Annex I to the 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: **-**

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Note on confidential information

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CONTENTS

1 PHYSICAL HAZARDS

1.1 Explosives

Reference: Comb, A.L., 2007

Test substance: S-abscisic acid, purity 97.0%, lot number 124-164-W9-00.

Methods (EEC method A14)

A Koenen test apparatus was used or determination of sensitivity to heat (flame), a fall hammer for determination of sensitivity of shock and a friction apparatus for determination of sensitivity to friction.

Definition

The test material is said to possess explosive properties, if a positive result is recorded on any one or all of the tests, which is defined as follows:

Thermal sensitivity (effect of flame): an audible explosion, with the steel tube blown into three or more fragments.

Mechanical sensitivity (shock): an audible explosion or if the material ignites.

Mechanical sensitivity (friction): an audible explosion, creparation or bursting into flame.

Thermal test apparatus

Koenen apparatus made according to BAM 785-004 with propane gas supply through calibrated flow meter. The four burners consume approximately 4 litres/ minute of propane gas.

Procedure of thermal test

VBC-30054 was packed into steel tubes 75 mm x 24 mm i.d. in three equal portions, tamping each portion with 80N force. The tubes were closed with an orfice plate (6 mm or 2 mm orfice) and heated at the specified rate on the Koenen apparatus for 5 minutes or until an explosion occurred.

Three tests were conducted using 6 mm orfice plates followed by three using 2 mm orfice plates.

Thermal test results

	T1 (seconds)	T2 (seconds)	observations
6 mm orfice			
Test 1	33	300	Yellow flame
Test 2	32	300	Yellow flame
Test 3	32	300	Yellow flame
2 mm orfice			

Test 1	38	300	Yellow flame
Test 2	35	300	Yellow flame
Test 3	37	300	Yellow flame

Where T1 = time from start of test to flame from nozzle

T2 = time from start of test to explosion, or end of test (300 seconds)

In each case, the tubes were recovered intact.

Mechanical sensitivity (shock) test apparatus

Impact hammer apparatus made according to BAM 782-0005 with 10 kg weight and specified sample dies.

Procedure of shock test

The dry substance was sieved (<500µm). A sample (40 mm^3) of sieved VBC-30054 was put in the dies assembly and placed on the anvil in the drop hammer apparatus. A 10 kg weight was released from a height of 0.4m. The test was performed six times using a different sample and die assembly on each occasion.

Shock test results

replicate	1	2	3	4	5	6
result	N	N	N	N	N	N

Where N = no evidence of ignition or explosion.

Mechanical sensitivity (friction) test apparatus

Friction apparatus made according to BAM 781-0000 and consisting of a fixed porcelain peg and a porcelain plate held in a carriage. The peg is loaded to a known force by attaching weights.

Procedure to the friction test

The dry test substance was sieved (<500 µm). A sample (10 mm^3) was placed on the porcelain plate, and the porcelain peg drawn once to and fro across with a loading of 360N. The test was performed six times, using a fresh sample on each occasion.

Friction test results

Replicate	1	2	3	4	5	6
Result	D	D	D	D	D	D

Where D = no sign of ignition or explosion but slight decomposition indicated by dark mark on porcelain plate.

Conclusion

VCB-30054 was not explosive.

1.2 Flammable gases (including chemically unstable gases)

Not relevant.

1.3 Oxidising gases

Not relevant.

1.4 Gases under pressure

Not relevant.

1.5 Flammable liquid

Not relevant.

1.6 Flammable solids

Reference: Comb, A.L., 2007

Test substance: S-abscisic acid, purity 97.0%, lot number 124-164-W9-00.

Method (EEC method A10)

The flammability of the test substance was determined using a test mould and an ignition source.

Definition and units

A substance is considered to be highly flammable when the time taken for a pile of test substance to burn a distance of 100 mm, after having burned a distance of 80 mm, is less than 45 seconds under the test conditions.

Procedure

The metal mould of triangular cross-section of 20 mm base and 10 mm height and 250 mm in length, was loosely filled with test substance and dropped three times from a height of 2 cm onto a solid surface. The mould was removed from its holder and excess substance scraped off with a straight edge. A ceramic tile was placed on the mould, the apparatus inverted, and the mould removed. The tile was placed behind a screen, across the line of draught in a fume cupboard.

A gas burner was used to apply a hot flame (minimum diameter 5 mm) to one end of the pile.

Results

VCB-30054 melted and burned with a yellow flame. The flame extinguished within 2 seconds of removing the heat source and did not propagate along the test pile.

Conclusion

VCB-30054 was not highly flammable.

1.7 Self-reactive substances

No data available.

1.8 Pyrophoric liquids

Not relevant.

1.9 Pyrophoric solid

No data available.

1.10 Self-heating substances

Reference: Comb, A.L., 2007

Test substance: S-abscisic acid, purity 97.0%, lot number 124-164-W9-00.

Method (EEC method A16)

Definition and units

If the heat developed wither by reaction of a substance with oxygen, or by exothermic decomposition, is not lost rapidly enough to the surroundings, self-heating, leading to self-ignition, occurs. Self-ignition therefore occurs when the rate of heat production exceeds the rate of heat loss.

The self-ignition temperature is defined as the minimum temperature, expressed in degrees Celsius (°C), at which a certain quantity of substance will ignite under defined conditions. In accordance with the directive, the self-ignition temperature in this test will be taken as the oven temperature at which the sample temperature reaches 400°C by self-heating.

Apparatus

Carbolite furnace with natural air circulation and explosion relief. Standard K-type thermocouples for temperature measurement.

Open toppes 20 mm cube made from stainless steel wire mesh with 0.045 mm openings. PICO ADC16 data logger with PICOLOG data logging software.

Calibration: the furnace controller display is regularly calibrated against a standard thermometer. Instrument readings collected by the software are scaled from data points manually entered.

Procedure

The mesh cube was filled with test substance by tapping gently and adding more until the cube was full. The cube was then suspended in the centre of the furnace at room temperature and a thermocouple placed in the centre of the cube. Another thermocouple was placed midway between the furnace wall and the cube in order to monitor the furnace temperature.

The temperature of the furnace and sample were then continuously recorded while the temperature of the furnace was increased at a rate of 0.5°C/min to 400°C.

Results

There was no exothermic reaction of VBC-30054 over the temperature range studies.

1.11 Substances which in contact with water emit flammable gases

Data lacking.

1.12 Oxidising liquids

Not relevant.

1.13 Oxidising solids

Reference: Comb, A. L., 2011

Test substance: S-abscisic acid, lot number 191-373-W9, purity 97.1% w/w

Method (EEC method A17)

Definition

The test substance to be tested and a defined combustible substance, cellulose, are mixed in various ratios. Each mixture is formed into a pile and ignited at one end. The maximum burning rate determined is compared with the maximum burning rate of the reference mixture of cellulose and barium nitrate.

A substance is considered as possessing oxidising properties when the maximum burning rate of the test mixtures is higher than, or equal to, the maximum burning rate of the reference mixture.

Procedure

The test substance, cellulose and barium nitrate used as a reference were sieved (125 µm), dried at 105°C and then stored in a desiccator prior to use.

Preliminary test

The dried test substance (2 parts) was thoroughly mixed with dried cellulose 91 part) and formed into a cone shaped pile of dimensions 3.5 cm 9diameter of base) x 2.5 cm (height). The cone, on a non-combustible, non-poroes surface was ignited with a gas burner, with a minimum flame diameter of 5 mm. the vigour and

duration of the combustion were observed, recorded and compared with that of a similarly prepared cone consisting of 3 parts of barium nitrate to 2 parts of cellulose.

As the sample cone failed to burn to completion, cones consisting of S-abscisic acid (1 part) and cellulose (1 and 2 parts) were prepared and tested.

Results

The following observations were noted for each of the test substance samples:

Test substance/ cellulose ratio	Duration of combustion (seconds)	Observations
2:1	116	Burned with a yellow, sooty flame. 1 mm at base remained unburned.
1:1	105	Burned with a yellow, sooty flame. 2 mm at base remained unburned.
1:2	100	Burned with a yellow, sooty flame. 2 mm at base remained unburned.

As neither mixture of 2;1, 1:1 or 1:2 test substance/ cellulose burned vigorously or to completion, then S-abscisic acid was considered to be non-oxidising. By comparison, the reference mixture burned vigorously to completion in 25 seconds. No further testing was therefore necessary.

Conclusion

S-abscisic acid was found not to possess oxidising properties.

1.14 Organic peroxides

Not relevant.

1.15 Corrosive to metals

Not relevant.

2 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

No data available.

3 HEALTH HAZARDS

Acute toxicity

3.1 Acute toxicity - oral route

3.1.1 Animal data

Study reference: B.6.2.1, STUDY 1

Characteristics

type of study	:	Acute oral toxicity study	exposure	:	Once by gavage
year of execution	:	2005	doses	:	5000 mg/kg bw
test substance	:	VBC-30054 (Technical Grade S-abscisic acid), batch no. 124-164-W9-00, purity 95%	vehicle	:	Corn oil
route	:	Oral	GLP statement	:	Yes
species	:	Rat (Sprague-Dawley)	guideline	:	See acceptability
group size	:	3 (females)	acceptability	:	Acceptable
			LD_{50}	:	> 5000 mg/kg bw

Study design

The study was performed in accordance with OECD 425 (Up-and-Down-Procedure), with some exceptions (see acceptability). The test substance was administered as a 30% w/w suspension in oil because the substance appeared not suspendable in distilled water not in an aqueous carboxymethylcellulose solution.

Results

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.

Acceptability

The study was performed in accordance with OECD 425, with some exceptions. Three (female) animals per dose level were used instead of five/sex. Furthermore, according to OECD 425, the maximum volume administered to rodents should not exceed 1 mL/100 g bw for non-aqueous vehicles. Table 1 of the study report indicated that the animals had initial body weights of 187, 218 and 208 gram and were administered 3.2, 3.7 and 3.5 ml, respectively, resulting in an administered volume of 1.7 mL/100 g bw. Since no signs of toxicity were observed in this study, it is considered acceptable to conclude that the results found in this study indicate that the LD50 is > 5000 mg/kg bw in rat.

Conclusions

The acute oral LD_{50} of S-abscisic acid (VBC-30054) was found to be greater than 5000 mg/kg bw in female rats.

3.1.2 Human data

No data available.

3.1.3 Other data

No data available.

3.2 Acute toxicity - dermal route

3.2.1 Animal data

Study reference: B.6.2.1, STUDY 1

Characteristics

type of study	:	Acute dermal toxicity study	exposure	:	24 hours on a skin area of about 39 cm^2 (semi-occlusive)
year of execution	:		doses	:	5000 mg/kg bw
test substance	:	VBC-30054 (Technical Grade S-abscisic acid), batch no. 124-164-W9-00, purity 95%	vehicle	:	Distilled water
route	:	Dermal	GLP statement	:	Yes
species	:	Rat (Sprague-Dawley)	guideline	:	In accordance with OECD 402 (limit test)
group size	:	5/sex	acceptability	:	Acceptable
			LD_{50}	:	> 5000 mg/kg bw

Study design

The study was performed in accordance with OECD 402.

Results

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.

Acceptability

The study is considered acceptable.

Conclusions

The acute dermal LD_{50} of S-abscisic acid (VBC-30054) was found to be greater than 5000 mg/kg bw in male and female rats.

3.2.2 Human data

No data available.

3.2.3 Other data

No data available.

