Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

(3a*S*,5*S*,6*R*,7a*R*,7b*S*,9a*S*,10*R*,12a*S*,12b*S*)-10-[(2*S*,3*R*,4*R*,5*R*)-3,4-dihydroxy-5,6-dimethylheptan-2-yl]-5,6-dihydroxy-7a,9a-dimethylhexadecahydro-3*H*-benzo[c]indeno[5,4-e]oxepin-3-one; 24-epibrassinolide

EC Number: -
CAS Number: 78821-43-9

CLH-O-0000006728-62-01/F

Adopted

5 December 2019

P.O. Box 400, FI-00121 Helsinki, Finland | Tel. +358 9 686180 | Fax +358 9 68618210 | echa.europa.eu

5 December 2020

CLH-O-0000006728-62-01/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: **(3a*S*,5*S*,6*R*,7a*R*,7b*S*,9a*S*,10*R*,12a*S*,12b*S*)-10-[(2*S*,3*R*,4*R*,5*R*)-3,4-dihydroxy-5,6-dimethylheptan-2-yl]-5,6-dihydroxy-7a,9a-dimethylhexadecahydro-3*H*-benzo[c]indeno[5,4-e]oxepin-3-one; 24-epibrassinolide**

EC Number: **-**

CAS Number: **78821-43-9**

The proposal was submitted by **Austria** and received by RAC on **10 January 2019.**

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

PROCESS FOR ADOPTION OF THE OPINION

Austria has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **21 January 2019**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **22 March 2019**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Miguel A. Sogorb**

Co-Rapporteur, appointed by RAC: **Ignacio de la Flor Tejero**

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 **5 December 2019** 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	No current Annex VI entry										
Dossier submitters proposal	TBD	(3a*S*,5*S*,6*R*,7a*R*,7b*S*,9a*S*,10*R*,12a*S*,12b*S*)-10-[(2*S*,3*R*,4*R*,5*R*)-3,4-dihydroxy-5,6-dimethylheptan-2-yl]-5,6-dihydroxy-7a,9a-dimethylhexadecahydro-3*H*-benzo[c]indeno[5,4-e]oxepin-3-one; 24-epibrassinolide		78821-43-9	Aquatic Chronic 4	H413		H413			
RAC opinion	TBD	(3a*S*,5*S*,6*R*,7a*R*,7b*S*,9a*S*,10*R*,12a*S*,12b*S*)-10-[(2*S*,3*R*,4*R*,5*R*)-3,4-dihydroxy-5,6-dimethylheptan-2-yl]-5,6-dihydroxy-7a,9a-dimethylhexadecahydro-3*H*-benzo[c]indeno[5,4-e]oxepin-3-one; 24-epibrassinolide		78821-43-9	Aquatic Chronic 4	H413		H413			
Resulting Annex VI entry if agreed by COM	TBD	(3a*S*,5*S*,6*R*,7a*R*,7b*S*,9a*S*,10*R*,12a*S*,12b*S*)-10-[(2*S*,3*R*,4*R*,5*R*)-3,4-dihydroxy-5,6-dimethylheptan-2-yl]-5,6-dihydroxy-7a,9a-dimethylhexadecahydro-3*H*-benzo[c]indeno[5,4-e]oxepin-3-one; 24-epibrassinolide		78821-43-9	Aquatic Chronic 4	H413		H413			

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

The substance 24-epibrassinolide is used as a plant protection product, more specifically, as an elicitor and plant activator in agriculture (viticulture, arable crops and vegetable production) and exerts no direct fungicidal or antagonic effect against harmful organisms since it acts by activating and enhancing the defence and immune system of plants.

A Member State Competent Authority (MSCA) considered that there is insufficient information for important hazards in the submitted CLH report and recommended not to assess the impact on human health and the resulting classification and labelling. This MSCA did not consider the arguments provided by the applicant and rapporteur member state for not providing this information (the presence of brassinosteroids in plants and a literature review do not raise concerns) as sufficient justification for non-submission of the required toxicological studies. Finally, this Member State Competent Authority stated that a proposal for requesting at least further studies on *in vivo* genotoxicity and *in vitro* endocrine disruption had been proposed.

