Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

[*S*-(*Z,E*)]-5-(1-hydroxy-2,6,6-trimethyl-4-oxocyclohex-2-en-1-yl)-3-methylpenta-2,4-dienoic acid ; *S*-abscisic acid

EC Number: 244-319-5
CAS Number: 21293-29-8

CLH-O-0000001412-86-286/F

Adopted

13 June 2019

13 June 2019

CLH-O-0000001412-86-286/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: **[*S*-(*Z,E*)]-5-(1-hydroxy-2,6,6-trimethyl-4-oxocyclohex-2-en-1-yl)-3-methylpenta-2,4-dienoic acid ; *S*-abscisic acid**

EC Number: **244-319-5**

CAS Number: **21293-29-8**

The proposal was submitted by **Netherlands** and received by RAC on **27 September 2018.**

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

PROCESS FOR ADOPTION OF THE OPINION

Netherlands 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 **29 October 2018**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **11 January 2019**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Katalin Gruiz**

Co-Rapporteur, appointed by RAC: **Julie Séba**

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 **13 June 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	607-RST-VW-Y	[*S*-(*Z*,*E*)]-5-(1-hydroxy-2,6,6-trimethyl-4-oxocyclohex-2-en-1-yl)-3-methylpenta-2,4-dienoic acid ; *S*-abscisic acid	244-319-5	21293-29-8	Aquatic Acute 1 Aquatic Chronic 1	H400 H410	GHS09 Wng	H410		M=1 M=1	
RAC opinion	607-RST-VW-Y	[*S*-(*Z*,*E*)]-5-(1-hydroxy-2,6,6-trimethyl-4-oxocyclohex-2-en-1-yl)-3-methylpenta-2,4-dienoic acid ; *S*-abscisic acid	244-319-5	21293-29-8	Aquatic Acute 1 Aquatic Chronic 1	H400 H410	GHS09 Wng	H410		M=1 M=1	
Resulting Annex VI entry if agreed by COM	607-RST-VW-Y	[*S*-(*Z*,*E*)]-5-(1-hydroxy-2,6,6-trimethyl-4-oxocyclohex-2-en-1-yl)-3-methylpenta-2,4-dienoic acid ; *S*-abscisic acid	244-319-5	21293-29-8	Aquatic Acute 1 Aquatic Chronic 1	H400 H410	GHS09 Wng	H410		M=1 M=1	

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

S-abscisic acid is used as an active substance in plant protection products as a plant growth regulator. It has no existing harmonised classification and labelling in Annex VI to CLP. The CLH proposal and this RAC opinion covers selected physical hazards, human health hazards and hazards to the aquatic environment with the exception of carcinogenicity, respiratory sensitisation, aspiration hazard and hazardous to the ozone layer.

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

S-abscisic acid is a white power, melting at 140 °C under atmospheric conditions, decomposing upon melting at 154.5 °C, not reacting with air until 400 °C, and not containing any chemical groups associated with explosive or any aggressively reactive properties.

Table: *Summary of the results*

Physical hazard	Method	Result	Reference	Conclusion
Explosive	EC A14	Non-explosive The substance does not contain chemical groups associated with explosive properties	Comb, 2007 2.1.4.3 of the CLP Regulation.	No classification
Flammable solid	EC A10	Not self-igniting, not highly flammable	Comb, 2007	No classification
Self-reactivity substance or mixture		Data not available, does not contain reactive chemical groups → not self-reactive	2.8.4.2 of the CLP Regulation.	No classification
Pyrophoric solid		No reports of self-ignition during experiments or use → not pyrophoric	2.10.4.1 of the CLP Regulation.	No classification
Self-heating substance or mixture	US EPA OPPTS 830 (EC A16)	Auto-ignition point > 400 °C >> 140 °C (CLP criterion)	Comb, 2010b	No classification
Substance or mixture which in contact with water emits flammable gas		Data measured by standard method are not available, but use experience proves no reaction and no gas production on the effect of water	2.12.4.1 of the CLP Regulation.	No classification

Oxidising solid	EC A17	Non-oxidizing The substance contains oxygen chemically bound to carbon only.	Comb, 2011 2.14.4.1 of the CLP Regulation.	No classification
Organic peroxide		The substance does not contain peroxide groups.	2.15.4.1 of the CLP Regulation.	No classification
Substance or mixture corrosive to metals		Data lacking		No classification due to lack of data.

Comments received during public consultation

No comments were received during the public consultation.

Assessment and comparison with the classification criteria

RAC agrees with the Dossier Submitter (DS) that no classification is warranted for any of the physical hazards.

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

Oral Route

One OECD TG 425 study in female rats is available for the oral route. Under the conditions of the study, the acute oral LD_{50} for *S*-abscisic acid was greater than 5 000 mg/kg bw for female rats as no mortalities were observed.

The DS did not propose a classification for *S*-abscisic acid for acute oral toxicity.

Inhalation Route

Two OECD TG 403 studies are available in rat for the inhalation route. Based on the results of these studies, the acute inhalatory LC_{50} for *S*-abscisic acid was greater than 5.13 mg/L for female and male rats as no mortalities were observed.