3.3 Acute toxicity - inhalation route

3.3.1 Animal data

3.3.1.1 STUDY 1 – Acute inhalation toxicity

Study reference: B.6.2.1, STUDY 1

Characteristics

type of study	:	Acute inhalation toxicity study	exposure	:	4 hours (nose only)
year of execution	:	2005	concentration	:	8.78 mg/L (nominal concentration); 2.06 mg/L (actual concentration); MMAD 3.8 µm with GSD 2.19.
test substance	:	VBC-30054 (Technical Grade S-abscisic acid), batch no. 124-164-W9-00, purity 95%	vehicle	:	None
route	:	inhalation	GLP statement	:	Yes
species	:	Rat (Sprague-Dawley)	guideline	:	In accordance with OECD 403 (limit test)
group size	:	5/sex/dose	acceptability	:	Acceptable
			LC_{50}	:	> 2.06 mg/L

Study design

The study was performed in accordance with OECD 403 (limit test). Maximum attainable concentration in this study was 2.06 mg/L.

Results

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.

Acceptability

The study is considered acceptable.

Conclusions

The acute inhalation LC_{50} of S-abscisic acid (VBC-30054) in male and female rats was found to be greater than 2.06 mg/L (maximum attainable concentration in this study).

3.3.1.2 STUDY 2 – Acute inhalation study

Study reference: B.6.2.1, STUDY 2

Characteristics

type of study	:	Acute inhalation toxicity study	exposure	:	4 hours (nose only)
year of execution	:	2009	concentration	:	27.41 mg/L (nominal concentration); 5.13 mg/L (actual concentration); MMAD 3.75 µm with GSD 2.21.
test substance	:	S-abscisic acid Technical grade active ingredient, batch no. 147-690-W9, purity 96%	vehicle	:	None
route	:	inhalation	GLP statement	:	Yes
species	:	Rat (Sprague-Dawley)	guideline	:	In accordance with OECD 403 (limit test)
group size	:	5/sex/dose	acceptability	:	Acceptable
			LC_{50}	:	> 5.13 mg/L

Study design

The study was performed in accordance with OECD 403 (limit test). Maximum attainable concentration of 5.13 mg/L were obtained by using adapted dust generation methods compared to the inhalation study performed in 2005 (study 1).

Results

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.

Acceptability

The study is considered acceptable.

Conclusions

The acute inhalation LC_{50} of S-abscisic acid (VBC-30054) in male and female rats was found to be greater than 5.13 mg/L (maximum attainable concentration in this study).

3.3.2 Human data

No data available.

3.3.3 Other data

No data available.

3.4 Skin corrosion/irritation

3.4.1 Animal data

Study reference: B.6.2.2, STUDY 1

Characteristics

type of study	:	Skin irritation study	exposure	:	4 hours, semi-occlusive, application area 6 cm^2
year of execution	:	2005	doses	:	0.5 g
test substance	:	VBC-30054 (Technical Grade S-abscisic acid), batch no. 124-164-W9-00, purity 95%	vehicle	:	Water
route	:	Dermal	GLP statement	:	Yes
species	:	Rabbit, New Zealand White	guideline	:	In accordance with OECD 404
group size	:	1 male, 2 females	acceptability	:	Acceptable
			Effect	:	Not irritating to skin

Study design

The study was performed in accordance with OECD 404.

Results

The results are summarised in table 3.4.1-1.

Table 3.4.1-1 Individual and mean skin irritation scores according to the Draize scheme

Time	Erythema			Oedema		
Animal number #	13973F	13974M	13975F	13973F	13974M	13975F
after 30-60 minutes	0	0	0	0	0	0
after 24 hours	0	0	0	0	0	0
after 48 hours	0	0	0	0	0	0
after 72 hours	0	0	0	0	0	0
mean score 24-72 h	0.0			0.0		

Acceptability

The study is considered acceptable.

Conclusions

S-abscisic acid (VBC-30054) was found to be non-irritating to rabbit skin.

3.4.2 Human data

No data available.

3.4.3 Other data

No data available.

3.5 Serious eye damage/eye irritation

3.5.1 Animal data

Study reference: B.6.2.2, STUDY 2

Characteristics

type of study	:	Eye irritation study	exposure	:	Single instillation in conjunctival sac
year of execution	:	2005	doses	:	0.1 ml (0.04 grams)
test substance	:	VBC-30054 (Technical Grade S-abscisic acid), batch no. 124-164-W9-00, purity 95%	vehicle	:	None
route	:	Ocular	GLP statement	:	Yes
species	:	Rabbit, New Zealand White	guideline	:	In accordance with OECD 405
group size	:	3 males	acceptability	:	Acceptable
			Effect	:	not irritating to eyes

Study design

The study was performed in accordance with OECD 405. It was not reported whether or not the treated eyes were washed after instillation of the test substance.

Results

The results are summarised in table 3.5.1-1.

Table 3.5.1-1 Individual scores for ocular irritation according to the Draize scheme

Scores observed after	1 hour	24 hours	48 hours	72 hours	mean score per animal(24-72 hours)
Cornea/opacity	0, 0, 0	1, 0, 0	1, 0, 0	0, 0, 0	0.7; 0.0; 0.0
Iris	1, 1, 1	0, 0, 0	0, 0, 0	0, 0, 0	0.0; 0.0; 0.0
Conjunctiva redness	2, 2, 2	1, 1, 1	0, 0, 0	0, 0, 0	0.3; 0.3; 0.3
Conjunctiva chemosis	1, 1, 1	0, 0, 0	0, 0, 0	0, 0, 0	0.0; 0.0; 0.0
Conjunctiva discharge	1, 1, 1	0, 0, 0	0, 0, 0	0, 0, 0	0.0; 0.0; 0.0

Acceptability

The study is considered acceptable.

Conclusions

S-abscisic acid (VBC-30054) was found to be not eye irritating in rabbits.

3.5.2 Human data

No data available.

3.5.3 Other data

No data available.

3.6 Respiratory sensitisation

3.6.1 Animal data

No data available.

3.6.2 Human data

No data available.

3.6.3 Other data

No data available.

3.7 Skin sensitisation

3.7.1 Animal data

Study reference: B.6.2.2, STUDY 3

Characteristics

type of study	:	Skin sensitisation study (Maximisation test)	exposure	:	intradermal and topical induction, topical challenge (occlusive, 48h)
year of execution	:	2005	doses	:	1% w/w intradermal induction; 55% w/w topical induction and 55% w/w challenge
test substance	:	VBC-30054 (Technical Grade S-abscisic acid), batch no. 124-164-W9-00, purity 95%	vehicle	:	Mineral oil or 50% v/v Complete Freund's Adjuvant in distilled water
route	:	Dermal	GLP statement	:	Yes
species	:	Guinea pig, Hartley albino	guideline	:	In accordance with OECD 406
group size	:	10 controls, 20 test animals, males and females	acceptability	:	Acceptable
			Effect	:	Not sensitising to skin

Study design

The study was performed in accordance with OECD 406 (1992) and conducted according to Magnusson and Kligman. Dose levels were based on a preliminary irritation test using 1%, 3% and 5% S-abscisic acid for intradermal injection in mineral oil or in 50% v/v Freund's Complete Adjuvant in water and 55% and 41% in mineral oil for topical application. For the challenge, 55%, 41%, 28% and 14% S-abscisic acid in mineral oil were tested. For intradermal induction, the 1% w/w mixture in mineral oil was selected. The concentration selected for topical induction producing faint irritation was a 55% w/w mixture in mineral oil. The challenge

phase was also done with the 55% w/w solution of S-abscisic acid in mineral oil. Concentrations in excess of 55% could not be tested as they were considered too dry to provide adequate skin contact.

Results

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 24 hours after challenge patch removal. Irritation persisted at one of these sites through 48 hours. Very faint to faint erythema (skin irritation score 0.5) was noted at 3 out of 10 sham control sites 24 hours after challenge patch removal. Sensitisation of this strain of animals was positively tested with alpha-hexylcinnamic aldehyde.

No mortality, symptoms of systemic toxicity or effect on body weight was noted.

Acceptability

The study is considered acceptable.

Conclusions

In this Magnusson and Kligman study, S-abscisic acid (VBC-30054) has no skin sensitising potential.

3.7.2 Human data

No data available.

3.7.3 Other data

No data available.

3.8 Germ cell mutagenicity

3.8.1 In vitro data

3.8.1.1 STUDY 1 – Ames test

Study reference: B.6.4.1, STUDY 1

Study design and results

Type of study: Ames test, plate incorporation method, with independent repeat assay

Indicator cells	Endpoint	Res. -act.	Res. +act.	Activation		Dose range
				Tissue	Inducer	
B: *S. typh.* TA 98 TA 100	 point mut. point mut.	 - -	 - -	rat liver	Arochlor 1254	33.3, 100, 333, 1000, 3.330 and 5000 µg/plate solvent: DMSO

Indicator cells	Endpoint	Res. -act.	Res. +act.	Activation		Dose range
				Tissue	Inducer	
TA 1535 TA 1537 B : *E.coli* WP2*uvr*A	point mut. point mut. point mut.	- - -	- - -			

Test substance: S-abscisic acid , lot no. 124-164-W9 , purity 96.2%,
Cytotoxicity observed at dose level: no cytotoxicity was observed.
Precipitation observed at dose level: The active substance remained in solution at all dilution prepared.
GLP statement: yes
According to OECD 471: yes
Adequate positive and negative controls were included.

Table 3.8.1.1-1 Plate incorporation assay with S-abscisic acid - Mean number of revertants

Strain	TA 98		TA 100		TA 1535		TA 1537		WP2*uvr*A	
Metabolic activation	**-S-9**	**+S-9**	**-S-9**	**+S-9**	**-S-9**	**+S-9**	**-S-9**	**+S-9**	**-S-9**	**+S-9**
Neg. control (DMSO)	10	13	69	98	7	9	5	6	17	16
S-abscisic acid										
33.3 µg/plate	11	13	72	89	9	8	4	7	11	19
100 µg/plate	6	15	62	100	8	10	6	3	13	15
333 µg/plate	11	15	72	91	12	14	3	6	9	18
1000 µg/plate	10	15	73	95	9	9	4	5	9	11
3330 µg/plate	11	16	62	88	7	12	3	7	13	12
5000 µg/plate	10	11	62	86	8	12	3	11	8	12
Pos. control	289	258	901	615	729	119	322	97	315	49

Positive control –S-9:
TA98: benzo(a)pyrene
TA100: 2-aminoanthracene
TA1535: 2-aminoanthracene
TA1537: 2-aminoanthracene
WP2*uvr*A: 2-aminoanthracene

Positive control +S-9:
TA98: 2-nitrofuorene
TA100: sodium azide
TA1535: sodium azide
TA1537: ICR-191
WP2*uvr*A: 4-nitroquinoliane-N-oxide

Table 3.8.1.1-2 Plate incorporation assay with S-abscisic acid - Mean number of revertants (independent repeat assay)

Strain	TA 98		TA 100		TA 1535		TA 1537		WP2uvrA	
Metabolic activation	**-S-9**	**+S-9**	**-S-9**	**+S-9**	**-S-9**	**+S-9**	**-S-9**	**+S-9**	**-S-9**	**+S-9**
Neg. control (DMSO)	9	22	71	99	10	10	5	10	6	10
S-abscisic acid										
33.3 µg/plate	9	15	67	90	7	12	6	8	8	12
100 µg/plate	9	19	74	101	11	8	4	5	9	8
333 µg/plate	12	16	72	94	10	9	5	9	9	7
1000 µg/plate	12	19	76	103	12	15	4	5	8	14
3330 µg/plate	9	22	71	93	8	14	5	8	8	13
5000 µg/plate	11	19	73	96	9	12	3	9	5	8
Pos. control	3288	321	941	866	635	156	374	144	548	67

Positive control –S-9:
TA98: benzo(a)pyrene
TA100: 2-aminoanthracene
TA1535: 2-aminoanthracene
TA1537: 2-aminoanthracene
WP2*uvr*A: 2-aminoanthracene

Positive control +S-9:
TA98:2-nitrofuorene
TA100: sodium azide
TA1535: sodium azide
TA1537: ICR-191
WP2*uvr*A: 4-nitroquinoliane-N-oxide

Acceptability

The study was considered acceptable.

Conclusions

S-abscisic acid showed no evidence of mutagenic activity in bacteria in either the presence or absence of metabolic activation.