The Dossier Submitter (DS) replied that the submission of an incomplete data package has been considered acceptable as the substance occurs naturally in food of plant origin and there is continuous lifetime exposure to phytosterols, including 24-epibrassinolide via the diet. Moreover, despite the fact that information in the public literature on 24-epibrassinolide is scarce, a wealth of literature on phytosterols and stanols is available, although the presentation of information about these substances was considered by the DS to be outside the scope of this specific assessment.

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

The DS proposed no classification of 24-epibrassinolide for physical hazards on the basis of the following considerations:

- The structural formula and the negative oxygen balance of 24-epibrassinolide suggest that the substance is not explosive;
- The results of an A.10 assay for testing flammability of solids showing that the substance cannot be ignited;
- The experience in manufacture and handling that demonstrates that the substance does not spontaneously ignite coming into contact with air at normal temperatures, hence it's not pyrophosphoric;
- The substance does not give exothermic reactions with oxygen until it melts and therefore is not self-heating;
- The structural formula and the negative oxygen balance suggest that 24-epibrassinolide is not an oxidising solid.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

RAC, in the absence of other relevant information, consider that **no classification of 24-epibrassinolide for physical hazards** is warranted.

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

The DS proposed no classification of 24-epibrassinolide for acute toxicity on the basis of the following results:

- An acute oral toxicity study in rats with an LD_{50} higher than 5000 mg/kg bw;
- An acute dermal toxicity study in rats with an LD_{50} higher than 2000 mg/kg bw;
- An acute inhalation toxicity study in rats with an LC_{50} higher than 1.08 mg/L.

Comments received during public consultation

See RAC general comments.

Assessment and comparison with the classification criteria

The table below overalls the main findings reported by the CLH dossier in the available acute toxicity studies.

Table: Summary of animal studies on acute toxicity with 24-epibrassinolide. *All studies summarised below were performed observing GLP procedures.*

Study	Dose level	Results	Reference
OECD TG 423 Oral route Wistar rats	5000 mg/kg bw 91.2 % purity Vehicle: refined groundnut oil	No mortalities No clinical signs No abnormalities at necropsy LD_{50} > 5000 mg/kg bw	Anonymous, 2017a
OECD TG 402 Dermal route Wistar rats 5 animals/sex	2000 mg/kg bw 91.2 % purity Vehicle: distillate water 24 hours semi-occlusive dressing	No mortalities No clinical signs No abnormalities at necropsy LD_{50} > 2000 mg/kg bw	Anonymous, 2017b
OECD TG 426 Inhalation route Wistar rats 3 animals/sex	Dust aerosol 1.08 mg/L air (maximum attainable concentration) 4 hours exposure 91.2 purity MMAD = 2.54 - 3.01 µm	No mortalities No clinical signs No abnormalities at necropsy LC_{50} > 1.08 mg/L	Anonymous, 2017c

MMAD= Median mass aerodynamic diameter

Comparison with the criteria

The substance has low solubility in water (3.8 mg/L); which suggests very high lipophilicity. Indeed, 24-epibrassinolide was dosed in refined groundnut oil in the acute oral toxicity study. The OECD TG 402 states that substances should be dosed in suitable vehicles to ensure good contact with the skin. Thus, RAC does not consider distilled water a suitable vehicle for 24-epibrassinolide in the acute dermal toxicity study, and therefore the result of this study could be considered as of limited value. Nevertheless, RAC also notes that no toxicity was reported up to 5000 mg/kg be in the acute oral toxicity study and therefore mortalities would not be likely after dermal exposure to 2000 mg/kg bw.

RAC notes that LD_{50} for acute oral and acute dermal toxicity must necessarily be above the cut-off points for triggering classification of 5000 and 2000 mg/kg bw; respectively. Therefore, RAC agrees with the DS' proposal for **no classification of 24-epibrassinolide for oral and dermal acute toxicity.**

The maximum concentration for triggering classification for acute inhalation toxicity is 5 mg/L. The only available study shows that exposure to 1.08 mg/L during of 24-epibrassinolide for 4 hours did not cause mortalities, clinical signs and necropsy alterations. RAC agrees with the DS's proposal for **no classification of 24-epibrassinolide for acute inhalation toxicity** since the maximum attainable concentration (1.08 mg/L) does not cause mortalities.

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

Summary of the Dossier Submitter's proposal

DS proposed no classification of 24-epibrassinolide for STOT SE.

Comments received during public consultation

See RAC general comments.

