the extrapolated guidance value. Due to the severity of the effect and as higher sensitivity of pregnant rabbits compared to pregnant humans has not been shown, the MS considered that STOT RE 1 could also be considered for this severe effect. This was agreed by the DS in their response to comments.

## Assessment and comparison with the classification criteria

Based on the available repeated-dose toxicity studies, the main effects of concern were liver toxicity and lethality.

### *Liver toxicity*

There were several studies available in rats and mice on the repeated-dose toxicity of silthiofam. In rats, liver toxicity was noted in the oral 28-day, 90-day and 2-year studies and in the dermal 21-day toxicity study above the guidance value level for classification as STOT RE 2. In the two-generation reproduction dietary study (1998), effects on liver consisted of increased organ weight and incidence of microscopic changes (hepatocyte vacuolation, bile duct hyperplasia). These effects occurred at 226 mg/kg bw/d in F0 males and at 273 mg/kg bw/d in F0 females which are also above the guidance values.

In mice, liver toxicity was observed in the feeding 28-day, 60-day or 18-month studies at doses above the guidance values.

In dogs, levels in ALP and γGT were consistently increased in both sexes after 28-day, 90-day or 1-year exposure at relevant dose levels for classification as STOT RE 2, indicating possible bile duct effects/cholestasis. Moreover, the increase in activated partial thromboplastin time and/or prothrombin time in the 28-day and 90-day studies might also support liver disease. An increase in absolute and relative liver weight was noticed at relevant dose levels only in the 28-day and 90-day toxicity studies. No histopathological findings were found in the 90-day and one-year studies (necropsy was not performed in the 28-day study). Overall, the increase in weight and the changes in enzyme activity are not considered sufficient for classification. This is supported by the absence of histopathological liver findings even at the highest dose level, at which marked general toxicity was shown in the one-year study. Thus, RAC agrees with the DS's proposal for no classification for liver.

### *Lethality*

In rats, treatment-related mortality in males was observed in the 90-day repeated-dose toxicity study above the relevant guidance value doses. No effect on survival was reported in the 28-day range-finding study or in the carcinogenicity study in rat. In the rat developmental toxicity study, a single death was considered treatment-related but occurred at a dose above the guidance values for classification (e.g. 1 000 mg/kg bw/d).

In mice, no deaths occurred in the 4-week range-finding study and in the 60-day feeding toxicity study. In the mouse 18-month study, a statistically significant decrease in survival was observed

in females only at 2 and 20 mg/kg bw/d. Nevertheless, as the effect was not dose-related and did not occur at higher dose levels, this finding was not considered treatment-related.

In rabbits, in the range-finding developmental toxicity study, 4/6 and 5/6 females died in the 100 and 150 mg/kg bw/d dose group, respectively. The deaths were all treatment-related, except one intubation error at 100 mg/kg bw/d and 150 mg/kg bw/d occurred between gestation days 13-16 and 15-22 in the respective groups. Clinical observations reported at 100 and 150 mg/kg in the decedents included hypoactivity/lethargy, decreased defecation, discoloured faeces and/or staining of body surfaces/cage bedding. Moreover, body weight losses and reduced food consumption was observed during treatment and post-treatment periods. Gross pathology performed on the decedents revealed in three dams red fluid in the contents of the urinary bladder. Red-fluid in the urinary bladder was considered treatment related based on similar observations made in non-pregnant rabbits at 150 mg/kg or greater in the five-day repeated dose toxicity study. Other findings noted in decedents included dark red contents in the stomach of two females, of the caecum in one female and/or trachea in one female. One female had also dark red lungs and a pale heart. The cause of death was not reported. As stated in the guidance on the application of CLP criteria, for exposure durations shorter than 9 days (GD7 to GD 13-16), the effect should be compared to a guidance value of 100 mg/kg bw/d ≤ STOT RE 2 ≤ 1 000 mg/kg bw/d. At 150 mg/kg bw/d effects were also within the criteria for classification as STOT RE 2 (70-100 ≤ STOT RE 2 ≤ 700-1 000 mg/kg bw/d for GD7 to GD15-22 with last day of treatment at GD 19). Although the effects were observed at doses just above the guidance value for STOT RE 1 at 100 mg/kg bw/d, lethality occurring at a higher dose level (150 mg/kg bw/d) was consistent with STOT RE 2.RAC agrees with the DS that a very steep dose-response curve exists for mortality as no effects were observed in the main study at up to 60 mg/kg bw/d in rabbits. Although no target organs were identified, no findings were observed to suggest that the pregnant rabbit would not be a relevant species for investigating toxicity in humans. Indeed, the substance is not irritating/corrosive and clinical signs and gross-necropsy did not reveal severe toxicity in the gastro-intestinal tract of rabbits. Thus, mortality did not appeared to be the result of a non-relevant higher sensitivity of this species.

In dogs, in the 28-day range-finding study, 2/2 males (days 12, 18) and 1/2 females (day 24) were sacrificed at 350 mg/kg bw/d. The dose was reduced to 250 mg/kg bw/d following the two first weeks of exposure in males and following 3 weeks of exposure in females. At this dose level, clinical signs were observed (emesis, diarrhoea, hypoactivity, pale integument, emaciation, dehydration and decreased defecation). Liver was identified as the target organ in this study. Taking into account Haber's rule, the deaths were observed at relevant dose levels for classification as STOT RE 2 (≤ 375-750 mg/kg bw/d for 12-24 days). In the 90-day study, one out of 5 females was sacrificed (day 50) at 125 mg/kg bw/d. The high dose was then reduced to 75 mg/kg bw/d in females because of excessive toxicity. Clinical signs noted in the female sacrificed *in extremis* were emesis, weight loss, hypothermia, hypoactivity, pale mucosa and decreased defecation. No other findings were reported. The cause of death was not reported. Using Haber's rule, this is in line with the guidance values for STOT RE 2 classification (18 ≤ STOT RE 2 ≤ 180 mg/kg bw/d for 50 days). No death occurred in males in the 90-day study. In the one-year dog study, no deaths were reported up to 80 mg/kg bw/d. Overall, mortality was observed in both sexes in the 28-day range-finding study and in females in the 90-day study, at doses below the guidance values for STOT RE 2. As observed in rabbits, a very steep dose-response existed for this effect and there are uncertainties as to whether Haber's rule could be applied here (no mortality in longer term studies: no mortality in males in the 90-day study and no mortality observed in the one-year dog study). Nevertheless, data on dogs could be used as supporting evidence for classification. Indeed, mortality observed in dogs suggested that this effect is not rabbit-specific due to a higher sensitivity of this species.