3.8.1.2 STUDY 2 – Chromosome aberration test

Study reference: B.6.4.1, STUDY 2

Study design and results

Type of study: mammalian cells *in vitro*, cytogenic assay, with independent repeat assay

Indicator cells	Endpoint	Res. -act.	Res. +act.	Activation		Dose range	Reference
				Tissue	Inducer		
Chinese hamster ovary (CHO) cells	chromosome aberration	-	-	rat liver	Arochlor 1254	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,	Murli, H., 2006

Indicator cells	Endpoint	Res. -act.	Res. +act.	Activation		Dose range	Reference
				Tissue	Inducer		
						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 solvent: DMSO at 1: v/v	

Test substance: S-abscisic acid , lot no. 124-164-W9 , purity % 96.2
Cytotoxicity observed at dose level: No cytotoxicity observed at the dose levels tested.
Precipitation observed at dose level: At a dosed concentration of 4590 µg/ml, a precipitate most of which went back into solution with gentle agitation was observed with some fine-sized particles that settled down in the dilution tube, with the culture medium becoming yellow, and pH was 6.5.
At 2300 and 1150 µg/ml, no precipitate was observed, but the culture medium became yellowish red and pH was 7.0 and 7.5 respectively.
GLP statement: yes
According to OECD 473: yes
Adequate positive and negative controls were included.

Scoring of slides

200 cells were scored for each dose level. The types of aberrations scored are: gaps, simple breaks, chromatid exchange, chromosome exchange, multiple aberrations, greater than 4. Additionally polyploidy and endoreduplication was scored. One hundred cells, if possible, from each replicate culture from 4 concentrations of the test substance, the vehicle, the negative, and one dose of the positive control cultures were analyzed for the different types of chromosomal aberration. The types of aberrations scored are: gaps, simple breaks, chromatid exchange, chromosome exchange, multiple aberrations, greater than 4. At least 25 cells were analyzed from those cultures that had greater than 25% of cells with one or more aberration. Mitotic index was evaluated from the negative and vehicle control, and a range of concentrations by analyzing the number of mitotic cells in 1000 cells and the ratio expressed as percentage of mitotic cells. Percent polyploidy and endoreduplication were also analyzed by evaluating at least 100 metaphases, if available. The average number of cells with chromosome aberrations vary between 0.5 – 6% (positive controls are 54-81%).

Table 3.8.1.2-1 Mitotic index assessment with and without metabolic activation – 3 hour treatment, ~ 20-hour harvest

Treatment		Dose	% Mitotic index			
			A culture	B culture	Index	Reduction
Without metabolic activation						
Negative control	Culture medium		7.3	7.4	7.4	-
Vehicle control	DMSO	10.0 µl/ml	8.6	7.5	8.1	0
Test article		672 µg/ml	9.0	8.2	8.6	0
		960 µg/ml	7.8	7.7	7.8	4
		1370 µg/ml	7.5	7.9	7.7	5
		1960 µg/ml	7.8	6.9	7.4	9
		2800 µg/ml	6.9	7.0	7.0	14
With metabolic activation						
Negative control	Culture medium		7.9	8.0	8.0	-
Vehicle control	DMSO	10.0 µl/ml	9.3	9.1	9.2	0
Test article		672 µg/ml	8.6	7.7	8.2	11
		960 µg/ml	9.0	8.3	8.7	5
		1370 µg/ml	10.4	8.5	9.5	0
		1960 µg/ml	8.0	8.7	8.4	9

	2800 µg/ml	7.5	8.8	8.2	11

Table 3.8.1.2-2 Mitotic index assessment with and without metabolic activation – 20 hour treatment, ~ 20-hour harvest, independent repeat assay

Treatment		Dose	% Mitotic index			
			A culture	B culture	Index	Reduction
Without metabolic activation						
Negative control	Culture medium		7.3	7.4	7.4	-
Vehicle control	DMSO	10.0 µl/ml	8.6	7.5	8.1	0
Test article		672 µg/ml	9.0	8.2	8.6	0
		960 µg/ml	7.8	7.7	7.8	4
		1370 µg/ml	7.5	7.9	7.7	5
		1960 µg/ml	7.8	6.9	7.4	9
		2800 µg/ml	6.9	7.0	7.0	14
With metabolic activation						
Negative control	Culture medium		14.1	12.2	13.2	-
Vehicle control	DMSO	10.0 µl/ml	13.9	14.3	14.1	0
Test article		1050 µg/ml	13.6	14.2	13.9	1
		1400 µg/ml	11.1	12.9	12.0	15
		2100 µg/ml	9.8	13.1	11.5	18
		2800 µg/ml	9.5	8.9	9.2	35

Results

Table 3.8.1.2-3 Chromosomal aberrations without metabolic activation (3-h treatment, 20-h harvest)

Assay No.: 27994-0-437OECD Trial No.: B1 Date: 01/17/06 Lab No.: CY011206 Test Article: Abscisic Acid, Technical Powder

				# Cells Scored for Aberrations	% Mitotic Index Reduction[a]	# Cells Scored for pp and er	# of pp Cells	# of er Cells	Judgement (+/-)[b]	Numbers and Percentages of Cells Showing Structural Chromosome Aberrations: gaps	simple breaks	clste	clsre	mab	Totals[c] -g	Totals[c] +g	Judgement (+/-)[d]
Controls																	
Negative:	McCoy's 5a		A	100		100	16	0		3					0	3	
			B	100		100	17	0		1			1		1	2	
			Total	200		200				4			1		1	5	
			Average	%	–		16.5	0.0		2.0			0.5		0.5	2.5	
Vehicle:	DMSO	10.0 µL/mL	A	100		100	6	0					2		2	2	
			B	100		100	18	0		2	1	2	1		4	6	
			Total	200		200				2	1	2	3		6	8	
			Average	%	0		12.0	0.0		1.0	0.5	1.0	1.5		3.0	4.0	
Positive:	MMC	0.750 µg/mL	A	50		100	12	0		3	12	14		28	45	45	
			B	36		100	18	1			4	5		19	25	25	
			Total	86		200				3	16	19		47	70	70	
			Average	%	–		15.0	0.5	-	3.5	18.6	22.1		54.7	81.4	81.4	+
Test Article		960 µg/mL	A	100		100	18	1					2		2	2	
			B	100		100	17	0			1		1		2	2	
			Total	200		200					1		3		4	4	
			Average	%	4		17.5	0.5	-		0.5		1.5		2.0	2.0	-
		1370 µg/mL	A	100		100	14	0							0	0	
			B	100		100	16	0			1	1		1	2	2	
			Total	200		200					1	1		1	2	2	
			Average	%	5		15.0	0.0	-		0.5	0.5		0.5	1.0	1.0	-
		1960 µg/mL	A	100		100	19	0		3	1	3			4	7	
			B	100		100	19	1		2	1		1		2	4	
			Total	200		200				5	2	3	1		6	11	
			Average	%	9		19.0	0.5	-	2.5	1.0	1.5	0.5		3.0	5.5	-
		2800 µg/mL	A	100		100	23	1			1		2		3	3	
			B	100		100	20	1			1	1	3	1	6	6	
			Total	200		200					2	1	5	1	9	9	
			Average	%	14		21.5	1.0	-		1.0	0.5	2.5	0.5	4.5	4.5	-

clte: chromatid exchange clre: chromosome exchange mab: multiple aberrations, greater than 4 aberrations pp: polyploidy er: endoreduplication

[a] % Mitotic index reduction as compared to the vehicle control.

[b] Significantly greater in % polyploidy and % endoreduplication than the vehicle control, $p \leq 0.01$.

[c] -g = # or % of cells with chromosome aberrations; +g = # or % of cells with chromosome aberrations + # or % of cells with gaps.

[d] Significantly greater in -g than the vehicle control, $p \leq 0.01$. McCoy's 5a = culture medium DMSO = dimethylsulfoxide MMC = Mitomycin C

Table 3.8.1.2-4 Chromosomal aberrations with metabolic activation (3-h treatment, 20-h harvest)

Assay No.: 27994-0-437OECD Trial No.: B1 Date: 01/17/06 Lab No.: CY011206 Test Article: Abscisic Acid, Technical Powder

				# Cells Scored for Aberrations	% Mitotic Index Reduction[a]	# Cells Scored for pp and er	# of pp Cells	# of er Cells	Judgment (+/-)[b]	Numbers and Percentages of Cells Showing Structural Chromosome Aberrations: gaps	simple breaks	chte	chre	mab	Totals[c] -g	Totals[c] +g	Judgment (+/-)[d]
Controls																	
Negative:	McCoy's 5a		A	100		100	18	0							0	0	
			B	100		100	13	0		1			1		1	2	
			Total	200		200				1			1		1	2	
			Average	%	--		15.5	0.0		0.5			0.5		0.5	1.0	
Vehicle:	DMSO	10.0 µL/mL	A	100		100	11	0		2	1				1	3	
			B	100		100	8	0		1		1			1	2	
			Total	200		200				3	1	1			2	5	
			Average	%	0		9.5	0.0		1.5	0.5	0.5			1.0	2.5	
Positive:	CP	7.50 µg/mL	A	38		48	4	0		1	8	14		6	24	25	
			B	50		100	5	0		1	7	18		3	24	25	
			Total	88		148				2	15	32		9	48	50	
			Average	%	--		6.1	0.0	-	2.3	17.0	36.4		10.2	54.5	56.8	+
Test Article		960 µg/mL	A	100		100	20	0		4					0	4	
			B	100		100	17	0		3		1			1	4	
			Total	200		200				7		1			1	8	
			Average	%	5		18.5	0.0	-	3.5		0.5			0.5	4.0	-
		1370 µg/mL	A	100		100	17	1		2	1		1		2	4	
			B	100		100	13	2		5	1				1	6	
			Total	200		200				7	2		1		3	10	
			Average	%	0		15.0	1.5	-	3.5	1.0		0.5		1.5	5.0	-
		1960 µg/mL	A	100		100	19	0		6		1	1		2	8	
			B	100		100	17	0		3	1	1			2	4	
			Total	200		200				9	1	2	1		4	12	
			Average	%	9		18.0	0.0	-	4.5	0.5	1.0	0.5		2.0	6.0	-
		2800 µg/mL	A	100		100	17	0		3		1			1	4	
			B	100		100	20	0		1	1				1	2	
			Total	200		200				4	1	1			2	6	
			Average	%	11		18.5	0.0	-	2.0	0.5	0.5			1.0	3.0	-

chte: chromatid exchange chre: chromosome exchange mab: multiple aberrations, greater than 4 aberrations pp: polyploidy er: endoreduplication

[a] % Mitotic index reduction as compared to the vehicle control.

[b] Significantly greater in % polyploidy and % endoreduplication than the vehicle control, $p \leq 0.01$.

[c] -g = # or % of cells with chromosome aberrations; +g = # or % of cells with chromosome aberrations + # or % of cells with gaps.