Assessment and comparison with the classification criteria

RAC notes that no clinical signs of toxicity were reported in the three acute toxicity studies and therefore no target organ toxicity could be identified; therefore the classification criteria for STOT SE Categories 1 or 2 are not fulfilled. Moreover, no narcotic effects or respiratory irritation were reported and therefore the classification of 24-epibrassinolide within Category 3 of STOT SE is not supported. In conclusion, RAC agrees with the DS's proposal for **no classification of 24-epibrassinolide as STOT SE.**

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

The DS proposed no classification of 24-epibrassinolide for skin corrosion/irritation since no dermal effects (no erythema/oedema) were observed in an OECD TG 404 study with rabbits.

Comments received during public consultation

See RAC general comments.

Assessment and comparison with the classification criteria

The table below overalls the main findings reported by the CLH dossier in the available dermal irritation/corrosion study.

Table: Summary of the skin corrosion/irritation study with 24-epibrassinolide.

Study	Dose level	Results	Reference
OECD TG 404 New Zealand White rabbits GLP 3 males	0.5 g moistened with distilled water 91.2 % purity 4 hours semi-occlusive exposure	No mortalities No clinical signs No abnormalities at necropsy All individual scores of oedema and erythema at 24, 48 and 72 hours were 0.	Anonymous, 2017d

RAC notes that no oedema and erythema was caused as consequence of the exposure to 24-epibrassinolide and therefore the criteria for supporting classification were not met. RAC agrees with the DS's proposal for **no classification of 24-epibrassinolide for skin corrosion/irritation.**

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

The DS proposed no classification of 24-epibrassinolide for serious eye damage/eye irritation since no ocular (cornea, iris or conjunctiva) damages were observed in an OECD TG 405 study with rabbits.

Comments received during public consultation

See RAC general comments.

Assessment and comparison with the classification criteria

The table below overalls the main findings reported by the CLH dossier in the available study on serious eye damage/eye irritation.

Table: Summary of the animal studies on serious eye damage/eye irritation with 24-epibrassinolide.

Study	Dose level	Results	Reference
OECD TG 405 New Zealand White rabbits GLP 3 males	0.1 g 91.2 % purity	No mortalities No clinical signs No abnormalities at necropsy All individual scores of opacity, iris, conjunctivae and chemosis were 0.	Anonymous, 2017e

RAC notes that no effects on cornea, iris or conjunctiva was caused as a consequence of the exposure to 24-epibrassinolide and therefore the criteria for classification were not met. RAC agrees with the DS's proposal for **no classification of 24-epibrassinolide for serious eye damage/eye irritation.**

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

The DS proposed no classification of 24-epibrassinolide for skin sensitisation since no reaction were observed after challenge exposure in an OECD TG 406 study with guinea pig.

Comments received during public consultation

See RAC general comments.

Assessment and comparison with the classification criteria

The table below overalls the main findings reported by the CLH dossier in the available study on skin sensitisation.

Table: Summary of the animal studies on skin sensitisation with 24-epibrassinolide.

Study	Dose level	Results	Reference
OECD TG 406 GLP Albino Dunkin Hartley Guinea pig Males 10 challenges 5 controls	Intradermal <u>Day 0</u> Intradermal induction: Injection 1: 1:1 (v/v) mixture of Freud's Complete Adjuvant and physiological saline Injection 2: 0.1 % 24-epibrassinolide (w/v) in propylene glycol Injection 3: 0.1 % 24-epibrassinolide in propylene glycol and in a 1:1 (v/v) mixture of Freud's Complete Adjuvant and physiological saline <u>Day 6</u> 0.5 mL of 10 % sodium lauryl sulphate in vaseline <u>Day 7</u> Epidermal induction: 48 hours under occlusion with 100 % 24-epibrassinolide, <u>Day 21</u> Challenge by epidermal application of 200 mg 100 % 24-epibrassinolide moistened with distilled water under occlusion	24 hours after intradermal injection 6/10 treated animals showed erythema (grade 1) 24 hours after epidermal induction 5/10 treated animals showed erythema (grade 1) No positive skin reactions were observed in both treated and control animals 24 hours and 48 hours after challenge with 100 % test item	Anonymous, 2017f