Moreover, silthiofam is a selective fungicide acting through the inhibition of the exportation of ATP from the mitochondrial matrix to the cytosol in fungi leading to cell death due to the disruption of energy-dependent processes. *In vitro*, in rats and human hepatocytes, decreased intracellular ATP production was indeed observed (confidential report, 2013). Reduction of ATP concentration in cells might affect all tissues. Thus, although clinical symptoms and findings at necropsy reported in the dossier in dogs and rabbits prior to death did not allow the identification of a target organ, mortality observed may be still of relevance to humans. Nevertheless, RAC noted that additional MOA data would be needed to confirm the relevance of the MOA in fungi to mammals.

Overall, RAC supports the DS proposal to **classify silthiofam as STOT RE 2** based on lethality observed in rabbits and supported by mortality observed in dogs. As no specific target organ was identified, no organ will be specified for the STOT RE 2 classification.

## RAC evaluation of germ cell mutagenicity

### Summary of the Dossier Submitter's proposal

In a battery of *in vitro* genotoxicity studies performed under GLP and OECD TG, silthiofam did not cause gene mutations or chromosome aberrations. *In vivo*, negative results were obtained in a micronucleus assay in mice (up to 2 000 mg/kg bw) and in an unscheduled DNA synthesis test (UDS). On this basis, no classification was proposed by the DS for germ cell mutagenicity.

### Comments received during public consultation

One MS commented that there was no information on proof of exposure to bone marrow in the *in vivo* micronucleus test. According to the MS, the negative results of the study should not be taken into account in the event that no such information was available. The DS responded that silthiofam was highly absorbed by oral route and was widely distributed. Therefore, the DS believed that the target tissue had been exposed to silthiofam.

### Assessment and comparison with the classification criteria

Silthiofam was negative in the three available *in vitro* assays (bacterial mutation assays, a mammalian gene mutation assay, a mammalian cytogenicity test) and in two *in vivo* assays (mouse micronucleus and UDS). RAC agrees with the comment that no proof of exposure was reported in the dossier and therefore the negative results obtained in the micronucleus test are difficult to interpret. Nevertheless, on the basis of the negative *in vitro* test and negative *in vivo* results, RAC agrees with the conclusion of the DS that silthiofam **did not meet the criteria for classification for germ cell mutagenicity**.

## RAC evaluation of carcinogenicity

### Summary of the Dossier Submitter's proposal

The carcinogenic potential of silthiofam was investigated in a 2-year rat study and in an 18-month mouse study. In addition, several mechanistic studies were available to investigate the potential tumour MoA.

In the carcinogenicity rat study, a treatment-related increase in the incidence of liver adenoma and carcinoma was observed at the mid and high dose male rats. The increases in adenoma and carcinoma were not statistically significant but were above concurrent and historical control data (HCD) (See the table below). Moreover, an increase in thyroid follicular adenoma and/or carcinoma was observed in male rats (not statistically significant) at the highest dose above the concurrent controls and at the upper range of the HCD.

In the mouse carcinogenicity study, a slight statistically significant positive trend (Peto analysis) in liver adenoma was observed in females at the highest dose (855 mg/kg bw/day; 5/50 animals, 10 %). The increase was not statistically significant by pairwise comparison. The incidence was above concurrent controls (1/50 animals, 2 %), historical control data ranges from the testing laboratory (maximum 3 %) and HCD ranges from Charles River laboratories for the same strain of mice (maximum 8 %). The tumours occurred in presence of severe liver toxicity (cell necrosis, chronic inflammation).

Mechanistic data were provided in the dossier to investigate a potential CAR/PXR-mediated MoA for liver tumours. Five mechanistic studies were conducted with silthiofam and the results were summarised by the DS as follows:

(1) 14 day *in vivo* rat study (silthiofam):
- Substantial induction of hepatic CYP2B1, CYP2B2 and (to a lesser extent) CYP3A1 (based on enzyme activity, mRNA expression and Western blot data);
- substantial increase in replicative DNA synthesis (cell proliferation) in the liver at 7 and 14 days;
- No evidence of activation of PPARα (as measured by enzyme activity, gene expression and Western blots);
- Increased induction of hepatic T4-UDPGT activity after 14 days of dosing.

(2) Rat wild-type hepatocyte *in vitro* study (phenobarbital and silthiofam tested):
- Silthiofam acted in a phenobarbital-like manner;
- Increased induction of CYP2B1, CYP2B2 and CYP3A1 (revealed by enzyme activity, mRNA expression);
- Increase in replicative DNA synthesis (cell proliferation);
- Cytotoxicity at silthiofam concentrations > 100 µM.

(3-4) Human hepatocyte *in vitro* studies (phenobarbital and silthiofam tested, one study with one male donor and one study with one female donor):
- Silthiofam acted in a phenobarbital-like manner;
- No increase in PROD activity (CYP2 marker), weak response in BROD and BQ activity (CYP2/ CYP3 and selective CYP3 markers respectively);
- Weak induction of CYP2 and CYP3 mRNA expression;
- No increase in replicative DNA synthesis (cell proliferation);
- Cytotoxicity at silthiofam concentrations > 100 µM.

(4) Rat CARKO/PXRKO hepatocyte *in vitro* study (phenobarbital and silthiofam tested):
- No increase in PROD, BROD or BQ enzyme activity relative to controls;
- Weak effect on CYP2B1 but no effect in CYP2B2 or CYP3A gene expression;
- No increase in cell proliferation;
- Cytotoxicity at silthiofam concentrations > 100 µM.

According to the DS the results were consistent with the proposed CAR/PXR-mediated effect on rodent liver which is not relevant for human health: activation of CAR/PXR, induction of CYP isoenzymes, increased hepatocellular proliferation leading to tumours in the liver. Some

uncertainties were noted by the DS as some MoA were not investigated (involvement of AhR) and as no *in vivo* studies with CAR/PXR knock out animals were performed.

Overall, no classification was proposed by the DS based on a weight-of-evidence analysis which took into account the following:

- Silthiofam was not genotoxic;
- Mechanistic data were supportive of a CAR/PXR-mediated effect on rodent liver not relevant for human health supporting no classification for liver tumours observed in rats;
- Thyroid follicular tumours observed in rats were not statistically significant and did not exceed the HCD. In addition, the increase in hepatic UDPGT activity observed in the *in vivo* mechanistic study suggested that these tumours were also not relevant to humans;
- Finally, hepatic tumours in mice at a very high dose did not progress to malignancy and did not affect survival. In addition, liver toxicity observed in mice suggested that tumours could be secondary to a regenerative hyperplasia from cytotoxicity. The rat mechanistic data indicated that a CAR/PXR nuclear receptor may also play a role.