[d] Significantly greater in -g than the vehicle control, $p \leq 0.01$. McCoy's 5a = culture medium DMSO = dimethylsulfoxide CP = Cyclophosphamide

Table 3.8.1.2-5 Chromosomal aberrations without metabolic activation (20-h treatment, 20-h harvest)

Assay No.: 27994-0-437OECD Trial No.: C1 Date: 02/23/06 Lab No.: CY030106 Test Article: Abscisic Acid, Technical Powder

				# Cells Scored for Aberrations	% Mitotic Index Reduction[a]	# Cells Scored for pp and er	# of pp Cells	# of er Cells	Judgment (+/-)[b]	Numbers and Percentages of Cells Showing Structural Chromosome Aberrations: gaps	simple breaks	chte	chre	mab	Totals[c] -g	Totals[c] +g	Judgment (+/-)[d]
Controls																	
Negative:	McCoy's 5a		A	100		100	0	0		1					0	1	
			B	100		100	1	0							0	0	
			Total	200		200				1					0	1	
			Average	%	--		0.5	0.0		0.5					0.0	0.5	
Vehicle:	DMSO	10.0 µL/mL	A	100		100	1	0		2					0	2	
			B	100		100	0	0		1					0	1	
			Total	200		200				3					0	3	
			Average	%	0		0.5	0.0		1.5					0.0	1.5	
Positive:	MMC	0.400 µg/mL	A	50		100	0	0		2	11	10		20	39	40	
			B	50		100	0	0		1	9	9		27	41	41	
			Total	100		200				3	20	19		47	80	81	
			Average	%	--		0.0	0.0	-	3.0	20.0	19.0		47.0	80.0	81.0	+
Test Article		350 µg/mL	A	100		100	0	0		4	1		1		2	5	
			B	100		100	0	0		3					0	3	
			Total	200		200				7	1		1		2	8	
			Average	%	3		0.0	0.0	-	3.5	0.5		0.5		1.0	4.0	-
		525 µg/mL	A	100		100	0	0		2			1		1	3	
			B	100		100	0	0		1					0	1	
			Total	200		200				3			1		1	4	
			Average	%	12		0.0	0.0	-	1.5			0.5		0.5	2.0	-
		700 µg/mL	A	100		100	1	0		2	2				2	4	
			B	100		100	0	0		2	1				1	3	
			Total	200		200				4	3				3	7	
			Average	%	32		0.5	0.0	-	2.0	1.5				1.5	3.5	-
		1050 µg/mL	A	100		100	0	0		1	1				1	2	
			B	100		100	1	0		3	1				1	4	
			Total	200		200				4	2				2	6	
			Average	%	57		0.5	0.0	-	2.0	1.0				1.0	3.0	-

chte: chromatid exchange chre: chromosome exchange mab: multiple aberrations, greater than 4 aberrations pp: polyploidy er: endoreduplication

[a] % Mitotic index reduction as compared to the vehicle control.

[b] Significantly greater in % polyploidy and % endoreduplication than the vehicle control, $p \leq 0.01$.

[c] -g = # or % of cells with chromosome aberrations; +g = # or % of cells with chromosome aberrations + # or % of cells with gaps.

[d] Significantly greater in -g than the vehicle control, $p \leq 0.01$. McCoy's 5a = culture medium DMSO = dimethylsulfoxide MMC = Mitomycin C

Table 3.8.1.2-6 Chromosomal aberrations with metabolic activation (20-h treatment, 20-h harvest)

Assay No.: 27994-0-437OECD Trial No.: C1 Date: 02/23/06 Lab No.: CY030106 Test Article: Abscisic Acid, Technical Powder

				# Cells Scored for Aberrations	% Mitotic Index Reduction[a]	# Cells Scored for pp and er	# of pp Cells	# of er Cells	Judgement (+/-)[b]	Numbers and Percentages of Cells Showing Structural Chromosome Aberrations: gaps	simple breaks	chte	chre	mab	Totals[c] -g	Totals[c] +g	Judgement (+/-)[d]
Controls																	
Negative:	McCoy's 5a		A	100		100	0	0			1				1	1	
			B	100		100	0	0							0	0	
			Total	200		200					1				1	1	
			Average	%	–		0.0	0.0			0.5				0.5	0.5	
Vehicle:	DMSO	10.0 µL/mL	A	100		100	0	0		4					0	4	
			B	100		100	0	0		2					0	2	
			Total	200		200				6					0	6	
			Average	%	0		0.0	0.0		3.0					0.0	3.0	
Positive:	CP	12.5 µg/mL	A	50		100	0	0		5	17	15	3	7	34	36	
			B	50		100	0	0		2	20	13	1	3	33	35	
			Total	100		200				7	37	26	4	10	67	71	
			Average	%	–		0.0	0.0	-	7.0	37.0	26.0	4.0	10.0	67.0	71.0	+
Test Article		1050 µg/mL	A	100		100	0	0		1					0	1	
			B	100		100	0	0			3		1		3	3	
			Total	200		200				1	3		1		3	4	
			Average	%	1		0.0	0.0	-	0.5	1.0		0.5		1.5	2.0	-
		1400 µg/mL	A	100		100	0	0		1					0	1	
			B	100		100	0	0		2					0	2	
			Total	200		200				3					0	3	
			Average	%	15		0.0	0.0	-	1.5					0.0	1.5	-
		2100 µg/mL	A	100		100	0	1		3	1		1		2	5	
			B	100		100	0	0		4	1		2		3	7	
			Total	200		200				7	2		3		5	12	
			Average	%	18		0.0	0.5	-	3.5	1.0		1.5		2.5	6.0	-
		2800 µg/mL	A	100		100	0	0		1		1			1	2	
			B	100		100	0	0			1				1	1	
			Total	200		200				1	1	1			2	3	
			Average	%	35		0.0	0.0	-	0.5	0.5	0.5			1.0	1.5	-

chte: chromatid exchange chre: chromosome exchange mab: multiple aberrations, greater than 4 aberrations pp: polyploidy er: endoreduplication

[a] % Mitotic index reduction as compared to the vehicle control.

[b] Significantly greater in % polyploidy and % endoreduplication than the vehicle control, $p \leq 0.01$.

[c] -g = # or % of cells with chromosome aberrations; +g = # or % of cells with chromosome aberrations + # or % of cells with gaps.

[d] Significantly greater in -g than the vehicle control, $p \leq 0.01$. McCoy's 5a = culture medium DMSO = dimethylsulfoxide CP = Cyclophosphamide

Acceptability

The study is considered acceptable.

Conclusions

S-abscisic acid did not induce structural chromosome aberrations in cultured Chinese hamster ovary cells in either the presence or absence of exogenous metabolic activation.

3.8.1.3 STUDY 3 - Gene mutation test

Study reference: B.6.4.1, STUDY 3

Study design and results

Type of study: mammalian cells *in vitro*, gene mutation test, with independent repeat assay

Indicator cells	Endpoint	Res. –act.	Res. +act.	Activation		Dose range
				Tissue	Inducer	
Mouse lymphoma cells L5178Y	gene mutations (TK)	-	-	rat liver	Arochlor 1254	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 Solvent: DMSO (1%)

Indicator cells	Endpoint	Res. –act.	Res. +act.	Activation		Dose range
				Tissue	Inducer	

Test substance: S-abscisic acid , lot no. 153-912-W9 , purity 98.3 %
Cytotoxicity observed at dose level: Cytotoxicity was observed at the highest concentration evaluated (2650 μg/ml)
Precipitation observed at dose level: freely soluble at all concentration.
GLP statement: yes
According to OECD 476: yes
Adequate positive and negative controls were included.

Initial assay: Those cultures treated at a concentration of 2650 μg/ml with and without S9 were discarded prior to selection, or excluded from evaluation of mutagenicity due to excessive cytotoxicity. Those treated with concentrations ≤125 μg/ml with S9 and ≤62.5 μg/ml without S9 were discarded because a sufficient number of higher concentrations were available.

Confirmatory assay: Those cultures treated at a concentration of 2650 μg/ml with and without S9 were discarded prior to selection, or excluded from evaluation of mutagenicity due to excessive cytotoxicity. Those treated with concentrations ≤125 μg/ml with S9 and ≤15.6 μg/ml and 1300 μg/ml without S9 were discarded because a sufficient number of higher or other concentrations were available.

Table 3.8.1.3-1 Gene mutation in mammalian cells – initial mutation assay with and without activation

Test condition	Total mutant colonies		Total viable colonies		Mutant frequency (x10^6)[a]	
4-hour exposure period, expression period 2 days.						
Metabolic activation	**-S-9**	**+S-9**	**-S-9**	**+S-9**	**-S-9**	**+S-9**
Vehicle (1% DMSO)	98	145	466	499	42.2	58.0
Vehicle (1% DMSO)	133	126	391	518	67.9	48.5
Vehicle (1% DMSO)	79	127	512	466	31.0	56.9
S-abscisic acid						
125 μg/mL	91	-	404		45.2	
250 μg/mL	81	147	334	455	48.2	64.6
500 μg/mL	74	109	451	525	32.6	41.5
750 μg/mL	101	151	422	532	47.8	56.6
1000 μg/mL	77	147	389	550	39.6	53.4
1300 μg/mL	72	166	382	532	37.9	62.4
1600 μg/mL	71	122	356	432	40.0	56.6
2000 μg/mL	92	148	326	468	56.7	63.3
2300 μg/mL	145	194	327	359	88.4	108.3
Positive control MCA						
2.0 μg/mL	363	478	189	223	384.9	428.7[b]
3.0 μg/mL	268	456	103	226	519.5	403.1[b]

[a] Mutant frequency = Total mutant colonies/total viable colonies x (2 x 10^{-4}) Decimal is moved to express the frequency in units of 10^{-6}

[b] Mutagenic exceeds minimum criterion of 137.0 x 10^{-6}

Table 3.8.1.3-2 Gene mutation in mammalian cells – repeat assay with and without activation

Test condition	Total mutant colonies		Total viable colonies		Mutant frequency ($x10^6$)[a]	
4-hour exposure period, expression period 2 days.						
Metabolic activation	**-S-9**	**+S-9**	**-S-9**	**+S-9**	**-S-9**	**+S-9**
Vehicle (1% DMSO)	50	120	415	513	24.0	46.7
Vehicle (1% DMSO)	64	114	428	619	29.9	36.8
Vehicle (1% DMSO)	97	87	387	593	50.3	29.2
S-abscisic acid						
31.3 µg/mL	46	-	398	-	23.2	-
62.5 µg/mL	41	-	336	-	24.7	-
125 µg/mL	76	-	275	-	55.2	-
250 µg/mL	56	119	251	592	44.3	40.1
500 µg/mL	79	134	287	588	55.4	45.6
750 µg/mL	79	100	286	483	55.6	41.6
1000 µg/mL	78	128	286	548	54.8	46.8
1200 µg/mL	128	-	327	-	78.3	-
1300 µg/mL	-	96	-	542	-	35.4
1400 µg/mL	95	-	338	-	56.1	-
1600 µg/mL	-	153	-	522	-	58.6
2000 µg/mL	-	101	-	513	-	39.3
2300 µg/mL	-	141	-	595	-	47.4
2500 µg/mL	-	152	-	449	-	67.5
Positive control MCA						
1.5 µg/mL	111	410	71	365	224.7	313.3
2.0 µg/mL	66	448	45	253	354.9	294.7

[a] Mutant frequency = Total mutant colonies/total viable colonies x (2 x 10^{-4}) Decimal is moved to express the frequency in units of 10^{-6}

Acceptability

The study is considered acceptable.

Conclusions

These results indicate S-abscisic acid was negative in the L5178Y $TK^{+/-}$ mouse lymphoma forward mutation assay.

3.8.2 Animal data

Study reference: B.6.4.1, STUDY 4

Study design and results

Type of study: mouse micronucleus test

Species	Endpoint	Result	Dose range	Reference
Mouse, CD-1 strain, 32.4 – 33.2 g (males) and 25.1 – 27.8 g (females) in dose range finding assay 31.1 – 34.9 g (males for micronucleus assay)	micronuclei (bone marrow)	-	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 vehicle: corn oil	Xu, Y., 2006
Test substance: S-abscisic acid, lot no. 124-164-W9 , purity 96.2% Toxicity observed at dose level: No toxicity observed at all dose level tested. GLP statement: yes According to OECD 474: yes				

Table 3.8.2-1 Micronucleus assay

Treatment	Dose	Harvest Time	% Micronucleated PCEs Mean of 200 per animal ± S.E. Males	Ratio PCE: NCE Mean ± S.E. Males
Vehicle	Corn Oil 10 mL/kg/d	24 h	0.05 ± 0.02	0.60 ± 0.14
Positive	CP 80 mg/kg	24 h	1.17 ± 0.17	0.64 ± 0.06
S-abscisic acid	500 mg /kg bw/d	24 h	0.04 ± 0.02	0.69 ± 0.06
	1000 mg /kg bw/d	24 h	0.03 ± 0.01	0.68 ± 0.08
	2000 mg /kg bw/d	24 h	0.04 ± 0.02	0.63 ± 0.04

CP = cylophospamide

PCE = Plychromatic erythrocyte

NCE = Normochromatic erythrocyte

Acceptability

The study is considered acceptable.