RAC notes that the substance is solid at room temperature. OECD TG 406 determines that skin sensitization of solids should be tested using the substance finely pulverised in a suitable vehicle. As was stated above, 24-epibrassinolide is a lipophilic substance and therefore water cannot be considered as a suitable vehicle. However, RAC notes that animals were challenged on day 21 with patches of filter papers saturated with 200 mg of 100 % non-irritating 24-epibrassinolide moistened with 0.2 mL of distilled water. Thus, the bioavailability of the substance might be compromised during the challenge and therefore RAC does not consider the results of this study as conclusive. Therefore, for all the above stated reasons, **RAC was unable to assess this hazard due to inconclusive data.**

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

Summary of the Dossier Submitter's proposal

As the highest concentration for warranting classification as STOT RE based on studies of 90 days of exposure was 100 mg/kg bw/d the DS proposed no classification of 24-epibrassinolide for this hazard on the basis of a 90-days oral toxicity study in rats with a NOAEL of 300 mg/kg bw/d.

Comments received during public consultation

See RAC general comments.

Assessment and comparison with the classification criteria

The table below overalls the main findings reported by the CLH dossier in the only available repeated dose toxicity study.

Table: Summary table for the only repeated dose available toxicity study with 24-epibrassinolide.

Method	Results	Reference
90-day repeated dose oral toxicity study OECD TG 408 GLP Wistar rats Gavage (vehicle 0.1 % sodium carboxymethyl cellulose) 10 animals/sex/group 91.2 % purity 0, 100, 300, 1000 mg/kg bw/d Two extra groups dosed with 0 and 1000 mg/kg bw/d for studying recovery during 4 weeks	1000 mg/kg bw/d Reductions in body weight in males (in all cases $p < 0.05$): by 5 % (days 71, 78 and 85). Reductions in body weight in male recovery group (in all cases $p < 0.05$): by 6 % (days 50 and 57); by 9 % (days 64, 71, 78, 87, 98, 105, 112 and 118). Reductions in body weight in female recovery group (in all cases $p < 0.05$): by 7 % (days 43 and 50); by 8 % (day 57); by 9 % (day 64); by 10 % (day 71); by 11 % (day 78); by 10 % (days 85 and 92); and by 9 % /days 98, 105, 112 and 118). Reduction by around 15 % of food consumption in males (weeks 1, 2, 3, 4, 5, 6 and 7) ($p < 0.05$ in all cases). Reduction by around 9 % of food consumption in females (weeks 10) ($p < 0.05$). Reduction by 7 % ($p < 0.05$) of prothrombin time in males.	Anonymous, 2017g

	Increase by 26 % ($p < 0.05$) of AST in males. Reduction by 60 % ($p < 0.05$ but within the historical control range) of bilirubin in females recovery group. Increases in epithelial cells (by 300 %) and pus (by 60 %) in urine of males of the recovery group (in both cases $p < 0.05$). Increases by 15 % in the spleen weight of males and thymus weight of females (both $p < 0.05$). <u>300 mg/kg bw/d</u> Reduction by around 15 % of food consumption in males (weeks 1, 2, 3, 4, 5, 6 and 7) ($p < 0.05$ in all cases) Two females with distended uterus with watery content (it was also found in three control females). <u>100 mg/kg bw/d</u> Reductions in body weight in males (in all cases $p < 0.05$): by 5 % (days 78 and 85). Reduction by around 5 % ($p < 0.05$) of food consumption in males (week 1) Reduction by around 9 % of food consumption in females (weeks 9) ($p < 0.05$)

RAC notes that the only effect at a dose that might warrant classification is the reduction of 5 % in body weight of males reported at 100 mg/kg bw/d. Other effects reported in the table above are of unclear toxicological relevance and appear at doses clearly above the limit dose for warranting classification with a 90-day repeated dose toxicity study. Therefore, RAC agrees the DS's proposal for **no classification of 24-epibrassinolide as STOT RE.**

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

The DS proposed no classification of 24-epibrassinolide for germ cell mutagenicity on the basis of three *in vitro* assays showing that the substance is not mutagenic in bacteria, does not induce gene mutations at the HPRT locus in V79 cells and does not induce chromosome aberrations in human peripheral blood lymphocytes.

Comments received during public consultation

See RAC general comments.

Assessment and comparison with the classification criteria

The table below overalls the results the available *in vitro* mutagenicity/genotoxicity studies with 24-epibrassinolide.

Table: Summary table for *in vitro* mutagenicity/genotoxicity studies with 24-epibrassinolide.

