CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation), Annex VI, Part 2

International Chemical Identification:

[*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**

Index Number: **-**

Contact details for dossier submitter:

Bureau REACH
National Institute for Public Health and the Environment (RIVM)
The Netherlands
bureau-reach@rivm.nl

Version number: 1 **Date: August 2018**

Note on confidential information

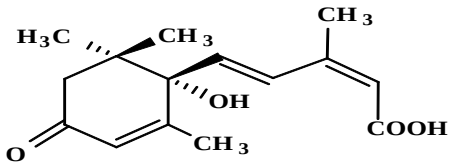
Please be aware that this report is intended to be made publicly available. Therefore it should not contain any confidential information. Such information should be provided in a separate confidential Annex to this report, clearly marked as such.

CONTENTS

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 1: Substance identity and information related to molecular and structural formula of the substance

Name(s) in the IUPAC nomenclature or other international chemical name(s)	(2Z,4E)-5-[(1S)-Hydroxy-2,6,6-trimethyl-4-oxo-2-cyclohexen-1-yl]-3-methyl-2,4-pentadienoic acid
Other names (usual name, trade name, abbreviation)	2,4-Pentadienoic acid, 5-[(1*S*)-1-hydroxy-2,6,6-trimethyl-4-oxo-2-cyclohexen-1-yl]-3-methyl-, (2*Z*,4*E*)- [CA name]
ISO common name (if available and appropriate)	No published ISO common name is available for S-abscisic acid.
EC number (if available and appropriate)	244-319-5
EC name (if available and appropriate)	[S-(Z,E)]-5-(1-hydroxy-2,6,6-trimethyl-4-oxocyclohex-2-en-1-yl)-3-methylpenta-2,4-dienoic acid
CAS number (if available)	21293-29-8
Other identity code (if available)	-
Molecular formula	$C_{15}H_{20}O_4$
Structural formula	
SMILES notation (if available)	-
Molecular weight or molecular weight range	264.3 grams/mole
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	Optical purity: Specific rotation of S-ABA was found to be + 396° (20 °C in ethanol, 10.0 mg/mL)
Description of the manufacturing process and identity of the source (for UVCB substances only)	Not applicable.
Degree of purity (%) (if relevant for the entry in Annex VI)	Min. 96%

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	**Concentration range (% w/w minimum and maximum in multi-constituent substances)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self-classification and labelling (CLP)**
S-abscisic acid	Min. 96%	-	

Table 3: Impurities (non-confidential information) if relevant for the classification of the substance

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling
Not applicable. The active substance does not contain any impurities that should be regarded as relevant.				

Table 4: Additives (non-confidential information) if relevant for the classification of the substance

Additive (Name and numerical identifier)	Function	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The additive contributes to the classification and labelling
Not applicable.					

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 5:

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	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	
Resulting Annex VI entry if agreed by RAC and 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	

Table 6: Reason for not proposing harmonised classification and status under public consultation

Hazard class	Reason for no classification	Within the scope of public consultation
Explosives	Data conclusive but not sufficient for classification	Yes
Flammable gases (including chemically unstable gases)	Hazard class not applicable	No
Oxidising gases	Hazard class not applicable	No
Gases under pressure	Hazard class not applicable	No
Flammable liquids	Hazard class not applicable	No
Flammable solids	No classification is proposed. Data is conclusive but not sufficient for classification.	Yes
Self-reactive substances	No classification is proposed. Data (chemical structure) is conclusive but not sufficient for classification.	Yes
Pyrophoric liquids	Hazard class not applicable	No
Pyrophoric solids	No classification is proposed. Data (experience in handling) is conclusive but not sufficient for classification.	Yes
Self-heating substances	No classification is proposed. Data is conclusive but not sufficient for classification.	Yes
Substances which in contact with water emit flammable gases	No classification is proposed. Data (experience in handling) is conclusive but not sufficient for classification.	Yes
Oxidising liquids	Hazard class not applicable	No
Oxidising solids	Data conclusive but not sufficient for classification	Yes
Organic peroxides	Hazard class not applicable	No
Corrosive to metals	Data lacking	No
Acute toxicity via oral route	Data conclusive but not sufficient for classification	Yes
Acute toxicity via dermal route	Data conclusive but not sufficient for classification	Yes
Acute toxicity via inhalation route	Data conclusive but not sufficient for classification	Yes
Skin corrosion/irritation	Data conclusive but not sufficient for classification	Yes
Serious eye damage/eye irritation	Data conclusive but not sufficient for classification	Yes
Respiratory sensitisation	Data lacking	No
Skin sensitisation	Data conclusive but not sufficient for classification	Yes
Germ cell mutagenicity	Data conclusive but not sufficient for classification	Yes
Carcinogenicity	Data lacking	No
Reproductive toxicity	Data conclusive but not sufficient for classification	Yes
Specific target organ toxicity-	Data conclusive but not sufficient for	Yes

Hazard class	Reason for no classification	Within the scope of public consultation
single exposure	classification	
Specific target organ toxicity-repeated exposure	Data conclusive but not sufficient for classification	Yes
Aspiration hazard	Data lacking	No
Hazardous to the aquatic environment	Harmonised classification proposed	Yes
Hazardous to the ozone layer	Data lacking	No

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

S-abscisic acid has not previously been assessed for harmonised classification by RAC or TC C&L.

S-abscisic acid is not registered under REACH (October, 2017).

According to the data presented in the DAR (2013), the classification of S-abscisic acid is: R50 “Very toxic to aquatic organisms”

The conclusions on the peer review of the pesticide risk assessment of S-abscisic acid was published as an EFSA scientific report (EFSA Journal 2013;11(8):3341). The classification was unchanged. The DAR can be requested via http://dar.efsa.europa.eu/dar-web/provision. EFSAs peer review is available via the EFSA website (https://www.efsa.europa.eu/en/efsajournal/pub/3341).

It should be noted that in the current CLH report classification with H400 and H410 is proposed. The classification proposed in the DAR was done according to Directive 99/45/EC (DPD). R50 corresponds to H400 under Regulation Dir. No. 1272/2008/EC. For the DSD classification only data were taken for acute toxicity and not for chronic toxicity and therefore classification for chronic aquatic toxicity was not assigned. However, based on the re-evaluation of the available data as done for the current CLH report, also chronic toxicity data were included.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

S-abscisic acid is an active substance in the meaning of Regulation EC 1107/2009 and therefore no justification is required.

5 IDENTIFIED USES

S-abscisic acid can control the growth of tomato seedlings in the greenhouse to extend the planting period, promote survival and growth in the field to increase yield, extend the market period of transplants by reducing dehydration during storage/shipping and provide chilling injury protection in the field.

S-abscisic acid also enhances development of grape berry colour, when environmental conditions have not reached a point to trigger the complete maturation of berries, giving the desired level of berry colour.

6 DATA SOURCES

This CLH report is compiled based on the data on S-abscisic acid that was submitted and evaluated in the DAR (2013).

7 PHYSICOCHEMICAL PROPERTIES

Table 7: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	TGAI 96.2%: white powder at 25 °C	Comb, A. L., 2010a	Measured (OPPTS 830.6302 and 830.6303)
	PAI 98.3%: off-white solid, Munsell 5Y 9/1 (fine powder) at 20 °C	Ponte, M., 2005	Measured (Munsell colour system)
Melting/freezing point	The test substance (TGAI, 96.2%) changed from a white powder to a yellowish-orange colour immediately before melting. The test substance decomposed before melting at 159 °C.	Ponte, M., 2005	Measured (EC A1)
	Two determinations (PAI, 98.3%) Sample I: 154.5 – 162.0 °C Sample II: 154.5 – 162.5 °C The test substance changed from an off-white powder to a yellow liquid during the test and became darker in colour by the end of melting. This change in colour was considered to be due to decomposition of the sample.	Comb, A. L., 2010a	Measured (EC A1 metal block method) The substance is considered to decompose upon melting at 154.5 °C
Boiling point	Two determinations (TGAI, 96.2%) A: 159.7 – 162.2°C B: 159.2 – 162.1°C	Ponte, M., 2005	Measured (EC A2) Decomposition prior to boiling
	Two determinations (PAI, 98.3%) Sample I: 154.5 – 162.0 °C Sample II: 154.5 – 162.5 °C The test substance changed from an off-white powder to a yellow liquid during the test and became darker in colour by the end of melting. This change in colour was considered to be due to decomposition of the sample.	Comb, A. L., 2010a	Measured (EC A1) The substance is considered to decompose upon melting at 154.5 °C
Relative density	1.21 g/cm3 at 25 °C (mean, n=2) (TGAI, 96.2%)	Ponte, M., 2005	Measured (ASTM 153 pycno-meter)
	D_4^{20} = 1.18 (mean, n=2) (PAI, 98.3%)	Comb, A. L., 2010a	Measured (EC A3 pycno-meter)
Vapour pressure	< 2.0 x10^{-6} Pa at 25 °C (detection limit) (PAI, 98.3%)	Ponte, M., 2006a	Measured (Gas saturation) An estimation using MPBPVP version 1.43 (EPI suite 4.11) was also performed: 5.8x10^{-7} Pa at 25 °C (Modified Grain method). The substance is not volatile.
Surface tension	1 g/L dilution: 57.5 mN/m at 20	Comb, A. L.,	Measured (EC A5 torsion

Property	Value	Reference	Comment (e.g. measured or estimated)
	°C (PAI, 98.3%)	2010a	balance)
Water solubility	3.10+/- 0.11 g/L in purified water at 20 °C Effect of pH at 20˚C Buffer pH: Solubility (g/L) 4: 3.80 ± 0.15 7: >250 10: >250	Comb,A., L., 2011	Measured (Shake flask; OECD TG 105)
Partition coefficient n-octanol/water	HPLC estimate (25 °C, 99.7 %): Non-ionised form: Log Pow = 1.8 (pH 2.5) Ionised form: Log Pow = 0.94 (pH 6.2) Calculation from individual solubilities: Solubility in *n*-octanol: 54.8g/L; Solubility in water: 3.1g/L 54.8g/L:3.1g/L=17.7, Log of 17.7= 1.25 Pow QSAR estimate: KOWWIN 1.67 estimation: 2.38	Ponte, M., 2006b	Calculated/ Estimated. The DAR contains different log K_{ow} estimates/calculations. The partition coefficient has been calculated from the individual solubilities in water and *n*-octanol. However, in the same study a log K_{ow} of 1.8 was estimated for the neutral molecule at pH 2.5. Considering that the surface tension of a 1g/L solution was just slightly below 60 mN/m, the HPLC study is considered valid, and the log K_{ow} of 1.8 (pH 2.5) is used for classification purposes.
Flash point	Not applicable. The melting point of the a.s. is > 40 °C	-	-
Flammability	Not highly flammable (TGAI, 97.0%)	Comb, A.L., 2007	Measured (EC A10)
Explosive properties	Not explosive (TGAI, 97.0%)	Comb, A.L., 2007	Measured (EC A14)
Self-ignition temperature	> 400 °C (TGAI, 97.1%)	Comb, A.L., 2010b	Measured (US EPA OPPTS 830, EC A16)
Oxidising properties	Not oxidising (TGAI, 97.1%)	Comb, A. L., 2011	Measured (EC A17)
Granulometry	No data available	-	-
Stability in organic solvents and identity of relevant degradation products	Solubility at 20°C (PAI, 99.7%): Methanol: 506.8 g/L Acetone: 290.2 g/L Ethyl acetate: 92.2 g/L 1,2-dichloroethane: 10.95 g/L Xylene: 0.265 g/L Octanol: 54.8 g/L	Schick, M., 2008a	Measured (US EPA OPPTS 830.7840, OECD 105, shake flash)
Dissociation constant	pKa = 4.61 (PAI, 99.7%) (mean of 3 determinations)	Ponte, M., 2006b	Measured (Titration)
Viscosity	No data available	-	-
Henry's law constant	3.79×10^{-8} Pa.m^3/mol at 25 °C $<1.7 \times 10^{-7}$ Pa.m^3/mol at 25 °C	Ponte, M., 2006a	Calculated. In the DAR the following was commented: Based on the EPI suite estimation at 25 °C and

Property	Value	Reference	Comment (e.g. measured or estimated)
		DAR/ EFSA conclusion document (2013)	water solubility of 3192 mg/L (distilled water, pH unknown). HENRYWIN 3.1 was also used, resulting in an estimated Henry's law constant of $2.98x10^{-9}$ $Pa.m^3/mol$. Considering a measured value is preferable over an estimated value, the Henry's law constant is recalculated by the RMS, based on a vapour pressure of < $2x10^{-6}$ Pa at 25 °C and a water solubility of 3102 mg/L (pH 4 buffered, at 20 °C). M = 264.3 g/mol. H<$1.7x10^{-7}$ $Pa.m^3/mol$ The substance is unlikely to evaporate from water surfaces.

8 EVALUATION OF PHYSICAL HAZARDS

8.1 Explosives

Table 8: Summary table of studies on explosive properties

Method	Results	Remarks	Reference
EC A14	Not explosive	-	Comb, A.L., 2007

8.1.1 Short summary and overall relevance of the information provided on explosive properties

A study performed according to EC A14 is available in which the explosive properties of S-abscisic acid were determined (Comb, A.L., 2007). Based on the test performed it was concluded that S-abscisic acid is not explosive.

8.1.2 Comparison with the CLP criteria

S-abscisic acid does not contain any chemical groups associated with explosive properties as given in section 2.1.4.2 of the CLP Regulation and therefore no classification is required.

8.1.3 Conclusion on classification and labelling for explosive properties

No classification is proposed. Data is conclusive but not sufficient for classification.

8.2 Flammable gases (including chemically unstable gases)

Hazard class not applicable (S-abscisic acid is not a gas).

8.2.1 Short summary and overall relevance of the provided information on flammable gases (including chemically unstable gases)

Not relevant.

8.2.2 Comparison with the CLP criteria

Not relevant.

8.2.3 Conclusion on classification and labelling for flammable gases

Hazard class not applicable.

8.3 Oxidising gases

Hazard class not applicable (S-abscisic acid is not a gas).

8.3.1 Short summary and overall relevance of the provided information on oxidising gases

Not relevant.

8.3.2 Comparison with the CLP criteria

Not relevant.

8.3.3 Conclusion on classification and labelling for oxidising gases

Hazard class not applicable.

8.4 Gases under pressure

Hazard class not applicable (S-abscisic acid is not a gas).

8.4.1 Short summary and overall relevance of the provided information on gases under pressure

Not relevant.

8.4.2 Comparison with the CLP criteria

Not relevant.

8.4.3 Conclusion on classification and labelling for gases under pressure

Hazard class not applicable (S-abscisic acid is not a gas).

8.5 Flammable liquids

Hazard class not applicable (S-abscisic acid is not a liquid).

