Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

benalaxyl (ISO); methyl *N*-(2,6-dimethylphenyl)-*N*-(phenylacetyl)-*DL*-alaninate

EC Number: 275-728-7
CAS Number: 71626-11-4

CLH-O-0000007053-82-01/F

Adopted

26 November 2021

26 November 2021

CLH-O-0000007053-82-01/F

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

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

Chemical name: **benalaxyl (ISO); methyl *N*-(2,6-dimethylphenyl)-*N*-(phenylacetyl)-*DL*-alaninate**

EC Number: **275-728-7**

CAS Number: **71626-11-4**

The proposal was submitted by **Romania** and received by RAC on **3 November 2020.**

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

PROCESS FOR ADOPTION OF THE OPINION

Romania 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 **7 December 2020**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **5 February 2021**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Michal Martínek**

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

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

The RAC opinion on the proposed harmonised classification and labelling was adopted on **26 November 2021** by **consensus**.

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

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	616-104-00-X	benalaxyl (ISO); methyl*N*-phenylacetyl-*N*-2,6-xylyl-*DL*-alaninate	275-728-7	71626-11-4	Aquatic Acute 1 Aquatic Chronic 1	H400 H410	GHS09 Wng	H410			
Dossier submitters proposal	616-104-00-X	benalaxyl (ISO); methyl *N*-(2,6-dimethylphenyl)-*N*-(phenylacetyl)-*DL*-alaninate	275-728-7	71626-11-4	**Retain** Aquatic Acute 1 Aquatic Chronic 1 **Add** Carc. 2 Acute Tox. 4 STOT SE 2	**Retain** H400 H410 **Add** H351 H302 H371 (nervous system)	**Retain** GHS09 Wng **Add** GHS08 GHS07	**Retain** H410 **Add** H351 H302 H371 (nervous system)		**Add** oral; ATE = 2000 mg/kg bw M = 1 M = 1	
RAC opinion	616-104-00-X	benalaxyl (ISO); methyl *N*-(2,6-dimethylphenyl)-*N*-(phenylacetyl)-*DL*-alaninate	275-728-7	71626-11-4	**Retain** Aquatic Acute 1 Aquatic Chronic 1 **Add** Acute Tox. 4	**Retain** H400 H410 **Add** H302	**Retain** GHS09 Wng **Add** GHS07	**Retain** H410 **Add** H302		**Add** oral; ATE = 1000 mg/kg bw M = 1 M = 1	
Resulting Annex VI entry if agreed by COM	616-104-00-X	benalaxyl (ISO); methyl *N*-(2,6-dimethylphenyl)-*N*-(phenylacetyl)-*DL*-alaninate	275-728-7	71626-11-4	Acute Tox. 4 Aquatic Acute 1 Aquatic Chronic 1	H302 H400 H410	GHS07 GHS09 Wng	H302 H410		oral; ATE = 1000 mg/kg bw M = 1 M = 1	

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

Benalaxyl is a fungicide which is currently classified as Aquatic Acute 1, H400 and Aquatic Chronic 1, H410 in Annex VI of the CLP Regulation. No M-factors have been set previously.

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

Explosives

A negative A.14 test (Constantini, 1995) is available for benalaxyl. The Dossier Submitter (DS) proposed no classification based on absence of chemical groups associated with explosive properties.

Flammable solids

The DS proposed no classification based on a negative A.10 test (Jonas, 1993). A negative result in an A.10 test can be used for classification under CLP (see Guidance on information requirements and chemical safety assessment, R.7.1.10.3).

Pyrophoric solids

The DS proposed no classification based on experience in handling and use.

Self-heating substances

An A.16 test (Constantini, 1995) showed no significant difference between sample and oven temperatures until the melting point (76.8°C). The DS proposed no classification based on a melting point below 160°C.

Oxidising solids

An A.17 test (Constantini, 1995) was positive. The maximum combustion velocity of benalaxyl was 0.78 mm/s, while the maximum combustion velocity of the reference mixture was 0.60 mm/s. The DS proposed no classification based on chemical structure (the substance contains oxygen, but the oxygen is chemically bonded only to carbon).

Comments received during consultation

A manufacturer supported no classification for physical hazards.

Assessment and comparison with the classification criteria

Explosives, Flammable solids, Pyrophoric solids, Self-heating substances

RAC agrees with the DS's assessment that **no classification** is warranted for the above hazard classes.

Oxidising solids

The substance contains oxygen atoms but these are only bonded to carbon. Thus, the substance meets the waiving criteria of the CLP based on structure (CLP, Annex I, 2.14.4.1)

In the A.17 test benalaxyl showed a combustion velocity comparable to that of the reference substance. The interpretation of the result according to the Risk Assessment Report (RAR) is that 'the test sample proved to be a weak oxidising substance'. However, the structure does not indicate oxidising properties and a follow-up test to exclude a false positive (with a non-combustible substance in place of cellulose, or in an inert atmosphere) is not available. Therefore, the result is considered equivocal rather than positive. Further, a result of an A.17 test cannot be directly compared with the CLP criteria.

As the only test available is an equivocal A.17 test and the CLP waiving criteria have been met, RAC agrees with the DS's proposal that **no classification** is warranted**.**

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute oral toxicity

Summary of the Dossier Submitter's proposal

Benalaxyl does not currently have a harmonized classification for acute toxicity. No mortality was observed at 2000 mg/kg bw in a standard acute oral toxicity study in rats (Anonymous 2013a; 5.2.1/01). However, lethality occurred at and below 2000 mg/kg bw in an acute neurotoxicity study and a respective range-finding study (Anonymous 2014c, 5.7.1/01; Anonymous 2014a, 5.7/02). Based on the mortality in the latter two studies the DS proposed classification with Acute Tox. 4; H302 and an ATE of 2000 mg/kg bw.

Comments received during consultation

Comments were received from 1 Member State Competent Authority (MSCA) and a manufacturer. The manufacturer agreed with the DS's proposal. The commenting MSCA considered the DS's proposal of Acute Tox. 4 and STOT SE 2 (nervous system) to represent a double classification. They preferred STOT SE 2 only, pointing out the lack of mortality in the standard acute toxicity study.

Assessment and comparison with the classification criteria

The studies relevant for the assessment of acute oral toxicity are summarized in the table below. In addition to the acute toxicity and acute neurotoxicity studies presented by the DS, the table also includes two other gavage studies in rats: a prenatal developmental (PNDT) toxicity study and a 5-week tolerability study.