## Comments received during public consultation

One MS disagreed with the DS's proposal and supported a Carc. 2 classification. Although the MS acknowledged that some evidence suggested a CAR/PXR mediated MoA, they considered that other potential MoA were not sufficiently excluded. The MS was of the opinion that hepatotoxicity and cytotoxicity of silthiofam might be the main potential MoA in the rat and mouse studies.

## Assessment and comparison with the classification criteria

### *Rat*

In the combined chronic toxicity and carcinogenicity study in rats, an increase in hepatocellular adenoma and carcinoma was observed in males. The increases were not statistically significant and were inside the relevant laboratory historical control range. Survival, mean body weight and body weight gain were not affected in the study in males. A treatment-related increase in absolute and relative liver weight was observed at the top dose in males (↑ 19 % in relative weight). Eosinophilic foci were increased in the liver at the high dose in both males and females. Centrilobular pallor was increased in both male and female rats at the mid and high dose levels. An increase in the incidence of cystic degeneration was also observed at the top dose in males. No degenerative changes were noted (See table below).

In this study, an increase in thyroid follicular cell tumours was also observed in males. The increase was not statistically significant but was observed at the upper range of HCD for this strain of rats and slightly above laboratory historical control data.

***Table**: Selected non-neoplastic findings at terminal sacrifice (carcinogenicity rat study, 1998)*

| | **n = 50** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Males** | | | | | **Females** | | | | |
| **Dose (mg/kg bw/d)** | **0** | **0.5** | **5** | **51** | **150** | **0** | **0.65** | **6.4** | **65** | **195** |
| Rel. liver wt (% bw) | 100 | 102 | 110 | 109 | 119** | 100 | 100 | 110 | 114 | 107 |
| Hypertrophy | 0 | 0 | 0 | 0 | 2 | - | - | - | - | - |
| Eosinophilic Foci | 7 | 12 | 7 | 9 | 29** | 5 | 4 | 10 | 10 | 23** |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Centrilobular pallor | 0 | 0 | 4 | 11* | 22** | 0 | 0 | 4 | 12** | 29** |
| Cystic degeneration | 11 | 20 | 22 | 20 | 30** | - | - | - | - | - |
| vacuolation | 3 | 1 | 2 | 5 | 24** | 0 | 4 | 1 | 11** | 24** |

*p≤0.05; **p≤0.01; -: not found; wt: weight

***Table:*** *Incidence of liver and thyroid tumours in male rats (carcinogenicity study, 1998)*

| **Tumour type** | **Tumour incidence (%) Dose (mg/kg bw/d)** | | | | | | |
|---|---|---|---|---|---|---|---|
| | **0** | **0.5** | **5** | **51** | **150** | **HCD*** | **HCD**** |
| **Liver** | | | | | | | |
| Adenoma | 8 | 6 | 4 | 10 | 14 | 0-8 | 0-18 |
| Carcinoma | 0 | 4 | 4 | 6 | 8 | 0-6.7 | |
| Adenoma and/or carcinoma | 8 | 10 | 8 | 16 | 20 | 0.9-10 | |
| **Thyroid follicular tumours** | | | | | | | |
| Adenoma | 6 | 0 | 0 | 2 | 10 | 1.7-12 | 0-8 |
| Carcinoma | 0 | 0 | 2 | 2 | 4 | 0.9-3.9 | 0-2 |
| Adenoma and/or carcinoma | 6 | 0 | 2 | 4 | 14 | 0-14 | |

*Charles river (SD)BR rat HCD (23 studies, 1995-2001), ** Historical control range from the laboratory

Mode of action of liver tumours

The human relevance framework has been used by the DS to assess the human relevance of the rodent tumours. Five mechanistic studies were performed in the dossier to investigate a potential CAR-mediated MoA in rodents. The postulated MoA was that the activation of CAR and PXR nuclear receptors in male rats results in the altered expression of a number of genes as well as an increase in hepatic cell proliferation leading to hepatocellular tumours.

**Is the WOE provided sufficient to establish the MoA in animals in the case of silthiofam?**

Two key events have been considered by the DS: the activation of CAR/PXR nuclear receptors and hepatocellular proliferation.

- Activation of CAR and PXR nuclear receptors

CAR activation has been investigated in an *in vivo* 14-day MoA study in male rats (2013). A single dose was tested which was equivalent to the top dose used in the carcinogenicity study.

BROD (a marker for CYP2B and CYP3A, CAR/PXR), PROD (a marker for CYP2B, CAR) and BQ (a marker for CYP3A, PXR) enzyme activities were increased 13×, 14× and 3×, respectively. Altered gene expression was also noted, as hepatic CYP2B1, CYP2B2 and CYP3A mRNA levels were increased about 1 200-fold, 62-fold and 3-fold, respectively.

The liver induction profile of silthiofam was thus consistent with CAR/PXR activation. Although no comparison with a positive control was performed in the study, CYP2B induction was higher than CYP3A activity as observed with CAR/PXR activators.

Associated events to CAR/PXR activation were also noted in the study. Indeed, increased liver weights and minimal to slight hepatocellular hypertrophy were observed in the 14-day rat study. Cytochrome P450 enzymes were not evaluated in longer term studies. However, liver weight was increased in both males and females in the carcinogenicity study and hepatocellular pallor was consistent with P450 enzyme induction. Nevertheless, RAC noted the absence of liver hypertrophy in both the 90-day and carcinogenicity studies (except in 2 out of 50 animals).

- Increased hepatocellular proliferation.

Hepatocellular proliferation as shown by BrDU labelling of hepatocytes was statistically significantly increased (about 7-fold after 7-day exposure and 9-fold after 14-day exposure) in the *in vivo* 14-day rat study (2013).

Although hepatocellular proliferation was not investigated in longer term studies, an increase in a pre-neoplastic lesion (altered foci) was observed in both males and females at the top dose in the rat carcinogenicity study, which was consistent with hepatocellular proliferation. As liver tumours were only increased in males, the cause of this sex difference is unknown.

It is unknown how long the cell proliferation was sustained but RAC agrees with the statement in the dossier that an early, short term burst of cell proliferation could be sufficient to cause liver tumours.