8.5.1 Short summary and overall relevance of the provided information on flammable liquids

Not relevant.

8.5.2 Comparison with the CLP criteria

Not relevant.

8.5.3 Conclusion on classification and labelling for flammable liquids

Hazard class not applicable.

8.6 Flammable solids

Table 9: Summary table of studies on flammable solids

Method	Results	Remarks	Reference
EC A10	Not highly flammable	-	Comb, A.L., 2007
US EPA OPPTS 830 (EC A16)	Auto-flammability: > 400 °C	-	Comb, A.L., 2010b

8.6.1 Short summary and overall relevance of the provided information on flammable solids

S-abscisic acid is a white solid at room temperature and decomposes upon melting at 154.5 °C (Comb, A. L., 2010a). Technical S-abscisic acid is a fine white powder, is not self-igniting (Comb, A.L., 2010b) or highly flammable (Comb, A.L., 2007) based on the available studies.

8.6.2 Comparison with the CLP criteria

Based on Regulation (EC) 1272/2008, the following criteria for flammable solids are defined:

Category 1: Burning rate test Substances and mixtures other than metal powders: (a) wetted zone does not stop fire and (b) burning time < 45 seconds or burning rate > 2,2 mm/s Metal powders: burning time ≤5 minutes

Category 2: Burning rate test Substances and mixtures other than metal powders: (a) wetted zone stops the fire for at least 4 minutes and (b) burning time < 45 seconds or burning rate > 2,2 mm/s Metal powders: burning time > 5 minutes and ≤ 10 minutes.

Information on the burning rate of S-abscisic acid is not available. However, considering that S-abscisic acid is not highly flammable and the auto-ignition temperature of >400 °C under atmospheric conditions, classification of S-abscisic acid as flammable or auto-flammable is not considered justified.

8.6.3 Conclusion on classification and labelling for flammable solids

No classification is proposed. Data is conclusive but not sufficient for classification.

8.7 Self-reactive substances

No data available.

8.7.1 Short summary and overall relevance of the provided information on self-reactive substances

No data available.

8.7.2 Comparison with the CLP criteria

No specific data has been provided. However, S-abscisic acid does not contain any chemical groups associated with explosive or self-reactive properties in accordance with section 2.8.4.2 of the CLP Regulation. Therefore, S-abscisic acid is concluded to not self-reactive.

8.7.3 Conclusion on classification and labelling for self-reactive substances

No classification is proposed.

8.8 Pyrophoric liquids

Hazard class not applicable (S-abscisic acid is not a liquid).

8.8.1 Short summary and overall relevance of the provided information on pyrophoric liquids

Not relevant.

8.8.2 Comparison with the CLP criteria

Not relevant.

8.8.3 Conclusion on classification and labelling for pyrophoric liquids

Hazard class not applicable.

8.9 Pyrophoric solids

Data lacking.

8.9.1 Short summary and overall relevance of the provided information on pyrophoric solids

Data lacking.

8.9.2 Comparison with the CLP criteria

Data on pyrophoric solids is lacking. However, S-abscisic acid has been handled in air within all studies available in the dossier and there are no reports of self-ignition (see references in all sections).

8.9.3 Conclusion on classification and labelling for pyrophoric solids

No classification is proposed. Data (experience in handling) is conclusive but not sufficient for classification.

8.10 Self-heating substances

Table 10: Summary table of studies on self-heating substances

Method	Results	Remarks	Reference
US EPA OPPTS 830 (EC A16)	Auto-flammability: > 400 °C	-	Comb, A.L., 2010b

8.10.1 Short summary and overall relevance of the provided information on self-heating substances

A test is available in which the self-ignition temperature of S-abscisic acid was determined (Comb, A.L., 2010b). This test was performed according to EEC method A16. Based on the study it was concluded that there was no exothermic reaction of S-abscisic acid at the tested temperatures (up to 400°C). the auto-ignition temperature of S-abscisic acid is > 400°C.

8.10.2 Comparison with the CLP criteria

According to table 2.11.1 the criteria for self-heating are as follows:

Category Criteria 1 A: positive result is obtained in a test using a 25 mm sample cube at 140 °C 2

Category 2:

(a) a positive result is obtained in a test using a 100 mm sample cube at 140 °C and a negative result is obtained in a test using a 25 mm cube sample at 140 °C and the substance or mixture is to be packed in packages with a volume of more than 3 m3; or

(b) a positive result is obtained in a test using a 100 mm sample cube at 140 °C and a negative result is obtained in a test using a 25 mm cube sample at 140 °C, a positive result is obtained in a test using a 100 mm cube sample at 120 °C and the substance or mixture is to be packed in packages with a volume of more than 450 litres; or

(c) a positive result is obtained in a test using a 100 mm sample cube at 140 °C and a negative result is obtained in a test using a 25 mm cube sample at 140 °C and a positive result is obtained in a test using a 100 mm cube sample at 100 °C.

Since the auto-ignition temperature of S-abscisic acid is above the criteria set for self-heating substances, it does not fulfill the criteria for classification.

8.10.3 Conclusion on classification and labelling for self-heating substances

No classification is proposed.

8.11 Substances which in contact with water emit flammable gases

No data available.

8.11.1 Short summary and overall relevance of the provided information on substances which in contact with water emit flammable gases

Not relevant.

8.11.2 Comparison with the CLP criteria

No specific data derived in accordance with the recommended test method in CLP has been provided. However, S-abscisic acid has been handled in water within many of the studies available in the dossier and there are no reports of violent reaction and emission of gas.

8.11.3 Conclusion on classification and labelling for substances which in contact with water emit flammable gases

No classification is proposed. Data (experience in handling) is conclusive but not sufficient for classification.

8.12 Oxidising liquids

Hazard class not applicable (S-abscisic acid is not a liquid).

8.12.1 Short summary and overall relevance of the provided information on oxidising liquids

Not relevant.

8.12.2 Comparison with the CLP criteria

Not relevant.

8.12.3 Conclusion on classification and labelling for oxidising liquids

Hazard class not applicable.

8.13 Oxidising solids

Table 11: Summary table of studies on oxidising solids

Method	Results	Remarks	Reference
EC A17	Not oxidising	-	Comb, A. L., 2011

8.13.1 Short summary and overall relevance of the provided information on oxidising solids

S-abscisic acid is non-oxidising based on the results from a study performed in accordance to EC method A17 (Comb, A. L., 2011).

8.13.2 Comparison with the CLP criteria

The available test performed to determine the oxidising properties of S-abscisic acid demonstrated that neither the 2:1, 1:1 nor 1:2 mixtures of the test item and cellulose burned vigorously or to completion. Based on these findings, no classification is proposed in accordance with the CLP Regulation.

8.13.3 Conclusion on classification and labelling for oxidising solids

Data conclusive but not sufficient for classification.

8.14 Organic peroxides

Hazard class not applicable (S-abscisic acid is not an organic peroxide).

8.14.1 Short summary and overall relevance of the provided information on organic peroxides

Not relevant.

8.14.2 Comparison with the CLP criteria

Not relevant.

8.14.3 Conclusion on classification and labelling for organic peroxides

Hazard class not applicable.

8.15 Corrosive to metals

Data lacking (S-abscisic acid is not a liquid).

8.15.1 Short summary and overall relevance of the provided information on the hazard class corrosive to metals

Not relevant.

8.15.2 Comparison with the CLP criteria

Not relevant.

8.15.3 Conclusion on classification and labelling for corrosive to metals

Data lacking.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Toxicokinetic studies

S-abscisic acid is a natural plant growth regulator that is ubiquitous present in plants. Levels of S-abscisic acid are reported to be 0.06-0.096 mg/kg in tomatoes and 0.535 mg/kg in banana fruit. The natural precursor is mevalonic acid (Noddle and Robinson, 1969). The total levels of bound and free S-abscisic acid in ripe strawberries were shown to be tenfold the levels in unripe strawberries (34.1 and 296.0 mg/kg, respectively ripe vs unripe) (Rudnicki and Pieniazek, 1971). In grains, levels increased during development from 0.31 to 2.56 ng/grain towards the end of grain development. Then, with grain water loss, the content of abscisic acid decreased with the formation of the two metabolites phaseic acid (PA) and dihydrophaseic acid (DPA) (King, 1979).

ADME studies are considered not necessary since S-abscisic acid is ubiquitous in plants, and mammals have been chronically exposed to natural levels of S-abscisic acid throughout history via their diet without adverse effects. Furthermore, S-abscisic acid is a normal component of a healthy diet and we know that humans are regularly exposed without obvious ill-effects. Additionally no toxic events are seen in any of the toxicity test and there is no indication that toxicological relevant metabolites will be formed after human exposure to S-abscisic acid.

Oral absorption

Not having a ADME study means that a clear picture of the oral absorption levels of S-abscisic acid is absent.

A literature search with comparative weak acids was performed:

Gobas et al (1988) did a compilation of available literature data on uptake efficiencies of hydrophobic, organic chemicals from food by fish. They showed a dependence of absorption efficiency on a chemical log Kow, with absorption efficiencies averaging about 50% for chemicals with log Kow between 4 and 7. The oral absorption then declined progressively with higher Kows. While further refinements have been made to this concept, such as by Nichols et al (2004), the basic concept has held. For S-abscisic acid the non-ionized form gave a Kow of 1.8, while the ionized form is substantially lower at 0.94 Kow, indicating that the oral absorption should easily be above 50%.

10 EVALUATION OF HEALTH HAZARDS

The mammalian toxicity studies of S-abscisic acid were assessed in the Draft Assessment Report (2013), addenda and Proposed Decision of the Netherlands prepared in the context of the approval (Reg. (EU) No. 151/2014), under Reg. (EC) 1107/2009. Studies considered valid in the DAR (reliability score of 1 or 2) have been included in this report and were considered for classification purposes. All studies were carried out under GLP unless indicated otherwise. The non-GLP studies were range-finding studies or mechanistic studies. Other than the mechanistic studies all studies reported in this section were carried out in accordance with OECD guidelines. Minor deviations were noted in some cases but these did not affect the overall reliability of the studies. The deviations are included in the summaries were relevant.

Acute toxicity

10.1 Acute toxicity - oral route

Table 12: Summary table of animal studies on acute oral toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Value LD_{50}	Reference
Acute oral toxicity OECD 425 (Up-and-Down-Procedure) Deviations: - 3 females/dose were used instead of five/sex - max. volume exceeded 1 mL/ 100 g bw (i.e. 1.7 mL/ 100 g bw	Rat (Sprague-Dawley) 3 females/ dose	VBC-30054 (Technical Grade S-abscisic acid), batch no. 124-164-W9-00, purity 95%	5000 mg/kg bw, single exposure	> 5000 mg/kg bw	B.6.2.1, STUDY 1

Table 13: Summary table of human data on acute oral toxicity

No data available.

Table 14: Summary table of other studies relevant for acute oral toxicity

No data available.

10.1.1 Short summary and overall relevance of the provided information on acute oral toxicity

A study is available in which the acute oral toxicity of S-abscisic acid in rats was determined. The available study was performed according to OECD 425. However, some deviations from the guideline were identified. In fact, instead of 5 animals/ sex/ dose, only 3 females/ dose were tested. Moreover, the administered volume was 1.7 mL/ 100 g BW while according to the OECD guideline the maximum volume should not exceed 1.0 mL/100 g bw. However, in the absence of any signs of toxicity, the study was considered acceptable and a LD50 > 5000 mg/kg bw/day was established.

10.1.2 Comparison with the CLP criteria

According to Regulation No. (EC) 1272/2008 (Table 3.1.1), S-abscisic acid cannot be allocated to one of the four hazard categories for oral acute toxicity since the LD50 was found to exceed the values that trigger classification.

10.1.3 Conclusion on classification and labelling for acute oral toxicity

No classification is proposed.

10.2 Acute toxicity - dermal route

Table 15: Summary table of animal studies on acute dermal toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Value LD_{50}	Reference
Acute dermal toxicity OECD 402	Rat (Sprague-Dawley), 5 animals/ sex/ dose	VBC-30054 (Technical Grade S-abscisic acid), batch no. 124-164-W9-00, purity 95%	5000 mg/kg bw/day, 24 hours on a skin area of about 39 cm2 (semi-occlusive)	> 5000 mg/kg bw	B.6.2.1, STUDY 1

Table 16: Summary table of human data on acute dermal toxicity

No data available.

Table 17: Summary table of other studies relevant for acute dermal toxicity

No data available.

10.2.1 Short summary and overall relevance of the provided information on acute dermal toxicity

An acute dermal toxicity study performed with S-abscisic acid in accordance to OECD 402 is available. In this study, Sprague Dawley rats were exposed to S-abscisic acid at a concentration of 5000 mg/kg bw/day during 24h under semi-occlusive conditions. Based on this study, no treatment-related effects were observed and the LD50 was determined to be > 5000 mg/kg BW.

10.2.2 Comparison with the CLP criteria

According to Regulation No. (EC) 1272/2008 (Table 3.1.1), S-abscisic acid cannot be allocated to one of the four hazard categories for dermal acute toxicity since the LD50 was found to exceed the values that trigger classification.

10.2.3 Conclusion on classification and labelling for acute dermal toxicity

No classification proposed.

10.3 Acute toxicity - inhalation route

Table 18: Summary table of animal studies on acute inhalation toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, , form and particle size (MMAD)	Dose levels, duration of exposure	Value LC_{50}	Reference
Acute inhalation test OECD 403	Rat (Sprague-Dawley) 5/sex/dose	VBC-30054 (Technical Grade S-abscisic acid), batch no. 124-164-W9-00, purity 95% MMAD 3.8 µm with GSD 2.19.	8.78 mg/L (nominal concentration) 2.06 mg/L (actual concentration) 4 hours (nose only)	> 2.06 mg/L (maximum attainable concentration)	B.6.2.1, STUDY 1
Acute inhalation toxicity OECD 403	Rat (Sprague-Dawley) 5/sex/dose	S-abscisic acid Technical grade active ingredient, batch no. 147-690-W9, purity 96% MMAD 3.75 µm with GSD 2.21	27.41 mg/L (nominal concentration) 5.13 mg/L (actual concentration) 4 hours (nose only)	> 5.13 mg/L (maximum attainable concentration)	B.6.2.1, STUDY 2

Table 19: Summary table of human data on acute inhalation toxicity

No data available.

Table 20: Summary table of other studies relevant for acute inhalation toxicity

No data available.

10.3.1 Short summary and overall relevance of the provided information on acute inhalation toxicity

Two studies are available in which the acute inhalation toxicity of S-abscisic acid was studied. Both studies are performed according to OECD guideline 403. In the first study, the maximum attainable concentration was 2.06 mg/L and in the second study this was 5.13 mg/L. In both studies no treatment related effects were observed and the LC50 value for acute inhalation toxicity is thus greater than 5.13 mg/L.