Overview of oral gavage studies relevant for acute toxicity assessment		
Species; year; reference number	**Method** (for further details see the CLH report)	**Mortality or severe toxicity**
Rat 20135.2.1/01	Acute oral toxicity, up-and-down procedure (OECD TG 425) Strain: Sprague-Dawley derived Vehicle: aqueous carboxymethyl cellulose 5 females, 2000 mg/kg bw	No mortality at 2000 mg/kg bw 1 animal showed hypoactivity and irregular respiration; no significant clinical signs in the remaining 4 animals
Rat 1979 5.2.1/02	Acute oral toxicity Pre-guideline, limited reporting Dose levels: 3750 and 4500 mg/kg bw 5/sex/group	LD_{50} = 4200 mg/kg bw Mortality at 3750 mg/kg bw: 4/10 Mortality at 4500 mg/kg bw: 6/10
Rat 2014 5.7.1/01	Acute oral neurotoxicity, dose range-finding study Strain: Sprague-Dawley (Crl:CD(SD)) Vehicle: aqueous methylcellulose Dose levels (main phase): 0, 200, 400, 600, 2000 mg/kg bw 3/sex/dose (except 2000 mg/kg bw: only 1 male and 2 females, dosing discontinued due to excessive toxicity) Additional phase (to confirm clonic convulsions and lethality): 2000 mg/kg bw, 2/sex Scheduled sacrifice 1 day after dosing	2000 mg/kg bw, main phase: all 3 animals died within 4.5 hours; clonic convulsions in 2 animals 2000 mg/kg bw, additional phase: both females euthanized due to clonic convulsions; both males survived (no convulsions in males) 600 mg/kg bw: 1 male clonic convulsions; no significant clinical signs in the remaining 5 animals
Rat 2014 5.7.1/02	Acute oral neurotoxicity (OECD TG 424) Strain: Sprague-Dawley (Crl:CD(SD)) Vehicle: aqueous methylcellulose Dose levels (Phase 1): 0, 200, 400, 1000 mg/kg bw; 10/sex/group Dose levels (Phase 2): 0, 50, 100 mg/kg bw; 10 females/group Scheduled sacrifice 15 days after dosing	1000 mg/kg bw: 4 animals found dead (2 m, 2 f) within 4.5 hours, 3 of them (1 m, 2 f) clonic convulsions; 1 female clonic convulsions and survived; no significant clinical signs in the remaining 15 animals 400 mg/kg bw: 1 female increased respiration, splayed hindlimbs and immobility, euthanized in extremis 3 h post-dosing; no significant clinical signs in the remaining 19 animals 200 mg/kg bw: 1 female clonic convulsions and found dead 4 h post-dosing; no significant clinical signs in the remaining 19 animals
Rat 2015 5.6.2/02	Prenatal developmental toxicity (OECD 414) Strain: Sprague-Dawley (Crl:CD(SD)) Vehicle: aqueous methylcellulose Dose levels (Phase 1): 0, 15, 50, 150 mg/kg bw/d Dose levels (Phase 2 – due to lack of maternal toxicity in Phase 1): 0, 450/300 mg/kg bw/d Dosing GD 6-19 25/group	450 mg/kg bw (administered to 10 animals): 3 animals found dead within 6 hours after the first dose The dose was then reduced to 300 mg/kg bw/d. 300 mg/kg bw/d: no mortality, no significant clinical sings
Rat 1982 5.3.1/01	5-week repeated dose, non-guideline Strain: Wistar (BOR:WISW) Vehicle: Traganth 0.5% Dose levels: 0, 10, 100, 800 mg/kg bw/d The top dose was gradually increased: after the 1st week to 1000 mg/kg bw/d, after the 2nd week to 1500 mg/kg bw/d, after the 3rd week to 2500 mg/kg bw/d, after the 4th week to 3500 mg/kg bw/d, in the middle of the 5th week to 4000 mg/kg bw/d 10/sex/group + 2-week recovery at 0 and 800/4000 mg/kg bw/d, 5/sex/group	800/4000 mg/kg bw/d: no mortality, no clinical signs

The GLP- and OECD guideline-compliant acute oral toxicity study (2013; 5.2.1/01) reported no mortality at 2000 mg/kg bw. Hypoactivity and irregular respiration were observed in 1 out of 5 animals.

No mortality or clinical signs were reported up to 4000 mg/kg bw/d in a non-guideline rat repeated dose study (1982; 5.3.1/01).

In contrast, all dosed animals (1 male, 2 females) died at 2000 mg/kg bw in the main phase of the range-finding acute neurotoxicity study (2014; 5.7.1/01). Two of them showed clonic convulsions. Since the standard acute toxicity study (2013; 5.2.1/01) conducted shortly before by a different laboratory had not found any mortality at this dose, 2 additional males and 2 females were dosed at 2000 mg/kg bw in the range-finding neurotoxicity study to confirm the severe toxicity. Both additional females had to be sacrificed *in extremis* due to clonic convulsions, whereas the males survived without convulsions.

A top dose of 1000 mg/kg bw was then selected for the main acute neurotoxicity study (2014; 5.7.1/02). At doses of 1000, 400 and 200 mg/kg bw, 4, 1 and 1 out of 20 animals per group died, respectively.

A single dose of 450 mg/kg bw was lethal to 3 out of 10 pregnant rats in a PNDT study (2015; 5.6.2/02) from the same laboratory as the acute neurotoxicity studies. No overt toxicity was observed after reduction of the top dose to 300 mg/kg bw/d. RAC notes that acute toxicity classification should preferably be based on standard acute toxicity studies using non-pregnant animals (Guidance on the application of the CLP criteria, 3.1.3.3.5, in combination with OECD TG 420), so this study is considered only supplementary in the assessment of acute toxicity.

RAC does not see any obvious explanation for the difference in sensitivity between the standard acute oral toxicity study and the acute oral neurotoxicity studies, besides presumably a different breeder. All these studies are recent, conducted under GLP, and do not show deficiencies. The result of the standard acute toxicity study (i.e. no mortality at 2000 mg/kg bw) is in line with an older gavage study reporting no mortality up to 4000 mg/kg bw/d. Lethality below 2000 mg/kg bw was not limited to a single study either, as it occurred in the rat PNDT study from the same laboratory. According to the CLP guidance (Guidance on the application of the CLP criteria, 3.1.2.3.2), the lowest ATE from a suitable study should be taken forward for classification.

Although the acute neurotoxicity studies were not standard acute toxicity studies, they used young adult animals (6-8 weeks old), females were non-pregnant and the top dose was 2000 mg/kg bw (equal to the limit dose for acute toxicity studies). The post-observation period was 2 weeks only in the main study (testing up to 1000 mg/kg bw), but the peak of effects occurred within a couple of hours, so the short 1-day observation period in the range-finding study is unlikely to compromise the mortality rates in this case. Therefore, RAC agrees with the DS that the acute neurotoxicity studies provide reliable information on acute toxicity of benalaxyl and can be used for classification of acute toxicity.