The DS did not classify *S*-abscisic acid for acute inhalatory toxicity.

Dermal Route

One OECD TG 402 study is available for the dermal route. Under the conditions of the study, the acute dermal LD_{50} for *S*-abscisic acid was greater than 5 000 mg/kg bw for female and male rats as no mortalities were observed.

The DS did not propose a classification for *S*-abscisic acid for acute dermal toxicity.

Comments received during public consultation

No comment was received for this endpoint during the public consultation.

Assessment and comparison with the classification criteria

Oral Route

In a GLP OECD TG 425 acute oral study, *S*-abscisic acid (purity 95 %) was administered as a single dose exposure of 5 000 mg/kg bw in corn oil to 3 females Sprague-Dawley rats (DAR B.6.2.1 Study 1). No mortalities or signs of toxicity were noted during the course of this study.

Some deviations from the OECD guideline were identified, including the number of tested animals (3 females/dose instead of 5 animals/sex/dose) and the administered volume (1.7 mL/100 g bw instead of max. 1.0 mL/100 g bw). However, RAC agrees with the DS that in the absence of any signs of toxicity, the study can be considered as acceptable.

The oral LD_{50} for rats was greater than 5 000 mg/kg bw.

Since relevant criteria in the CLP Regulation were not met, RAC agrees with the DS's proposal for **no classification of *S*-abscisic acid regarding acute oral toxicity.**

Inhalation Route

In a GLP OECD TG 403 acute inhalation study, *S*-abscisic acid (purity 95 %) was administered via the inhalation route (nose only) at an actual concentration of 2.06 mg/L (maximum attainable concentration) to 5 males and 5 females Sprague-Dawley rats for a single 4-hour period (DAR B.6.2.1 Study 1). The mean mass median aerodynamic diameter (MMAD) was 3.8 µm with GSD 2.19 µm. No mortality or treatment-related effects were reported.

In a second OECD TG 403 study in Sprague-Dawley rat (5/sex/dose), dust generation methods were adapted to obtain an actual maximum concentration of 5.13 mg/L (DAR B.6.2.1 Study 2). The mean mass median aerodynamic diameter (MMAD) was 3.8 µm with GSD 2.19 µm and the purity was 96 %. After a 4-hour exposure, no mortality or treatment-related findings were observed. The mean body weights increased throughout the study.

Since relevant criteria in the CLP Regulation were not met, RAC agrees with the DS's proposal for **no classification of *S*-abscisic acid regarding acute inhalation toxicity.**

Dermal Route

In a GLP OECD TG 402 acute dermal study, *S*-abscisic acid (purity 95 %) was administered as a single dose of 5 000 mg/kg bw directly to the skin of 5 male and 5 female Sprague-Dawley rats, covering approximately 39 cm^2 of each animal's body surface area with a semi-occluded dressing for 24 hours (DAR B.6.2.1 Study 2). All animals survived. The animals exhibited no clinical signs of toxicity or macroscopic pathological abnormalities and the mean body weights increased throughout the study.

The dermal LD_{50} for rats was greater than 5 000 mg/kg bw. Since relevant criteria in the CLP Regulation were not met, RAC agrees with the DS's proposal for **no classification of *S*-abscisic acid regarding acute dermal toxicity.**

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

Summary of the Dossier Submitter's proposal

The DS did not propose a classification for *S*-abscisic acid for specific target organ toxicity after single exposure due to the absence of findings indicating such hazardous properties following a single administration by oral, dermal or inhalation route.

Comments received during public consultation

No comments were received for this endpoint during the public consultation.

Assessment and comparison with the classification criteria

No functional disturbance, morphological change or other significant/severe toxicity were reported in any of the available acute animal studies through oral, dermal or inhalation exposures. There were neither indications of respiratory tract irritation nor narcotic effects. Therefore, RAC is of the opinion that a **classification for STOT SE is not warranted**.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

DS proposed no classification for *S*-abscisic acid as a skin irritant based on negative results in a GLP compliant OECD TG 404 study in New Zealand White (NZW) rabbits. *S*-abscisic acid produced no skin irritation in rabbits at 24 and 72 hours following a 4-hour dermal exposure. All individual scores were 0.

Comments received during public consultation

No comments were received for this endpoint during the public consultation.

Assessment and comparison with the classification criteria

In a GLP compliant OECD TG 404 study, *S*-abscisic acid (purity 95 %) was applied as a single 500 mg dermal dose to the skin of two female and one male NZW rabbits (DAR B.6.2.2 Study 1). The test substance moistened with water was applied on a 6 cm^2 skin area and covered by a semi-occlusive dressing.

After a 4-hour exposure, the test substance was removed. No signs of erythema or oedema were reported in any of the animals at 30-60 minutes or 24, 48 and 72 h after the patch removal.

Since relevant criteria in the CLP Regulation were not met, RAC agrees with the DS's proposal that **no classification of *S*-abscisic acid regarding skin corrosion/irritation is warranted.**

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

The DS proposed no classification for s-abscisic acid for serious eye damage/irritation based on a GLP compliant OECD TG 405 study in NZW rabbit. Results after 24 hours showed that *S*-abscisic acid produced only slight conjunctival redness in all rabbits, which had reversed within 48 hours.