10.3.2 Comparison with the CLP criteria

According to Regulation No. (EC) 1272/2008 (Table 3.1.1), S-abscisic acid cannot be allocated to one of the four hazard categories for oral inhalation toxicity since the LC50 was found to exceed the values that trigger classification.

10.3.3 Conclusion on classification and labelling for acute inhalation toxicity

No classification proposed.

10.4 Skin corrosion/irritation

Table 21: Summary table of animal studies on skin corrosion/irritation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results -Observations and time point of onset -Mean scores/animal -Reversibility	Reference
Skin irritation OECD 404	Rabbit, New Zealand White 1 male, 2 females/ dose	VBC-30054 (Technical Grade S-abscisic acid), batch no. 124-164-W9-00, purity 95%	4 hours, semi-occlusive, application area 6 cm^2 Dose: 0.5 g	Observations made at 30-60 minutes, 24, 48, 72 h Mean scores at 24, 48 and 72h: Erythema: 0 Oedema: 0 Reversibility: not applicable	B.6.2.2, STUDY 1

Table 22: Summary table of human data on skin corrosion/irritation

No data available.

Table 23: Summary table of other studies relevant for skin corrosion/irritation

No data available.

10.4.1 Short summary and overall relevance of the provided information on skin corrosion/irritation

The potential of skin irritation caused by S-abscisic acid was tested in New Zealand white rabbits. The available study is performed according to OECD 404. Based on the results obtained, S-abscisic acid was found to be non-irritating to rabbit skin.

10.4.2 Comparison with the CLP criteria

According to Regulation No. (EC) 1272/2008 a substance should be classified as skin irritant if:

(1) Mean score of ≥ 2,3 - ≤ 4,0 for erythema/eschar or for oedema in at least 2 of 3 tested animals from gradings at 24, 48 and 72 hours after patch removal or, if reactions are delayed, from grades on 3 consecutive days after the onset of skin reactions; or

(2) Inflammation that persists to the end of the observation period normally 14 days in at least 2 animals, particularly taking into account alopecia (limited area), hyperkeratosis, hyperplasia, and scaling; or

(3) In some cases where there is pronounced variability of response among animals, with very definite positive effects related to chemical exposure in a single animal but less than the criteria above.

S-abscisic acid does not fulfil the criteria for skin irritation as the scores for erythema and oedema were 0 in all animals at all time points and no signs of inflammation were observed.

10.4.3 Conclusion on classification and labelling for skin corrosion/irritation

No classification proposed.

10.5 Serious eye damage/eye irritation

Table 24: Summary table of animal studies on serious eye damage/eye irritation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results - Observations and time point of onset - Mean scores/animal - Reversibility	Reference
Eye irritation OECD 405	Rabbit, New Zealand White 3 males/ dose	VBC-30054 (Technical Grade S-abscisic acid), batch no. 124-164-W9-00, purity 95%	Single instillation in conjunctival sac Dose: 0.1 ml (0.04 grams)	Observations made at 1, 24, 48, 72 h Mean scores at 24, 48 and 72h: Cornea/opacity: 0.7; 0.0; 0.0 Iris: 0.0; 0.0; 0.0 Conjunctiva redness: 0.3; 0.3; 0.3 Conjunctiva chemosis: 0.0; 0.0; 0.0 Conjunctiva discharge: 0.0; 0.0; 0.0 Reversibility: all effects reversible after 72h	B.6.2.2, STUDY 2

Table 25: Summary table of human data on serious eye damage/eye irritation

No data available.

Table 26: Summary table of other studies relevant for serious eye damage/eye irritation

No data available.

10.5.1 Short summary and overall relevance of the provided information on serious eye damage/eye irritation

Based on a study in which a single installation of S-abscisic acid was applied to the conjunctival sac of 3 male White New Zealand rabbits, S-abscisic acid was not found to be irritating to the eyes. Corneal opacity was found in one animal at 24 and 48 h after application which was not present after 72h. In all animals treated with s-abscisic acid, conjunctival redness was observed at 24 h after installation, but were absent after 48 and 72 h. Although the study is performed according to OECD 405 it remains unclear whether the treated eyes were washed after instillation of s-abscisic acid.

10.5.2 Comparison with the CLP criteria

According to Regulation No. (EC) 1272/2008 a single hazard category (Category 1) is adopted for substances that have potential to seriously damage the eyes. For such substances the following criteria apply:

A substance that produces:

(a) in at least one animal effects on the cornea, iris or conjunctiva that are not expected to reverse or have not fully reversed within an observation period of normally 21 days; and/or

(b) in at least 2 of 3 tested animals, a positive response of:

(i) corneal opacity ≥ 3 and/or

(ii) iritis > 1,5

calculated as the mean scores following grading at 24, 48 and 72 hours after installation of the test material.

According to Regulation No. (EC) 1272/2008 a single hazard category (Category 2) is adopted for substances that have potential for eye irritation. For such substances the following criteria apply:

Substances that produce in at least in 2 of 3 tested animals, a positive response of:

(a) corneal opacity ≥ 1 and/or

(b) iritis ≥ 1, and/or

(c) conjunctival redness ≥ 2 and/or

(d) conjunctival oedema (chemosis) ≥ 2

calculated as the mean scores following grading at 24, 48 and 72 hours after installation of the test material, and which fully reverses within an observation period of 21 days

S-abscisic acid resulted in a positive response of corneal opacity of 0.7 in one animal and conjunctival redness of 0.3 was observed in all animals. These effects were fully reversible after 48h. S-abscisic acid does therefore not meet the CLH criteria for classification for eye irritation.

10.5.3 Conclusion on classification and labelling for serious eye damage/eye irritation

No classification proposed.

10.6 Respiratory sensitisation

No data available.

10.6.1 Short summary and overall relevance of the provided information on respiratory sensitisation

Not relevant.

10.6.2 Comparison with the CLP criteria

Not relevant.

10.6.3 Conclusion on classification and labelling for respiratory sensitisation

No classification proposed due to lack of data.

10.7 Skin sensitisation

Table 27: Summary table of animal studies on skin sensitisation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results	Reference
Skin sensitisation (Maximisation test)	Guinea pig, Hartley albino	VBC-30054 (Technical Grade S-abscisic acid), batch	intradermal and topical induction, topical challenge (occlusive, 48h)	Not sensitising	B.6.2.2, STUDY 3

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results	Reference
OECD 406	Control group: 5/ sex Treatment: 10/ sex/dose	no. 124-164-W9-00, purity 95%	1% w/w intradermal induction; 55% w/w topical induction and 55% w/w challenge Mineral oil or 50% v/v Complete Freund's Adjuvant in distilled water		

Table 28: Summary table of human data on skin sensitisation

No data available.

Table 29: Summary table of other studies relevant for skin sensitisation

No data available.

10.7.1 Short summary and overall relevance of the provided information on skin sensitisation

S-abscisic acid was tested in a Magnusson and Kligman study according to OECD 406. The results demonstrate that topical induction with 55% w/w S-abscisic acid in mineral oil caused faint erythema (skin irritation scores 1) in all animals treated. Very faint to faint erythema (skin irritation score 0.5 – 1) was noted during the topical induction phase at all sham control sites one hour after patch removal. Following challenge with 55% w/w S-abscisic acid in mineral oil, very faint erythema (skin irritation score 0.5) was noted for seven of twenty test sites (equal to 35%) 24 hours after challenge patch removal. Irritation persisted at one of these sites through 48 hours. Very faint erythema (skin irritation score 0.5) was noted at 3 out of 10 sham control sites (equal to 30%) 24 hours after challenge patch removal. The observed irritation was found to be resolved within 48 hours. Sensitisation of this strain of animals was positively tested with alpha-hexylcinnamic aldehyde. It should be noted that the study deviates from the OECD TG 406 since the same concentration of S-abscisic acid was used or topical induction and topical challenge whereas according to the TG the highest dose to cause mild irritation should be selected for induction and the highest non-irritating dose should be used for the challenge phase. However, the study authors noted that concentrations in excess of 55% could not be tested as they were considered too dry to provide adequate skin contact. This argumentation is plausible, considering that the observed response was comparable between the sham control animals and challenge dose-tested animals. The deviation from the guideline therefore causes some uncertainties about the reliability of the results and validity of the study.

10.7.2 Comparison with the CLP criteria

According to Regulation No. (EC) 1272/2008 a single hazard category (Category 1) is adopted for substances that have potential for skin sensitisation. For such substances the following criteria apply:

Category 1: *Substances shall be classified as skin sensitisers (Category 1) where data are not sufficient for sub-categorisation in accordance with the following criteria: (a) if there is evidence in humans that the substance can lead to sensitisation by skin contact in a substantial number of persons; or (b) if*

there are positive results from an appropriate animal test (see specific criteria in paragraph 3.4.2.2.4.1).

Sub-category 1A: *Substances showing a high frequency of occurrence in humans and/or a high potency in animals can be presumed to have the potential to produce significant sensitisation in humans. Severity of reaction may also be considered.*

Sub-category 1B: *Substances showing a low to moderate frequency of occurrence in humans and/or a low to moderate potency in animals can be presumed to have the potential to produce sensitisation in humans. Severity of reaction may also be considered.*

For S-abscisic acid no human data and comparison with CLP criteria can therefore only be done based on animal data. The following CLP criteria apply:

Animal test results for sub-category 1A:

Assay Criteria Local lymph node assay	*EC3 value ≤ 2 %*
Guinea pig maximisation test	*≥ 30 % responding at ≤ 0,1 % intradermal induction dose or ≥ 60 % responding at > 0,1 % to ≤ 1 % intradermal induction dose*
Buehler assay	*≥ 15 % responding at ≤ 0,2 % topical induction dose or ≥ 60 % responding at > 0,2 % to ≤ 20 % topical induction dose*

Animal test results for sub-category 1B

Assay Criteria Local lymph node assay	*EC3 value > 2 %*
Guinea pig maximisation test	*≥ 30 % to < 60 % responding at > 0,1 % to ≤ 1 % intradermal induction dose or ≥ 30 % responding at > 1 % intradermal induction dose*
Buehler assay	*≥ 15 % to < 60 % responding at > 0,2 % to ≤ 20 % topical induction dose or ≥ 15 % responding at > 20 % topical induction dose*

After challenge, 7 out of 20 animals (equal to 35%) treated with 55% w/w/ S-abscisic acid showed very faint erythema. In the sham control animals a comparable response of 30% was observed (3/10 animals). The response rate of 5% is thus not sufficient for classification since a positive response of 30% or more was not observed. In addition, it should be noted that very faint erythema is considered only a mild response. Based on the available guinea pig maximisation test (Magnusson and Kligman), it can be concluded that S-abscisic acid does not fulfil the criteria for skin sensitisation. However, as indicated above, some uncertainties exist about the reliability of the study.

10.7.3 Conclusion on classification and labelling for skin sensitisation

No classification proposed.

10.8 Germ cell mutagenicity

Table 30: Summary table of mutagenicity/genotoxicity tests in vitro

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
Ames test (Point mutation) OECD 471	S-abscisic acid , lot no. 124-164-W9, purity 96.2%	Organism/ strain: *S. typh.* (TA 98, TA 100, TA 1535, TA 1537) and *E.coli* (WP2uvrA) Concentration tested: 33.3, 100, 333, 1000, 3.330 and 5000 µg/plate Positive control: –S-9: benzo(a)pyrene (TA98), 2-aminoanthracene (TA100, TA1535, TA1537, WP2uvrA) +S-9: 2-nitrofuorene (TA98), sodium azide (TA100), sodium azide (TA1535), ICR-191 (TA1537), 4-nitroquinoliane-N-oxide (WP2uvrA)	Results without activation: - Results with activation: - No cytotoxicity was observed.	B.6.4.1, STUDY 1
chromosome aberration OECD 473	S-abscisic acid, lot no. 124-164-W9, purity % 96.2	Organism/ strain: Chinese hamster ovary (CHO) cells Concentration tested: Initial assay: -S9: 19.0, 27.1, 38.8, 55.4, 79.1, 113, 161, 231, 329, 471, 672, 960, 1370, 1960, 2800 µg/ml +S9: 19.0, 27.1, 38.8, 55.4, 79.1, 113, 161, 231, 329, 471, 672, 960, 1370, 1960, 2800 µg/ml Confirmatory assay: -S9: 43.8, 87.5, 131, 175, 263, 350, 525, 700, 1050, 1400, 2100 and 2800 µg/ml +S9: 700, 1050, 1400, 2100 and 2800 µg/ml Positive control: -S9: Mitomycin C +S9: cyclophosphamide	Results without activation: - Results with activation: - No cytotoxicity was observed.	B.6.4.1, STUDY 2
gene mutations (TK) OECD 476	S-abscisic acid, lot no. 153-912-W9, purity 98.3 %	Organism/ strain: Mouse lymphoma cells L5178Y Concentration tested: Initial assay: +/- S9: 7.81, 15.6, 31.3, 62.5, 125, 250, 500, 750, 1000, 1300, 1600, 2000, 2300, and 2650 µg/ml Confirmatory assay: -S9: 7.81, 15.6, 31.3, 62.5, 125, 250, 500, 750, 1000, 1200, 1300, 1400, 1500, and 1600 µg/ml +S9: 31.3, 62.5, 125, 250, 500, 750, 1000, 1300, 1600, 2000, 2300, 2500, and 2650 µg/ml Positive control:	Results without activation: - Results with activation: - Cytotoxicity was observed at the highest concentration evaluated (2650 µg/ml)	B.6.4.1, STUDY 3

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
		-S9: methyl methanesulfonate +S9: methylcholanthrene		

Table 31: Summary table of mutagenicity/genotoxicity tests in mammalian somatic or germ cells in vivo

Method, guideline, deviations if any	Test substance	Relevant information about the study (as applicable)	Observations	Reference
In vivo micronucleus OECD 474	S-abscisic acid, lot no. 124-164-W9, purity 96.2%	Organism/ strain: Mouse, CD-1 strain Concentrations tested: 500, 1000 and 2000 mg/kg bw/d, administered orally three times at 24 hour intervals by oral gavage; sacrifice at 24 after last dose. Positive control: cyclophosphamide	No induction of micronuclei in bone marrow cells	B.6.4.2, STUDY 4

Table 32: Summary table of human data relevant for germ cell mutagenicity

No data available.

10.8.1 Short summary and overall relevance of the provided information on germ cell mutagenicity

S-abscisic acid is not mutagenic in the Ames test. S-abscisic acid did not induce structural chromosome aberrations in CHO cells and showed no mutagenic potency in vitro in the ML cell/TK assay.