The range-finding study reported a 100% mortality of females (4/4) and 33% mortality of males (1/3) at 2000 mg/kg bw. Females appeared to be somewhat more sensitive than males. The next lower dose of 1000 mg/kg bw resulted in a 20% mortality in both sexes (males 2/10, females 2/10). The male LD_{50} appears to lie around 2000 mg/kg bw, whereas the female LD_{50} somewhere between 1000 (20% mortality) and 2000 mg/kg bw (100% mortality). A dose causing 50% mortality in females cannot be determined from these data as one of the mortality values is 100%. In the absence of a reliable LD_{50} estimate for females, a conservative ATE of 1000 mg/kg bw is proposed by RAC.

In conclusion, RAC considers that benalaxyl warrants to be classified as **Acute Tox. 4; H302** with an **ATE** of **1000 mg/kg bw** based on mortality of female rats in acute oral neurotoxicity studies.

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

Summary of the Dossier Submitter's proposal

The DS proposed classification with STOT SE 2; H371 (nervous system) based on clinical sings of neurotoxicity observed in the main acute neurotoxicity study in rats (5.7.1/02), occurring not only below the proposed ATE for acute oral toxicity (2000 mg/kg bw), but also below the cut-off for classification as Acute Tox. 4 (300 mg/kg bw).

Comments received during consultation

Comments were received from 1 MSCA and a manufacturer. The manufacturer was of the view that the mortality in the acute neurotoxicity study may not have been due to neurotoxicity. Further, they mentioned inconsistency with other studies and lack of neurotoxicity in the subchronic neurotoxicity study. Still, they supported the DS's proposal.

The MSCA, who also commented on acute toxicity (see above), questioned whether the mortality in the acute neurotoxicity studies was a consequence of clonic convulsions or simply concomitant with the convulsions. They pointed out that the convulsions should not be used for classification in two hazard classes (acute toxicity and STOT SE).

Assessment and comparison with the classification criteria

The following table provides a detailed overview of findings related to neurotoxicity in the acute neurotoxicity studies (5.7.1/01, 02) below the proposed oral ATE of 1000 mg/kg bw. Description of effects at ≥ 1000 mg/kg bw can be found in the acute toxicity section.

Neurotoxicity-related findings below 1000 mg/kg bw in the acute neurotoxicity studies			
Dose (mg/kg bw); study (dose range-finding or main)	**No. of animals per group**	**Mortality; clinical sings in animals found dead or sacrificed *in extremis***	**Findings potentially related to neurotoxicity in survivors**
600 (drf)	3 m, 3 f	–	1 m clonic convulsions, decreased respiratory rate, gasping, drooping eyelids, slightly impaired mobility, dragging body, low arousal (2-4 h post-dosing)
400 (drf)	3 m, 3 f	–	–
400 (main)	10 m, 10 f	1 f: increased respiration, splayed hindlimbs and immobility at 2 h, euthanized at 3 h	Reduced no. of rearing counts (f 3.2 vs 9.3), increased motor activity (m, f) (day 0)
200 (drf)	3 m, 3 f	–	–
200 (main)	10 m, 10 f	1 f: clonic convulsions and vocalization at 2 h, found dead at 4 h	Reduced no. of rearing counts (f 3.9 vs 9.3), increased motor activity (f) (day 0)
100 (main)	10 f	–	3 f repetitive movement of the mouth and jaws, 2 f salivation (2 h post-dosing)
50 (main)	10 f	–	–

m = male, f = female

One male (out of 3) showed clonic convulsions and other clinical signs of toxicity at 600 mg/kg bw in the range-finding study. This animal survived to the scheduled sacrifice. However, 600 mg/kg is relatively close to the proposed acute oral ATE of 1000 mg/kg bw (causing 20% mortality in males), so the clinical signs at 600 mg/kg bw are considered to be of limited relevance for a STOT SE classification.

One female (out of 13) at 400 mg/kg bw was killed *in extremis* due to increased respiration, splayed hindlimbs and immobility. Death, preceded by convulsions, also occurred in 1 female (out of 13) at 200 mg/kg bw. As both animals died, clinical signs in these two animals are considered to be covered by the acute toxicity classification.

In the rest of the animals no notable clinical signs of toxicity were observed at 200, 400 or 600 mg/kg bw.

Repetitive movement of the mouth and jaws, possibly representing small-scale convulsions, was reported in 3 out of 10 females at a non-lethal dose of 100 mg/kg bw. Repetitive movement of the mouth and jaws was also noted in one female with convulsions at 2000 mg/kg bw (range-finding study, additional phase) but not at other doses (200-1000 mg/kg bw). Although this finding might indicate neurotoxicity, the absence of this finding at 200 and 400 mg/kg bw makes the interpretation difficult.

Further, an increase in total motor activity in the latter part of the testing session was observed from 400 mg/kg bw in males and from 200 mg/kg bw in females. The increase was attributed to a change in the pattern of habituation. In addition, the number of rearing counts was considerably reduced in females from 200 mg/kg bw. The alterations in locomotor activity and rearing, although suggestive of neurotoxicity, are not considered sufficiently severe to trigger classification.

No evidence of neurotoxicity was observed in a 90-day dietary neurotoxicity study in rats (5.3.2/03) conducted by the same laboratory as the acute toxicity studies. The top dose in this study was 10,000 ppm (677/745 mg/kg bw/d m/f).

A gavage prenatal developmental toxicity (PNDT) study, in rats (2015; 5.6.2/02) of the same strain from the same laboratory and breeder (Charles River) but in a different location of the breeder, reported no clinical signs of toxicity at a dose of 300 mg/kg bw, just below that causing mortality after a single dose (450 mg/kg bw) in this repeated dose study.

Overall, the acute neurotoxicity studies provide some indications of neurotoxicity at doses below those causing mortality. However, these are not considered sufficiently consistent or adverse to warrant a STOT SE classification in addition to the proposed acute oral toxicity classification (Acute Tox. 4, ATE = 1000 mg/kg bw). Further, the available studies do not provide evidence of other specific target organ effects relevant for a STOT SE classification in categories 1, 2 or 3. Therefore, RAC considers that for STOT SE **no classification** is warranted.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

The carcinogenic potential of benalaxyl has been investigated in a 2-year study in rats and an 18-month study in mice. The DS proposed classification in Category 2. The basis for this proposal is not very clear from the CLH-report, but astrocytomas in the rat study appear to have played a role in the reasoning.

Comments received during consultation

Comments were received from 1 MSCA and a manufacturer. The commenting MSCA supported Carc. 2 based on a low incidence of astrocytoma in the 2-year rat study, noting that this proposal is in line with the EFSA conclusion (EFSA, 2020).