Comments received during public consultation

No comments were received for this endpoint during the public consultation.

Assessment and comparison with the classification criteria

In a GLP compliant OECD TG 405 eye irritation study, *S*-abscisic acid (purity 95 %) was applied as a single dose of 0.04 g (equivalent to 0.1 mL) into the conjunctival sac of the eye of 3 NZW male rabbits (DAR B.6.2.2 Study 2). It was not reported whether or not the treated eyes were washed after instillation of the test substance.

Conjunctival redness occurred in all animals (score 2) but the effect had completely reversed by 72 h. Also slight iritis, conjunctival discharge and chemosis were observed after 1 h, but the effects had reversed by 24 hours. Finally, slight corneal opacity was noted in one animal at 24 and 48 hours only. Mean individual scores are presented in the Table below.

Table: *Mean individual eye irritation scores in an irritation study in rabbit*

Scores observed after	1 hour	24 hours	48 hours	72 hours	Mean score per animal (24-72 hours)
Corneal opacity	0, 0, 0	1, 0, 0	1, 0, 0	0, 0, 0	0.7, 0.0, 0.0
Iritis	1, 1, 1	0, 0, 0	0, 0, 0	0, 0, 0	0.0, 0.0, 0.0
Conjunctival redness	2, 2, 2	1, 1, 1	0, 0, 0	0, 0, 0	0.3, 0.3, 0.3
Conjunctival chemosis	1, 1, 1	0, 0, 0	0, 0, 0	0, 0, 0	0.0, 0.0, 0.0
Conjunctival discharge	1, 1, 1	0, 0, 0	0, 0, 0	0, 0, 0	0.0, 0.0, 0.0

Since only slight conjunctival redness was observed in at least 2 of 3 tested animals after 24 hours with a mean score lower than 2 and all effects being completely reversed by 72 hours, the CLP criteria for serious eye damage and eye irritation are not met. RAC therefore agrees with the DS's proposal that **no classification of *S*-abscisic acid for serious eye damage/irritation is warranted.**

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

No classification was proposed for *S*-abscisic acid as a skin sensitiser on the basis of a Magnusson-Kligman Maximisation study in guinea pig. The DS concluded that based on this test, *S*-abscisic acid did not fulfil the criteria for skin sensitisation, although noting that there were some uncertainties regarding the reliability and validity of the study.

Comments received during public consultation

No comments were received for this endpoint during the public consultation.

Assessment and comparison with the classification criteria

The dermal sensitisation potential of s-abscisic acid (purity 95 %) was evaluated by the GLP OECD TG 406 maximisation test in male and female Hartley albino guinea pigs (DAR B.6.2.2 Study 3).

Based on the results of a preliminary irritation testing, 20 animals were induced by intradermal injections of the test substance (1 % w/w mixture in mineral oil) and topically induced with a 55 % w/w mixture of test substance in mineral oil. Ten animals were selected for the test vehicle control. Alpha-hexylcinnamic aldehyde was used as a positive control to validate the sensitisation potential of this strain of animals.

Challenging phase consisted of an occlusive application of 55 % w/w mixture of the test substance in mineral oil during 48 h. Very faint erythema (skin irritation score 0.5) was reported for 7/20 test sites (equal to 35 %) 24 hours after patch removal and persisted at one of the sites through 48 hours. In control animals, very faint erythema was noted at 3 out of 10 sham control sites (equal to 30 %) 24 hours after challenge patch removal but reversed within 48 hours.

Possible deviations from the OECD TG 406 included the use of the same concentration of *S*-abscisic acid for topical induction and topical challenge. The TG indicates that topical induction dose should be based on the highest dose to cause mild irritation whereas the highest non-irritating dose should be selected for the challenging phase.

Dose levels were based on a preliminary irritation test using 1 %, 3 % and 5 % *S*-abscisic acid for intradermal injection in mineral oil or in 50 % v/v Freund's Complete Adjuvant in water and 55 % and 41 % in mineral oil for topical application. The concentration of 55 % w/w mixture in mineral oil was shown to produce faint irritation. For the challenge, 55 %, 41 %, 28 % and 14 % *S*-abscisic acid in mineral oil were tested and the highest dose was retained for the challenging phase. The study authors indicated that concentrations in excess of 55 % could not be tested as they were considered too dry to provide adequate skin contact.

RAC acknowledges that this deviation might question the reliability of the study. However, considering that the response was of similar magnitude between the tested animals and the control animals, RAC is of the opinion that the results of the guinea pig maximization are conclusive for classification.

In conclusion, following challenge with 55 % w/w of *S*-abscisic acid, no animal showed significant skin sensitizing effect (all skin irritation scores < 1, response rate of 5 %) in a Magnusson-Kligman Maximisation study in guinea pig. Since the CLP criteria were not met, RAC agrees with the DS's proposal that **no classification of *S*-abscisic acid for skin sensitisation is warranted.**

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

Summary of the Dossier Submitter's proposal

The DS assessed several repeated dose toxicity studies through oral and dermal routes as well as reproductive toxicity studies in rat and concluded that none of the repeated dose toxicity studies available demonstrated adverse effects that trigger STOT RE classification.