S-abscisic acid did not show any chromosomal damage in mouse bone marrow cells in an in vivo cytogenetic test up to and including 2000 mg/kg bw. Although the study was considered acceptable for evaluation in the DAR, it remains unclear whether the bone marrow was reached. The study report does not address bone marrow exposure to s-abscisic acid. In the available study, plasma and/or blood levels of S-abscisic acid were not determined and a depression of the ratio immature to mature erythrocytes was not observed. Additionally, ADME data is not available. Therefore, it cannot be concluded if bone marrow exposure occurred and the study is considered supplemental. However, considering that all *in vitro* studies were negative it can be concluded that S-abscisic acid has no mutagenic or clastogenic potential.

10.8.2 Comparison with the CLP criteria

According to Regulation EC No 1272/2008 (CLP), Table 3.5.2.2, classification in Category 1 mutagen is based on:

Substances known to induce heritable mutations or to be regarded as if they induce heritable mutations in the germ cells of humans. Substances known to induce heritable mutations in the germ cells of humans.

The classification in Category 1A is based on positive evidence from human epidemiological studies. Substances to be regarded as if they induce heritable mutations in the germ cells of humans.

The classification in Category 1B is based on: – positive result(s) from in vivo heritable germ cell mutagenicity tests in mammals; or – positive result(s) from in vivo somatic cell mutagenicity tests in mammals, in combination with some evidence that the substance has potential to cause mutations to germ cells. It is possible to derive this supporting evidence from mutagenicity/genotoxicity tests in germ cells in vivo, or by demonstrating the ability of the substance or its metabolite(s) to interact with the genetic material of germ cells; or – positive results from tests showing mutagenic effects in the germ cells of humans, without demonstration of transmission to progeny; for example, an increase in the frequency of aneuploidy in sperm cells of exposed people.

Classification in Category 2 mutagen is based on:

- *Positive evidence obtained from experiments in mammals and/or in some cases from in vitro experiments, obtained from:*
    - *Somatic cell mutagenicity tests in vivo, in mammals; or*
    - *Other in vivo somatic cell genotoxicity tests which are supported by positive results from in vitro mutagenicity assays*

Based on the results of an adequate range of negative in vitro studies (gene mutation tests with bacterial and mammalian cells, a chromosome aberration test with mammalian cells and a gene mutation test) and an in vivo study (micronucleus test in mice), it is concluded that S-abscisic acid does not fulfill the criteria for classification for germ cell mutagenicity.

10.8.3 Conclusion on classification and labelling for germ cell mutagenicity

No classification proposed.

10.9 Carcinogenicity

10.9.1 Short summary and overall relevance of the provided information on carcinogenicity

No long-term toxicity and/or carcinogenicity studies are available.

10.9.2 Comparison with the CLP criteria

Not relevant.

10.9.3 Conclusion on classification and labelling for carcinogenicity

No classification proposed.

10.10 Reproductive toxicity

10.10.1 Adverse effects on sexual function and fertility

Table 33: Summary table of animal studies on adverse effects on sexual function and fertility

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
2-generation study rat, Crl:CD (SD), 30/ sex/ dose OECD 416	Test substance: S-abscisic acid, lot no. 283-912-W9-00, purity ≥98%, and lot no. 183-912-W9, purity ≥98% Exposure: continuously through the study period Dose: 0, 10000, 15000, 20000 ppm (equal to 684, 1031 and 1360 mg/kg bw/day)	NOAEL Parental: ≥1360 mg/kg bw/d Fertility: ≥1360 mg/kg bw/d Critical effects: no adverse effects.	B.6.6.1, STUDY 1

Table 34: Summary table of human data on adverse effects on sexual function and fertility

No data available.

Table 35: Summary table of other studies relevant for toxicity on sexual function and fertility

No data available.

10.10.2 Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility

The potential of S-abscisic acid to cause reproductive effects was tested in a 2-generation study in Crl:CD(SD) rats in accordance with OECD 416. Administration of S-abscisic acid continuously in the diet to F0 and F1 animals resulted in non-adverse organ weight changes, including lower seminal vesicles/coagulating glands/accessory fluid weight and higher liver weight in the 20,000 ppm F0 group males and higher liver weight in the 15,000 and 20,000 ppm F0 group females and 20,000 ppm F1 group females. In the absence of any histologic correlates in the seminal vesicles and liver, these organ weight changes were not considered to be adverse. In the F0 generation, a lower mean testis sperm concentration (79.3 million/g (88% of controls)) and a related lower mean sperm production (13 million/g per day (88% of controls)) was observed in the highest dose tested. Nevertheless, these values were found to be within the historical control data (79.8 million/g and 13.1 million/g/day, respectively). These historical control data was derived in the performing lab from 165 studies performed during the years 2000 and 2009 with Crl:CD(SD) rat. The observed effects on sperm concentration and sperm production were not observed in the F1 generation. Therefore, this effect was considered not treatment-related.

The no-observed-adverse-effect level (NOAEL) for F0 and F1 parental toxicity was considered to be 20,000 ppm.

Based on the absence of effects on F0 reproductive performance (mating, fertility, copulation and conception indices, oestrous cyclicity and spermatogenic endpoints) and on the F1 and F2 litters and offspring, an exposure level of 20,000 ppm was considered to be the NOAEL for F0 and F1 reproductive toxicity, as well as F1 and F2 neonatal toxicity of S-abscisic acid when administered continuously in the diet to Crl:CD(SD) rats.

10.10.3 Comparison with the CLP criteria

According to Regulation EC No 1272/2008 (CLP), Table 3.7.2.2, classification as for effects on fertility is based on:

Category 1A:

Known human reproductive toxicant

Category 1B:

Presumed human reproductive toxicant largely based on data from animal studies

- *clear evidence of an adverse effect on sexual function and fertility in the absence of other toxic effects, or*
- *the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects*

Category 2:

Suspected human reproductive toxicant

- *some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility and*
- *where the evidence is not sufficiently convincing to place the substance in Category 1 (deficiencies in the study).*
- *the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects*

According to the CLP criteria classification as Repr. 1A is based on human data. No human data is available for S-abscisic acid and therefore, classification as Repr 1A is not justified. Moreover, the available animal data show that no adverse effects occurred up to 1360 mg/kg bw/ day (the highest dose tested) in the available 2-generation test and the available repeated dose toxicity tests and therefore, S-abscisic acid also does not fulfil the requirements of Repr. 1B or 2.

10.10.4 Adverse effects on development

Table 36: Summary table of animal studies on adverse effects on development

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
2-generation study	Test substance: S-abscisic acid, lot no. 283-912-W9-00, purity ≥98%, and lot no. 183-912-W9, purity	NOAEL Parental: ≥1360 mg/kg bw/d	B.6.6.1, STUDY 1

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
rat, Crl:CD (SD), 30/ sex/ dose OECD 416	≥98% Exposure: continuously through the study period Dose: 0, 10000, 15000, 20000 ppm (equal to 684, 1031 and 1360 mg/kg bw/day)	Development: ≥1360 mg/kg bw/d Critical effects: no adverse effects.	
Teratogenicity study Rat, Crl:CD (SD), 25 females/ dose OECD 414	Test substance: S-abscisic acid, lot no. 124-164-W9-00, purity 97.0% Exposure duration: Days 6-19 of gestation, gavage Dose: 0, 500, 750 and 1000 mg/kg bw/d	NOAEL: Maternal: > 1000 mg/kg bw/day Developmental: > 1000 mg/kg bw/day Critical effects: no adverse effects	B.6.6.2, STUDY 1

Table 37: Summary table of human data on adverse effects on development

No data available.

Table 38: Summary table of other studies relevant for developmental toxicity

No data available.

10.10.5 Short summary and overall relevance of the provided information on adverse effects on development

A developmental toxicity study was performed in which rats were exposed to S-abscisic acid during days 6-19 of gestation. The study was performed according to OECD 414. Daily oral administration of up to 1000 mg/kg/day of S-abscisic acid technical to pregnant Crl:CD(SD) rats during Day 6 to 19 of gestation showed no effect on maternal bodyweight, bodyweight gain and gross necropsy.

No test substance-related effects were seen on postimplantation loss, live litter size, mean foetal weights and foetal sex rates. There were no foetal malformations or developmental variations attributed to the test substance.

Based on the lack of adverse test substance-related effects on maternal animals and on prenatal development, a dosage level of 1000 mg/kg/bw/day was considered to be the NOAEL for maternal toxicity and prenatal developmental toxicity. Under the conditions of this study, it was concluded that S-abscisic acid was not teratogenic to rats.

10.10.6 Comparison with the CLP criteria

Annex I (3.7.2.4.2) of the CLP criteria states the following: '*Based on pragmatic observation, maternal toxicity may, depending on severity, influence development via non-specific secondary mechanisms, producing effects such as depressed foetal weight, retarded ossification, and possibly resorptions and certain malformations in some strains of certain species. However, the limited*

numbers of studies which have investigated the relationship between developmental effects and general maternal toxicity have failed to demonstrate a consistent, reproducible relationship across species. Developmental effects which occur even in the presence of maternal toxicity are considered to be evidence of developmental toxicity, unless it can be unequivocally demonstrated on a case-by-case basis that the developmental effects are secondary to maternal toxicity. Moreover, classification shall be considered where there is a significant toxic effect in the offspring, e.g. irreversible effects such as structural malformations, embryo/foetal lethality, significant post-natal functional deficiencies.'

According to Regulation EC No 1272/2008 (CLP), Table 3.7.2.2, classification as for effects on development is based on:

Category 1A:

Known human reproductive toxicant

Category 1B:

Presumed human reproductive toxicant largely based on data from animal studies

- *clear evidence of an adverse effect on development in the absence of other toxic effects, or*
- *the adverse effect on development is considered not to be a secondary non-specific consequence of other toxic effects*

Category 2:

Suspected human reproductive toxicant

- *some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on development and*
- *the evidence is not sufficiently convincing to place the substance in Category 1 (deficiencies in the study).*
- *the adverse effect on development is considered not to be a secondary non-specific consequence of the other toxic effects*

According to the CLP criteria classification as Repr. 1A is based on human data. No human data is available for S-abscisic acid and therefore, classification as Repr 1A is not justified. Since no developmental effects were observed in the available teratogenicity study up to 1000 mg/kg bw/day (the highest dose tested), classification as Cat. 1B or 2 is not justified.

It is considered not necessary to classify S-abscisic acid for developmental toxicity.

10.10.7 Adverse effects on or via lactation

According to Regulation EC No 1272/2008 (CLP), Table 3.7.2.2.2, classification for lactation effects is based on:

(a) human evidence indicating a hazard to babies during the lactation period; and/or

(b) results of one or two generation studies 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.

The 2-generation study did not report any adverse findings occurring via lactation.

10.10.8 Conclusion on classification and labelling for reproductive toxicity

No classification is proposed.

10.11 Specific target organ toxicity-single exposure

Table 39: Summary table of animal studies on STOT SE

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
Acute oral toxicity OECD 425 (Up-and-Down-Procedure) Deviations: - 3 females/dose were used instead of five/sex - max. volume exceeded 1 mL/ 100 g bw (i.e. 1.7 mL/ 100 g bw	Test substance: VBC-30054 (Technical Grade S-abscisic acid), batch no. 124-164-W9-00, purity 95% Organism/ strain: Rat (Sprague-Dawley), 3 females/ dose Dose: 5000 mg/kg bw, single exposure	Mortality: No mortality was observed at 5000 mg/kg bw. Symptoms of toxicity: No treatment related findings were observed. Body weight: The mean body weights increased throughout the study period. There was no treatment related effect. Pathology: No macroscopic pathologic abnormalities were observed.	B.6.2.1, STUDY 1
Acute dermal toxicity OECD 402	Test substance: VBC-30054 (Technical Grade S-abscisic acid), batch no. 124-164-W9-00, purity 95% Organism/ strain: Rat (Sprague-Dawley), 5 animals/ sex/ dose Dose: 5000 mg/kg bw/day, 24 hours on a skin area of about 39 cm2 (semi-occlusive)	Mortality: No mortality was observed at 5000 mg/kg bw. Symptoms of toxicity: No treatment related findings were observed. Body weight: The mean body weights increased throughout the study period. There was no treatment related effect. Pathology: No macroscopic pathologic abnormalities were observed.	B.6.2.1, STUDY 1

Acute inhalation test OECD 403	Test substance: VBC-30054 (Technical Grade S-abscisic acid), batch no. 124-164-W9-00, purity 95% Organism/ strain: Rat (Sprague-Dawley), 5/ sex/ dose Dose: 8.78 mg/L (nominal concentration); 2.06 mg/L (actual concentration) MMAD 3.8 µm with GSD 2.19 Exposure duration: 4 hours (nose only)	Mortality: No mortality occurred at a concentration of 2.06 mg/L. Symptoms of toxicity: No treatment related findings were observed. Body weight: The mean body weights increased throughout the study period. There was no treatment related effect. Pathology: No macroscopic pathologic abnormalities were observed.	B.6.2.1, STUDY 1
Acute inhalation toxicity OECD 403	Test substance: S-abscisic acid Technical grade active ingredient, batch no. 147-690-W9, purity 96% Organism/ strain: Rat (Sprague-Dawley), 5/ sex/ dose Dose: 27.41 mg/L (nominal concentration); 5.13 mg/L (actual concentration) MMAD 3.75 µm with GSD 2.21	Mortality: No mortality occurred at a concentration of 5.13 mg/L. Symptoms of toxicity: No treatment related findings were observed. Body weight: The mean body weights increased throughout the study period. There was no treatment related effect. Pathology: No macroscopic pathologic abnormalities were observed.	B.6.2.1, STUDY 2

Table 40: Summary table of human data on STOT SE

No data available.

Table 41: Summary table of other studies relevant for STOT SE

No data available.

10.11.1 Short summary and overall relevance of the provided information on specific target organ toxicity – single exposure

S-abscisic acid did not induce clinical signs of toxicity or evidence of any pharmacologic or behavioral effects shortly after acute dosing, particularly after oral gavage administration, in animals treated at dose levels up to 5000 mg/kg bw/day for oral and dermal exposure and up to 5.13 mg/L for inhalation exposure.

10.11.2 Comparison with the CLP criteria

Table 3.8.1, defines specific target organ toxicity single exposure, cat. 1 as follows:

'Substances that have produced significant toxicity in humans or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following single exposure Substances are classified in Category 1 for specific target organ toxicity (single exposure) on the basis of: a. reliable and good quality evidence from human cases or epidemiological studies; or b. observations from appropriate studies in experimental animals in which significant and/or severe toxic effects of relevance to human health were produced at generally low exposure concentrations. Guidance dose/concentration values are provided below (see 3.8.2.1.9) to be used as part of weight-of-evidence evaluation.'