Conclusions

The test substance did not induce micronuclei in mouse bone marrow cells.

3.8.3 Human data

No data available.

3.8.4 Other data

No data available.

3.9 Carcinogenicity

3.9.1 Animal data

No data available.

3.9.2 Human data

No data available.

3.9.3 *In vitro* data (e.g. in vitro germ cell and somatic cell mutagenicity studies, cell transformation assays, gap junction intercellular communication tests)

No data available.

3.9.4 Other data (e.g. studies on mechanism of action)

No data available.

3.10 Reproductive toxicity

3.10.1 Animal data

3.10.1.1 STUDY 1 - Reproductive toxicity

Study reference: B.6.6.1, STUDY 1

Characteristics

type of study	:	2-generation study	exposure	:	continuously through the study period
year of execution	:	2009-2011	doses	:	0, 10000, 15000, 20000 ppm(Equivalent to: See Table 3.10.1.1-1)
test substance	:	S-abscisic acid, lot no. 283-912-W9-00, purity ≥98%, and lot no. 183-912-W9, purity ≥98%	vehicle	:	none
route	:	oral	GLP statement	:	yes
species	:	rat, Crl:CD(SD)	guideline	:	in accordance with OECD 416
group size	:	30/sex/dose	acceptability	:	acceptable
			NOAELpar	:	1360 mg/kg bw/d
			NOAELdev	:	1360 mg/kg bw/d
			reproductive effects	:	Not observed

Study design

The study was performed in accordance with OECD 416, with the deviation that paired organs were weighed together. Dietary concentrations of 0 (basal feed only), 10,000, 15,000, and 20,000 ppm were selected based on the previous prenatal development toxicity study in rats (Fleeman 2007, WIL-505004) where no adverse test substance-related effects on maternal animals and on prenatal development were noted at a dosage level of 1000 mg/kg/day.

Table3.10.1.1-1Mean calculated F_0 test substance consumption (mg/kg/day)[a]

Target dietary concentration (ppm)	Males		Females		
	Prior to mating	After mating	Prior to mating	Gestation	Lactation[b]
10,000	684	490	787	667	1613
15,000	1031	738	1159	1010	2338
20,000	1360	985	1569	1310	3108

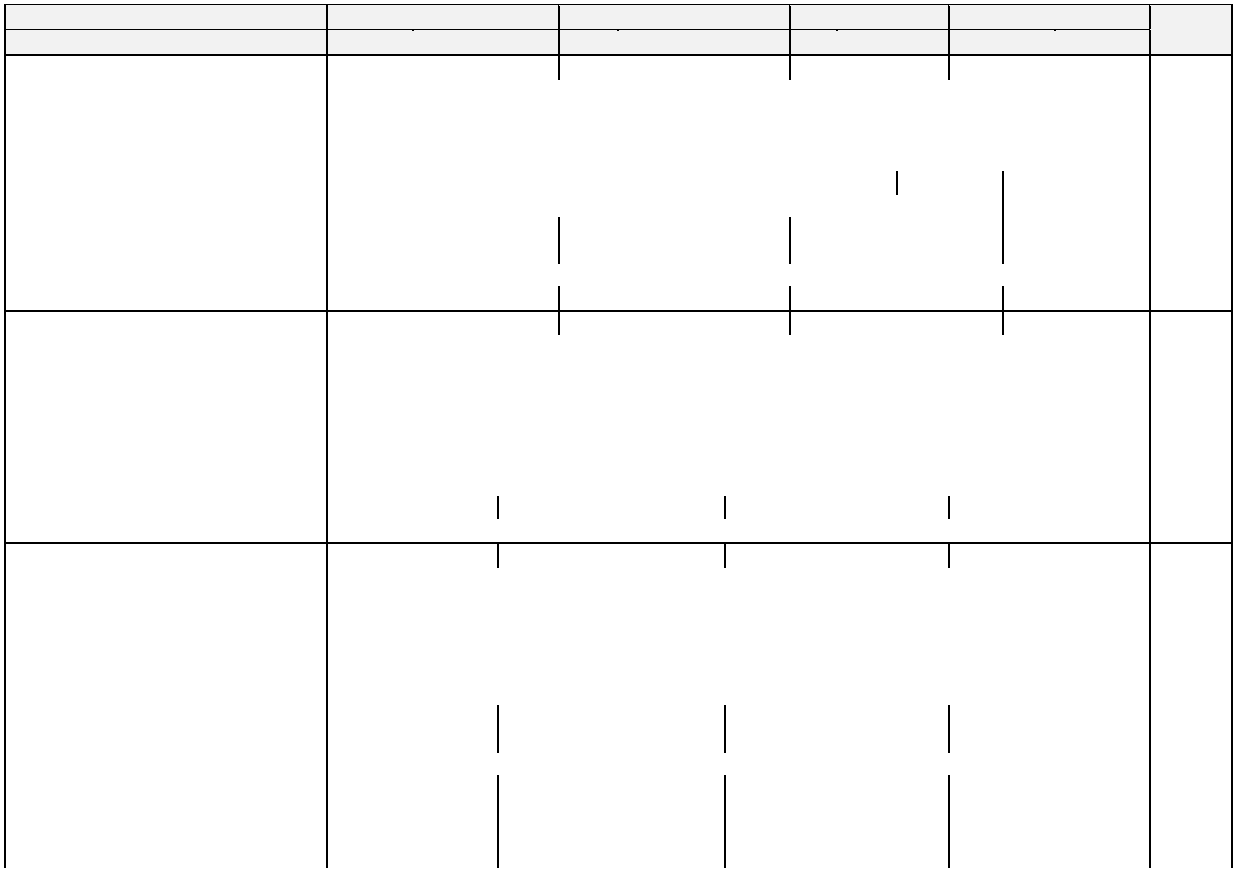
[a]: Summation of mean test substance consumption for the specified interval/Number of days or intervals assessed

[b]: Food consumption generally increases during lactation due to milk production and direct consumption of diet by offspring in the latter portion of the lactation period

Results

The results are summarised in tables 3.10.1.1-1, 3.10.1.1-2 and3.10.1.1-3.

Table 3.10.1.1-2.



Dose (ppm)	**0**		**10000**		**15000**		**20000**		**dr**
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**	
F0 animals									
Mortality	1	1	1	0	0	0	0	2	
Clinical signs	0	0	0	2	0	0	0	0	
Body weight (mean ± SD)	593 ±46.5	306± 23.5	594± 46.5	304± 22.8	585± 51.1	309± 23.1	590± 48.0	304± 24.0	
Food consumption (g/ kg /day)	46± 3.1	71± 5.7	46± 2.2	70± 8.2	47± 3.9	70± 8.2	48*± 3.0	68± 13.3	
Mating/fertility/gestation									
- mating index (%)	100	100	100	100	96.7	96.7	100	100	
- fertility index (%)	90	90	83.3	83.3	96.7	96.7	83.3	83.3	
- copulation/ conception index (%)	90	90	83.3	83.3	100	100	83.3	83.3	
- estrogous cycle length (days)		5.6		5.1		4.0*		4.2	
- pre-coital interval (days)		2.2		2.6		2.6		2.8	
Sperm evaluation									
- mean left testis sperm concentration (millions/gram)	89.6		85.9		91.6		79.3*		
- mean sperm production	14.7		14.1		15.0		13.0*		
Organ weight									
Liver									
- absolute (%)			↑5.5	↑6.3	↑4.3	↑8.9*	↑7.7	↑11.8**	
- relative to body weight (%)			↑3.0	↑4.9	↑5.8	↑7.2*	↑8.6**	↑11.5**	
Seminal vesicles/ coagulating glands/ accessory fluid									
- absolute (%)			↓4.7		↓3.9		↓13.4**		
- relative to body weight (%)			↓9.5		↓9.7		↓12.6**		
Pathology									
macroscopy	No treatment-related findings								
microscopy									
- kidney mineralisation	0/29	17/29	0/29	0/3	1/30	0/3	11/30[dc/ic]	12/28	
minimal	-	17	-	-	-	-	10	11	
mild	-	0	-	-	-	-	1	1	
F1 pups									
Litter size (number born)	13.2 ±2.8		13.0±2.46		13.2±3.32		13.4±3.38		
Survival index (live litter size)	13.0±2.90		12.9±2.48		13.1±3.42		13.2±3.42		
Sex ration (% male per litter)	46.4±14.44		48.2±12.47		43.4±15.70		52.6±16.75		
Body weight	233.6 ± 25.00	110.5± 11.68	234.9± 24.82	109.9± 12.99	239.1± 25.27	113.9± 15.91	230.2± 19.54	122.4** ± 18.18	
Organ weights									
Balanopreputial separation	45.5± 2.66		45.4± 3.09		47.0± 3.63		45.5± 3.08		
Vaginal patency		34.0± 2.31		33.8± 1.94		34.3± 1.75		35.3± 2.46	
Pathology									

macroscopy									
Kidneys (cysts)	0/25	1/26	0/25	0/25	0/28	0/28	1/24	0/24	
Spleen (accessory)	0/25		0/25		0/28		1/24		
Thymus (dark red areas)	1/25	0/26	0/25	2/25	1/28	0/28	0/24	0/24	
Urinary bladder (distended)	0/25		0/25		0/28		1/24		
F1 animals									
Mortality	0	0	0	0	2	0	0	0	
Clinical signs	0	0	0	0	0	0	0	1	
Body weight (gain)	548± 69.2	339± 19.30	530± 57.0	346± 2.1	519± 63.1	339± 0.0	510± 71.4	353± 4.1	
Mating/fertility/gestation									
- mating index (%)	93.1	93.1	90.0	90.0	96.4	96.7	100	100	
- fertility index (%)	93.1	93.1	90.0	90.0	89.3	90.0	90.0	90.0	
- copulation/ conception index (%)	100	100	100	100	92.6	93.1	90.0	90.0	
- estrogous cycle length (days)		4.6		4.4		4.4		4.3	
- pre-coital interval (days)		2.9		2.4		3.6		3.2	
- mating index (%)	93.1	93.1	90.0	90.0	96.4	96.7	100	100	
- fertility index (%)	93.1	93.1	90.0	90.0	89.3	90.0	90.0	90.0	
Sperm evaluation	No treatment-related findings								
Organ weight									
liver									
- absolute (%)								↑11.2*	
- relative to body weight (%)								↑9.5**	
Pathology									
macroscopy	No treatment-related findings								
microscopy									
- kidney mineralisation	1/30	15/29	1/30	0/1	4/28	1/3	11/30	14/30	
minimal	1	15	1	-	4	1	10	14	
mild	0	0	0	-	0	0	1	0	
F2 pups									
Litter size (number born)	11.8± 4.14		12.6± 3.41		13.3± 2.40		12.3± 2.77		
Survival index	11.8± 4.16		12.3± 3.49		13.1± 2.40		12.3± 2.77		
Sex ratio (% males per litter)	51.0± 14.8		52.3± 15.02		43.9± 11.64		54.1± 15.07		
Organ weight									
spleen									
-Absolute (%)	↓0.2334	↓0.2219	↓0.1989*	↓0.2085	↓0.191	↓0.1854*	↓0.197	↓0.192	
-Relative to body weight (%)	↓0.434	↓0.445	↓0.398	↓0.420	8**	↓0.378**	9*	3	
- Relative to brain (%)					↓0.375		↓0.385	↓0.398	
	↓15.663	↓15.434	↓13.579*	↓14.557	**	↓12.938**	**	*	
					↓12.914**		↓13.195**	↓13.154*	
Pathology									
macroscopy	No treatment-related findings								

dr dose related

dc/ic statistically significantly decreased/increased compared to the controls

d/i decreased/increased, but not statistically significantly compared to the controls

a/r absolute/relative organ weight

\+ present in one/a few animals

++	present in most/all animals
*	significantly different from the control group at 0.05 using Dunnett's test
**	significantly different from the control group at 0.01 using Dunnett's test

Table 3.10.1.1-3. Organ weight changes in the F0 generation

Parameter	Direction and magnitude of change	Exposure level (ppm)	Sex
Liver			
Absolute	↑ 7.7%		
Relative to body weight	↑ 8.6%**	20,000	M
Relative to brain weight	↑ 8.2%*		
Liver			
Absolute	↑ 8.9%*		
Relative to body weight	↑ 7.2%*	15,000	F
Relative to brain weight	↑ 8.8%*		
Liver			
Absolute	↑ 11.8%**		
Relative to body weight	↑ 11.5%**	20,000	F
Relative to brain weight	↑ 11.3%**		
Seminal Vesicles/ Coagulating Glands/ Accessory Fluid			
Absolute	↓ 13.4%**	20,000	M
Relative to body weight	↓ 12.6%**		
Relative to brain weight	↓ 12.7%**		

* = Significantly different from the control group at 0.05 using Dunnett's test.
** = Significantly different from the control group at 0.01 using Dunnett's test.
M = Male.
F = Female.