Comments received during public consultation

No comments were received for this endpoint during the public consultation.

Assessment and comparison with the classification criteria

The Table below summarises the available oral and dermal repeated-dose toxicity studies in animals.

Table: *Summary table for oral and dermal repeated dose toxicity studies in animals with S-abscisic acid*

Method	Results	Reference
OECD TG 407 GLP study 28 days Sprague-Dawley Rat 5 animals/sex/dose Oral, diet 0, 2 000, 6 000 or 20 000 mg/kg bw/day (equivalent to 0, 215.8-233.6, 660.1-660.2 or 2 171 mg/kg bw/day for males and females, respectively) Purity 97 %	No adverse effects **NOAEL ≥ 2 171 mg/kg bw/day (m, f)** **No LOAEL**	DAR B.6.3.1 Study 1
OECD TG 408 **GLP study** 90 days Sprague-Dawley rat 10 animals/sex/dose Oral, diet 0, 2 000, 6 000 or 20 000 mg/kg food (equivalent to 0, 137.6-164.1, 407.8-498 or 1 420-1 752 mg/kg bw/day for males and females, respectively) Purity: 97 %	No adverse effects **NOAEL ≥ 1 420-1 752 mg/kg bw/day (m, f)** **No LOAEL**	DAR B.6.3.3 Study 1

OECD TG 410 **GLP study** 21-days Sprague-Dawley rat 5 animals/sex/dose Dermal 0, 100, 300 or 1 000 mg/kg bw/day 6h/day, semi-occlusive 10 % body surface area Purity: 97 %	No adverse effects **NOAEL > 1 000 mg/kg bw/day** **No LOAEL**	DAR B.6.3.2 Study 1

Oral repeated-dose toxicity studies

Oral 28-day study

In an oral 28-day repeated-dose toxicity study, rats were administered S-abscisic acid at 0, 2 000, 6 000 and 20 000 mg/kg food (equivalent to 0, 215.8-233.6, 660.1-660.2 and 2 171 mg/kg bw/day for males and females, respectively).

No treatment-related mortality, clinical signs, histopathology or neurotoxicity were observed. A minor reduction in body weight gain was observed in males and females at the highest tested dose.

Statistically significant increases in epididymitis weights were reported in males in the low- and mid-dose groups (9.9 and 9.7 % increases compared to controls, respectively) but not in the high-dose group. A non-significant increase in salivary gland weight was observed in males in the mid-dose group and males and females in the high-dose groups. Finally, kidney weight was significantly increased in females in the low-dose group only. No histopathology was associated with these findings.

Females of all dose groups showed decreased reticulocytes (77 %, 67 % and 77 % of the control in the low-, mid- and high-dose group, respectively) without a dose-response relationship. Neutrophils were also notably decreased in females (0.77, 0.87, 0.74 in the low-, mid- and high-dose group, respectively) compared to the control group (1.49). This result was considered by the DS to be caused by two high control values (2.17 and 1.92). However, RAC notes that the mean neutrophils values were similar between male and female control groups (1.50 vs 1.49, respectively). Moreover, neutrophil cells were only measured in 4/5 females in the control group. Therefore, RAC is of the opinion that the mean neutrophil values in the female control group are considered valid.

At the highest dose, slight but statistically significant decreases in the mean cell haemoglobin (MHC) and activated partial thromboplastin (APTT) values were observed in males (96 % and 90 % of the control, respectively).

Clinical chemistry indicated statistically significant and dose-related decreases in the blood glucose levels in males in all dose-groups (83 %, 80 %, 72 % of the control in the low-, mid- and high-dose group, respectively). Without other associated effects and considering the normal variations of blood glucose levels, this finding is considered of no toxicological significance.

Without the observation of any adverse effects, the NOAEL of *S*-abscisic acid in this study was set at 2 171 mg/kg bw/day for both males and females.

Oral 90-day studies

In an OECD TG 408 repeated-dose toxicity study, rats were administered *S*-abscisic acid at 0, 2 000, 6 000 and 20 000 mg/kg food during 90 days (equivalent to 0, 137.6-164.1, 407.8-498 or 1 420-1 752 mg/kg bw/day for males and females, respectively). All the selected doses were above the guidance values for STOT RE 2 classification.

Statistically non-significant body weight gain reductions were seen in males and females in the highest dose group after 13 weeks of treatment (89 % and 90 % of control values, respectively). Non-significant modifications were reported in various organ weights and are considered to be not treatment-related. Slight, statistically non-significant increases in lymphocytes and white blood cell were seen in females whereas cholesterol and triglycerides were statistically non-significantly altered in males and females.

A small number of males were also reported to have a reduced response to tactile stimulus at all tested doses and a small number of females were noted to have an increased exaggerated reaction or jerks around/away response to tactile stimulus. The observations being contradictory, these effects are not considered to be indicative of toxicity. The NOAEL in this study was set at 1 420 mg/kg bw/day for males and 1 752 mg/kg bw/day for females.