Regulation EC No 1272/2008 (CLP), section 3.8.1 states that:

"Acute toxicity refers to lethality and STOT-SE to non-lethal effects. However, care should be taken not to assign both classes for the same toxic effect, essentially giving a "double classification", even where the criteria for both classes are fulfilled. In such case the most appropriate class should be assigned. "

In addition to category 1 and 2 for specific target organ toxicity, category 3 concerns transient organ effects. Table 3.8.2.3 defines category 3 as follows:

This category only includes narcotic effects and respiratory tract irritation. These are target organ effects for which a substance does not meet the criteria to be classified in Categories 1 or 2 indicated above. These are effects which adversely alter human function for a short duration after exposure and from which humans may recover in a reasonable period without leaving significant alteration of structure or function.

S-abscisic acid did not show toxic effects after acute dosing and therefore classification with STOT SE is not justified.

10.11.3 Conclusion on classification and labelling for STOT SE

No classification proposed.

10.12 Specific target organ toxicity-repeated exposure

Table 42: Summary table of animal studies on STOT RE

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
28-day oral toxicity study Rat, Sprague-Dawley, 5/ sex/ dose OECD 407	Test substance: VBC-30054 (Technical Grade S-abscisic acid), batch no. 124-164-W9-00, purity 97% Exposure: 28 days, diet Dose: 0, 2000, 6000 or 20000 mg/kg food	NOAEL: > 2171 mg/kg bw/day (m, f) Critical effects: no adverse effects.	B.6.3.1, STUDY 1

21-day dermal toxicity study Rat (Sprague Dawley), 5/ sex/ dose	Test substance: VBC-30054 (Technical Grade S-abscisic acid), batch no. 124-164-W9-00, purity 97% Exposure: 21 days, 6 h/d, semi-occlusive (10% of the total body surface area) Dose: 0, 100, 300, 1000 mg/kg bw/d	NOAEL: > 1000 mg/kg b/day (m, f) Critical effects: no adverse effects	B.6.3.2, STUDY 1
90-day oral toxicity study Rat, Sprague-Dawley, 10/ sex/ dose OECD 408	Test substance: VBC-30054 (Technical Grade S-abscisic acid), batch no. 124-164-W9-00, purity 97% Exposure: 90 days, in diet Dose: 0, 2000, 6000 or 20000 mg/kg food	NOAEL: 1420 mg/kg bw/day (m), 1752 mg/kg bw/day (f) Critical effects: no adverse effects	B.6.3.3, STUDY 1
2-generation study rat, Crl:CD (SD), 30/ sex/ dose OECD 416	Test substance: S-abscisic acid, lot no. 283-912-W9-00, purity ≥98%, and lot no. 183-912-W9, purity ≥98% Exposure: continuously through the study period Dose: 0, 10000, 15000, 20000 mg/kg food	NOAEL Parental: ≥1360 mg/kg bw/d Fertility: ≥1360 mg/kg bw/d Development: ≥1360 mg/kg bw/d Critical effects: no adverse effects.	B.6.6.1, STUDY 1
Teratogenicity study Rat, Crl:CD (SD), 25 females/ dose OECD 414	Test substance: S-abscisic acid, lot no. 124-164-W9-00, purity 97.0% Exposure duration: Days 6-19 of gestation, gavage Dose: 0, 500, 750 and 1000 mg/kg bw/d	NOAEL: Maternal: > 1000 mg/kg bw/day Developmental: > 1000 mg/kg bw/day Critical effects: no adverse effects	B.6.6.2, STUDY 1

Table 43: Summary table of human data on STOT RE

No data available.

Table 44: Summary table of other studies relevant for STOT RE

No data available.

10.12.1 Short summary and overall relevance of the provided information on specific target organ toxicity – repeated exposure

Several dose repeated toxicity studies relevant for classification with STOT RE are available:

28-day oral toxicity study

Rats were given 0, 2000, 6000 and 20000 mg/kg food (equivalent to 0, 215.8, 660.1 and 2171 mg/kg bw/d for males and 0, 233.6, 660.2 and 2171 mg/kg bw/d for females) during 28 days. A minor reduction in body weight gain was observed in males and females treated with the highest dose tested. However, these effects were without statistical significance when compared to the control.

Slightly lower levels of mean cell haemoglobin (MHC) and activated partial thromboplastin (APTT) were found in males in the high dose group. A slight decrease in lymphocytes was found in males and females in the high dose group; however, this was without any statistical significance. Effects on reticulocytes were seen in females of all dose groups in the absence of a dose response effect.

A dose-related and significant decrease of blood glucose levels was observed. No other toxicologically relevant effects were observed. Because blood glucose levels do normally vary within relative broad ranges and no other effects were observed, the decrease in blood glucose levels were considered of no toxicological significance. No histological findings were observed. Therefore, the NOAEL in this study is set at 2171 mg/kg bw/d for males and females.

21-day dermal toxicity study

Rats were exposed via the dermal route to 100, 300 or 1000 mg/kg bw/day for 21 consecutive days.

Very slight erythema was observed at the dose site in females exposed to the mid- and highest dose tested and in males exposed to the highest dose tested. Moreover, an increase in white blood cell count and associated parameters was observed in male at 1000 mg/kg bw/day. However, blood film smears revealed normal white cell morphology throughout the treated groups and histological findings were absent. Activated partial thromboplastin time was statistically significant increased in males in the mid- and high-dose groups, however without showing a dose-response curve. Because of the small magnitude of changes and the lack of dose responses, the changes were considered not to be toxicologically significant. Therefore, the NOAEL in this study is set at 1000 mg/kg bw/day.

90-day oral toxicity study

Rats were given 0, 2000, 6000 and 20000 mg/kg food (0, 137.6, 407.8, and 1420 mg/kg bw/d for males and 0, 164.1, 497, and 1752 mg/kg bw/d for females) during 90 days. Non-statistical body weight gain reductions were seen in males and females in the highest dose group. Contradicting findings on tactile stimuli were observed as in males a small number showed a reduced response whereas in females an increased response was observed. Cholesterol was slightly increased showing a dose-dependent trend in males, but without statistical significance. Triglyceride levels were slightly decreased showing a dose-dependent trend in both males and females, but also without statistical significance. The variation within the individual values would indicate that these values are not toxicologically relevant. Moreover, there were no treatment related effects in liver pathology. Some differences were noted in the organ weights of thyroid, liver, pituitary, spleen and adrenal glands. However, these differences were found to be without statistical significance and were considered to be not of toxicological relevance. Therefore, the NOAEL in this study is set at 1420 mg/kg bw/d for males and 1752 mg/kg bw/d for females.

Reproduction and developmental toxicity

Based on the available studies, no treatment related effects were observed at the highest dose levels (please refer to section 10.10).

10.12.2 Comparison with the CLP criteria

The following CLP criteria are laid down for STOT RE:

Category 1 (H372):

Substances that have produced significant toxicity in humans or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following repeated exposure.

Substances are classified in Category 1 for target organ toxicity (repeat exposure) on the basis of:

reliable and good quality evidence from human cases or epidemiological studies; or observations from appropriate studies in experimental animals in which significant and/or severe toxic effects, of relevance to human health, were produced at generally low exposure concentrations.

Category 2 (H373)

Substances that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to be Harmful to human health following repeated exposure.

Substances are classified in Category 2 for target organ toxicity (repeat exposure) on the basis of observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were produced at generally moderate exposure concentrations.

As presented above, several dose repeated studies are available relevant for the STOT RE classification. However, none of the dose-repeated toxicity studies available demonstrated adverse effects that trigger STOT RE classification.

10.12.3 Conclusion on classification and labelling for STOT RE

No classification proposed.

10.13 Aspiration hazard

No data available.

10.13.1 Short summary and overall relevance of the provided information on aspiration hazard

No data available.

10.13.2 Comparison with the CLP criteria

Not relevant.

10.13.3 Conclusion on classification and labelling for aspiration hazard

No classification proposed.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

The environmental hazards of S-abscisic acid were assessed in the Draft Assessment Report (DAR) (2013), addenda and Proposed Decision of the Netherlands prepared in the context of the approval (Reg. (EU) No. 151/2014), under Reg. (EC) 1107/2009. Studies considered valid in the DAR (reliability score of 1 or 2) have been included in this report and were considered for classification purposes. The study summaries as presented in the DAR are included in Annex 1. All studies were carried out under GLP unless indicated otherwise. Studies were carried out in accordance with relevant test guidelines. Minor deviations were noted in some cases which have been included in the study summaries bellow. The deviations did not affect the overall acceptability of the studies.

11.1 Rapid degradability of organic substances

Table 45: Summary of relevant information on rapid degradability

Method	Results	Remarks	Reference

Method	Results	Remarks	Reference
Ready biodegradability OECD 301F (1992) EU 92/69/EEC C.4 (1992); activated sludge, assessment of ready biodegradability by respirometry S-abscisic acid, purity 98.2%	Readily biodegradable 10% after 4 days, 60% after 8 days and 89% after 28 days (based on mean oxygen consumption)	The study was performed in accordance with guideline OECD 301F and is acceptable. Klimisch score: 1	Dickinson, R.A., 2010; STUDY IIA, 7.7/001
Hydrolysis US EPA OPPTS 835.2110 s-abscisic acid, purity 99.7%	S-abscisic acid is stable at pHs 7 and 9. At pH 4 and at 40 °C, minor degradation is observed, but hydrolysis is not considered a main route of degradation in the environment. S-abscisic acid is considered hydrolytically stable.	The study is considered acceptable. Klimisch score: 1	Schick, 2008; STUDY IIA, 2.9.1
Degradation in surface water OECD 309 (2004); non-radiolabelled test S- abscisic acid S-abscisic acid, chemical purity not reported, radiochemical purity 97.1%. (dark) & 97.6% (irradiated)	$DT50_{water}$ (20.2-22.5°C): 3.3 hours (geometric mean)	The active substance was only tested in a surface water system and not in a water-sediment system. In the DAR the following was noted: There are some deviations from OECD 309 including: - the tested concentrations only showed a difference factor two from each other; - the criterion that the lowest concentration should not exceed 10 µg/L was met; - no labelled S-abscisic acid was used in the test; - mineralization of the test substance is also not measured when using unlabelled test-substance; but overall the study is considered acceptable (see Annex I for details) Klimisch score: 2	Dickinson, R.A., 2011; STUDY IIA, 7.8.3/001
Degradation in soil OECD 307 (2002); 14C-S-abscisic acid, purity 97%, tested in sandy loam,	DT50 (20 °C) for different soil types: - Bromsgrove: 2.1 days - Elmton: 0.66 days[a] - Fladbury: 0.95 days - Empingham: 1.1 days[a]	The study is considered acceptable. Klimisch score: 1	Jones, A., 2010; STUDY IIA, 7.1.1/001

Method	Results	Remarks	Reference
sandy clay and 2 clay loam soils	[a] DT90/3.32		
Photochemical oxidative degradation in air Theoretical estimation	DT50 of 0.874 hours (0.036 days)	Value derived by the Atkinson model (AOPWIN version 1.92). OH (12 h) concentration assumed = 1.5 x 10^6 OH radicals/cm^3	-
Aqueous photolysis OECD 316; 14C-S-abscisic acid, purity > 97%	DT50 (25°C): 1.2 – 2.5 days Estimated environmental DT50 will range from 0.93 – 1.09 d in summer to 3.92 – 4.61 d in winter at 40 °N.	The photolytic degradation study is acceptable and performed under sterile conditions. The obtained endpoints are reliable and acceptable. Klimisch score: 1	Kane, T., 2011; STUDY IIA, 7.6/001
Soil photolysis OPPTS 835-2410 (2008) SETAC (1995) OECD guideline for soil photolysis; 14C-S-abscisic acid 100 %, tested in sandy clay loam	DT50 (20°C) of 2.3 OECD solar days in irradiated system DT50 (20°C) of 5.2 days in dark control	The study is considered reliable and acceptable. Klimisch score: 1	Ponte, M., 2012; STUDY IIA, 7.1.3/002

11.1.1 Ready biodegradability

The ready biodegradability of S-abscisic acid (purity 98.2%) was studied in an oxygen consumption test according to OECD 301F (Dickinson, R.A. 2010). At test initiation (Day 0), the test substance (nominally 222.5 mg) was weighed into a 500 ml volumetric flask containing mineral salts medium (MSM) and the preparations treated with ultrasound. Sodium benzoate was used as positive control. Cumulative levels of oxygen consumption by the controls after 28 days (15.43 and 15.03 mgO_2 per 500 mL, equivalent to 30.86 and 30.06 mgO_2 / L) were considered to be in accordance with the guidelines. Degradation of sodium benzoate reached 36% on day 1, and 59% at day 3 indicating the viability of the inoculum. In the presence of S-abscisic acid the degradation of sodium benzoate achieved 60% after 3 days indicating that the test substance was not inhibitory to the microbial inoculum. These results confirm that S-abscisic acid was not inhibitory to the activity of the microbial inoculum and that the test was valid. Biodegradation of S-abscisic acid based on oxygen consumption amounted to 10% after approximately 4 days, 60% after approximately 8 days and 89% at the end of the test (Day 28). Therefore, as ≥ 60% mineralization within the 10-day window was met, S-abscisic acid can be considered as readily biodegradable.

The study is considered reliable with a Klimisch score of 1, and data are used for classification purposes.

11.1.2 BOD_5/COD

No data available.

11.1.3 Hydrolysis

The aqueous photolysis was tested in accordance to OECD 316 (Schick, 2008). S-abscisic acid was tested in a range of environmentally relevant pHs (pH 4-9). A preliminary test was conducted at a concentration of approximately 100 ppm and incubated for up to 5 days at 50 ± 0.2°C that showed that

S-abscisic acid did not hydrolyse significantly in pH 7 or 9. No definite study was performed at pH 7 or 9. In the pH 4 buffered set, greater than 10% degradation was observed. Therefore, a definite study was conducted with the test substance in sterile 0.05 M aqueous pH 4 buffer solution for up to 32 days at 25 ± 0.1°C and 40 ± 0.13°C.

All test solutions were quantified by high performance liquid chromatography (HPLC) analysis of duplicate injections. In the definitive pH 4 set, recovery of S-abscisic acid represented averages of 97.2% and 87.1% following 32 days of incubation at 25 and 40°C, respectively, when comparing concentrations in test solutions with the concentration in the dose solution at time 0.

The degradation rate constant and half-life of S-abscisic acid in buffer solutions was determined using pseudo-first order kinetics, and were 791.6 days and 161.9 days for 25°C and 40°C, respectively.

S-abscisic acid is stable under sterile hydrolytically conditions at all tested pHs and different temperatures. The study is considered reliable and has a Klimisch score of 1, and data are used for classification purposes.

11.1.4 Other convincing scientific evidence

No data available.

11.1.4.1 Field investigations and monitoring data (if relevant for C&L)

No data available.

11.1.4.2 Inherent and enhanced ready biodegradability tests

No data available.