Method	Test system	Tested concentrations	Results	Remarks	Reference
Bacterial reverse mutation test OECD TG 471 GLP 3 replicates/condition	*Salmonella typhimurium* TA 1535, TA 1537, TA 98, TA 100 and TA 102	0.0125, 0.0396,0.1252, 0.3956 and 1.25 mg 24-epibrassinolide /plate, both in the presence (+S9) and absence (-S9) of metabolic activation 91.2 % purity Positive controls -S9: sodium azide, 4-nitro-o-phenylenediamine, methyl methane sulfonate Positive control +S9: 2-aminoianthracene	For both +S9 and -S9: No substantial increase in revertant colony numbers in any of the tested strains was observed following treatment at any dose level. The solvent and positive controls induced the appropriate responses 24-epibrassinolide did not show a mutagenic potential in bacteria	Two independent experiments Slight cytotoxicity at 1.25 mg/plate	Srilatha, 2017
In vitro mammalian cell gene mutation test (HPRT) OECD TG 476 GLP	Chinese hamster V79 lung fibroblast cells	0, 1.6, 3.1, 6.3, 12.5, 25 and 50 µg 24-epibrassinolide /mL in the absence of S9 0, 3.1, 6.3, 12.5, 25, 50 and 100 µg 24-epibrassinolide /mL in the presence of S9 91.2 % purity Positive control +S9: 7,12-dimethylbenz(a)anthracene Positive control -S9: ethylmethane sulfonate	No substantial and reproducible dose dependent increase of the mutation frequency was observed Positive controls induced a distinct increase in mutant colonies	Precipitation was observed after 4 hours of exposure in cells exposed to 25 and 50 µg/mL (-S9) Precipitation was observed after 4 hours of exposure in cells exposed to 25, 50 and 100 µg/mL (+S9)	Wollny, 2017

RAC notes the absence of *in vivo* results and agrees with the DS that the available *in vitro* studies do not warrant classification of 24-epibrassinolide for germ cell mutagenicity**.** However, **RAC was unable to conclude on this hazard due to lack of *in vivo* data.**

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

No guideline-compliant studies on long term toxicity/carcinogenicity for 24-epibrassinolide were available. The applicant did not consider conducting these studies due to the ubiquitous presence of brassinosteroids in plant material and therefore the continuous lifetime exposure via food and feed. The applicant did not identify any studies addressing potential long term toxicity or carcinogenicity of 24-epibrassinolide. The DS proposed no classification due to lack of data.

Comments received during public consultation

See RAC general comments.

Assessment and comparison with the classification criteria

RAC was unable to assess this hazard due to lack of data.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

No studies on fertility and sexual function were submitted or considered necessary by the applicant due to the ubiquitous presence of 24-epibrassinolide in food resulting in continuous lifetime exposure. A developmental toxicity study reporting no adverse effect at 1000 mg/kg bw/d was available. Overall, the DS proposed no classification of 24-epibrassinolide for reproductive toxicity.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

No studies on sexual function and fertility were available. The table below overalls the results of a developmental toxicity study with 24-epibrassinolide.

Table: Summary table for the only available developmental toxicity study with 24-epibrassinolide.

Method	Results	Reference
Prenatal developmental toxicity study OECD TG 414	Maternal toxicity No clinical signs, no mortalities, no reductions in body weights, no gross macroscopic alterations during necropsy.	Anonymous, 2017h

Wistar rats Gavage (vehicle 0.1 % sodium carboxymethyl cellulose) 0, 100, 300 and 1000 mg/kg bw/d 91.2 % purity Exposure: Gestation days 5-19 24 animals/group	<u>Caesarean data</u> No effects on gravid uterine, placental and ovarian weights, total live foetuses, number of implantations, early and late resorptions, post implantations loss and dams with resorptions. Significant reduction ($p < 0.05$) in pre-implantation loss in animals treated with 1000 mg/kg/bw (1.91 ± 3.15 versus 3.00 ± 2.61). No significant differences were observed in foetus weight, anogenital distance and sex ratio. <u>Developmental toxicity</u> The variations observed in external, visceral and skeletal findings were randomly distributed across the groups and of no toxicological significance.

Comparison with the criteria

RAC notes that the results of the study summarised in the table above do not raise concerns for development, although RAC also highlights the lack of information on a second species.