Reproduction toxicity studies

In an OECD TG 416 study in rat (DAR B.6.6.1 Study 1), *S*-abscisic acid was administered at doses of 684, 1 031 and 1 360 mg/kg bw/day continuously through the study period. The observations included increased liver weight in the high dose F0 group males and in the mid- and high-dose F0 group females. Increased liver weight was also reported in the high-dose F1 group females without any correlated histological change. These effects are considered not relevant for STOT RE classification.

In the OECD TG 414 teratogenicity study in rat (DAR B.6.6.2 Study 1), no effects were reported on maternal parameters at doses up to and including 1 000 mg/kg bw/day.

Dermal repeated-dose toxicity studies

In an OECD TG 410 dermal repeated-toxicity study, rats (5/sex/dose) were exposed via the dermal route to 100, 300 or 1 000 mg/kg bw/day of *S*-abscisic acid for 21 days. All males and 3 females in the high-dose group and 3 males and 2 females in the mid-dose groups showed very slight erythema at the site of application. White blood cell count and lymphocytes were statistically significantly increased (166 % and 166 % of the control, respectively) in males at 1 000 mg/kg bw/day. APTT was statistically significantly increased in males in the mid- and high-dose groups (124 % and 118 % of the control, respectively) and prothrombin time was statistically significantly decreased in females in the high-dose group (93 % of the control).

Conclusion

Slight increase in APTT was observed in males in the dermal repeated-dose toxicity study at doses within the guidance value range for STOT RE 2. Moreover, in the 28-day oral toxicity study in rat, slight decreases in reticulocytes and neutrophils in females were also observed within the guidance value range for STOT RE 2. However, RAC considers that these observations are not sufficient to trigger a STOT RE classification due to their minimal toxicological importance.

As a general conclusion, there were no significant or severe toxic effects observed at doses warranting classification according to the CLP criteria in the available repeated-dose toxicity

studies or reproductive toxicity studies in rat. Therefore, RAC agrees with the DS proposal that **no classification is warranted for *S*-abscisic acid for STOT-RE.**

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

S-abscisic acid was negative in a complete battery of *in vitro* genotoxicity tests with or without metabolic activation. An *in vivo* mouse micronucleus study was also negative.

The *in vitro* studies included one bacterial mutation assays, a mammalian chromosome aberration test in Chinese hamster ovary (CHO) cells and a mammalian cell gene mutation (TK) test in mouse lymphoma cells L5178Y. All studies were performed under GLP in accordance with OECD TG.

In vivo, *s*-abscisic acid did not induce micronucleus formation in bone marrow cells in a GLP OECD TG 474 mouse micronucleus test. Therefore, no classification was proposed by the DS.

Comments received during public consultation

No comments were received for this endpoint during the public consultation.

Assessment and comparison with the classification criteria

In vitro studies

In a bacterial reverse mutation test (GLP, OECD TG 471), *S*-abscisic acid (96.2 % purity) was tested for mutagenicity in *S. typhimurium* strains TA98, TA100, TA1535, and TA1537 and in *E. coli* strain WP2 uvrA at nominal concentrations of 33.3, 100, 333, 1 000, 3 330, and 5 000 µg/plate (DAR B.6.4.1 Study 1). No positive mutagenic responses were observed in any of the tested strains with or without S9 metabolic activation. No appreciable toxicity was observed at doses up to and including 5 000 µg/plate.

The clastogenic potential of *S*-abscisic acid (96.2 % purity) was tested in the mammalian chromosome aberration test (GLP, OECD TG 473) using CHO cells (DAR B.6.4.1 Study 2). Based on the findings in a preliminary cytotoxicity assay, the chosen doses for the chromosome aberration assay ranged from 43.8 to 2 800 µg/mL for 3- and 20-hour treatment groups with and without metabolic activation. No cytotoxicity was observed at the tested dose levels. No statistically significant increases in structural chromosome aberrations, polyploidy or endoreduplication were observed at any of the tested concentrations in the initial or confirmatory assays.

A TK gene mutation assay (GLP, OECD TG 476) was also performed with and without exogenous metabolic activation (DAR B.6.4.1 Study 3). Mouse lymphoma cells L5178Y were exposed for 4 hours to *S*-abscisic acid (98.3 % purity) at concentrations ranging from 31.3 to 2 500 µg/mL. A concentration of 2 650 µg/mL was excluded from the evaluation of mutagenicity due to cytotoxicity. There was no indication of gene mutation by *S*-abscisic acid in the presence and absence of an exogenous metabolic activation system in the *in vitro* ML (mouse lymphoma) cell/TK (thymidine kinase) gene mutation assay.

In vivo studies

In vivo, a mouse micronucleus assay (GLP, OECD TG 474) was performed on the bone marrow of male CD-1 mice (DAR B.6.4.2 Study 4). A single dose of vehicle, or 500, 1 000 or 2 000 mg *S*-abscisic acid/kg bw (purity 96.2 %) was given by oral gavage three times at 24-hour intervals.

Bone marrow was removed approximately 24 hours after the last dosing at all dose levels. No significant decrease in the PCE/NCE ratio was found at any dose level or sacrifice time, indicating an absence of target cell toxicity. No statistically significant increases in micronucleated polychromatic erythrocytes frequency were reported at any time point up to the limit dose of 2 000 mg/kg bw *S*-abscisic acid. RAC however agrees with the DS that it remains unclear whether the bone marrow was reached by the test compound.