Table 3.10.1.1-4. Organ weight changes in the F2 generation

Parameter	Direction and magnitude of change	Exposure level (ppm)	Sex
Spleen			
Absolute	↓ 14.8%*		
Relative to body weight	↓ 8.3%	10,000	M
Relative to brain weight	↓ 13.3%*		
Absolute	↓ 17.8%**		
Relative to body weight	↓ 13.6%**	15,000	M
Relative to brain weight	↓ 17.6%**		
Absolute	↓ 15.2%**		
Relative to body weight	↓ 11.3%**	20,000	M
Relative to brain weight	↓ 15.8%**		
Absolute	↓ 16.4%*		
Relative to body weight	↓ 15.1%**	15,000	F
Relative to brain weight	↓ 16.2%**		
Absolute	↓ 13.3%		
Relative to body weight	↓ 10.6%*	20,000	F
Relative to brain weight	↓ 14.8%*		

* = Significantly different from the control group at 0.05.
** = Significantly different from the control group at 0.01.
M = Male.
F = Female.

Table 3.10.1.1-5: Historical control op pup spleen weight (PND21)

	Mean	S.D.	Range	N
Males	0.2197	0.04442	0.1471-0.3641	42
Females	0.2154	0.04131	0.1401-0.3099	42

Species: Rat, Strain Crl:CD(SD), age 21 days, Number of Studies/Number Data Sets: 165/223, Range of study dates: 10/00 – 10/09.
N = Count of study means from control groups.

In the F0-generation, no treatment-related effects were observed on mortality, clinical signs, body weight (gain), food consumption, reproductive performance, gestation length and parturition and macroscopic examinations.
A significantly lower mean testis sperm concentration was observed in the high-dose group (79.3 million/g (88% of controls)) and a related lower mean sperm production (13 million/g per day (88% of controls)). However, these values were similar to means in the WIL reproductive historical control data (79.8 million/g and 13.1 million/g/day, respectively) and the significance achieved in this group was likely due to control group values which were near the maximum mean values in the WIL reproductive historical control data. Furthermore, this phenomenon was not observed in the F1. Therefore, this effect was considered not treatment-related.
Absolute and relative liver weights were significantly increased in females in the mid- and high-dose groups and relative liver weights were significantly increased in males in the high-dose group. Coagulating gland weight was significantly decreased in males in the high-dose group. These changes in organ weights were considered related to treatment with the test substance. However, since these changes were not accompanied by histophatological changes, they were considered to be non-adverse. Renal mineralisation in was characterized by the presence of basophilic aggregates within renal tubules at the junction of the inner and outer stripes of the outer renal medulla. Although the renal mineralization was considered to be test substance-related, it was considered to be non-adverse based on the limited severity of the lesion and its similarity in character and severity to spontaneous background renal mineralization in female rats for which no whole-kidney functional alterations are observed (Al-Modhefer, 1986). Additionally renal mineralisation showed no substance related effect in females and mineralisation was not found in any other toxicity test.
In the F1-pups, no treatment-related effects were observed on litter size, survival index, sex ration, body weight (gain), organ weights, balanopreputial separation, vaginal patency and macroscopic pathology. In the F1 –animals, no treatment-related effects were observed on mortality, clinical signs, body weight (gain), food consumption, reproductive performance, gestation length and parturition, sperm evaluation and macroscopic examinations. An absolute and relative increase in liver weight was observed in female in the high-dose group (111% and 109% of control, respectively). This change was considered treatment-related. Based on the absence of accompanying histopathological changes, this increase in liver weight was considered to be non-adverse. Renal mineralisation was characterised by the presence of basophilic aggregates within renal tubules often at the junction of the inner and outer stripes of the outer renal medulla. Although the renal mineralization was considered to be test substance-related, it was considered to be non-adverse based on the limited severity of the lesion and its similarity in character and severity to spontaneous background renal mineralization in female rats for which no whole-kidney functional alterations are observed (Al-Modhefer, 1986). Additionally renal mineralisation showed no substance related effect in females and mineralisation was not found in any other toxicity test.

In the F2-pups, no treatment-related effects were observed on litter size, survival index, sex ration, body weight (gain) and macroscopic pathology. Lower mean absolute and relative (to body and brain weight) spleen weights were noted for F2 males and females in all dose groups. No dose-related pattern was seen. Furthermore, the absolute and relative spleen weights were within the WIL reproductive historical control data range (0.1471 g – 0.3641 g, males; 0.1401 g – 0.3099 g, females). Therefore, the differences were considered to be spurious and unrelated to maternal test substance exposure.

Acceptability

The study is considered acceptable.

Conclusions

Based on the absence of effects on F0 and F1 reproductive performance and on the F1 and F2 litters and offspring, an exposure level of 20000 ppm (equal to 1360 mg/kg bw/day) was considered to be the NOAEL for F0 and F1 reproductive toxicity as well as F1 and F2 neonatal toxicity of S-abscisic acid.

3.10.1.2 STUDY 2 – Developmental study

Study reference: B.6.6.2, STUDY 1

Characteristics

type of study	:	Teratogenicity study	exposure	:	Days 6-19 of gestation, gavage
year of execution	:	2007-2008	doses	:	0, 500, 750 and 1000 mg/kg bw/d
test substance	:	S-abscisic acid, lot no. 124-164-W9-00, purity 97.0%	vehicle	:	Methylcellulose
route	:	Oral	GLP statement	:	Yes
species	:	Rat, Crl:CD (SD)	guideline	:	In accordance with OECD 414
group size	:	25 females/dose	acceptability	:	Acceptable
			NOAELmat	:	> 1000 mg/kg bw/d
			NOAELdev	:	> 1000 mg/kg bw/d
			teratogenic effects	:	not observed

Study design

The study was performed in accordance with OECD 414 (2001). Dose levels were selected based on results from previous studies provided by the sponsor representative and the limit dose per OECD guideline 414 and EPA guideline OPPTS 870.3700, 1000 mg/kg body weight.

Results

Table 3.10.1.2.

Dose (mg/kg bw/day)	0	500	750	1000	dr
Maternal effects					
Mortality (n=25)	0	0	0	0	
Clinical signs		No treatment-related effects (see text)			
Pregnant animals (n=25)	25	25	25	25	
Abortions (n=25)	0	0	0	0	
Initial body wt.	247	249	250	247	
Terminal body weight	399	401	397	396	
Gravid uterine weight	78.6	85.6 +8.9%	83.9 +6.7%	86.2 +9.6%	
Net. body weight	319.2	315.7 -1.1%	313.4 -1.8%	309.6 -3%	
Net. body weight gain[a]	72.3	66.6 -7.9%	63.3 -12.4%*	62.3 -13.8%*	
Food consumption		No treatment-related effects			
Gravid uterine weight		No treatment-related effects			

Dose (mg/kg bw/day)	**0**	**500**	**750**	**1000**	**dr**
Pathology					
macroscopy	No treatment-related effects				
Litter response					
Viable foetuses/dam	13.7	14.7	14.2	15.1	
Dead foetuses/dam	0.0	0.0	0.0	0.0	
Resorptions/dam					
- early	1.1	1.0	1.1	1.0	
- late	0.1	0.0	0.1	0.0	
Implantation loss/dam					
- pre	1.4	1.2	1.3	0.5	
- post	1.2	1.0	1.2	1.0	
Corpora lutea (mean)	16.3	16.9	16.6	16.6	
Fetal weight	3.7	3.8	3.9	3.8	
Sex ratio	No treatment-related effects				
Examination of the foetuses					
External observations (litters/maternal animal)			0	0	
- short tail	1/1				
- spina bifida		1/1			
- fetal anasarca		1/1			
- macrophthalmia		1/1			
- microphthalmia and/or anophthalmia		1/1			
Skeletal findings	0	0	0	0	
Visceral findings					
- persistent truncis anophthalmia		1			

* Significantly different from the control group at 0.05 using Dunnett's test

a HCD: 69.0 g (range 58.9-77.1) based on 10 recent studies (from the same lab)

Acceptability

The individual uterine distribution was numbered arbitrarily in one of the control animals, instead of in a consecutive fashion as is prescribe in section 7.8 of the guideline. However, this deviation is considered not negatively impacting the quality or integrity of the study. Therefore, the study is considered acceptable.

Conclusions

All females in all dose groups survived to the scheduled necropsy on gestation day 20. Dried yellow material on the anogenital area was noted in the high-dose group in 5 animals with a total occurrence of 11 times and in the mid-dose group in 1 animal with a total occurrence of 2 times. Dried yellow material was also observed in the mid-dose group at the right and left inguinal area and the urogenital area; all three observations were done once in a single animal. Dried red material was found in the high-dose group at the anogenital area in 3 animals at 3 occasions and at the ventral abdominal area in 1 animal at a single occasion. Sporadic incidences of clear material around the mouth in the mid- and high-dose group and red material around the mouth in the high-dose group were noted approximately 1 hour following dose administration. Since the material noted on various body surfaces was generally slight and no other test article-related clinical findings were noted, these findings were considered not adverse.

Lower mean net body weight gains were noted in all test article-treated groups, which was statistically significant in the mid- and high-dose groups (88%, 86%, respectively). The change was considered to be the result of a higher net body weight change in the control group (mean: 72.3 g; SD: 8.79). The mean net body weight change in the control group for this study was greater than the WIL historical control mean of 10 recent studies (mean 68.0 g; SD: 5.7) but within the range (59.9-77.1 g).Therefore, this observation was considered not considered treatment-related.

Malformations were observed in 1(1) and 4(4) foetuses (litters) in the control and low-dose, respectively. Visceral findings were only seen in 1(1) foetus in the low-dose animal. Since no fetal malformations were observed in the mid- and high-dose groups, these observations were considered not treatment-related.

Based on the results of this teratogenicity study in rat, the NOAEL for both maternal toxicity and for prenatal developmental toxicity is set at > 1000 mg/kg bw/day.

3.10.2 Human data

No data available.

3.10.3 Other data (e.g. studies on mechanism of action)

No data available.

3.11 Specific target organ toxicity – single exposure

3.11.1 Animal data

Please refer to sections 3.1 – 3.3.

3.11.2 Human data

No data available.

3.11.3 Other data

No data available.

3.12 Specific target organ toxicity – repeated exposure

3.12.1 Animal data

3.12.1.1 STUDY 1 - 28-day oral studies

Study reference: B.6.3.1, STUDY 1

Characteristics

type of study	:	28-day oral toxicity study	exposure	:	28 days, diet

year of execution	:	2006	dose	:	0, 2000, 6000 or 20000 mg/kg food
test substance	:	VBC-30054 (Technical Grade S-abscisic acid), batch no. 124-164-W9-00, purity 97%	vehicle	:	None
route	:	Oral	GLP statement	:	Yes
species	:	Rat, Sprague-Dawley	guideline	:	In accordance with OECD 407
group size	:	5/sex/dose	acceptability	:	Acceptable
			NOAEL	:	20000 mg/kg food (2171 mg/kg bw/d for males and females)

[1] 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.