RAC was unable to assess the effects on sexual function and fertility and lactation due to lack of data.

ENVIRONMENTAL HAZARD EVALUATION

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

Degradation

<u>Hydrolysis</u>

Hydrolysis of Brassinolide was tested in three buffer solutions (pH 5, 7 and 9) at two temperatures (25 °C and 50 °C). The substance's highest degradation rate is at a pH of 9 and a temperature of 50 °C. At 25 °C, Brassinolide has hydrolysis half-life values of 24.1 days at pH 5, 19.6 days at pH 7 and 16.4 days al pH 9. The Rapporteur Member State for the plant protection product approval also included the temperature normalised (for 20°C) value at pH 7 corresponding to 31.5 days. No degradation products were analysed during this study.

<u>Photolysis</u>

Photolysis is not expected to contribute significantly to the degradation of 24-epibrassinolide due to the low light absorbance of the active substance at a wavelength of 295 nm.

<u>Ready biodegradation</u>

No specific study on ready biodegradability was available.

Bioaccumulation

No experimentally determined BCF is available.

24-Epibrassinolide has a measured log Pow = 2 obtained in a test done according to Guideline OECD TG 117. This value is below the cut-off value of log Pow = 4. Therefore, a high potential for bioaccumulation was not expected.

Aquatic hazards

Summary of the available data for aquatic hazards:

Method	**Species**	**Results mg/L**		**Reference**
OECD TG 203 Acute 96h (static)	Zebrafish (*Danio rerio*)	Mortality LC_{50} > 5 (nom)	Key study	Anonymous (2017)
Acute 48h (static)	*Daphnia magna*	Mortality EC_{50}>2.86 (mm)	Key study	Matlock & Moore (2017)

Two acute aquatic studies are available in the CLH Dossier. No chronic studies are available.

The acute fish toxicity study in freshwater fish (*Danio rerio*) with 24-Epibrassinolide was performed following OECD TG 203. Based on the test item solubility and range finding experiment results, a limit test was conducted with the concentration of 5 mg/L. Ten fresh water fish were exposed for 96 hours to the limit test concentration (5 mg/L). A concurrent control group with ten fish was also maintained. No mortality or sub-lethal effects were observed in the control or fish throughout the experimental period. Thus the percent mortality at the end of 96 hour was recorded to be 0% in control and 5 mg/L concentration. Therefore, the endpoint was determined to be: LC_{50} > 5 mg/L**.**

The acute toxicity of 24-Epibrassinolide to *Daphnia magna*, was determined in a static, 48-hour test. Treatments consisted of a dilution water control and the nominal concentrations of 0.250, 0.500, 1.00, 2.00, 4.00 mg a.s./L. Geometric mean concentrations were control, 0.218, 0.501, 0.982, 1.85, and 2.86 mg a.s./L. Results are based on geometric mean measured test concentrations. The 48-hour EC_{50} was > 2.86 mg a.s./L, based on geometric mean measured test concentrations.

The DS proposed to consider 24-epibrassinolide as not rapidly degradable and to have a low potential for bioaccumulation. Based on this and the available acute aquatic toxicity data, the DS proposed no classification for acute aquatic toxicity. Although no chronic data were available, the DS proposed to classify as Aquatic Chronic 4, based on a concern in algae and aquatic plants resulting from the substance being a plant growth hormone.

Comments received during public consultation

There were two comments during public consultation.

One MSCA agreed with the proposal of classification for environmental hazards as Aquatic Chronic 4; H413.

The second MSCA pinpointed that in the DAR 'adverse effects to algae posed by 24-Epibrassinolide are considered unlikely' and that the data waiver to not conduct toxicity to algae / aquatic plants is acceptable (consequently, there is no chronic algal or plant data in the CLH/DAR dossier). On the contrary the CLH proposal for Aquatic Chronic 4 which is based on a concern for algal toxicity in the absence of experimental E_rC_{50}/NOE_rC data. Hence, the commenting MSCA concluded that in the case of 24-Epibrassinolide, the concern needs to be clarified before the application of Chronic 4 can be considered.