11.1.4.3 Water, water-sediment and soil degradation data (including simulation studies)

Surface water

An aerobic natural water simulation study was performed with unlabelled S-abscisic acid (chemical purity not reported) for 48 hours based on OECD TG 309 (Dickinson, R.A. 2011). Temperature ranged 20 to 25ºC. The behaviour of S-abscisic acid was evaluated in one surface water system (River Gipping, Sufflok), at two different concentrations (5 µg m/L and 10 µg m/L). The lowest concentration is higher than recommended in OECD Test Guidance 309. Therefore a difference of a factor 5-10, as proposed in the Guidance, is not achieved, the conclusion that the rate constant is independent of the concentration cannot be obtained from the results presented in the report. Actual concentrations were determined at 0, 6, 12, 18, 24, 30, 36, 42 and 48 hours by UPLC-MS/MS and GC-MS. At the start of the test, actual concentrations were 92%-99% and 95%-103% of nominal for 5 µg m/L and 10 µg m/L, respectively. The results of the surface water degradation study indicate that the active substance is quickly degraded in a surface water system: after a lag period of about 26 to 30 h the substance was rapidly degraded with degradation half-lives between 2.6 – 4.3 hours. The RMS determined a geometric mean of 3.3 hours for log-linear plot (best fit), after a lag-phase of 30 hours. No metabolites could be identified nor could a degradation route be established using this non-radiolabelled test substance study. It was also not possible to determine mineralization. Therefore, it cannot be excluded that part of disappearance is due to adsorption, Moreover, there were some limitations because of the concentrations used, i.e. only factor 2 spacing instead of a factor 5 to 10 as prescribed by OECD TG 309. The study is considered sufficiently reliable with a Klimisch score of 2, and data are used for classification purposes.

Degradation in soil

The route and rate of degradation of ^{14}C-S-abscisic acid (purity 97%) was studied in 4 soil types (1 sandy loam, 1 sandy clay and 2 clay loam soils) at a nominal application of 1.67 mg/kg (equivalent to a use rate of 1250 g a.s. ha^{-1}) (Jones, A., 2010). The available study was performed according to OECD

307 (2002). Soil vessels were incubated for a period of 120 days. Samples were taken after 0, 1, 2, 3, 7, 14, 59 and 120 days of incubation and were analysed by LSC and chromatography to determine the level of radioactivity. Recoveries of applied radioactivity in all four soils were in the range 87.7 – 108.9% (% AR). S-abscisic acid was quickly degraded in aerobic soil concentrations decreased to 0.8 – 2.0%AR after 120 days. Unextractable residues became larger as S-abscisic acid disappeared. DT_{50} values ranged from 0.44 to 2.2 days at 20°C. DegT50 values for these soils, calculated using the guidance given by FOCUS (2006) were similar; 0.43 to 2.1 days. The degradation of S-abscisic acid resulted in the formation of carbon dioxide and the incorporation of residues into bound fractions. Mineralization reached after 120 days of incubation 58.2, 42.4, 49.3, and 45.5% in the sandy loam, clay loam (Elmton), sandy clay and clay loam (Empingham) soils, respectively. Low levels of some intermediate products (<6.3% AR) were also detected although not identified. Based on this study, it is concluded that S-abscisic acid is quickly degraded in soil under aerobic conditions. Under the test conditions in the laboratory soil degradation study no major metabolites were detected (*i.e.* no metabolites were observed exceeding the 10 percent AR nor exceeding 5% AR at two consecutive time points). The study is considered reliable with a Klimisch score of 1, and data are used for classification purposes.

11.1.4.4 Photochemical degradation

Aqueous:

A study is available in which solutions of S-abscisic acid (purity > 97%) in sterile buffer at pH 4, 7 and 9 were irradiated, at ca 25°C, using a xenon arc light source for periods up to eight days (Kane, T., 2011). S-abscisic acid is rapidly transformed in an aqueous photo degradation test under all studied pH conditions. The DT50 values were between 1.2 and 2.5 days and the quantum yield was between 0.015 and 0.018 resulting in a theoretical half-life of 0.93 – 1.09 days in summer and 3.92 – 4.61 days in winter. Three major metabolites were formed from S-abscisic acid. The parent is rapidly isomerised to trans-trans abscisic acid (component 9A) and two major components formed during photolysis. The other two metabolites were identified as (Z,exo)-1',3',3'-trimethyl-2',5'-dioxobicyclo[4.1.0]heptan-7'-yl)but-3-enoic acid (component 9B) and (Z,endo)-1',3',3'-trimethyl-2',5'-dioxobicyclo[4.1.0]heptan-7'-yl)but-3-enoic acid (component 9C).

The study is considered reliable with a Klimisch score of 1, and data are used for classification purposes.

Terrestrial:

The photo degradation of S-abscisic acid (purity 100%) was investigated in a sandy clay loam soil with continuous exposure to artificial sunlight for periods up to nine days (216 hours) (Ponte, M., 2012). Two-mm-thick layers of soil were treated with 14C-S-abscisic acid at a nominal rate of 79 µg/g equivalent to a field rate of approximately 1250 g/ha and maintained at a soil moisture content equivalent to pF 2.0.

Total recoveries of radioactivity from irradiated and dark control samples lay in the range 91.4 – 109% of the applied radioactivity (AR).

S-abscisic acid degraded rapidly in irradiated soil with a DT50 of 2.3 OECD solar days. S-abscisic acid underwent photo-induced isomerization to trans-ABA in irradiated samples. The DT50 of trans-ABA was calculated as 2.6 OECD solar days.

S-abscisic acid degraded extensively in the dark control samples with a DT50 of 5.2 days. No isomerization was observed in the dark control samples.

The study is considered reliable with a Klimisch score of 1, and data are used for classification purposes.

11.2 Environmental transformation of metals or inorganic metals compounds

Not relevant for this dossier.

11.2.1 Summary of data/information on environmental transformation

Not relevant for this dossier.

11.3 Environmental fate and other relevant information

Volatility

S-abscisic acid has a vapour pressure of < 2.0 $x10^{-6}$ Pa at 25 °C and Henry's Law Constant was determined at <1.7$x10^{-7}$ $Pa.m^3.mol^{-1}$ (Ponte, M., 2006a). This indicates that S-abscisic acid has a low volatility.

Adsorption studies

Batch adsorption studies were according to guideline OECD 106 performed for R-abscisic acid (Corden, 2010). The obtained results can be used to describe the adsorption behaviour of S-abscisic acid.

The sorption of abscisic acid onto soil was investigated in a laboratory sorption study with five European soils, varying in pH, carbon content and other soil properties within the limits of the Guidance. A preliminary test was conducted showing no sorption to the glass tubes. A soil:solution ratio of 1:1 and an equilibrium time of 24 hours was used in the test. Two soils showed a low (<80%) total recovery of radioactivity, likely to be the result of degradation to carbon dioxide, which was not measured.
The measured Kf,oc values ranged from 2.69 mL g-1 to 77.0 mL g-1, with an arithmetic mean Kf,oc value of 27.6 mL g-1 (corresponding to a Kf,om value of 16.0 mL g-1). Individual Freundlich exponent varied significantly (0.65 -1.38), however the arithmetic mean Freundlich exponent 1/n of 1.04 derived from five soils was within the normal range (0.7-1.1). these values were considered for PECgw calculations. The adsorption of 14C-abscisic acid is pH independent.

The study is considered reliable with Klimisch of 1 and data are used for classification purposes.

11.4 Bioaccumulation

Table 46: Summary of relevant information on bioaccumulation

Method	Results	Remarks	Reference
Calculation from individual solubilities	Solubility in n-octanol: 54.8g/L; Solubility in water: 3.1g/L Pow = 54.8g/L:3.1g/L=17.7, Log Pow = 1.25		Ponte, M., 2006b

Method	Results	Remarks	Reference
HPLC estimate	(25 °C, purity: 99.7 %) Non-ionised form: Log Pow = 1.8 (pH 2.5) Ionised form: Log Pow = 0.94 (pH 6.2) (25 °C, 99.7 %)	Although the surface tension of a 1g/L solution is slightly below 60 mN/m, the study is considered valid. Key data is the log K_{ow} of 1.8 determined for the neutral molecule.	Ponte, M., 2006b

11.4.1 Estimated bioaccumulation

In the DAR reference is made to a KOWWIN (v.167) estimated log K_{ow} of 2.38. However, since the log K_{ow} was also experimentally determined (see section below), the QSAR analysis is not used for classification purposes.

11.4.2 Measured partition coefficient and bioaccumulation test data

The log P_{ow} of S-abscisic acid was estimated using the HPLC method at pH 2.5 and 6.2 yielding log P_{ow}values of 0.94 and 1.8 for the ionised and unionised form, respectively (Ponte, 2006). Considering that the surface tension of a 1g/L solution was just slightly below 60 mN/m, the HPLC study is considered valid. The value of 1.8 determined for the neutral molecule is considered key data for classification purposes. This is supported by the partition coefficient of S-abscisic acid that was calculated from the individual solubilities in water and n-octanol, and that amounted to log K_{ow} 1.25 (Ponte, 2006). The available experimentally determined log K_{ow} values are all below the trigger value of 4 and thus indicate a low potential for bioaccumulation. There are no experimentally determined BCF values. S-abscisic acid is considered to have a low bioaccumulation potential.

11.5 Acute aquatic hazard

Table 47: Summary of relevant information on acute aquatic toxicity

Method	Species	Test material*	Results	Remarks	Reference
Acute toxicity study semi-static OECD 203, OPPTS 850.1075	Rainbow trout *(Oncorhynchus mykiss)*	VBC-30054, lot no. 124-164-W9, purity 97%	96-hour LC_{50} >121 mg a.s./L	Mean measured Validity criteria according OECD guideline 203 are met and the study is considered acceptable.	STUDY IIA, 8.2.1/01
Acute toxicity study Static OECD 202, OPPTS 850.1010	*Daphnia magna*	VBC-30054, lot no. 124-164-W9, purity 97%	48-hour EC50 >116 mg a.s./L	Mean measured Validity criteria according OECD guideline 202 are met and the study is considered acceptable.	Palmer S.J. et al. (2007b); STUDY IIA, 8.3.1/01

Algae growth inhibition OECD 201 (2006)	*Pseudokirchneriella subcapitata*	VBC-30054, lot no. 183-912-W9-00, purity 98%	72-hour EbC50, ErC50 and EyC50 >95.3 mg/L 72-hour NOEbC, NOErC and NOEyC 29.3 mg/L Mean measured concentrations	The study was performed according to OECD 201 (2006) and is acceptable.	Biester A.M. (2010a); STUDY IIA, 8.4/01
Algae growth inhibition Static OECD 201 (2006)	*Navicula pelliculosa*	VBC-30054, lot no. 183-912-W9-00, purity 98%	72-hour EbC50, ErC50 and EyC50 >90.1 mg/L; 72-hour NOEbC, NOErC and NOEyC 90.1 mg/L	The study was performed according to OECD 201 (2006) and is acceptable.	Biester A.M. (2010b); STUDY IIA, 8.4/02
Duckweed growth inhibition test Semi-static; stock solution in dilution water OECD 221 (2006)	Duckweed (*Lemna gibba*)	S-abscisic acid, batch no. 183-912-W9-00, purity 98%	**7-day EbC50 (frond number): 0.024 mg/L** 7-day ErC50 (frond number): 0.20 mg/L **7-day NOEC 0.0025 mg/L (frond number)** ErC50 (dry-weight): >0.26 mg/L	The test was performed in agreement with OECD 221 and is acceptable. No results from a test with a reference substance were reported, but the growth in the control satisfied the validity criterion and the endpoints were based on measured concentrations. Mean measured concentrations **Key data**	Biester A.M. (2010c); STUDY IIA, 8.6/1

* VBC-30054 = S-abscisic acid

11.5.1 Acute (short-term) toxicity to fish

A 96-hour acute toxicity limit test in rainbow trout (*Oncorhynchus mykiss*) was performed under semi-static conditions (STUDY IIA, 8.2.1/01). Rainbow trout was exposed to S-abscisic acid (purity 97%) at a concentration of 0 or 120 mg a.s./L during 96 hours. Test solutions were prepared by direct addition of the test substance to the dilution water followed by mixing. 3 Replicates of 10 fish were performed. The measured concentrations of S-abscisic acid were found to range between 97% and 104% of nominal, with a mean measured concentration of 101% of nominal i.e. 121 mg a.s./L. The mortality in the control was found to be ≤10% and the oxygen concentration was found to be at least 60% of saturation. Therefore, validity criteria according OECD guideline 203 are met. The same conclusion is drawn for the water quality parameters. In the fish treated with 0 (control) and 120 mg a.s./L (treatment) no mortality was observed under the experimental conditions applied. Based on this study, a-abscisic acid has not shown acute toxicity to Rainbow trout and with an LC_{50} value of >121 mg a.s./l (the single limit test concentration).

11.5.2 Acute (short-term) toxicity to aquatic invertebrates

A 48-hour acute toxicity limit test in *Daphnia magna* was performed in accordance with OECD 202 under semi-static conditions (Palmer S.J. et al. (2007b), STUDY IIA, 8.3.1/01). In this test *Daphnia magna* were exposed to S-abscisic acid (purity 97%) for 48 hours to 0 or 120 mg a.s./L S-abscisic acid. Test solutions were prepared by direct addition of the test substance to the dilution water followed by sonication and mixing. Measured concentrations between 97% and 104% of nominal were found with the mean measured concentration being 97% of nominal (equal to 116 mg a.s/L). The immobilisation of the control group was below 10% and the dissolved oxygen concentration was ≥3 mg/L and as a result the validity of OECD guideline 202 are met. The water quality criteria were also found to be in accordance with guideline requirements. No immobility occurred in the treated and control replicates. For *Daphnia* an EC50 value of >116 mg a.s./L (the single limit test concentration) was determined indicating a low toxicity.

11.5.3 Acute (short-term) toxicity to algae or other aquatic plants

Two growth inhibition tests in algae are available.

In the first study, green algae (*Pseudokirchneriella subcapitata*) (3 replicates per test concentration, each containing 1 x 104 cells/mL at the start) was conducted with S-abscisic acid according to OECD 201 (2006) at nominal test concentrations of 1.0, 3.2, 10, 32 and 100 mg/L, with an untreated control tested in 6 replicates (Biester A.M. (2010a); STUDY IIA, 8.4/01). Measured concentrations were found to range between 110-113% and 71-81% of nominal at test initiation and test termination, respectively. The mean measured concentrations were 0.885, 2.95, 9.08, 29.3 and 95.3 mg/L at nominal test concentrations of 1.0, 3.2, 10, 32 and 100 mg/L, respectively. Water quality criteria for pH and temperature were in accordance with OECD 201 and the validity criteria were also met (i.e. the growth factor in the control was 176 within 72 hours, the mean coefficient of variation of the daily growth rates in the control was 16.4% (0-72 hours) and the coefficient of variation of the average specific growth rates in the replicates of the controls was 1.72%). Based on this study, the 72-h EbC50, ErC50 and EyC50 was determined to be >95.3 mg/L. The 72-h NOEbC, NOErC and NOEyC values were 29.3 mg/L.