Study design

The study was performed in accordance with OECD 407 (1995), including neurotoxicity clinical observations, motor activity, detailed functional observations, urinalysis and ophtalmoscopy examinations. Dose levels were based on the results of a preliminary study carried out at Charles River Laboratories, Edinburgh (Charles River Laboratories Study No. 457850). Dose levels took into account the limit dose in the test model.

Results

The results are summarised in table 3.12.1.1-1.

Table 3.12.1.1-1

Dose (mg/kg food)	0		2000		6000		20000		dr
	m	f	m	f	m	f	m	f	
Mortality	No mortality								
Clinical signs	No treatment-related findings								
Neurotoxicity clinical observations	No treatment-related findings								
Motor activity	No treatment-related findings								
Detailed functional observations	No treatment-related findings								
Body weight gain							d	d	
Food consumption	No treatment-related findings								
Ophthalmoscopy	No treatment-related findings								
Haematology									
- mean cell haemoglobin							dc		
- reticulocytes				dc		dc		dc	
- lymphocytes							d	d	
- neutrophils				dc		dc	d	dc	
- activated partial thromboplastin							dc		
Clinical chemistry									
- urea							d	d	
- glucose			dc		dc		dc		m
Urinalysis									
- specific gravity							d		
Organ weights									
- epididymis			ic^r		ic^r				
- testis							i		
- salivary glands					i^r		i^r	i^r	
- thyroid glands								i	

Dose (mg/kg food)	**0**		**2000**		**6000**		**20000**		**dr**
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**	
- kidney				ic					
Pathology									
macroscopy	No treatment-related findings								
microscopy	No treatment-related findings								

dr dose related

dc/ic statistically significantly decreased/increased compared to the controls

d/i decreased/increased, but not statistically significantly compared to the controls

a/r absolute/relative organ weight

\+ present in one/a few animals

++ present in most/all animals

Table 3.12.1.1-2 Body weight gain

Group/Dose Level (ppm)		**Body Weight Gain (g) male** (Days 0 - 28)	**Body Weight Gain (g) female** (Day 0 - Day 28)
1 (0)	Number Mean SD	5 204 23	5 96
2 (2000)	Number Mean SD	5 201 32	9 5 97
3 (6000)	Number Mean SD	5 186 30	5 96
4 (20,000)	Number Mean SD	5 178 16	5 89 11

Acceptability

The study is considered acceptable.

Conclusions

No substance related mortality was observed upon dietary exposure of rat to 0, 2000, 6000 or 20000 mg VBC-30054 per kg food for 28 days. No treatment-related clinical signs and neurotoxicity clinical signs were observed. No treatment-related changes in motor activity, detailed functional behaviour, ophthalmoscopy, pathology and histopathology were seen. There were sporadic incidences where the food consumption in treated groups was higher or lower when compared to their respective controls, however, no pattern was established and therefore it was thought not to be a treatment related effect. After 4 weeks of treatment, a slight reduction in group mean body weight gain was seen in males and females in the high-dose groups when compared to the controls without statistical significance. Specific gravity in males in the high-dose group was slightly lower when compared to the controls, but without statistical significance.

Covariant epididymitis weights were slightly higher in males in the low- and mid-dose groups compared to controls with statistical significance. Testis weights were slightly increased in the high-dose group without statistical significance. A slight increase in the covariant salivary glands was observed in males in the mid-dose group and males and females in the high-dose groups. Kidney weight was significantly increased in females in the low-dose group; however no notable findings were seen in the other dose-groups.

Mean cell haemoglobin (MCH) and activated partial thromboplastin (APTT) were slightly decreased in males in the high-dose group (96% and 90% of control, respectively). Although these decreases were significant, the slight decreases were considered of no toxicological concern. Lymphocytes were slightly decreased in males and females in the high-dose group, but without statistical significance. Retilocytes were significantly decreased in females in all treated groups (77%, 67% and 77% of control in the low-, mid- and high-dose group, respectively), but no dose response curve was seen. Also, neutrophils were significantly decreased in females in all dose-groups (0.77, 0.87, 0.74 in the low-, mid- and high-dose group, respectively) compared to the control group (1.50), but this was probably due to two high control values (2.17 and 1.92).

Urea was slightly decreased in males and females in the high-dose group (87% and 79% of control, respectively), but without statistical significance. Glucose was significantly and dose-related decreased in males in all dose groups (83%, 80%, 72% of control in the low-, mid- and high-dose group, respectively). Since normal blood glucose levels may have a wide range of variation and since no other signs of toxicity are observed, these findings are considered of no toxicological relevance.

In conclusion, the NOAEL of S-abscisic acid (VBC-30054) in this study is set at 2171 mg/kg bw/d for both males and females.

3.12.1.2 STUDY 2 - 21/28-day dermal studies

Study reference: B.6.3.2, STUDY 1

Characteristics

type of study	:	21-day dermal toxicity study	exposure	:	21 days, 6 h/d, semi-occlusive (10% of the total body surface area)
year of execution	:	2007	dose	:	0, 100, 300, 1000 mg/kg bw/d
test substance	:	VBC-30054 (Technical Grade S-abscisic acid), batch no. 124-164-W9-00, purity 97%	vehicle	:	0.5% high viscosity carboxymethylcellulose (CMC)
route	:	dermal	GLP statement	:	yes
species	:	Rat (Sprague Dawley)	guideline	:	in accordance with OECD 410
group size	:	5/sex/dose	acceptability	:	acceptable
			NOAEL	:	1000 mg/kg bw/d

Study design

The study was performed in accordance with OECD 410. Dose levels were based on the results of a preliminary study carried out by the Sponsor at Charles River Laboratories, Edinburgh (Study number 458194). Dose levels accounted for the limit dose in the test model.

Results

The results are summarised in table 3.12.1.2-1.

Table 3.12.1.2-1

Dose (mg/kg bw/d)	0	100	300	1000	dr

	m	f	m	f	m	f	m	f	
Mortality	No mortality								
Clinical signs									
- very slight erythema (week3)		1/5			2/5	1/5	4/5		
- stained fur		1/5		1/5		2/5		4/5	
- discharge from the eye(s)		2/5				2/5		3/5	
- darkened skin								2/5	
Body weight (gain)	No treatment-related findings								
Food consumption	No treatment-related findings								
Ophthalmology	No treatment-related findings								
Haematology									
- white blood cell count							ic		
- lymphocytes							ic		
- neutrophils							i		
- basophils							ic		
- activated partial thromboplastin					ic		ic		
- prothrombin time								dc	
Clinical chemistry	No treatment-related findings								
Urinalysis	No treatment-related findings								
Organ weights									
- epididymis					ic[a/r]				
Pathology									
macroscopy	No treatment-related findings								
microscopy	No treatment-related findings								

dr dose related

dc/ic statistically significantly decreased/increased compared to the controls

d/i decreased/increased, but not statistically significantly compared to the controls

a/r absolute/relative organ weight

\+ present in one/a few animals

++ present in most/all animals

Acceptability

The study is considered acceptable.

Conclusions

No treatment-related mortality was noted. No treatment-related changes in body weight, body weight gain, food consumption, ophthalmology, clinical chemistry, urinalysis, pathology and histopathology were observed.

All males and 3 females in the high-dose group and 3 males and 2 females in the mid-dose groups showed very slight erythema at the dose site on a maximum of 3 occasions during the treatment period. Stained fur and a discharge from the eye(s) were noted in 4 females in the high-dose group and 2 females in the mid-dose group. Two females in the high-dose group showed darkened skin. However, due to the fact that many of the signs were also seen in the control group and the lack of corroborating evidence, these signs were considered not to be treatment related.

In males in the high-dose group, white blood cell count and lymphocytes were increased statistically significant (166% and 166% of control, respectively) and neutrophils were increased non-statistically

significant (173% of control). Due to the increased white blood cell count, EDTA blood film smears were evaluated, which revealed normal white cell morphology throughout the treated groups. Many values in the low- and mid-dose group were lower than in the control group, and no dose dependent trend was observed. Therefore, these changes in blood parameters were considered not toxicologically significant.

Activated partial thromboplastin time was statistically significant increased in males in the mid- and high-dose groups (124% and 118% of control, respectively). Prothrombin time was statistically significant decreased in females in the high-dose group (93% of controls). However, due to the small magnitude of changes and the lack of dose responses, the changes were considered not to be toxicologically significant.

In conclusion, the NOAEL of S-abscisic acid (VBC-30054) in this study is set at 1000 mg/kg bw/day.

3.12.1.3 STUDY 3 - Semichronic oral studies

Study reference: B.6.3.3, STUDY 1

Characteristics

type of study	:	90-day oral toxicity study	exposure	:	90 days, in diet
year of execution	:	2007	doses	:	0, 2000, 6000 or 20000 mg/kg food[1]
test substance	:	VBC-30054 (Technical Grade S-abscisic acid), batch no. 124-164-W9-00, purity 97%	vehicle	:	None
route	:	oral	GLP statement	:	Yes
species	:	Rat, Sprague-Dawley	guideline	:	In accordance with OECD 408
group size	:	10/sex/dose	acceptability	:	Acceptable
			NOAEL	:	20000 mg/kg food (1420 mg/kg bw/d for males and 1752 for females)

[1] Equivalent to 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.

Study design

The study was performed in accordance with OECD 408 (1998). Dose levels were based on the 28-day, oral study in rat (see paragraph 6.3.1).

Results

The results are summarised in table 3.12.1.3-1.

Table 3.12.1.3-1

Dose (mg/kg food)	0		2000		6000		20000		dr
	m	f	m	f	m	f	m	f	
Mortality		1/10	1/10					1/10	
Clinical signs	No treatment-related findings								
Neurotoxicity clinical observations									
- reduced response to tactile stimulus			1/10		1/10		2/10		
- exaggerate reaction to tactile stimulus								3/10	
- jerks around/away to tactile stimulus				1/10		3/10			

Dose (mg/kg food)	**0**		**2000**		**6000**		**20000**		**dr**
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**	
Motor activity	No treatment-related findings								
Detailed functional observations	No treatment-related findings								
Body weight gain							d	d	
Food consumption	No treatment-related findings								
Ophthalmoscopy	No treatment-related findings								
Haematology									
- lymphocytes								i	
- white blood cell count								i	
Clinical chemistry									
- cholesterol			i			i		i	m
- triglycerides			d	d	d	d	d	d	m, f
Urinalysis	No treatment-related findings								
Organ weights									
- liver					i^{r}		i^{r}	i^{r}	
- spleen			$d^{a/r}$		$d^{a/r}$		$d^{a/r}$		
- adrenal glands			dc^{r}		dc^{r}		dc^{r}		
- thyroid glands						i^{r}		i^{r}	
- thymus			i^{r}		i^{r}		i^{r}		
- ovary				i^{r}		i^{r}		i^{r}	
Pathology									
macroscopy	No treatment-related findings								
microscopy	No treatment-related findings								

dr dose related

dc/ic statistically significantly decreased/increased compared to the controls

d/i decreased/increased, but not statistically significantly compared to the controls

a/r absolute/relative organ weight

\+ present in one/a few animals

++ present in most/all animals

Table 3.12.1.3-2Body weight gain

Group/Dose Level (ppm)		**Body weight gain (g) (Week 0 - 13) m**	**Body weight gain (g) (Week 0 - 13) f**
1 (0)	Number	10	9
	Mean	346	**130**
2 (2000)	Number	9	**10**
	Mean	322	**134**
3 (6000)	Number	10	**10**
	Mean	340	**131**
4 (20,000)	Number	10	**9**
	Mean	309	**117**

Acceptability

The study is considered acceptable.