The manufacturer proposed no classification, arguing that astrocytoma is a spontaneous brain tumour with a high incidence in Sprague-Dawley (SD) rats. They provided historical control data (HCD) from publicly available sources (summarised under 'additional key elements') since reliable HCD from the performing laboratory is not available.

Assessment and comparison with the classification criteria

As the presentation of the data in the CLH-report is not very clear, the RAC analysis is primarily based on the full study reports to the two carcinogenicity studies and on the RAR (draft Renewal Assessment Report, 2018).

2-year dietary study in rats (1985; 5.5/01)

The rats (Crl:CD(SD), 65/sex/group) were administered benalaxyl at dietary levels of 0, 4, 100 and 1000 ppm (top dose equivalent to 44/56 mg/kg bw/d m/f). 10 animals per sex and group were sacrificed after 1 year.

The treatment had no effect on survival, body weight, clinical signs, haematology, clinical chemistry parameters or organ weights. As to histopathology, the study authors concluded that there was no evidence of a treatment-related non-neoplastic or neoplastic effects. The brain tumour incidence is shown in the table below.

Brain tumours in the 2-year rat study				
Dose (ppm)	**0**	**4**	**100**	**1000**
Dose (mg/kg bw/d) m/f	0	0.18/0.23	4.4/5.6	44/56
No. examined, males	54	52	55	54
Astrocytoma, males; (day of death)	0	1 (647)	1 (630)	2 (654, 582)
Ependymoma, males	0	0	0	0
No. examined, females	54	55	54	55
Astrocytoma, females	0	0	0	0
Ependymoma, females (day of death)	0	0	0	1 (500)

Malignant astrocytoma was observed in 0, 1, 1, and 2 males in the control, low, mid and high dose group respectively. Astrocytoma was considered to be the cause of death in all 4 affected males, the same applies to the single top dose female with ependymoma (sacrificed moribund on day 500). The time to death or unscheduled sacrifice due to moribund condition in top dose males with astrocytomas does not appear to be shortened compared to the lower dose groups.

HCD from the performing laboratory is not available. No astrocytoma was observed in control males. A single astrocytoma occurred in males at the low dose, a dose so low (0.2 mg/kg bw/d) that it could almost be considered a "second control". In this regard, RAC notes that the dose spacing in this study is too wide (10 to 25-fold) and does not follow OECD TG 453.

The published HCD for male Crl:CD(SD) rats provided by industry, although of limited relevance, indicate a mean background incidence of 1-2% and a maximum incidence of about 5% (see 'additional key elements'). Thus, 1 astrocytoma per group is well within the spontaneous background incidence. The incidence of 2 at the top dose may slightly exceed the normal background, but the increase is not statistically significant on a pairwise comparison, the dose-response relationship is not particularly strong, and the incidence lies within the broader (although less relevant) HCD range. RAC concludes that there is insufficient evidence of treatment-related increase in brain tumours in rats.

The rationale for top dose selection is not provided in the study report. RAC notes that the top dose of 1000 ppm did not cause general toxicity. The main effects at a 10-fold higher dose of 10000 ppm in 90-day rat studies (5.3.2/02, /03) were slightly reduced body weight (<10%), increased cholesterol (by about 50%), increased liver weight (by ca. 40-55%), hepatocellular hypertrophy and steatosis, and thyroid follicular cell hypertrophy. This information indicates that a dose higher than 1000 ppm could have been tested without inducing excessive toxicity in a long-term rat study. Consequently, the available information on carcinogenic potential in rats is considered inconclusive due to low dosing.

18-month dietary study in mice (1985; 5.5/02)

Swiss mice (60/sex/group) were administered benalaxyl at dietary levels of 0, 250, 1000 and 3000 ppm (top dose equivalent to 559/522 mg/kg bw/d m/f).

The survival of males was reduced at the mid- and high-dose (statistically significant in a trend test). Female survival was not affected. There was no remarkable effect on body weight, clinical signs, haematology or clinical chemistry parameters. An increase in liver weight was detected in top dose females (relative by 27%). The treated males showed a higher incidence of amyloidosis (spleen, kidneys) and nephritis in the histopathological examination. A correlation between mortality rate and frequency of amyloidosis appears to exist in males. The top dose selection was based on a 90-day range-finding study where 5000 ppm (803/908 mg/kg bw/d m/f) caused a liver weight increase in both sexes (relative by 33%/53% m/f).

The only potentially treatment-related tumour in this study was urinary bladder proliferative lesion in 3 top dose males. These lesions were initially identified as transitional cell carcinomas by the study pathologist, but later, on a review by an external pathology working group (2001; 5.5/05), they were re-classified as submucosal mesenchymal tumours. This revised diagnosis was accepted in the EFSA assessment (EFSA, 2020). All three lesions were found in animals found dead during the study; two were detected on microscopic examination only (time of death weeks 46 and 55) and one also on gross pathology (week 68; a 4-mm nodule). Relevant HCD is not available. The background incidence of this lesion is generally difficult to determine due to a wide variety of diagnostic terms used in the past (including 'vegetative change' or 'decidual-like reaction') and differences in trimming procedure (often found in the trigone on microscopic examination, may be missed on cross-sectioning).

The nature and neoplastic potential of these mesenchymal proliferative lesions are controversial (Frazier *et al.*, 2012). They may be considered benign tumours and human relevance cannot be excluded (cf. the analysis in the RAC opinion on bifenthrin, 2011). Nevertheless, the incidence of these tumours in the mouse study with benalaxyl was low and occurred in presence of general toxicity. Therefore, this finding is not considered to warrant classification.

Histopathological findings and survival in the 18-month mouse study				
Dose (ppm)	**0**	**250**	**1000**	**3000**
Dose (mg/kg bw/d) m/f	0	45/43	181/174	559/522
Survival (%), males	62	58	32	25
Survival (%), females	72	83	85	70
No. examined, males	60	60	60	60
Kidney amyloidosis, males	10	12	28**	21**
Spleen amyloidosis, males	9	22**	29**	26**
Amyloidosis in any organ, males	14	24	37**	37**
Kidney abscesses and nephritis, males	13	15	14	24*
Urinary bladder submucosal mesenchymal tumour, males	0	0	0	3

Statistically significant difference from control: *, $p \leq 0.05$; **, $p \leq 0.01$

Conclusion

The available studies did not show any neoplastic effects warranting classification. However, the carcinogenicity potential of benalaxyl has not been fully investigated due to low dosing in the rat carcinogenicity study. Consequently, RAC concludes that **no classification** for carcinogenicity is warranted based on **inconclusive data**.