The Dossier Submitter responded that the waiver for algae and macrophytes in Vol. 3 CA B9 B.9.2.6/ B.9.2.7 is accepted in the EFSA pesticide review process in the view of considering a low risk under environmental conditions (consequently, there is no chronic algal or plant data in the CLH/DAR dossier). The proposal for a classification as "aquatic chronic 4" (safety net) is based on considering the potential hazard under standard laboratory toxicity studies (due to possible effects on growth under semi-static exposure laboratory conditions). Such potential hazard effects under tier 1 standard laboratory study conditions can't be fully excluded since no studies are presented and the active substance is considered as not readily biodegradable.

Assessment and comparison with the classification criteria

Degradation

The substance is considered not rapidly degradable. In a hydrolysis test the substance was not primarily degraded in the aquatic environment with a half-life <16 days (corresponding to a degradation of > 70 % within 28 days). No other relevant tests are presented for degradation assessment.

Bioaccumulation

The substance has a low bioaccumulation potential. 24-Epibrassinolide has a measured log Pow = 2 according to OECD TG 117. This value is below the cut-off value of log Pow = 4. Therefore, the substance has a low potential for bioaccumulation.

Aquatic Toxicity

There are two studies available for acute toxicity in fish and invertebrates, with L(E)C_{50} for *Danio rerio* and *Daphnia magna* not indicating any toxicity up to the solubility limit (which is > 1 mg/L). However, there is no data available for the potentially most sensitive trophic level (algae and aquatic plants). Hence, RAC agrees with the DS that 24-epibrassinolide does not warrant classification for acute aquatic hazards. However, RAC notes that as data is missing for the most sensitive trophic level (plants and algae), acute aquatic hazards cannot be fully assessed and that the conclusion is effectively based on insufficient data.

There are no chronic studies available in the dossier and as the available acute data indicates no effects up to the water solubility, the surrogate approach cannot be used. According to the CLP regulation a 'safety net' classification (referred to as category Chronic 4 in the CLP Regulation) can be used when the data available do not allow classification under the formal criteria for acute 1 or chronic 1 to 3 but there are nevertheless some grounds for concern. 24-epibrassinolide is a phytohormone whose mode of action and effects on plants and algae can be concentration-dependant boosting or inhibiting growth depending on the concentration (see in depth analysis by RAC – below).

In conclusion, since there is no algae or macrophyte data available to indicate lack of effects, the substance is not rapidly degradable, and is of low solubility, RAC agrees with the DS and considers that **aquatic chronic category 4 (H413, may cause long-lasting harmful effects to aquatic life)** is warranted for 24-Epibrassinolide based on the concern for algae and aquatic plants.

Additional references

Bajguz, A. 2000. Effect of brassinosteroids on nucleic acids and protein content in cultured cells of Chlorella vulgaris. Plant Physiol. Biochem. 38 (3), 209−215

Czerpak R, Piotrowska A, Szulecka K (2006) Jasmonic acid affects changes in the growth and some components content in alga Chlorella vulgaris. Acta Physiol Plant 28:195–203

Mekhalfi, M., Avilan, L., Lebrun, R., Botebol, H., Gontero, B. (2012). Consequences of the presence of 24-epibrassinolide, on cultures of a diatom, Asterionella Formosa. Biochimie 94 (2012) 1213e1220.

Piotrowska-Niczyporuk, A and Bajguz, A. 2014. The effect of natural and synthetic auxins on the growth, metabolite content and antioxidant response of green alga Chlorella vulgaris (Trebouxiophyceae). Plant Growth Regulation 73:57–66.

Salama ES, Kabra AN, Ji MK, Kim JR, Min B, Jeon BH (2014) Enhancement of microalgae growth and fatty acid content under the influence of phytohormones. Bioresour Technol 172:97–03

Talarek-Karwel, M., Bajguz, A., Piotrowska-Niczyporuk, A., Rajewska, I. 2018. The effect of 24-epibrassinolide on the green alga Acutodesmus obliquus (Chlorophyceae). Plant Physiology and Biochemistry 124 (2018) 175–183.

Tate, J.J., Gutierrez-Wing, M.T., Rusch, K.A., Benton, M.G., 2012. The Effects of plant growth substances and mixed cultures on growth and metabolite production of green algae Chlorella sp.: a review. J. Plant Growth Regul. 32, 417–428.

Utami D., Kawahata A., Sugawara, M., Jog, R. N., Miwa K., Morikawa, M. 2018. Effect of Exogenous General Plant Growth Regulators on the Growth of the Duckweed Lemna minor. Front. Chem. 6:251.

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).