- Exclusion of alternative MOA

The *in vitro* rat CARKO/PXRKO double knockout study (Chatham, 2015a) showed that the presence of functional CAR and/or PXR appeared essential for the initial hepatic proliferative response from both silthiofam and phenobarbital. Indeed, in contrast with the results observed in the *in vitro* study performed with wild-type rat hepatocytes, no cell proliferation was observed at non-cytotoxic concentrations either with silthiofam or phenobarbital. PROD, BROD and BQ activities were in all silthiofam-treated groups comparable or lower than control. Gene expression of CYP2B2 and CYP3A1 mRNA were either comparable to control or decreased. Nevertheless, a statistically significant dose-related 5-fold increase in mRNA expression was observed for CYP2B1 (no change was observed with phenobarbital).

Silthiofam was not genotoxic.

In the carcinogenicity study, liver tumours were observed in the absence of significant liver toxicity such as necrosis, fibrosis or inflammation. Cystic degeneration in liver was observed in males. This non neoplastic lesion is derived from altered ito cells and was not considered as evidence of pre-neoplastic lesions by RAC. Indeed, the finding was also statistically significantly increased in male mice but did not lead to tumours. Clinical chemistry changes such as $\gamma$GT and ALP were elevated in the study indicating possible bile duct effects but the increase was not statistically significant. In the 90-day rat repeated-dose toxicity study, liver toxicity was observed but necrosis was not found. In this study, histopathological findings consisted of hepatocyte vacuolation, hypertrophy/fibrosis of the bile duct, portal inflammation and/or pigment in Küpffer cells. In the 14-day *in vivo* rat study, hepatocellular cell proliferation was observed in the absence of significant hepatotoxicity. Although excessive cytotoxicity was observed in the *in vitro* studies at ≥ 100 µM in males and females, there was no toxicokinetic data in the dossier to compare this concentration to *in vivo* dose levels. Therefore, cytotoxicity may not be the main MoA for rat liver tumours.

No evidence of activation of PPAR$\alpha$ was noted in the 14-day study. Therefore, peroxisomal proliferation can be ruled out.

Nevertheless, CYP1A1 was not tested in the study, thus AhR activation cannot be ruled out.

There is no data in the dossier suggesting that other MoA such as Porphyria, statins/altered cholesterol synthesis, estrogenic activity and immunosuppression would be likely for silthiofam.

Overall, RAC agrees that the proposed MoA could be plausible in male rats. Nevertheless, the following uncertainties remained:
- Absence of dose-response data for CAR/PXR activation (only a single dose tested);
- No decrease in apoptosis as a consequence of alterations in gene expression was noted;
- No hypertrophy was observed in rats in the carcinogenicity study; this finding would also have been expected;

- No *in vivo* studies using CAR/PXR knock out animals were performed to confirm the *in vitro* results;
- No exclusion of AhR activation;
- Sex differences in tumour induction have not been investigated. Indeed, no mechanistic data in female rats (*in vitro* and *in vivo*) have been provided.

**Evaluate if the human relevance of the proposed MoA can be reasonably excluded on the basis of qualitative/quantitative differences in key events between animals and humans**

The two *in vitro* studies in human hepatocytes used cells from one male and one female donor. Silthiofam did not cause hepatocellular proliferation. Although to a lesser extent than in the *in vitro* rat study, silthiofam activated PROD, BROD (2-fold) and BQ in human hepatocytes. In these studies, treatment with EGF resulted in an increase in replicative DNA synthesis, demonstrating the suitability of the system for assessing cell proliferation.

***Table** : Comparative in vitro studies in human and rat wild-type hepatocytes*

| | **Human hepatocytes** | | | | **Male rat hepatocytes** | |
|---|---|---|---|---|---|---|
| | **Male donor** | | **Female donor** | | | |
| Concentrations tested (µM) | Silthiofam (1-30µM) | Phenobarbital (10-1 000µM) | Silthiofam (1-30µM) | Phenobarbital (100-1 000µM) | Silthiofam 30-100 µM | Phenobarbital 10-1 000µM |
| Cell proliferation (by BrdU incorp.) | - | - | - | - | 2× | 2× |
| PROD activity (Cyp2b) | - | 2× | - | 1.5× | 2× | 4× |
| BROD activity (Cyp2b/Cyp3a) | 3× | 8× | - | 1.6× | 2× | 5× |
| BQ activity (Cyp3a) | 2× | 5× | - | 1.9× | 5× | 4× |
| CYP2B mRNA | 5× | 9× | 2.5× | 3.7× | 13× | 36× |
| CYP3A mRNA | 4× | 9× | 1.9× | 6.8× | 97× | 34× |

In grey cells, results obtained with silthiofam

These studies showed that the increase in cell proliferation observed in rat was not observed in human donors. One limitation was the use of only one male and one female donor in the *in vitro* experiments. Moreover, in the dossier there is no information on the male donor and only little information on the female donor (a 68-year old female). Overall, these studies showed that there were quantitative differences in the activation of CAR by silthiofam in rats and humans. Indeed, activation of human CAR is lower than rat CAR, and cell proliferation was not detected in human hepatocytes while this was observed in the rat. These two differences are consistent with the lack of relevance of the CAR activation mechanism in humans.

<u>Mode of action of thyroid tumours</u>

These tumours were not considered relevant by the DS based on the increase in hepatic UDGPT observed in the *in vivo* 14-day mode of action rat study. In the absence of data on thyroid hormone levels (TSH, T4, T3) and further mechanistic data to support this hypothesis, thyroid tumours need to be considered relevant to humans.

***Mouse***

In the mouse carcinogenicity study (50 mice/sex/group), an increase in the incidence of hepatocellular adenoma was observed in females at the top dose (855 mg/kg bw/d) which was above concurrent controls and historical control data from the laboratory or CD-1 mice from Charles River laboratories. The two hepatocellular carcinoma observed at the top dose was inside the historical control data from the laboratory. In this study, no treatment-related effects on survival, clinical signs or body weight was observed. Liver toxicity included a treatment-related increase in absolute and relative liver weight in males and females and elevated serum AST and ALT in males only at the top dose. Liver hypertrophy was significantly increased in both males and females at the top dose. Additionally, at the top dose, liver foci (mixed cell focus) were significantly increased in males. Although not statistically significant, foci were also observed in females (mixed cells, basophilic and eosinophilic focus). Necrosis (individual cells) was observed in both males and females and was associated with karyomegaly, and cystic degeneration in males.