ENVIRONMENTAL HAZARD EVALUATION

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

Benalaxyl is a fungicide which is currently classified as Aquatic Acute 1, H400 and Aquatic Chronic 1, H410 in Annex VI of the CLP Regulation. No M-factors have been set. The DS proposed to add M-factor of 1 to both acute and chronic classification based on the 48-hour EC_{50} for *Daphnia magna* of 0.59 mg/L ($0.1 < EC_{50} \leq 1$ mg/L) and on the 21-day NOEC of 0.03 mg/L ($0.01 <$ NOEC ≤ 0.1 mg/L, not rapidly degradable) for *Daphnia magna*.

Degradation

In an OECD TG 111 study, the buffered aqueous solutions of 1.5 to 15 mg/L benalaxyl (99.2% purity) were incubated in the dark at pH 4, 7 and 9 for a period of 5 days at 50ºC and 70º C. Benalaxyl was stable to hydrolysis at pH 4 and 7. At pH 9, DT_{50} values were 55 days at 50°C and 19 hours at 70°C. DT_{50} values of 157 days and 86 days at 20°C and 25ºC, respectively, were extrapolated from the results at the higher temperatures. The main hydrolysis product was identified as benalaxyl acid (M9= DL-alanine, N-2,6-xylyl-N-phenylacetyl).

Benalaxyl was not easily photolysed under natural sunlight conditions during June – August at 45º 28' N, 3º 10' W coordinates since 60% Applied Radioactivity (AR) was still present as benalaxyl after 64 days of exposure. At least 15 unidentified compounds were detected but none of them individually represented more than 5% of the initially applied radioactivity.

In an aerobic surface water simulation test (GLP, OECD TG 309), degradation, transformation and mineralisation of ^{14}C-benalaxyl at two concentrations was studied in natural pond water. Natural water, pH 8.2, was treated with ^{14}C-benalaxyl at 10.5 µg/L and 106.7 µg/L and incubated in the dark at 23.4 ± 0.9°C for 62 days. Sterile controls and bio-controls were also set up. Duplicate test samples were taken at day 0, 7, 14, 21, 28, 42 and 62. Mineralisation of benalaxyl was negligible accounting for only 0.3% to 0.4% AR at the low- and high-test concentration, respectively. Insignificant transformation/degradation of benalaxyl was observed throughout the study period and therefore no DT_{50}/DT_{90} values could be derived. It was concluded that benalaxyl does not undergo any significant biodegradation/mineralisation in natural waters within the study period of 62 days.

In a GLP OECD TG 301 F Manometric respiratory test, ready biodegradability of benalaxyl (96.68% radiochemical purity) was investigated over a period of 28 days at 22ºC in the dark at a concentration of 30 mg suspended solids per litre. After 28 days of exposure the degradation rate of benalaxyl was –2.1. The DS considered that benalaxyl was not degraded by the activated sludge and can therefore be considered as "not readily biodegradable".

In a water/sediment study evaluated during the first EU review benalaxyl was observed to dissipate from the water compartment in two natural water/sediments according to a biphasic process. In the original evaluation benalaxyl was concluded to dissipate from the water phase with 1st order DT_{50} values of 5 and 10 days, respectively for the Pond and River systems, for the first phase. The second phase was slower and the corresponding 1st order DT_{50} values were 32 and 61 days for the Pond and River systems. No CO_2 was detected in the River system. In the Pond system CO_2 was only detected sporadically and reached a maximum of 0.4% AR at the end of the study. The main degradation products found in both systems were identified M1 (methyl-N-(2,6-xylyl)-N-malonyl alaninate) (max. < 10% AR) and M9 (benalaxyl acid) (< max. 10% AR). DT_{50} values in the whole sediment/water systems have been recalculated according to FOCUS (2006, 2011) guidance. Benalaxyl degraded with SFO DT_{50} values in the total system of 141.9 to 199.4 days at 20°C.

The DS presented summaries of the soil degradation studies in support of the original approval (Draft Assessment Report (DAR), 2000, 2003) in the CLH-Report. Due to concerns regarding possible shortcomings in the existing soil metabolism studies the route of degradation of benalaxyl was examined in four soils (loamy sand, clay, loam and silt loam) under aerobic conditions for 117 days according to OECD TG 307 (GLP). ^{14}C-benalaxyl degraded to 1.1% AR to 45.4% at the end of the study. Total non-extractable radioactivity reached a maximum of 22% to 50%. The RAR 2018 (Vol.3-Annex B.8) specified that $^{14}CO_2$ ranged from 3.5% AR to 17.1% AR in all four soils and organic volatiles were less than 0.1% AR. Two degradation products occurred at >5% at any sampling interval and were identified as M9 (10.1% AR) and M1 (45.2% AR).

Bioaccumulation

Benalaxyl has a log P_{OW} of 3.54 at 20°C and at pH=6.1 (Method EEC A 8, shake-flask method) which indicates a low bioaccumulation potential.

Bioconcentration potential of benalaxyl in fish was investigated in the non-GLP study using in house method complying with US updated requirements. Bluegill sunfish were exposed at a concentration of 0.0524 mg as/L under flow-through conditions for up to 28 days. Benalaxyl concentration in fish reached a plateau level within 3 days of exposure corresponding to a whole fish BCF value of 57. As the study was conducted according to an in-house method the validity of the study could not be assessed. Due to lack of information and deficiencies in complying with the OECD TG 305 conditions DS was of the opinion that the validity of the study according to current guidelines cannot be confirmed.