Conclusion

All the available *in vitro* and *in vivo* mutagenicity studies showed negative results. Therefore, since the CLP criteria were not met, RAC agrees with the DS's proposal that **no classification is warranted for *S*-abscisic acid for germ cell mutagenicity.**

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

The evaluation of reproductive toxicity by *S*-abscisic acid is based on a GLP-compliant two-generation OECD TG 416 reproductive toxicity study and an OECD TG 414 teratogenicity study in rats.

Adverse effects on sexual function and fertility

In an OECD TG 416 study in rat, changes in the weights of seminal vesicles/coagulating glands/accessory fluid (high-dose F0 males) and liver (mid-dose F0 females, high-dose F0 males and females, high-dose F1 females) were concluded as non-adverse by the DS on the basis of an absence of any correlated histological changes.

In the F0 males, a slightly lower mean testis sperm concentration and a related lower mean sperm production were also observed at the highest dose, but not in the F1 generation. These findings were considered not as treatment related and no classification was proposed for adverse effects on sexual function and fertility.

Adverse effects on development

Regarding the 2-generation study, the DS concluded that no treatment-related effects were observed on litter size, survival index, sex ratio, body weight (gain), organ weights, balanopreputial separation, vaginal patency and macroscopic pathology in the F1 pups. In the F2-pups, no treatment-related effects were observed on litter size, survival index, sex ratio, body weight (gain) and macroscopic pathology. Lower mean absolute and relative (to body and brain weight) spleen weights were noted for F2 males and females in all dose groups, but no dose-related pattern was seen. Furthermore, the absolute and relative spleen weights were within the WIL reproductive historical control data range and therefore the differences were considered not to be treatment-related.

In the OECD TG 414 teratogenicity study in rat, no effects were reported on maternal parameters at doses up to and including 1 000 mg/kg bw/day. No foetal malformations or other developmental effects were attributed to the test-substance. Based on the lack of adverse test

substance-related effects on development, the DS concluded that no classification was warranted for adverse effects on development for *S*-abscisic acid.

Adverse effects on or via lactation

The DS concluded that the 2-generation study did not report any adverse findings occurring via lactation.

DS Conclusion

Based on the absence of any adverse reproductive effects in a two-generation study and teratogenicity study in rat, the DS did not propose any classification for reproductive toxicity for *S*-abscisic acid.

Comments received during public consultation

No comments were received for this endpoint during the public consultation.

Assessment and comparison with the classification criteria

The Table below summarises the available reproductive toxicity studies with animals.

Table: *Summary table for reproductive toxicity studies in animals with S-abscisic acid*.

Method	Exposure	Doses tested	NOAELs/LOAELs	Reference
Two-generation reproduction study OECD TG 416 GLP Rat Crl:CD(SD) 30/sex/dose	Oral, feeding Continuously through the study period	0, 10 000, 15 000 and 20 000 ppm dietary (corresponding to 684, 1 031 and 1 360 mg/kg bw/day) Purity ≥ 98 %	No adverse effects **Adults:** NOAEL ≥ 1 360 mg/kg bw/day No LOAEL **Development**: NOAEL ≥ 1 360 mg/kg bw/day No LOAEL **Sexual function and fertility:** NOAEL ≥ 1 360 mg/kg bw/day No LOAEL	DAR B.6.6.1 Study 1
Teratogenicity study OECD TG 414 GLP Oral, gavage Rat Crl:CD(SD) 25 females/dose	Oral, gavage Days 6-19 of gestation	0, 500, 750 and 1 000 mg/kg bw/d Purity 97.0 %	No adverse effects **Maternal:** NOAEL ≥ 1 000 mg/kg bw/day No LOAEL **Developmental**: NOAEL ≥ 1 000 mg/kg bw/day No LOAEL	DAR B.6.6.2 Study 1

In an OECD TG 416 GLP-compliant two-generation reproductive toxicity study in Crl:CD(SD) rats, *S*-abscisic acid was administered in the diet to 30 rats/sex/dose at concentrations of 0, 10 000, 15 000 and 20 000 ppm (corresponding to 684, 1 031 and 1 360 mg/kg bw/day).

Slightly increased liver weights were observed at 20 000 ppm in the F0 males and F1 females and from 15 000 ppm in the F0 females without associated histological findings. In the F0 males, statistically significantly lower absolute and relative seminal vesicles/coagulating glands/accessory fluid weights (13.4 % and 12.6 % below the control, respectively) in the highest dose did not correlate with any histological observation in the seminal vesicles. Minimal renal mineralisation was also reported in the high dose F0 males but not in the females.

In the high-dose group, lower mean testis sperm concentration (79.3 million/g, i.e. 88 % of the control) and a related lower mean sperm production (13 million/g/day, i.e. 88 % of the control) were reported in the F0 animals. The WIL historical control data were 79.8 million/g and 13.1 million/g/day for mean testis sperm concentration and mean sperm production, respectively (data is from 165 studies performed during the years 2000-2009). No effect on sperm concentration or sperm production were observed in the F1 generation. In F0 females, oestrogenic cycle length was slightly decreased at 15 000 and 20 000 ppm (4.0 and 4.2 days respectively, 5.6 days in the control).