***Table:*** *Selected neoplastic and non-neoplastic liver findings in mice at terminal sacrifice (mouse carcinogenicity study, 1998)*

| | **Males** | | | | | **Females** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Dose (mg/kg bw/d)** | **0** | **1.4** | **13.7** | **14 1** | **564** | **0** | **2.03** | **20.7** | **203** | **855** |
| No. of animals | 38 | 41 | 41 | 30 | 34 | 39 | 38 | 28 | 34 | 39 |
| Rel. liver wt (% bw) | 10 0 | 100 | 96 | 100 | 128** | 100 | 102 | 105 | 107 | 117** |
| Hypertrophy | 0 | 0 | 0 | 2 | 25** | 0 | 0 | 0 | 0 | 12** |
| Focus<br>- Mixed cell<br>- Basophilic<br>- Eosinophilic | <br>0<br>0<br>0 | <br>-<br>-<br>- | <br>0<br>0<br>0 | <br>0<br>2<br>0 | <br>7*<br>0<br>0 | <br>0<br>0<br>0 | <br>0<br>0<br>0 | <br>0<br>0<br>0 | <br>0<br>0<br>0 | <br>2<br>2<br>1 |
| Necrosis | 3 | - | 3 | 3 | 40** | 5 | 1 | 0 | 3 | 19** |
| Cystic degeneration | 0 | 0 | 0 | 0 | 16** | 1 | 0 | 0 | 0 | 1 |
| Karyomegaly | 11 | - | 10 | 11 | 36** | 6 | 3 | 8 | 5 | 15 |
| Hepato-cellular adenoma | 6 | - | 4 | 9 | 6 | 2 | 0 | 2 | 0 | **10** |
| Hepato-cellular carcinoma | 6 | - | 2 | 2 | 1 | 0 | 0 | 0 | 0 | 2 |

**: statistically significant; In grey cells, bold text: statistically significant according to the DS, not stated in the study summaries available in the DAR and RAR for silthiofam.

The table shows that the increase in the incidence of adenoma correlated with increased body weight, preneoplastic lesions (foci) and liver hypertrophy. Nevertheless, although these non-neoplastic lesion were observed in male rats (with higher incidences than in females), no neoplastic findings were observed.

Tumours were observed in presence of hepatotoxicity, as necrosis was observed in both males and females at the top dose. The DS considered that tumours observed in female mice were mainly secondary to cytotoxicity. RAC further noted that in the 90-day study, centrilobular hypertrophy, vacuolation and degeneration and/or individual hepatocyte necrosis were already observed in males and females at 707/1 132 mg/kg bw/d (males/females, respectively). However, although higher cytotoxicity was observed in males compare to females, no tumours were observed in males and therefore cytotoxicity may not explain the observed tumours. The DS considered that the rat mechanistic data supported the argument that CAR/PXR mechanism

may play a role. Nevertheless, this potential MoA is not supported by RAC, as no mechanistic data were available in mice.

***Comparison with criteria***

Liver tumours have been observed in male rats and female mice. Additionally an increase in thyroid tumours has been observed in male rats.

The increases in the incidences of liver adenoma and carcinoma observed at the top dose in males were not statistically significant and were within the HCD range provided by the laboratory. Although liver toxicity was observed in the study, there was no clear evidence of a link between liver non-neoplastic findings and tumour induction as similar findings were observed in both males and females without tumour induction in females. Based on the mechanistic data available in the dossier, a CAR/PXR mediated effects are plausible although uncertainties have been noted by RAC. Overall, RAC considers this finding of increased top-dose liver male tumours to be insufficient evidence to support classification.

Thyroid follicular adenoma and/or carcinoma in male rats were not statistically significant. Although the tumours were slightly outside the range of laboratory HCD, the incidences did not exceed the HCD incidence for this strain of rat (Charles River HCD). Overall, RAC agrees with the DS that the evidence is insufficient to support classification.

With regard to the mouse liver tumours, no mechanistic data were provided and therefore human relevance cannot be excluded. There is no clear evidence that tumours could be secondary to cytotoxicity. Indeed, tumours where not seen in males having higher liver cytotoxicity. The increases in mouse liver tumours were only observed in females, at the top dose only and did not progress to malignancy. Moreover, silthiofam was not genotoxic. Overall, no classification is proposed based on this type of tumour.

In conclusion, RAC agrees with the DS's proposal **not to classify silthiofam for carcinogenicity.**

## RAC evaluation of reproductive toxicity

### Summary of the Dossier Submitter's proposal

***Fertility***

The DS based its evaluation on a 2-generation reproductive toxicity study in rats (GLP-compliant, OECD TG 416) from 1998. In this study, no effects on parameters relevant for sexual function and fertility were observed. Therefore, no classification was proposed by the DS.

***Developmental toxicity***

Two developmental toxicity studies were considered by the DS, one in rats and one in rabbits.

In the range-finding developmental toxicity study in rabbits, dose levels exceeded the maternal maximum tolerated dose at the two highest dose levels (lethality). No maternal toxicity was observed at the next lower doses (≤ 50 mg/kg bw/d). In this study no malformations or variations were observed. Nevertheless, the evaluation of developmental toxicity was limited at 100 and 150 mg/kg bw/d due to the low number of litters (only one litter from the 150 mg/kg bw/d group).

In the main developmental toxicity rabbit study, no maternal toxicity was observed up to the top dose of 60 mg/kg. No developmental effects were observed at this dose range. Nevertheless,

assessment of specific developmental toxicity was difficult in rabbits as the test substance went from no maternal effect at 60 mg/kg bw/d to fully lethal at 100 mg/kg bw/d.

In the rat developmental study, significant adverse effects on development were seen at the top dose level of 1 000 mg/kg bw/d:

- Statistically significantly reduced foetal weight (↓ 25 %);
- Skeletal variations: reduced ossification of centrum number 1 and sternebrae 1-4; increased 7th cervical rib;
- Increased incidences of dead foetuses (very rare in historical control data);
- External malformations: cleft palate observed in two litters: 1/15 foetuses and 8/22 foetuses. One foetus per litter was within historical control data. Nevertheless, the occurrence of a second litter with cleft palate raised the possibility of a treatment-related effect.

This top dose was clearly toxic to dams (one death, clinical signs, significant decrease in net body weight and food consumption, liver and kidney toxicity). The DS considered that some of the above-mentioned findings may have been secondary to maternal toxicity (delayed ossification, reduced weight, and skeletal variations) but that the cleft palate and dead foetuses occurring in two litters may have been treatment-related. Thus, the DS proposed to classify silthiofam as Repr. 2; H361d.

## Comments received during public consultation

### *Fertility*

One MS commented that the control in the 2-generation study should be considered invalid as fertility was lower than 70 % in this group. The DS still considered the study valid as no effects on treatment groups were observed.