Aquatic toxicity

Acute Aquatic toxicity

***Table**: Summary of reliable information on acute aquatic toxicity*

Method	Species	Test material (purity)	Results	Reference
Fish				
96-hour (semi- static) OECD TG 203, EC C.1 GLP	*Oncorhynchus mykiss*	Benalaxyl technical (98.4%)	LC_{50} = 4.8 mg/L (1 mm no solvent used, saturated solutions	RAR B.9.2.1. CA 8.2.1/06 Anonymous (2014a)
96-hour (semi- static) OECD TG 203, EC C.1 GLP	*Oncorhynchus mykiss*	Benalaxyl Isomer R (98.6%)	LC_{50} = 4.9 mg/L mm no solvent used, saturated solutions	RAR B.9.2.1. CA 8.2.1/07 Anonymous (2014b)
96-hour (semi- static) OECD TG 203, EC C.1 GLP	*Oncorhynchus mykiss*	Benalaxyl Isomer S (98.3%)	LC_{50} = 5.0 mg/L mm no solvent used, saturated solutions	RAR B.9.2.1. CA 8.2.1/08 Anonymous (2014c)
Aquatic invertebrates				
48 hours (static) OECD TG 202, EC C.2 GLP	*Daphnia magna*	Benalaxyl technical (98.4%)	EC_{50} = 15 mg/L measured (2 no solvent used, saturated solutions	DRAR B.9.2.4.1 CA 8.2.4.1/02 Anonymous (2014a)
48 hours (static) OECD TG 202, EC C.2 GLP	*Daphnia magna*	Benalaxyl Isomer R (98.6%) R/S ratio: 99.8/0.2	EC_{50} = 13 mg/L measured (3 no solvent used, saturated solutions	RAR B.9.2.4.1 CA 8.2.4.1/03 Anonymous (2014b)
48 hours (static) OECD TG 202, EC C.2 GLP	*Daphnia magna*	Benalaxyl Isomer S (98.3%) (R/S ratio: 0/100)	EC_{50} = 17 mg/L measured (4 no solvent used, saturated solutions	RAR B.9.2.4.1 CA 8.2.4.1/04 Anonymous (2014c)
48 hours (static) OECD TG 202, Part I (1984) GLP	*Daphnia magna*	Benalaxyl (96.6 %)	**EC_{50} = 0.59 mg/L** nominal, measured conc. not available acetone used as solvent (*	RAR B.9.2.4.1 CA 8.2.4.1/01 Anonymous (1993)
Algae				
72 hours (static) OECD TG 201, EC C.3 GLP	*Pseudokirchneriella subcapitata*	Benalaxyl technical (98.4%)	E_rC_{50} = 3.5 mg/L gmm (13-20% of nominal) no solvent used, saturated solutions	RAR B.9.2.6.1 CA 8.2.6.1/02 Anonymous (2014a)
72 hours (static) OECD TG 201, EC method C.3 GLP	*Pseudokirchneriella subcapitata*	Benalaxyl Isomer R (98.6%)	E_rC_{50}= 3.4 mg/L gmm (21-40% of nominal) no solvent used, saturated solutions	RAR B.9.2.6.1 CA 8.2.6.1/03 Anonymous (2014b)
72 hours (static) OECD TG 201, EC method C.3 GLP	*Pseudokirchneriella subcapitata*	Benalaxyl Isomer S (98.3%)	E_rC_{50}= 3.4 mg/L (5 measured no solvent used, saturated solutions	RAR B.9.2.6.1 CA 8.2.6.1/04 Anonymous (2014c)

(* the details concerning the dose preparation technique were taken from the DAR 2018 Volume 3CA B-9
gmm = based on geometric mean measured concentrations
mm = based on mean measured concentrations
(1 geomean of the highest concentration causing no mortalities and the lowest concentration causing 100% mortality
(2 There was no significant change < 80% (98.3 to 106.2%) in the measured concentrations at 48 hours and so the results are based on 0-Hour measured test concentrations only.
(3 There was no significant change < 80% (99.4 to 104.2%) in the measured concentrations at 48 hours and so the results are based on 0-Hour measured test concentrations only.
(4 There was no significant change < 80% (96.4 to 104.4%) in the measured concentrations at 48 hours and so the results are based on 0-Hour measured test concentrations only.
(5 There was no significant change < 80% (93 to 102 %) in the measured concentrations at 72 hours and so the results are based on 0-Hour measured test concentrations only.

In three OECD TG 203 fish studies performed with benalaxyl technical, benalaxyl Isomer R and benalaxyl Isomer S the 96-hour LC_{50} values were 4.8, 4.9 and 5.0 mg/L, respectively. The dose preparation included dispersion of nominal amounts of test item in test water with the aid of a propeller stirrer, filtration of undissolved test item and pooling the preparations to give the 100% v/v saturated solution test concentration which was used to give the test concentrations as v/v saturated solution. The results were based on mean measured concentrations.

For invertebrates there were four OECD TG 202 studies available. Three of the tests used a similar dose preparation technique, based on saturated concentrations, as in the fish studies. The 48-hour EC_{50} values for benalaxyl technical, benalaxyl Isomer R and benalaxyl Isomer S were 15, 13 and 17 mg/L, respectively. The results were based on initial measured concentrations.

The fourth invertebrate test was performed according to Part I of the OECD TG 202 (1984) with benalaxyl. The study was thought as screening test for the reproduction test on *Daphnia magna*. The 48-hour EC_{50} was 0.59 mg/L (nominal). Dose preparation in this test was based on using acetone as solvent. Control and solvent control using 100 mL acetone/L (used in the highest concentration of the test substance) were performed. There were deficiencies in reporting of the study conditions, but the validity criteria of the OECD TG 202 were fulfilled. However, as test concentrations were not measured, the study was included in the DAR as supportive information only. The DS considered the study to be reliable and used this study, showing the highest toxicity for aquatic organisms, as the basis for aquatic acute classification proposal.

The 72-hour E_rC_{50} values for algae, *Pseudokirchneriella subcapitata* were 3.5, 3.4 and 3.4 mg/L for to benalaxyl technical, benalaxyl Isomer R and benalaxyl Isomer S, respectively, in OECD TG 201 tests. The results are based on measured concentrations. All three tests used a similar dose preparation technique, based on saturated concentrations, as in the fish studies.

Chronic aquatic toxicity

***Table**: Summary of reliable information on chronic aquatic toxicity*

Method	Species	Test material	Results	Reference
Fish				
30 days (ELS; flow-through) OECD TG 210 (2013) GLP	*Danio rerio*	Benalaxyl Technical (98.4%)	NOEC = 0.079 mg/L (body weight) mm (>80% of nominal) DMF (1 used as solvent (*	RAR B.9.2.2.1 CA 8.2.2.1/02 Anonymous (2014)
Invertebrates				
21 days (flow though) OECD TG 202, Part II, (1984) GLP	*Daphnia magna*	Benalaxyl Technical (96.6%)	**NOEC = 0.03 mg/L** mm (78%±25% of nominal)	RAR B.9.2.5.1 CA 8.2.5.1/01 Anonymous

Method	Species	Test material	Results	Reference
			acetone used as solvent (*	(1992b)
28 days (spiked water) OECD TG 207 and BBA-Guideline proposal (1995) GLP	*Chironomus riparius*	Benalaxyl Technical (96.68% ± 0.95%)	NOEC = 3.13 mg/L (2 nominal acetone used as solvent (*	RAR B.9.2.5.3 CA 8.2.5.3/01 Anonymous (1998)
Algae				
72 hours (static) OECD TG 201 and EC C.3 GLP	*Pseudokirchneriella subcapitata*	Benalaxyl technical (98.4%)	E_rC_{10} = 0.33 mg/L NOEC = 0.066 mg/L (gmm 13-20% of nominal) no solvent used, saturated solutions	RAR B.9.2.6.1 CA 8.2.6.1/02 Anonymous (2014a)
72 hours (static) OECD TG 201 and EC C.3 GLP	*Pseudokirchneriella subcapitata*	Benalaxyl Isomer R (98.6 %)	E_rC_{10} = 0.49 mg/L NOEC = 0.35 mg/L (gmm 21-40% of nominal) no solvent used, saturated solutions	RAR B.9.2.6.1 CA 8.2.6.1/03 Anonymous (2014b)
72 hours (static) OECD TG 201 and EC C.3 GLP	*Pseudokirchneriella subcapitata*	Benalaxyl Isomer S (98.3 %)	E_rC_{10} = 0.12 mg/L (3NOEC < 0.042 mg/L measured no solvent used, saturated solutions	RAR B.9.2.6.1 CA 8.2.6.1/04 Anonymous (2014c)