No effects on body weight (gain), litter size, survival index, sex ratio or macroscopic pathology were reported in F1 and F2 pups with the exception of a slight increase in F1 female pup body weight at the highest dose. Balanopreputial separation and vaginal patency remained similar to controls in F1 pups. Mean absolute and relative (to body and brain weight) spleen weights were slightly decreased in all F2 dose groups without clear dose-dependency.

The NOAELs for parental effects, sexual function and fertility and developmental toxicity were 20 000 ppm. No LOAEL was determined for these endpoints.

Table: *Spleen weights of F2 pups in the rat two-generation study with s-abscisic acid*

F2 pups spleen weight	**0 ppm**		**10 000 ppm**		**15 000 ppm**		**20 000 ppm**	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Absolute (g)	0.2334	0.2219	0.1989* (-14.8 %)	0.2085 (-6.0 %)	0.1918** (-17.8 %)	0.1854* (-16.4 %)	0.1979* (-15.2 %)	0.1923 (-13.3 %)
Relative to bw (%)	0.434	0.445	0.398 (-8.3 %)	0.420 (-5.6 %)	0.375** (-13.6 %)	0.378** (-15.1 %)	0.385** (-11.3 %)	0.398* (-10.6 %)
Relative to brain	15.663	15.434	13.579* (-13.3 %)	14.557 (-5.7 %)	12.914** (-17.6 %)	12.938** (-16.2 %)	13.195** (-15.8 %)	13.154* (-14.8 %)
HCD pup spleen weight	Males : mean 0.2197 ± 0.04442 g, range 0.1471 g – 0.3641 g Females : mean 0.2154 ± 0.04131 g, range 0.1401 g – 0.3099 g *Crl:CD(SD) rat; PND 21; 165 studies; 223 data sets; range of study dates 10/2000 - 10/2009*							

* Significantly different from the control group at 0.05
** Significantly different from the control group at 0.01

In the developmental toxicity study, rats were exposed to 0, 500, 750 or 1 000 mg/kg bw/day *S*-abscisic acid during gestation days 6 to 19. No mortality or clinical signs of toxicity were reported in the dams. Slight but statistically significantly decreased maternal mean body weight gains were reported in the mid- and high-dose groups (12.4 % and 13.8 % lower as compared to control, respectively).

No test substance-related effects were reported on post-implantation loss, live litter size, mean foetal weights and foetal sex rates. No treatment-related foetal malformations or developmental variations were observed.

Based on the absence of adverse test substance-related effects on maternal animals and on prenatal development, a dosage level of 1 000 mg/kg bw/day, the NOAEL for maternal toxicity and prenatal developmental toxicity was considered to be 1 000 mg/kg bw/day.

Fertility

In a two-generation study, effects on fertility were limited to a slight decrease in the F0 mean testis sperm concentration and mean sperm production (88 % of the control for both values). Moreover, the observed effects on sperm parameters were not reproduced in the F1 generation and they were only observed in the F0 high-dose group. Therefore, these values are considered of limited relevance and not sufficient for classification for adverse effects on sexual function and fertility.

The lower seminal vesicles/coagulating glands/accessory fluid weights observed at the highest dose are considered non-averse based on an absence of any correlated histological observation in the seminal vesicles. The effect was only observed in F0 males and it is considered of limited magnitude and therefore not relevant for classification.

Overall, RAC is of the opinion that the observed effects on sexual function and fertility are not sufficient to warrant classification.

Developmental toxicity

No adverse effects on pups were seen in the rat teratogenicity study at doses up to 1 000 mg/kg bw/day. In the two-generation study in rat, slight effects on the spleen weight of F2 pups were noted in all dose groups. The effects were of limited magnitude, without associated histopathological findings and without clear dose-dependency. Moreover, no similar effects were reported in F1 pups. Therefore, this finding is considered not relevant for classification.

In conclusion, RAC concludes that a classification for developmental toxicity is not warranted.

Lactation

According to the CLP Regulation, a lactation classification can be assigned on the:

(a) "Human evidence indicating a hazard to babies during the lactation period; and/or
(b) Results of one or two generations in animals which provide clear evidence of adverse effect in the offspring due to transfer in the milk or adverse effect on the quality of the milk; and/or
(c) Absorption, metabolism, distribution and excretion studies that indicate the likelihood that the substance is present in potentially toxic levels in breast milk."

No adverse findings on lactation were reported in the available 2-generation study in rat. No human evidence or toxicokinetic study is available to support a classification for effects on or via lactation. Therefore, RAC is of the opinion that a classification for lactation is not warranted.

In conclusion, RAC recommends **no classification of abscisic acid for toxicity to reproduction**.

ENVIRONMENTAL HAZARD EVALUATION

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

According to the data presented in the DAR (2013), *S*-abscisic acid is very toxic to aquatic organisms.