### *Developmental toxicity*

One MS supported Repr. 2, H361d but asked for individual data to better understand whether dams where foetal mortality and cleft palate occurred were particularly affected by treatment. The MS also asked if historical control data for the cluster of litters with cleft palate were available. The DS responded that markedly reduced weight and ossification were observed in the litters having cleft palate and marked maternal toxicity above the maximum tolerated dose (MTD) was observed in the dams. The DS reviewed the published papers provided during public consultation by industry supporting the argument that cleft palate could be associated with foetal weight retardation but highlighted that marked toxicity is not always associated with cleft palate caused by a non-specific mechanism. HCD on a cluster of malformations were not available but the DS pointed out that on a litter basis, 2 litters were outside the range of historical control data supplied. The DS also noted that the severity of the effects may warrant classification but that a strong argument can also be made for non-classification.

One MS requested more information on the malformations occurring in the rabbit study before taking a position on classification. The DS provided tabulated data for the rabbit developmental toxicity study but did not considered this study to be relevant for classification.

One MS supported the classification as Repr. 2 but further noted that classification for effects on or via lactation need to be considered based on the clear decreases in pup weight from GD 4 onwards in the 2-generation study from both generations. Moreover, the MS noted that based on the physico-chemical properties of the substance (e.g. log $P_{ow}$), transfer to milk may be possible.

Two comments from industry disagreed with the proposal to classify silthiofam as a reproductive toxicant and provided additional references on the link between maternal toxicity and cleft palate. The marked toxicity observed in dams of which the litters exhibited cleft palate and dead foetuses explained the observed effects. One of the industry comments also noted that although the incidences of dead foetuses were increased (not statistically significant), no concurrent decrease in viable foetuses and post-implantation losses were noted. The DS responded that based on severe and rare malformations in conjunction with pup deaths and the increased incidence of 7th cervical ribs, classification is warranted. Nevertheless, the DS acknowledged that maternal and foetal toxicity is relevant and important to the discussion of the proposal for classification in category 2.

## Assessment and comparison with the classification criteria

### *Fertility*

Effects in the multigenerational study, available in rats, which could be possibly linked to fertility, were changes in absolute ovary weight in F0 rats which were within historical control ranges and in relative weight in both F0 and F1 generations. Moreover, in F0 an increased in ovarian cysts was observed at the top dose level (but not in F1). As the effects did not correlate with fertility effects, these changes are not considered to be of sufficient concern for classification. No effects were observed in the reproductive organs in other repeated-dose toxicity studies in any species (rats, dogs or mice).

Small prostate and seminal vesicles were found at the top dose in the rat 90-day repeated-dose toxicity study but they occurred in presence of marked general toxicity and were not observed in other studies.

In conclusion, RAC agrees with the DS's proposal of no classification for fertility.

### *Developmental toxicity*

In the developmental prenatal toxicity study performed in rabbits, no effects relevant for classification were observed. Nevertheless, due to severe toxicity (mortality), developmental toxicity may not have been identified.

In the rat developmental toxicity study, three main findings were highlighted by the DS: severe malformations (cleft palate, dead foetuses) and increased incidence of the 7th cervical rib (variations).

<u>Cleft palate</u>

The increase in malformations was primarily due to cleft palates (entire length). This severe malformation was observed in 8 out of 11 foetuses in one litter and in one foetus in a second litter. The incidence was above the HCD on both a foetus and litter basis (max: 7 foetuses in 7 litters; 0-0.3 % per litter). Eight out of the 9 foetuses were clustered to a single litter. Moreover, in this litter, all foetuses were malformed or late resorptions. This may reflect a total failure of foetal developmental in this dam. According to the study authors, these malformations were related to developmental delay as demonstrated by the severely reduced foetal body weight in this litter (46 % of the dose group mean and 33 % of the control group mean). Moreover, cleft palates were associated with reduced ossification (absence of ossification of the entire sternum).

<u>Dead foetuses</u>

Dead foetuses were observed in two litters at 1 000 mg/kg bw/d. No dead foetuses were reported either in the control and above the historical control data provided (0-0.3 % per litter). In these litters a low mean weight of the foetuses was noted (↓ 43-49 % of mean of controls).

7th cervical ribs

An increase in the 7th cervical ribs (pinpoint to intermediate) was observed at 1 000 mg/kg bw/d. This type of variations was observed in 13 foetuses in 7 litters (maximum 4 per litter, 5.1 % per litter) which was inside the highest value observed in the HCD (9 foetuses in 6 litters, 5.6 % per litter).

Maternal toxicity

At 1 000 mg/kg bw/d, considerable maternal toxicity was observed, exceeding the MTD, including mortality (one death), clinical signs, and significant effects on body weight and body weight gain, food consumption and organ weight changes (e.g. liver). Nevertheless, in some dams with comparable marked general toxicity (litter 43102), no malformations, dead foetuses or 7th cervical ribs were observed suggesting that maternal toxicity might not completely explain the occurrence of malformation at this dose level.

Comparison with criteria

RAC agrees that reduced foetal weight, reduced ossification and increased incidence of variations (7th cervical ribs) may be explained by the marked maternal toxicity observed at 1 000 mg/kg bw/d. There is some concern from potential developmental effects such as cleft palate and dead foetuses observed at the high dose level in rats. Nevertheless, cleft palates were mainly clustered in one dam that had total failure in foetal development. The occurrence of one cleft palate in the other litter is insufficient for classification. With regard to dead foetuses, although rarely occurring, this finding may have been secondary to the high maternal toxicity observed in the dams.

Overall, RAC considers that **no classification is warranted for developmental toxicity**.

***Effects on or via lactation***

In the 2-generation rat study, a reduction in mean pup weight (over LD4-21) was observed in both generations at the top dose that may indicate an effect on or *via* lactation (> 10 %, see table 54 of CLH report). At this dose, reduced body weight was observed but body weight loss was statistically significantly less than controls in both generations between lactation days 14-21. No data are available in the concentration of silthiofam and its metabolites in the milk. ADME data showed a wide distribution of silthiofam including fat suggesting that transfer to milk may be possible. Nevertheless, the reduced mean pup weight may also coincide with the beginning of ingestion of the chow containing the test material. Therefore, **no classification is proposed for lactation**.

# ENVIRONMENTAL HAZARD EVALUATION

## RAC evaluation of aquatic hazards (acute and chronic)

### Summary of the Dossier Submitter's proposal

***Degradation***

The dossier submitter proposed to consider silthiofam as not rapidly degradable for classification purposes. The basis for this proposal is that silthiofam is only rapidly hydrolysed at pH 4 (half-life at 20 °C > 73 hours) but must be considered to be stable at the more environmentally relevant pH 7 and pH 9 (half-life at 20 °C > 77 years).