(*the details concerning the dose preparation technique were taken from the DAR 2018 Volume 3CA B-9
(1 dimethylformamide
(2 Results from the analysis of overlying water, pore water and sediment are reported in a separate study (DAR 2018, Volume 3 CA B9, study: Benalaxyl dissipates from the water and remains absorbed to sediment after 28 days)
(3 There was no significant change < 80% (93 to 102%) in the measured concentrations at 72 hours and so the results are based on 0-Hour measured test concentrations only.

There was one chronic study available on fish *Danio rerio* performed according to OECD TG 210 early-life stage test. Fertilised zebrafish eggs were exposed to the test item in a flow-through system to mean measured concentrations of 0.079, 0.26, 0.85, 2.68 and 8.33 mg/L, control, and a solvent control for a duration of 30 days after hatching. Dimethylformamide (DMF) was used as a solvent (100 µL/L) in dose preparation. The concentration of the solvent was in a range recommended in the test guideline. The eggs and larvae were observed daily for any sublethal effects and mortality. The NOEC for hatching success of zebrafish eggs was determined to be 8.33 mg/L. The NOEC values for survival, length and body weight (both wet- and dry-) were 2.68, 0.85, 0.079 mg/L respectively.

For invertebrates there were two studies available, one for *Daphnia* and one for *Chironomus riparius* (OECD TG 207, spiked water). Results from the analysis of overlying water, pore water and sediment of the *Chironomus* study showed that benalaxyl dissipates from the water and remains absorbed to sediment after 28 days.

In the OECD TG 202, Part II study toxicity of benalaxyl to *Daphnia magna* was determined over 21 days under flow-through conditions. *Daphnia* were exposed to nominal concentrations of 0.041, 0.122, 0.34, 1.1 and 3.3 mg/L. Acetone (165 µL/L) in the highest concentration of the test

substance was used as the solubilising agent. The concentration of the solvent exceeded the highest concentration allowed in the current TG for chronic tests on daphnids. A control and solvent control were also prepared. The number of immobilised daphnids, observations of abnormal behaviour and the number of young daphnids (F1) were recorded on Days 1, 4, 7, 9, 11, 14, 16, 18 and 21. The samples for analytical control were taken at days 1, 11 and 21. The mean measured concentrations represented 78% ± 25% of nominal values for the test duration. Based on measured average concentrations, the 21-day EC_{50} (immobilisation, 21d) of 0.12 mg/L, EC_0 (immobilisation, 21d) of 0.03 mg/L, and EC_0 (reproduction, 21d) of 0.03 mg/L and an overall NOEC of 0.03 mg/L were established. The DS used this study, showing the highest toxicity for aquatic organisms, as the basis for aquatic chronic classification.

In addition, in the full study report available to the RAC, it was further explained that a test without the use of solubilising agent was performed. The stock solution was dispersed mechanically (ultrasonic bath). The nominal concentrations were 0.21, 1.04, 5.2 26 and 130 µg/L. No effects were seen in this test.

In the three algae studies (OECD TG 201) for benalaxyl technical, benalaxyl-R and benalaxyl-S 72-hour NOEC values of 0.066, 0.35 and <0.042 mg/L were, respectively, established based on measured concentrations. The corresponding E_rC_{10} values were 0.33, 0.49 and 0.12 mg/L, respectively. The dose preparation in each of the studies was done by dispersing the test item with the aid of propeller stirring and removing the undissolved test item by filtration to get a 100% v/v saturated solution. A series of dilutions were made to give v/v stock solutions which were then inoculated to algal suspension to give the required test concentrations in v/v saturated solutions.

Comments received during consultation

The manufacturer supported retaining the classification Aquatic Acute 1, H400 and Aquatic Chronic 1, H410.

Comments were made by two MSCAs and one National Authority (NA). A MSCA thought that it was not clear why the aquatic acute classification was based on the study CA 8.2.4.1/01 (Daphnia, Part I of OECD TG 202) which was originally included in the DAR as supplementary information only. Due to lack of chemical analysis further explanation for identifying this study as a key study was requested. The DS gave no further explanation in response. Both MSCAs pointed out a mistake in Table 24 of the CLH report concerning nominal/measured concentrations in this study. The other MSCA supported the proposed classification with M-factor of 1 for both acute and chronic classification.

The NA also paid attention to the lack of analytical verification in the aquatic acute key study CA 8.2.4.1/01. They considered supporting information to be necessary to define the reliability of the *Daphnia magna* study. In their opinion the study could be accepted because the physico-chemical properties support that the substance would remain stable in the aquatic phase and because in other ecotoxicity studies the measured concentrations are generally within 80-120% of the initial measured concentrations. In addition, most of the study parameters were comparable to those recommended in OECD TG 202 (2004) and the control data met the validity criteria. The NA agreed to the proposed chronic classification. The DS thanked for the comments.

Assessment and comparison with the classification criteria

Comparison with the criteria

Degradation

RAC agrees with the DS conclusion that benalaxyl is not rapidly degradable:

- benalaxyl was not degraded in the OECD TG 301 F ready biodegradability test in 28 days.
- in the aerobic surface water simulation test (OECD TG 309) mineralisation of benalaxyl was negligible accounting for only 0.3% to 0.4% AR at the low- and high-test concentration, respectively. Benalaxyl did not undergo any significant biodegradation/mineralisation in natural waters within the study period of 62 days.
- In the OECD TG 111 hydrolysis study benalaxyl was stable to hydrolysis at pH 4 and 7. At pH 9 DT_{50}s of 157 days and 86 days at 20°C and 25ºC, respectively, were extrapolated from the results at the higher temperatures.
- In the water/sediment study benalaxyl degraded with SFO DT_{50}s of 141.9 to 199.4 days at 20°C in the total system. Mineralisation was negligible.

Bioaccumulation

RAC agrees with the DS conclusion that benalaxyl has a low potential for bioaccumulation.