The DS assessed all existing relevant data and proposed to classify abscisic acid as Aquatic Acute 1 and Aquatic Chronic 1 (M = 1 for both), based on toxicity to *Lemna gibba*.

Degradation

Hydrolysis

The substance is not expected to hydrolyse in water: DT_{50} = 791.6 days and 161.9 days for 25 °C and 40 °C, respectively.

Biodegradation

The substance is rapidly degradable for classification purposes based on the results of two studies:

1) Ready biodegradability study, reliable, Klimisch 1 (OECD TG 301F, Dickinson, 2010): 10 % degradation after 4 days, 60 % after 8 days and 89 % after 28 days (based on mean oxygen consumption).

2) Degradation in surface water, Klimisch 2 (OECD TG 309, Dickinson, 2011): primary degradation based on the disappearance time $DT_{50water}$ (20.2–22.5 °C) = 3.3 hours (geometric mean).

S-abscisic acid is rapidly degradable in soil (DT_{50soil} = 0.66–2.1 days) and is also susceptible to photodegradation (DT_{50} = 0.874 hours = 0.036 days).

Aquatic bioaccumulation

No measured bioaccumulation data are available. Key information is the log K_{ow} of 1.8, a measured value, determined for the neutral molecule.

Based on the CLP Regulation trigger value of log K_{ow} = 4, the value of 1.8 indicates a low potential for bioaccumulation.

Aquatic acute and chronic toxicity results

Method	Results	Reliability	Reference
Fish acute toxicity, OECD TG 203 *Onchoryhnchus mykiss*	LC_{50} (96h) > 121 mg/L (mean measured)	Acceptable	STUDY IIA, 8.2.1/01
Invertebrates acute toxicity, OECD TG 202 *Daphnia magna*	LC_{50} (48h) > 116 mg/L (mean measured)	Acceptable	Palmer *et al.*, 2007b STUDY IIA, 8.3.1/01
Algae growth inhibition, OECD TG 201 *Pseudokirchneriella subcapitata*		Acceptable	Biester, 2010a STUDY IIA, 8.4/01
Acute toxicity	72-hour E_bC_{50}, E_rC_{50}, E_yC_{50} > 95.3 mg/L		
Chronic toxicity	72-hour NOE_bC, NOE_rC, NOE_yC = 29.3 mg/L mean measured		
Algae growth inhibition, OECD TG 201 *Navicula pelliculosa*		Acceptable	Biester, 2010b STUDY IIA, 8.4/02
Acute toxicity	72-hour E_bC_{50}, E_rC_{50}, E_yC_{50} > 90.1 mg/L;		
Chronic toxicity	72-hour NOE_bC, NOE_rC, NOE_yC = 90.1 mg/L		
Aquatic plant growth inhibition, OECD TG 221 *Lemna gibba*		Acceptable	Biester, 2010c STUDY IIA, 8.6/1

Acute toxicity	7-day E_bC_{50} (frond number) = 0.024 mg/L 7-day E_rC_{50} (frond number) = 0.20 mg/L		
Chronic toxicity	7-day NOEC (frond number) = 0.0025 mg/L		

Lemna gibba is the only test organism that exhibited effects (growth inhibition) after exposure to *S*-abscisic acid. Therefore, classification is based on the acute and chronic toxicity on *Lemna gibba*.

Comments received during public consultation

One Member State Competent Authority agreed with the Classification and the M factor values proposed by the dossier submitter in the CLH report.

Assessment and comparison with the classification criteria

S-abscisic acid is water soluble, physico-chemically stabile, does not hydrolyse, but it is photodegradable.

Evidence from a test following OECD TG 301F indicates that *S*-abscisic acid is rapidly degradable for classification purposes as 89 % was degraded after 28 days, which is more than the CLP criterion of 70 %. The 10-day window was also met.

No measured BCF is available. Based on measured log K_{ow}=1.8 (below the cut off value of log K_{ow} = 4), it has a low potential for bioaccumulation.

S-abscisic acid does not show acute toxicity to fish, invertebrates and algae, (LC_{50} >> 1 mg/L), and no chronic toxicity to algae (NOEC >> 1 mg/L), but is acutely and chronically toxic to the aquatic plant *Lemna gibba* (duckweed) (based on frond number):

Acute aquatic hazard: E_rC_{50} = 0.20 mg/L <1 mg/L (the criterion of aquatic acute 1)

Acute M-factor = 1 (0.1 < LC_{50} = 0.20 < 1.0)

Chronic aquatic hazard: NOEC = 0.0025 mg/L < 0.01 mg/L (the criterion of aquatic chronic 1 for rapidly degradable substances)

Chronic M-factor = 1 (0.001 < NOEC = 0.0025 < 0.01)

Chronic data for fish and invertebrates are not available. However, as *S*-abscisic acid is considered rapidly degradable and to have a low potential for bioaccumulation, the surrogate approach (using acute toxicity data in combination with environmental fate information (CLP Table 4.1.0(b)(iii)) does not result in classification for chronic toxicity.

Based on the available information, RAC agrees with the DS that *S*-abscisic acid warrants classification as **Aquatic Acute 1 and Aquatic Chronic 1, both with an M-factor of M=1.**

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).