A study on aqueous photolysis (Lewis, 1997) found that silthiofam was photodegraded in pH 7 aqueous solution under artificial sunlight with a $DT_{50}$ value of 16 days but it was not shown that under relevant environmental conditions the photochemical degradation could be expected to be above the criteria for classification.

In an OECD TG 301B test system, silthiofam degraded less than 2 % after 28 days and therefore is not readily biodegradable.

In a study (Lewis, 1997) with two different water/sediment systems, silthiofam dissipation resulted mainly in the formation of bound residues. It disappeared from the water phase with $DisT_{50}$ of 5 and 52 days and dissipated extremely slowly in whole systems with $DegT_{50}$s of 269 and 147 days (for the pond and run-off systems respectively). By the end of the study (100 days) the levels of silthiofam detected in the total system accounted for 74 % and 58 % of applied radioactivity for the pond and run-off systems, respectively. The amount of $^{14}$C-carbon dioxide was not reported by the dossier submitter.

In a second study (Irmer, 2013), two aquatic systems (river and pond) under aerobic conditions were investigated at 20 °C in the dark. Silthiofam dissipation from water was mainly due to the adsorption to the sediment layers. By the end of the study (118 days), silthiofam in the total system still accounted for 52.2 % and 50.2 % of applied radioactivity for the river and pond systems, respectively. The formation of $^{14}$C-carbon dioxide and other volatile products was insignificant.

The findings of two aerobic metabolism studies in soil under laboratory conditions (Lewis, 1996a and Goodyear, 1999a) indicate that silthiofam forms bound residues and may degrade to a certain degree in soil.

***Aquatic Bioaccumulation***

The dossier submitter proposed to not consider silthiofam as being bioaccumulative in the aquatic environment for classification purposes. The basis for this proposal is a log $P_{ow}$ of 3.72 and a measured steady-state bioconcentration factor (BCF) (total wet weight/normalised to 5 % lipid content) for fish of 98 L/kg.

***Acute Aquatic Toxicity***

The dossier submitter proposed to not classify silthiofam as acute toxic for the aquatic environment, as all acute toxicity values are above the threshold value of 1 mg/L. Thus, the basis for this proposal is that the available acute toxicity studies indicate that silthiofam is slightly toxic to fish and daphnia and moderately toxic to green algae. The most sensitive species tested was *Selenastrum capricornutum*, with a 72 h $EC_{50}$ (biomass) of 8.6 mg a.s/L and a 72 h $E_rC_{50}$ (growth rate) of 13 mg/L. All aquatic metabolites tested (MON 65513, MON 65533, MON 65534 and MON 65561) were of lower toxicity relative to silthiofam in all aquatic trophic levels (fish, daphnia, green algae).

***Table**: Summary of relevant information on aquatic toxicity*

| Method | Results | Remarks | Reference |
|---|---|---|---|
| **Fish** | | | |
| Silthiofam: A 96-hour static acute toxicity test with the rainbow trout (*Oncorhynchus mykiss*)<br><br>*OECD TG 203 and FIFRA Chapter 72-1 Subdivision E.* | $LC_{50}$ (mg a.s./L) = 14 (95 % CI: 13 - 16)<br><br>LOEC(mg a.s./L) = 5.3<br><br>NOEC(mg a.s./L) = 3.2 | Performed according to GLP criteria. | Anonymous (1996). MON 65500: A 96-hour static acute toxicity test with the rainbow trout |

| | | | |
|---|---|---|---|
| | Silthiofam is only slightly toxic to rainbow trout up to the water solubility limit. | | (*Oncorhynchus mykiss*) |
| Silthiofam: A 96-hour static acute toxicity test with the Bluegill (*Lepomis macrochirus*)<br><br>*OECD TG 203 and FIFRA Chapter 72-1 Subdivision E.* | $LC_{50}$ (mg a.s./L) = 11 (95 % CI: > 8.4)<br><br>LOEC(mg a.s./L) = 6.0<br><br>NOEC(mg a.s./L) = 3.7<br><br>Silthiofam is slightly toxic to bluegill sunfish up to the water solubility limit. | Performed according to GLP criteria. | Anonymous (1996). MON 65500: A 96-hour static acute toxicity test with the Bluegill (*Lepomis macrochirus*) |
| Silthiofam: An Early Life-Stage Toxicity Test with the Fathead Minnow (*Pimephales promelas*).<br><br>*ASTM Standard E-1241-05; FIFRA Subdivision E, Section 72-4a; OECD TG 210; OPPTS 850.1400* | Fathead minnows (*Pimephales promelas*) were exposed to silthiofam at mean measured concentrations ranging from 0.12 to 1.8 mg/L under flow-through conditions for 33 days (a 5-day hatching period plus a 28-day post-hatch growth period).<br><br>There were no significant treatment-related effects on hatching success or survival at concentrations ≤ 1.8 mg/L. Growth, measured as total length, wet and dry weight, was the most sensitive biological endpoint measured in this study.<br><br>Fathead minnows exposed to silthiofam at concentrations ≥ 1.8 mg/L had statistically significant reductions in total length, wet weight and dry weight in comparison to the pooled controls.<br><br>Consequently, the NOEC, based on growth, was 0.89 mg/L. The LOEC was 1.8 mg/L and the MATC was calculated to be 1.3 mg/L. | Performed according to GLP criteria. | Anonymous (2014). MON 65500: An Early Life-Stage Toxicity Test with the Fathead Minnow (*Pimephales promelas*) |
| **Aquatic Invertebrates** | | | |
| Silthiofam: A 48-hour static toxicity test with the cladoceran (*Daphnia magna*).<br><br>*OECD TG 202, FIFRA Chapter 72-2 Subdivision E.* | $EC_{50}$ (mg a.s./L) = 14.0 (95% CI: 12 – 16)<br><br>LOEC (mg a.s./L) = 7.8<br><br>NOEC (mg a.s./L) = 4.9<br><br>Up to the limit of water solubility, silthiofam is only slightly toxic to the waterflea, *Daphnia magna*. | Performed according to GLP criteria. | Graves W.C. and Swigert J. P. (1996). MON 65500: A 48-hour static toxicity test with the cladoceran (*Daphnia magna*) |