The available fish bioconcentration study has been considered not reliable by the DS; RAC agrees with this assessment.

In the octanol/water-coefficient study using shake-flask method a log K_{OW} of 3.54 at 20°C and at pH=6.1 was measured. Benalaxyl can, however, be considered as a surface-active substance because of the surface tension of 47.0 mN/m and thus the shake-flask method is not applicable. The KOWWIN v1.68 estimated log K_{ow} of 3.69 is, however, in the same order of magnitude. The log K_{ow} values are below the classification cut-off value of 4.

Aquatic toxicity

RAC notes that when reviewing the aquatic toxicity studies, one possible explanation to the over one order of magnitude difference in toxicity study results for the same species might be the dose preparation technique used. This can, obviously, be noted only when there are studies on the same trophic level using different dose preparation techniques. The used techniques are either based on the use of a solvent or the use of stirring followed with filtration of undissolved test item to get saturated test solutions. Both techniques are seen appropriate for poorly soluble substances in the OECD Guidance Document 23.

RAC also used ECOSAR v1.11 QSAR model to estimate the toxicity of benalaxyl. The model used prediction for esters, amides and baseline toxicity but neither of the estimations fitted the test results available for classification. RAC concludes that QSAR predictions for toxicity are not relevant in this case.

Acute

In the three reliable fish studies saturated solutions were used for dose preparation. The lowest 96-hour LC_{50} was 4.8 mg/L (measured) for benalaxyl technical.

For invertebrates three of the four studies on *Daphnia magna* used saturated solutions for dose preparation. The tests are considered reliable by RAC. The lowest 48-hour EC_{50} was 13 mg/L (measured) for benalaxyl-R. The fourth test was performed using acetone as solvent and the 48-hour EC_{50} was 0.59 mg/L (nominal). Despite the lack of measured concentrations, RAC considers this study reliable based upon the evaluation of the full study report. The study was a screening test for the reproduction study and led to the choice of the tested concentrations according to the OECD TG 202 (1984). The physico-chemical profile of the substance does not indicate any potential mechanism that would cause the test concentrations decline during the test. No information on tests using solvent, and which had measured concentrations for 48-hours, was available, therefore no comparison with tests using saturated solution method was possible.

In the three reliable algae studies saturated solutions were used for dose preparation. The lowest 72-hour E_rC_{50} was 3.4 mg/L for benalaxyl-R and benalaxyl-S.

RAC considers that the lowest acute toxicity value is a 48-hour EC_{50} of 0.59 mg/L (nominal) for *Daphnia*. The EC_{50} is in the range of 0.1 < EC_{50} ≤1 and thus M-factor of 1 is warranted.

Chronic

There is only one reliable chronic fish test available. Dimethylformamide was used as solvent in the test which gave a 30-day NOEC of 0.079 mg/L (measured) for benalaxyl technical.

For invertebrates there were two chronic studies available. The full study report available to RAC provided more detailed information of the *Daphnia magna* study. After evaluating the full study report RAC agrees with the DS to consider the study valid and reliable. RAC notes that for dose preparation acetone was used as a solvent. The concentration of acetone used exceeded (165 µL/L) the highest concentration allowed in the current TG for chronic tests on daphnids (0.1 mL/L in OECD TG 211). The 21-day NOEC (body weight) for benalaxyl technical was 0.03 mg/L (measured).

The *Chironomus* study is not in this case seen relevant for classification since the conclusion of the additional study concluded that benalaxyl dissipates from the water and remains absorbed to sediment after 28 days. Therefore, the exposure route in the study cannot be confirmed.

There are three reliable chronic algae studies available for benalaxyl technical, benalaxyl-R and benalaxyl-S. The lowest NOEC and E_rC_{10} values are < 0.042 and 0.12 mg/L, respectively, for benalaxyl-S. EC_{10} values are preferred by RAC as these are statistically derived from the entire dataset, and less dependent on test design considerations than the NOEC.

RAC considers that the lowest chronic toxicity value is a 21-day NOEC of 0.03 mg/L (measured) for *Daphnia*. The NOEC is in the range of 0.01 < NOEC ≤ 0.1 and, for a not rapidly degradable substance, an M-factor of 1 is warranted.

RAC, thus, agrees with the DS proposal to classify benalaxyl with **Aquatic Acute 1, H400, M = 1 and Aquatic Chronic 1, H410, M = 1**.

RAC considers, however, that the classification of benalaxyl might have to be revisited in case

- new acute invertebrate toxicity data based on measured concentrations become available;
- toxicity data on blue-green algae (cyanobacteria) become available. As a fungicide benalaxyl can be considered to have anti-microbial activity and blue-green algae are potentially more sensitive than green-algae to anti-microbials. With the current difference in sensitivity between algae and daphnid of less than a factor of 10, this could influence the current classification;
- the reason for over one order of magnitude differences in aquatic toxicity studies on the same species find an explanation.

Additional references

Baldrick (2005) Carcinogenicity evaluation: comparison of tumor data from dual control groups in the Sprague-Dawley rat. Toxicologic Pathology 33:283-291

Bertrandt *et al.* (2014): Incidence of spontaneous central nervous system tumors in CD-1 mice and Sprague-Dawley, Han-Wistar, and Wistar rats used in carcinogenicity studies. Toxicologic Pathology: 42:1168-1173

EFSA (2020) Peer review of the pesticide risk assessment of the active substance benalaxyl. EFSA Journal 18:5985. Online: https://www.efsa.europa.eu/en/efsajournal/pub/5985 (last accessed 05/08/2021)

Frazier *et al.* (2012) Proliferative and nonproliferative lesions of the rat and mouse urinary system. Toxicologic Pathology 40:14S-86S

Giknis and Clifford (2004) Compilation of spontaneous neoplastic lesions and survival in Crl:CD®(SD) rats from control groups. Charles River Laboratories. Online: https://www.criver.com/products-services/find-model/cd-sd-igs-rat?region=3616 (last accessed 10/08/2021)

Gopinath (1986) Spontaneous brain tumours in Sprague-Dawley rats. Food and Chemical Toxicology 24:113-120

Nagatani *et al.* (2013) Occurrence of spontaneous tumors in the central nervous system (CNS) of F344 and SD rats. Journal of Toxicologic Pathology 26:263-273

RAC (2011) Opinion proposing harmonised classification and labelling at Community level of bifenthrin. CLH-O-0000001740-81-01/F. Adopted 24 May 2011

Romania (RMS) and Portugal (Co-RMS) (October 2018) Benalaxyl – Draft Renewal Assessment Report under Regulation (EC) 1107/2009. Online: https://www.efsa.europa.eu/en/consultations/call/161125 (last accessed 05/08/2021)

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).