In the second study, *Navicula pelliculosa* (3 replicates per test concentration, each containing 1 x 10^4 cells/mL at the start) was exposed to S-abscisic acid at nominal test concentrations of 1.0, 3.2, 10, 32 and 100 mg/L (Biester A.M. (2010b); STUDY IIA, 8.4/02). Measured concentrations of S-abscisic acid ranged between 100-109% of nominal at test initiation and at test termination this was 75-81%. The mean measured concentrations were 0.880, 2.92, 9.02, 28.7 and 90.1 mg/L at nominal test concentrations of 1.0, 3.2, 10, 32 and 100 mg/L, respectively. Water quality criteria were in accordance with the criteria laid down in OECD guideline 201. Moreover, the validity criteria were met as the growth factor in the control was 104 within 72 hours, the mean coefficient of variation of the daily growth rates in the control was 26.6% (0-72 hours) and the coefficient of variation of the average specific growth rates in the replicates of the controls was 1.67% (0-72 hours. The 72-h EbC50, ErC50 and EyC50 values were >90.1 mg/L and the 72-h NOEbC, NOErC and NOEyC values were 90.1 mg/L.

Two 7-day semi-static toxicity tests with S-abscisic acid (98.0% pure) on the growth of duckweed (*Lemna gibba*) were performed (Biester A.M. (2010c); STUDY IIA, 8.6/1). A second test was performed since in the first test with renewal after 3 and 5 days it was demonstrated that the concentrations of the test substance had decreased in the test solutions within three days. Therefore the second test was performed with daily renewals. Only the results of the second test are discussed below. The test was performed according to OECD 221 under semi-static conditions in triplicate. The measured concentrations ranged between 94-133% of nominal with a range of 0-91% of nominal in 1-day aged solutions. The mean measured concentrations were 0.0088, 0.025, 0.0072, 0.023, 0.085 and 0.26 mg/L at nominal concentrations of 0.001, 0.0032, 0.01, 0.032, 0.1 and 0.32 mg/L, respectively. Test concentrations were not maintained between 80% and 120% of nominal but endpoints were initially based on nominal concentrations without providing a justification. Therefore, additional calculations were made were the mean measured concentrations were used to

calculate the endpoints based on individual replicate values for frond number and frond dry weight. Both the water quality criteria and the validity criteria were in accordance with OECD 221. Phytotoxic effects occurred at 0.01-0.32 mg/L of nominal. Based on the results it was concluded that the lowest EC50(frond number) equals 0.024 mg/L and the lowest NOEC (frond number) 0.0025 mg/L (all based on mean measured concentrations); recovery occurred within 7 days after transfer to untreated medium. These data are considered reliable (Klimisch score of 1), and are used for classification purposes.

11.5.4 Acute (short-term) toxicity to other aquatic organisms

No data available.

11.6 Long-term aquatic hazard

Table 48: Summary of relevant information on chronic aquatic toxicity

Method	Species	Test material	Results	Remarks	Reference
Duckweed growth inhibition in microcosms Modified OECD 221 (2006), OPPTS 850.4400; static;	Duckweed (*Lemna gibba*)	S-abscisic acid, batch no. 185-473-W9, purity 98.2%	NOEC: <3.31 µg/L	The study is not acceptable for classification purposes.	Finnegan M. (2011); STUDY IIA, 8.6.2/01
Duckweed growth inhibition test Semi-static; stock solution in dilution water OECD 221 (2006)	Duckweed (*Lemna gibba*)	S-abscisic acid, batch no. 183-912-W9-00, purity 98%	**7-day NOEC 0.0025 mg/L (frond number)**	The test was performed in agreement with OECD 221 and is acceptable. No results from a test with a reference substance were reported, but the growth in the control satisfied the validity criterion and the endpoints were based on measured concentrations. Mean measured concentrations **Key data**	Biester A.M. (2010c); STUDY IIA, 8.6/1
Algae growth inhibition OECD 201 (2006)	*Pseudokirchneriella subcapitata*	VBC-30054, lot no. 183-912-W9-00, purity 98%	72-hour NOEbC, NOErC and NOEyC 29.3 mg/L	The study was performed according to OECD 201 (2006) and is acceptable. Mean measured concentrations	Biester A.M. (2010a); STUDY IIA, 8.4/01
Algae growth inhibition	*Navicula pelliculosa*	VBC-30054, lot no. 183-912-W9-00,	72-hour NOEbC, NOErC and NOEyC 90.1 mg/L	The study was performed according to OECD 201 (2006)	Biester A.M. (2010b); STUDY IIA, 8.4/02

Static OECD 201 (2006)		purity 98%		and is acceptable. Mean measured concentrations	

11.6.1 Chronic toxicity to fish

No chronic data is available for fish.

11.6.2 Chronic toxicity to aquatic invertebrates

No chronic data is available for aquatic invertebrates.

11.6.3 Chronic toxicity to algae or other aquatic plants

Algae

See toxicity studies on algae and other aquatic plants. The following NOE_rCs were reported 29.3 mg/L for *Pseudokirchneriella subcapitata* and 90.1 mg/L for *Navicula pelliculosa*.

Lemna gibba

A microcosm study with *Lemna gibba* is available (Finnegan M. (2011); STUDY IIA, 8.6.2/01). In a pond study, three concentrations of S-abscisic acid (purity 98.2%) were tested. The microcosm consisted of sediment, natural pond water, macrophytes and any invertebrates naturally found in the water or the sediment. Static conditions were applied for a period of 14 days. The nominal test concentrations were 4.08, 20.4 and 102 µg/L. The measured concentrations of individual replicates ranged between 81-118% of nominal in fresh solutions. For one of the replicates a value 55% of nominal was found caused by a lack of homogeneity. The initial mean measured concentrations were 3.31, 23.6 and 108 µg/L at nominal concentrations of 4.08, 20.4 and 102 µg/L, respectively. Concentrations declined to <50% of nominal within 48 hours, and to <10% of nominal within 8 days. The growth of *Lemna gibba* was found to be reduced during day 0-3 resulting in a comparable to or higher growth rate in the treated microcosm compared with the control. Reductions in frond number (10-25%) were observed at test termination and are most likely caused by the reduced growth during the first days (day 0-3). It was reported that the effects on frond number were slight and transient and effects were reversible within 14 days. Effects on biomass were observed at 23.6 µg/L (i.e. statistically significant reduction of 17-19%) which were considered to be an indirect effect of the reduced growth during day 0-3. At a concentration of 108 µg/L also a reduction of frond counts was noted (10%) with a reduced total biomass of 20%. This observation could not solely be related to the reduced growth observed at day 0-3 but are considered to be caused by reduced frond development apparent at the highest concentration from day 5 onwards (small and gibbous fronds), resulting in a reduced final biomass per frond (statistically significant). No NOEC could be determined., It was further claimed that full recovery took place within 14 days at 23.6 µg/L (initial measured), and that this could be considered as EAC. In the EFSA conclusion document on the peer review of the pesticide risk assessment of S-abscisic acid it was concluded that: "*No NOEC could be determined from that study. The study indicates a potential for recovery at 23.6 µg/L (NOAEC), reasonably within 8 weeks. However, the application regime did not account for multiple exposures and was therefore not considered suitable for the representative use*". The Dossier Submitter would like to note that this study was not conducted according to OECD TG 221, that the static test design is inadequate (substance was fully degraded after 8 days), and that if the data were to be used effect concentrations should be based on mean measured test concentrations, and not initially measured

concentrations as proposed. Considering, a reliable semi-static *Lemna gibba* study is available that reports a NOEC of 0.0025 mg/L based on mean measured test concentrations, the microcosm study will not be used for classification purposes.

11.6.4 Chronic toxicity to other aquatic organisms

No data available.

11.7 Comparison with the CLP criteria

11.7.1 Acute aquatic hazard

The criteria for Category Acute 1 in line with Table 4.1.0 (a) from the Guidance on the Application of the CLP Criteria are:

96 hr LC50 (for fish)	*≤ 1 mg/l and/or*
48 hr EC50 (for crustacea)	*≤ 1 mg/l and/or*
72 or 96 hr ErC50 (for algae or other aquatic plants)	*≤ 1 mg/l*

In the available studies performed with fish, the lowest LC_{50} value was found to be >121 mg/L and is thus higher than 1 mg/L. The acute toxicity to crustacea and algae was also above 1 mg/L. However, the EC50 for *Lemna gibba* is < 1 mg/L (E_rC50 = 0.20 mg/L; E_bC50 = 0.024 mg/L).

Based on the ErC_{50} of 0.20 mg/L for *Lemna gibba*, S-abscisic acid should be classified as Aquatic Acute 1; H 400, with an M-factor of 1.

11.7.2 Long-term aquatic hazard (including bioaccumulation potential and degradation)

S-abscisic acid is readily biodegradable based on a guideline study performed according to OECD 301F. Therefore, S-abscisic acid is considered rapidly degradable for classification purposes.

There are no bioaccumulation data for S-abscisic acid. Experimentally derived log K_{ow} values are available. The key data is the HPLC estimated log K_{ow} of 1.8 determined at pH 2.5 where the substance is neutrally charged. This value is supported by the partition coefficient of S-abscisic acid that was calculated from the individual solubilities in water and n-octanol, and that amounted to log K_{ow} 1.25. As the log K_{ow} values are below the trigger value of 4,S-abscisic acid is considered to have a low potential for bioaccumulation.

No chronic data are available for fish and aquatic invertebrates. Based on the CLH criteria a surrogate approach can be followed for classification if the substance is not rapidly degradable and/or the experimentally determined BCF ≥ 500 (or, if absent, the log Kow ≥4). S-abscisic is considered rapidly degradable and having a low bioaccumulation potential. Therefore, the surrogate approach cannot be used. Two guideline studies in algae are available in which NOEC values of > 1 mg/L were derived. For L*emna gibba* the lowest reliable 7day-NOEC was determined to be 0.0025 mg/L.

The criteria for Category Chronic 1, 2 and 3 in the CLP Guidance for rapidly degradable substances for which adequate chronic toxicity data are available are (Table 4.1.0 (b) (ii)):

<u>*Category Chronic 1:*</u>	
Chronic NOEC or ECx (for fish)	*≤0,01 mg/l and/or*
Chronic NOEC or ECx (for crustacea)	*≤0,01 mg/l and/or*
Chronic NOEC or ECx (for algae or other aquatic plants)	*≤0,01 mg/l*

Category Chronic 2:	
Chronic NOEC or ECx (for fish)	*> 0.01 to ≤0,1 mg/l and/or*
Chronic NOEC or ECx (for crustacea)	*> 0.01 to ≤0,1 mg/l and/or*
Chronic NOEC or ECx (for algae or other aquatic plants)	*> 0.01 to ≤0,1 mg/l*
Category Chronic 3:	
Chronic NOEC or ECx (for fish)	*> 0.1 to ≤1 mg/l and/or*
Chronic NOEC or ECx (for crustacea)	*> 0.1 to ≤1 mg/l and/or*
Chronic NOEC or ECx (for algae or other aquatic plants)	*> 0.1 to ≤1 mg/l.*

The *L. gibba* 7 day-NOEC of 0.0025 mg/L is considered sufficient for chronic classification, as from the acute toxicity data it is clear that *L. gibba* is by far the most sensitive species. Considering that S-abscisic acid is rapidly degradable and has a low potential for bioaccumulation, classification for chronic toxicity is warranted as Aquatic Chronic 1; H 410, with an M-factor of 1.

11.8 CONCLUSION ON CLASSIFICATION AND LABELLING FOR ENVIRONMENTAL HAZARDS

Acute (short-term) aquatic hazard: category Acute 1, M-factor: 1.

Long-term aquatic hazard: category Chronic 1, M-factor: 1.

12 EVALUATION OF ADDITIONAL HAZARDS

12.1 Hazardous to the ozone layer

No data available.

12.1.1 Short summary and overall relevance of the provided information on ozone layer hazard

Not relevant.

12.1.2 Comparison with the CLP criteria

Not relevant.

12.1.3 Conclusion on classification and labelling for hazardous to the ozone layer

Data lacking.

13 ADDITIONAL LABELLING

None.

14 REFERENCES

A reference list for the relevant studies from the DAR is included below. In addition, the following reference from public literature was used in this CLH report:

Nichols, J.W., Patrick N., Fitzsimmons and Whiteman, F. W. 2004 A physiologically based toxicokinetic model for dietary uptake of hydrophobic organic compounds by fish II. Simulation of chronic exposure scenarios, Toxicological Sciences Volume 77, Issue 2. 219-229. Non-GLP, published

Physical and chemical properties (Annex IIA 2; Annex IIIA 2)

Annex point / reference number	**Author(s)**	**Year**	**Title** **Source (where different from company)** **Company, Report No** **GLP or GEP status (where relevant)** **Published or not**
IIA 2.1.1, 2.1.3, 2.2, 2.4, 2.14	Comb, A. L.	2010a	S-abscisic acid (pure grade) Physico-chemical properties Laboratory no. ZAB0120 Valent Biosciences Corporation GLP, unpublished
IIA 2.3	Ponte, M.	2006a	Vapour Pressure of S-Abscisic Acid PTRL West, Inc. Laboratory no. 1436W Valent Biosciences Corporation GLP, unpublished
IIA 2.4	Ponte, M.	2005	Physical Properties of S-Abscisic Acid Technical Grade Active Ingredient PTRL West, Inc. Laboratory no. 1438W Valent Biosciences Corporation GLP, unpublished
IIA 2.5	Zhang, H.	2004	Certification of S-ABA Reference Standard (Lot # 030806D1) deCODE chemistry Laboratory no. REP-RC-2004-048 Valent Biosciences Corporation GLP, unpublished
IIA 2.5	Comb, A.L.	2011	S-Abscisic Acid Characterisation Huntingdon Life Sciences Ltd Report no. ZAB 0156 Valent Biosciences Corporation GLP, Unpublished
IIA 2.6, 2.8.1, 2.9.5	Ponte, M.	2006b	S-Abscisic Acid: Dissociation constant in water, water solubility and n-octanol/water partition coefficient PTRL West, Inc. Laboratory no. 1437W Valent Biosciences Corporation Report no. GLP, unpublished
IIA 2.7	Schick, M.	2008a	Solubility of S-Abscisic Acid in Organic Solvents PTRL West, Inc. Laboratory no. 1730W Valent Biosciences Corporation GLP, unpublished
IIA, 2.9.1	Schick, M.	2008b	Hydrolysis of S-abscisic acid at pH 4, 7 and 9 PTRL West, Inc. Laboratory no. 1729W Valent Biosciences Corporation GLP, unpublished
IIA, 2.9.2	Kane, T.	2011	Abscisic Acid Photo degradation in water and determination of the quantum yield Huntingdon Life Sciences Ltd Report no., ZAB0144 Valent Biosciences Corporation GLP, unpublished