| | | | |
|---|---|---|---|
| Silthiofam: A semi-static life-cycle toxicity test with the cladoceran (*Daphnia magna*).<br><br>*OECD TG 211; ASTM Standard E1193-87* | LOEC (mg a.s./L) survival = 3.7<br><br>LOEC (mg a.s./L) reproduction = 3.7<br><br>LOEC (mg a.s./L) growth = 0.96<br><br>NOEC (mg a.s./L) survival= 1.8<br><br>NOEC (mg a.s./L) reproduction = 1.8<br><br>NOEC (mg a.s./L) growth = 0.47<br><br>Silthiofam has a moderate chronic toxicity to *Daphnia magna* with a NOEC of 0.47 mg a.i./L. | Performed according to GLP criteria. | Drottar, K.R., Kendall, T.Z. and Kreuger, H.O. (2000). MON 65500: A semi-static life-cycle toxicity test with the cladoceran (*Daphnia magna*) |
| **Algae** | | | |
| Silthiofam: A five-day toxicity test with the freshwater alga (*Selenastrum capricornutum*).<br><br>*OECD TG 201, EEC method C3 and FIFRA Chapter 123-2.* | There were no statistically significant reductions in cell density, area under the growth curve or growth rate of *Selenastrum capricornutum* exposed to silthiofam (MON 65500) at concentrations of 2.3 mg a.s./L.<br><br>Day 3 $EC_{50}$ values for biomass and growth rate were: $E_bC_{50}$ (0-72 h) = 8.6 (confidence limits: 2.9 and 11) and $E_rC_{50}$ (0-72 h) = 13 (confidence limits: 13 and 13), respectively.<br><br>$NOE_bC$ = 4.6 mg a.s./L<br><br>$NOE_rC$ = 2.3 mg a.s./L<br><br>Therefore, silthiofam is slightly to moderately toxic to the green algae, *Selenastrum capricornutum.* | Performed according to GLP criteria. | Drottar K.R. and Krueger H.O. (1998). MON 65500: A five-day toxicity test with the freshwater alga (*Selenastrum capricornutum*) |

### *Chronic Aquatic Toxicity*

The basis for the dossier submitter's proposal for chronic aquatic toxicity is that silthiofam is considered to be not rapidly degradable and to have a low bioaccumulation potential. Furthermore, the chronic aquatic toxicity studies indicate silthiofam is moderately toxic to fish (*Pimephales promelas*) and aquatic invertebrates (*Daphnia magna*). The 28-day Early Life-Stage Toxicity Test with the Fathead Minnow (*Pimephales promelas*) resulted in a NOEC (growth) of 0.89 mg a.s./L and the 21 day semi-static life-cycle toxicity test with the Waterflea (Daphnia magna) in a NOEC (growth) of 0.47 mg a.i./L. Thus, the dossier submitter proposed to classify silthiofam as toxic in Category Chronic 2; H411, based on the criteria set in Table 4.1.0 (b)(i).

## Comments received during public consultation

Four MSCAs commented on the proposals for environmental classification, all agreeing with the proposed classification as Category Chronic 2; H411.

Industry in their comments from June 2018 disagreed with the proposed classification. They argued that in the early life-stage toxicity test with the Fathead Minnow (*Pimephales promelas*) the $EC_{10}$ of 1.12 mg/L (mean measured) is more appropriate than the NOEC (growth) of 0.89 mg a.s./L for long-term environmental classification. In addition, they claim that for the OECD TG 211 semi-static life-cycle toxicity test with *Daphnia magna* the NOEC (reproduction) of 1.8 mg/L (mean measured) should be used instead of the NOEC (growth, length) of 0.47 mg a.i./L. They stressed that the results in the study report are only given as NOEC and LOEC information; no corresponding $EC_{10}$ values based on a dose-response curve are reported.

The DS noted the comments but did not provide any additional response.

## Assessment and comparison with the classification criteria

### *Degradation*

RAC agrees with the proposal of the dossier submitter to consider silthiofam as not rapidly degradable for classification purposes, based on the overall evidence from the hydrolysis, ready biodegradability and simulation studies.

### *Aquatic Bioaccumulation*

RAC agrees with the proposal of the dossier submitter to not consider silthiofam as being bioaccumulative in the aquatic environment for classification purposes, based on an experimentally derived BCF value for fish of 98 L/kg and a measured log $P_{ow}$ of 3.

### *Acute Aquatic Toxicity*

RAC agrees with the proposal of the dossier submitter to not classify silthiofam as acute toxic for the aquatic environment, based on no acute toxicity below the CLP threshold value of 1 mg/L.

### *Chronic Aquatic Toxicity*

RAC agrees with the comment by Industry that for long-term environmental classification in general the $EC_{10}$ value is more appropriate than the NOEC. The reason for this is that the $EC_{10}$ as a regression-based estimate is less influenced by dose selection and makes full use of the dose response curve. In general, the value of the $EC_{10}$ is smaller than the value of the NOEC and leads to a more stringent classification. In the case of the early life-stage toxicity test with the Fathead Minnow (*Pimephales promelas*) the $EC_{10}$ value is larger than the NOEC, which can be explained by the chosen test concentration intervals and by concentration-response modelling. The original study report does not provide an $EC_{10}$ value. In their comments from June 2018, the Applicant provided an $EC_{10}$ value of 1.12 mg/L (mean measured) while RAC has recalculated the $EC_{10}$ value using the Software ToxRat Professional Version 3.2.1 and found an $EC_{10}$ of 1.059 mg/L. Both values are close to the upper limit of the classification criteria for a not rapidly degradable substance. RAC concludes to take the uncertainty expressed by a lower NOEC than the $EC_{10}$ values into account and not to base the classification on the results from the early life-stage toxicity test with the Fathead Minnow (*Pimephales promelas*).

RAC notes, that the fish species Fathead Minnow (*Pimephales promelas*) used for the chronic endpoint is not represented in the acute fish data set. However, the surrogate approach using the acute data from the other two fish species (Rainbow Trout (*Oncorhynchus mykiss*) and Bluegill Sunfish (*Lepomis macrochirus*)) would not result in a more stringent classification.

In response to the comment by industry, RAC notes that OECD TG 211 states that growth measurements are highly desirable since they provide information on possible sub-lethal effects, which may be useful in addition to reproduction measures alone; the measurement of the length of the parent animals (i.e. body length excluding the anal spine) at the end of the test is recommended. The reporting may include any appropriate justification. Moreover, following the same guideline, a justification is not obligatory, which means that a missing justification does not invalidate the result as such. The endpoint growth based on length *per se* is a relevant endpoint for the purpose of classification. RAC concludes that for the OECD TG 211 semi-static life-cycle toxicity test with *Daphnia magna* the endpoint growth based on length is relevant for the purpose of classification.

RAC agrees with the proposal of the dossier submitter to classify silthiofam as **Aquatic Chronic 2; H411** based on the NOEC (growth) of 0.47 mg a.i./L from the 21 day test with the Waterflea (*Daphnia magna*) and silthiofam being not rapidly degradable.

## ANNEXES:

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

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