Annex point / reference number	**Author(s)**	**Year**	**Title** **Source (where different from company)** **Company, Report No** **GLP or GEP status (where relevant)** **Published or not**
IIA, 2.11.1, 2.13	Comb, A. L.	2007	VBC-30054: Physicochemical properties Huntingdon Life Sciences Ltd Laboratory no. ZAB/0083 Valent Biosciences Corporation GLP, unpublished
IIA, 2.11.2	Comb, A. L.	2010b	S-abscisic acid: Relative self-ignition temperature for solids Huntingdon Life Sciences Ltd Laboratory no. ZAB0143 Valent Biosciences Corporation GLP, unpublished
IIA, 2.15, 2.17	Ponte, M. and Schick, M.	2007	Stability of S-Abscisic Acid active ingredient to normal and elevated temperatures, metals, and metal ions, and oxidizing reducing properties of S-abscisic acid active ingredient PTRL West, Inc. Laboratory no. 1618W, 1618W-1 Valent Biosciences Corporation GLP, unpublished
IIA, 2.15	Comb, A.L.	2011	S-abscisic acid Oxidising properties Huntingdon Life Sciences Ltd Report no. ZAB 0158 Valent Biosciences Corporation GLP, unpublished
IIA, 2.16	Ponte, M.	2006c	Physical properties: pH determination of S-abscisic acid technical grade active ingredient in water PTRL West, Inc. Laboratory no. 1558W Valent Biosciences Corporation GLP, unpublished

Toxicology and metabolism (Annex IIA 5; Annex IIIA 7)

OECD data point number / reference number	**Author(s)**	**Year**	**Title** **Source (where different from company)** **Company, Report no.** **GLP or GEP status (where relevant),** **Published or not**
IIA, 5.1	Noddle, R.C. and Robinson, D.R.	1969	Biosynthesis of abscisic acid: incorporation of radioactivity from [2-^{14}C]mevalonic acid by intact fruit Biochem. J. (1969), 112, 547-548 Not GLP, published
	Rudnicki, R. and Pieniazek, J.	1971	Free and bound abscisic acid in developing and ripe strawberries Bulletin de l'Academie Polonaise des Sciences Serie des Sciences Biologiques (1971), 19(6), 421-3 Not GLP, published
	King, R.W.	1979	Abscisic acid synthesis and metabolism in wheat ears Aust. J. Plant Physiol., 1979, 6, 99-108 Not GLP, published
	Gobas, F. A. P. C, Muir, D. C. G., and Mackay, D.	1988	Dynamics of dietary bioaccumulation and fecal elimination of hydrophobic organic chemicals in fish. Chemosphere 17**,** 943–*962* Non-GLP, published

OECD data point number / reference number	Author(s)	Year	Title Source (where different from company) Company, Report no. GLP or GEP status (where relevant), Published or not
	Nichols, J.W., Patrick N., Fitzsimmons and Whiteman, F. W.	2004	A physiologically based toxicokinetic model for dietary uptake of hydrophobic organic compounds by fish II. Simulation of chronic exposure scenarios, Toxicological Sciences Volume 77, Issue 2. 219-229 Non-GLP, published
	Chan, T. Y.	1996	Medicated oil and severe salicylate poisoning: quantifying the risk based on methyl salicylate content and bottle size. Vet Hum Toxicol 38:133-4 Non-GLP, published
	Kurosaki, Y., Toshihito, T., Hidekatsu, N., Taiji, N. and Toshikiro, K.	1991	Regional variation in oral mucosal drug absorption: Permeability and degree of keratinization in hamster oral cavity, Pharmaceutical Research Volume 8, Number 10, 1297-1301 Non-GLP, published
	Dalvi S.S., Gupta K. C, Pohujani S. M., Vaidya A. B and Satoskar R. S.	1985	Bioavailability of aspirin after oral and rectal administration in volunteers and patients with fever. J Postgrad Med 1985; 31:192. Non-GLP, published
	Unknown	1972a	US FDA (1972a) GRAS (Generally Recognized As Safe) food ingredients: benzoic acid and sodium benzoate. Washington, DC, US Food and Drug Administration.
	Unknown	1973	US FDA (1973) Evaluation of the health aspects of benzoic acid and sodium benzoate as food ingredients. Bethesda, MD, US Food and Drug Administration, Life Sciences Research Office (PB-223 837).
	Kubota, K., Horai, Y., Kuhida, K. and Ishizaki, T.	1988	Determination of Benzoic acid and hippuric acid in human plasma and urine by high-performance liquid chromatography, Journ of Chrom, 425, 67 - 75
	Kubota, K. and Ishizaki, T.	1991	Dose-dependent pharmacokinetics of benzoic acid following oral administration of sodium benzoate to humans, Eur J Clin Pharmacol, 41: 363 - 368 Non-GLP, published
	Knopp, D. and Schiller, F.	1992	Oral and dermal application of 2,4-dichlorophenoxyacetic acid sodium and dimethylamine salts to male rats: Investigations on absorption and excretion as well as induction of hepatic mixed-function oxidase activities, *Arch Toxicol*. 66:170-174 Non-GLP, published
	Sauerhoff M. W., Braun W. H., Blau G. E., Gehring P. J.	1977	The fate of 2,4-dichlorophenoxyacetic acid (2,4-D) following oral administration to man, *Toxicology* 1977, 8**:**3-11 Non-GLP, published
IIA, 5.2.1	XXXX	2005a	VBC-30054 (ABA): Acute oral toxicity up and down procedure in rats XXXX Laboratory no. 16974 Valent BioSciences Corporation GLP, unpublished

OECD data point number / reference number	**Author(s)**	**Year**	**Title** **Source (where different from company)** **Company, Report no.** **GLP or GEP status (where relevant),** **Published or not**
IIA, 5.2.2	XXXX	2005b	VBC-30054 (ABA): Acute dermal toxicity study in rats – Limit test XXXX Laboratory no. 16975 Valent BioSciences Corporation GLP, unpublished
IIA, 5.2.3.1	XXXX	2005c	VBC-30054 (ABA): Acute inhalation toxicity study in rats – Limit test XXXX Laboratory no. 17515 Valent BioSciences Corporation GLP, unpublished
IIA, 5.2.3.2	XXXX	2009	S-abscisic acid technical grade active ingredient: Acute inhalation toxicity study in rats XXXX Laboratory no. 27098 Valent BioSciences Corporation GLP, unpublished
IIA, 5.2.4	XXXX	2005d	VBC-30054 (ABA): Primary skin irritation study in rabbits XXXX Laboratory no. 16977 Valent BioSciences Corporation GLP, unpublished
IIA, 5.2.5	XXXX	2005e	VBC-30054 (ABA): Primary eye irritation study in rabbits XXXX Laboratory no. 16976 Valent BioSciences Corporation GLP, unpublished
IIA, 5.2.6	XXXX	2005f	VBC-30054 (ABA): Dermal sensitization study in guinea pigs (Magnusson-Kligman Method) XXXX Laboratory no. 16978 Valent BioSciences Corporation GLP, unpublished
IIA, 5.3.1	XXXX	2008a	VBC-30054: 4 week toxicity study in rats with administration by the diet XXXX Laboratory no. 27720 Valent BioSciences Corporation GLP, unpublished

OECD data point number / reference number	**Author(s)**	**Year**	**Title** **Source (where different from company)** **Company, Report no.** **GLP or GEP status (where relevant),** **Published or not**
IIA, 5.3.2	XXXX	2008b	VBC-30054: 13 week toxicity study in rats with administration by the diet XXXX Laboratory no. 28084 Valent BioSciences Corporation GLP, unpublished
IIA, 5.3.7	XXXX	2008a	VBC-30054: Three week dose range finding study in rats with dermal administration XXXX Laboratory no. 27971 Valent BioSciences Corporation GLP, unpublished
IIA, 5.4.1	Mecchi, M. S.	2006	*Salmonella-Escherichia coli*/mammalian-microsome reverse mutation assay with a confirmatory assay with ABA Technical – VBC 30054 Covance Laboratories Inc., Laboratory no. 7194-101 Valent BioSciences Corporation GLP, unpublished
IIA, 5.4.2	Murli, H.	2006	Chromosomal aberrations in Chinese hamster ovary (CHO) cells Covance Laboratories Inc., Laboratory no. 7194-102 Valent BioSciences Corporation GLP, unpublished
IIA, 5.4.3	Stankowski, L. F.	2010	L5178Y $TK^{+/-}$ mouse lymphoma forward mutation study with confirmatory assay Covance Laboratories Inc., Laboratory no. 8226728 Valent BioSciences Corporation GLP, unpublished
IIA, 5.4.4	XXXX	2006	*In vivo* mouse bone marrow micronucleus assay XXXX Laboratory no. 7194-103 Valent BioSciences Corporation GLP, unpublished
IIA, 5.6.1	XXXX	2011	A dietary two-generation reproductive toxicity study of S-abscisic acid in rats XXXX Laboratory no. WIL-505006 Valent BioSciences Corporation GLP, unpublished
IIA, 5.6.9	Saito, K.	2008	Reporter gene assay for abscisic acid (ABA) using human estrogen and androgen receptors Environmental Health Sciences Laboratory Laboratory no. VBC-SCC ABA 12-14-07 Valent BioSciences Corporation GLP, unpublished

OECD data point number / reference number	**Author(s)**	**Year**	**Title** **Source (where different from company)** **Company, Report no.** **GLP or GEP status (where relevant),** **Published or not**
IIA, 5.6.10	XXXX	2008	A prenatal developmental toxicity study in S-abscisic acid in rats XXXX Laboratory no. WIL-505004 Valent BioSciences Corporation GLP, unpublished

Environmental fate and behaviour (Annex IIA 7; Annex IIIA 9)

Annex point/ reference number	Author(s)	Year	Title, Source, Company, Report No, GLP or GEP status (where relevant), Published or not
II A 7.1.1 II A 7.2.1	Jones, A.	2010	Abscisic acid: Transformation in aerobic soil Huntingdon Life Sciences Laboratory no. ZAB0119 Valent BioSciences Corporation GLP, unpublished
II A 7.1.1 II A 7.2.1	Hartung, W.	1996	Abscisic acid in soils: What is its function and which factors and mechanisms influence its concentration?, Plant and Soil 184, p 105 – 110 Not GLP, published
II A 7.1.3/002	Ponte, M.	2012	Photo degradation of [^{14}C]S-Abscisic acid on soil by artificial light PTRL West (a division of EAG Inc.) Laboratory no. 2272W-1 Valent BioSciences Corporation GLP, unpublished
II A 7.4.1	Corden, M. T.	2010	Abscisic acid: Adsorption / desorption in five soils Huntingdon Life Sciences Laboratory no. ZAB0111 Valent BioSciences Corporation GLP, unpublished
II A 7.6	Kane, T.J.	2011	Abscisic Acid Photo degradation in Water and Determination of the Quantum Yield Huntingdon Life Sciences Laboratory no. ZAB0144 Valent BioSciences Corporation GLP, unpublished
II A 7.7	Dickinson, R.A.	2010	Abscisic acid: Assessment of ready biodegradability by respirometry Huntingdon Life Sciences Laboratory no. ZAB0131 Valent BioSciences Corporation GLP, unpublished
II A 7.8.3	Dickinson, R.A.	2011	S-abscisic acid: Aerobic mineralisation in surface water Huntingdon Life Sciences Laboratory no. ZAB0142 Valent BioSciences Corporation GLP, unpublished

Ecotoxicology (Annex IIA 8; Annex IIIA 10)

OECD point	Author	Year	Title Source / Company Report No GLP or GEP status (where relevant), Published or not
IIA, 8.1.1.1	XXXX	2007	Abscisic acid (ABA), (VBC-30054):: An acute oral toxicity study with the Northern Bobwhite XXXX GLP, unpublished
IIA, 8.1.1.3 & IIA, 8.2.1.2 & IIA, 8.7.2	Zhu, Z, Shan, Z., and Cai D.	2000	Environmental safety assessment of natural Abscisic acid Nanjing Institute of Environmental Sciences Laboratory no. Not stated Valent BioSciences Corporation Non-GLP, unpublished
IIA, 8.2.1.1	XXXX	2007a	Abscisic acid (ABA), (VBC-30054):: A 96-hour static-renewal acute toxicity test with the rainbow trout (Oncorhynchus mykiss) XXXX GLP, unpublished
IIA, 8.3.1.1	Palmer, S. J., Kendall T. Z., and Krueger, H. O.	2007b	Abscisic acid (ABA), (VBC-30054):: A 48-hour static-renewal acute toxicity test with Cladoceran (Daphnia magna) Wildlife International Ltd Laboratory no. 529A-103 Valent BioSciences Corporation GLP, unpublished
IIA, 8.4.1	Biester, M. A.	2010a	S-abscisic acid: Growth inhibition test with Pseudokirchneriella subcapitata (syn. Selenastrum capricornutum) under static conditions Springborn Smithers Laboratories (Europe) Laboratory no. 1042.022.430 Valent BioSciences Corporation GLP, unpublished
IIA, 8.4.2	Biester, M. A.	2010b	S-abscisic acid: Growth inhibition test with Navicula pelliculosa under static conditions Springborn Smithers Laboratories (Europe) Laboratory no. 1042.022.440 Valent BioSciences Corporation GLP, unpublished

OECD point	**Author**	**Year**	**Title** **Source / Company** **Report No** **GLP or GEP status (where relevant),** **Published or not**
IIA, 8.6	Biester, M. A.	2010c	S-abscisic acid: Growth inhibition test with freshwater duckweed (Lemna gibba) under semi-static conditions Smithers Viscient AG formerly Springborn Smithers Laboratories (Europe) Laboratory no. 1042.022.410 Valent BioSciences Corporation GLP, unpublished
IIA, 8.6/02	Lustig J.H. & Heiman F.D	2012	Chemical synthesis report; Preparation of photolysis products of (S)-cis-,trans-abscisic acid
IIA, 8.6/03	Dobbins L.L. et al	2012	S-ABA (With and Without Photolysis): A 7-DAY TOXICITY TEST WITH DUCKWEED (*Lemna gibba* G3) WILDLIFE INTERNATIONAL, LTD. PROJECT NUMBER: 529P-103 GLP; unpublished
IIA, 8.6.2	Finnegan, M.	2011	Assessment on the impact of S-abscisic acid on the growth of Lemna gibba in a higher tier laboratory study Huntingdon Life Sciences Ltd., Suffolk, England Laboratory no. ZAB0145 Valent BioSciences Corporation GLP, unpublished

15 ANNEXES

The study summaries from the DAR of S-abscisic acid have been included in Annex I.