Conclusions

There were 3 premature decedents during the period of treatment, one in the control group, one in the low-dose group and one in the high dose group. The animal in the control group showed blood in the right eye with an abnormal shape. In addition, the mandibular lymph nodes were reddened. The eye damage was indicated by the study authors to be the cause of death due to orbital sinus blood collection in week 13. The animal treated with the low dose (2000 mg/kg) showed mandibular lymph nodes, enlarged hind feet, dark liver, marked arthritis in the ankle joints, a hunched body, limping and piloerection. In the animal in the high dosed group, the spleen, stomach and pancreas adhered to each other. The liver, adrenal glands and spleen were enlarged with the spleen having a pale focus. Moderate atrophy was seen in the stifle joint and capsular adhesion and moderate inflammation of cell foci in the spleen. The animal had a hunched body, intermittently dragged the hind limps, swollen hind limbs, a discoloured skin and piloerection. There were no indications that the observed mortality was related to the treatment. All three animals were killed *in extremis*.

No treatment-related clinical signs were observed and no treatment-related changes in motor activity, detailed functional behaviour, food consumption, ophthalmoscopy, urinalysis, pathology and histopathology were seen. During the week 12 observations, a small number of males were noted to have reduced response to tactile stimulus and a small number of females were noted to have an increased exaggerated reaction or jerks around/away response to tactile stimulus. As these findings were contradictory and there were no other signs indicative of neurotoxicity in the animals, the observations seen were not considered to be indicative of toxicity.

After 13 weeks of treatment males and females had slightly reduced group mean body-weight gains (89% and 90% of control, respectively), but not statistical significant.

Slightly increased lymphocytes and white blood cell count were seen in females in the high-dose group (132% and 128% of control, respectively), however, without statistical significance.

Cholesterol was slightly increased in males without statistical significance in a dose-related manner (106%, 117% and 122% of control, in the low-, mid-, and high-dose group, respectively). In females, cholesterol was slightly increase in the high-dose group only (122% of control), without statistical significance. Triglyceride levels were slightly decreased without statistical significance in a dose-related manner in both males and females (95%, 80% and 75% of control in males and 78%, 68% and 63% of control in females, in the low-, mid-, and high-dose group, respectively). The variation within the individual values would indicate that these values are not toxicologically relevant.

Relative liver weights were slightly increased in male in the mid- and high-dose group (104% and 110% of controls, respectively) and in females in the high-dose group (108% of control), without statistical significance.

The absolute and relative spleen weights were slightly decreased in males in all dose groups (absolute: 88%, 86% and 83% of control, and relative: 91%, 86% and 88% of control, in the low-, mid-, and high-dose group, respectively), without statistical significance. Relative adrenal gland weights were statistical significant decreased in males in all dose groups (86%, 80% and 82% of control, in the low-, mid-, and high-dose group, respectively), but not in a dose-related pattern. Relative thyroid gland weights were increased in females in the mid- and high-dose groups (128% and 119% of control, respectively), but not statistically significant. Relative thymus weights were slightly increased in males in the low-, mid- and high-dose groups (116%, 118% and 113% of control, respectively), but not statistically significant. Relative ovary weights were slightly increased in the low-, mid- and high-dose groups (117%, 110% and 110% of control, respectively), but not statistically significant. The slightly increased relative thymus weights in males and ovary weights in females were thought to be due to lower covariant weights in the control animals and therefore were considered not to be of toxicological significance.

In conclusion, the NOAEL of S-abscisic acid (VBC-30054) is set at 1420 mg/kg bw/d for males and 1752 mg/kg bw/d for females.

3.12.2 Human data

No data available.

3.12.3 Other data

No data available.

3.13 Aspiration hazard

3.13.1 Animal data

No data available.

3.13.2 Human data

No data available.

3.13.3 Other data

No data available.

4 ENVIRONMENTAL HAZARDS

4.1 Degradation

4.1.1 Ready biodegradability (screening studies)

Study reference: STUDY IIA, 7.7/001

Characteristics

year of execution	:	2010	test system	:	activated sludge, assessment of ready biodegradability by respirometry
GLP statement	:	yes	incubation time	:	28 d
guideline	:	OECD 301F (1992) EU 92/69/EEC C.4 (1992)	nominal concentration	:	38 $mgO_2 L^{-1}$
test substance	:	S-abscisic acid	temperature	:	20-24°C
purity	:	98.2%	result	:	readily biodegradable
			acceptability	:	**acceptable**

The ready biodegradability of S-abscisic acid was studied in an oxygen consumption test according to OECD 301F.

Study design

Filtered sludge (mesh size 1 mm^2) was left to stand for approximately 30 minutes to allow the sewage solids to settle to give a solids level of 30 mg/L in test cultures. 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 for 3 minutes. The preparation was made up to volume with MSM to give a nominal stock solution (445 mg L^{-1}). The pH of the stock was measured and adjusted to 7.3 with 5N NaOH. Aliquots (20 ml of the stock were then added to the test and test plus reference bottles to give a final, nominal, concentration of 38.8 mgO_2 L^{-1} (19.4 mgO_2/500 mL).

In order to ensure similarity in the preparation of all mixtures, MSM (20 mL) was added to the control cultures. Sodium benzoate (20 mL) was added as an aqueous stock solution (0.750 g L^{-1}) in MSM to the reference and inhibition assay cultures to give a final nominal concentration of 50 mgO_2 L^{-1} (25 mgO_2 500 mL^{-1}).

The pH of the cultures was measured and no adjustment was necessary. The CO_2 absorber was replaced in each cell (to ensure the maximum absorption capacity in the test) and the cultures were sealed and returned to the water bath to equilibrate. The cells were connected to the computer-controlled system and the test was initiated. A record of the cumulative oxygen demand made by each cell was printed at, typically, hourly intervals. The correct operation of the magnetic stirrers and the temperature of the water bath were recorded at appropriate intervals during the test. On Day 28 the test was halted and the pH of each mixture was measured.

Results

Calculation of the theoretical oxygen demand (ThOD)

The theoretical oxygen content of the mixtures containing the test substance and sodium benzoate were calculated to be 19.4 mgO_2 per 500 mL (38.8 mgO_2 L^{-1}) and 25 mgO_2 per 500 mL (50 mgO_2 L^{-1}), respectively.

Findings

The percentage biodegradation of S-abscisic acid and references substances are given in Table 4.1.1-1.

The blank-corrected oxygen demanded by the culture containing the reference substance had achieved 16.85 mgO_2 per 500 mL or 67% of the ThOD (25 mgO_2 per 500 mL) after 4 days of incubation and 23.69 mgO_2 per 500 mL or 95% by Day 28. In the presence of S-abscisic acid, degradation of sodium benzoate had achieved 60% by Day 3. 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^{-1}) were considered to be acceptable for this assay system. These results confirm that S-abscisic acid was not inhibitory to the activity of the microbial inoculum and that the test was valid.

Mean oxygen consumption in biotic mixtures containing S-abscisic acid was equivalent to 10% of the theoretical value (19.4 mgO_2 per 500 mL) after approximately 4 days, 60% after approximately 8 days and 89% at the end of the test (Day 28).

Table 4.1.1-1 Percentage biodegradation of S-abscisic acid and reference substances

Day	% Biodegradation

	S-abscisic acid (38.8 $mgO_2 L^{-1}$)			**Sodium Benzoate (50 $mgO_2 L^{-1}$)**	**Sodium benzoate (50 $mgO_2 L^{-1}$) in the inhibition assay**
	Test 1	**Test 2**	**Mean**		
1	1	0	0	36	43
2	1	0	0	53	53
3	0	0	0	59	60
4	2	2	2	67	-
5	29	31	30	72	-
6	44	46	45	75	-
7	50	51	50	77	-
8	56	59	58	79	-
9	61	63	62	81	-
10	65	67	66	82	-
11	68	70	69	83	-
12	71	73	72	85	-
13	73	75	74	86	-
14	76	77	76	87	-
15	77	79	78	87	-
16	78	80	79	88	-
17	80	81	81	89	-
18	81	82	82	90	-
19	82	83	83	90	-
20	84	84	84	91	-
21	84	85	85	91	-
22	85	85	85	92	-
23	86	86	86	92	-
24	86	87	86	93	-
25	87	87	87	93	-
26	88	88	88	94	-
27	89	88	88	94	-
28	89	89	89	95	-

Conclusions

Under the conditions of this study, S-abscisic acid can be considered as readily biodegradable, as O_2 consumption was ≥ 60% of the theoretical value within 10 days of the level achieving 10%. 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.

Remarks

The study was performed in accordance with guideline OECD 301F and is acceptable.

4.1.2 BOD_5/COD

No data available.

4.1.3 Aquatic simulation tests

No data available.

4.1.4 Other degradability studies

4.1.4.1 STUDY 1 - Hydrolysis

Study reference: STUDY IIA, 2.9.1

Test substance: S-abscisic acid, lot/ batch no. 030806D1, purity 99.7%

Procedures

The procedures were based on the methods described in OPPTS guideline 8352110.

Summary

First, a preliminary study was conducted with S-abscisic acid in sterile 0.05 M aqueous solutions buffered at pH 4, 7, and 9. The preliminary test was conducted at a concentration of approximately 100 ppm and incubated for up to 5 days at 50 ± 0.2°C. The preliminary study showed that S-abscisic acid did not degrade significantly in pH 7 or 9 buffered solutions 9greater than 90% S-abscisic acid remaining in solution). As a result, 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.

Sample set	Degradation rat constant ($days^{-1}$)	Half-life	R^2
pH 4 buffer set, 25°C	-0.00088	791.6 days	0.63988
pH 4 buffer set, 40°C	-0.0043	161.9 days	0.9039

4.1.4.2 STUDY 2 - degradation in water-sediment system

Reference: STUDY IIA, 7.8.3/001

Characteristics

year of execution	:	2011	incubation time	:	48 hours
GLP statement	:	yes	nominal concentration	:	5 – 10 µg/L
guideline	:	OECD 309 (2004) OECD 308 (2002) OECD 307 (2002) ISO/DIS 14592/1	temperature	:	20.2 – 22.5°C
test substance	:	S-abscisic acid	DT50	:	see results
purity	:	Chemical purity not reported, radiochemical purity 97.1%. (dark) & 97.6% (irradiated).			
test system	:	River Gipping, Needham Market, Suffolk, UK (TM0940054900)	acceptability	:	acceptable

Justification surface water degradation instead of water/sediment degradation study

Adsorption, diffusion, hydrolysis, and biodegradation processes are important in controlling the behaviour of pesticides in water-sediment systems

Partitioning of S-abscisic acid to the sediment will be insignificant due to the very low K_{oc} values

The degradation rate of S-abscisic acid in the soil is very rapid with DT_{50} values of *ca* 0.44 to 2.2 days.

The active substance S-abscisic acid has also been seen to be readily biodegradable

Literature clearly indicates microbial degradation of S-abscisic acid

Hydrolysis is not a route of degradation

Thus for S-abscisic acid degradation from a water-sediment system will be principally microbial and in the water column. A surface water degradation study (based on guideline OECD 309) has been conducted to determine a DT_{50} within the water system.

Study design

The behaviour of S-abscisic acid was studied in surface water systems. The definitive test was based on the results of the preliminary assessments. Surface water was filtered to remove coarse particles and parameters measured (Table 4.1.4.2-1). Stock solutions of S-abscisic acid (5 µg mL^{-1} and 10 µg mL^{-1}) and aniline (10 µg mL^{-1}; positive control) were prepared in ultra-pure water.

Table 4.1.4.2-1 Physico-chemical characterisation of surface water determined at the time of sampling

Parameter	Location
	River Gipping, Needham Market, Suffolk (TM0940054900)
pH (at sampling time)	8.11
Dissolved oxygen	11.85 mg O_2/L
Dissolved organic carbon	7.218 mg C/L

Total nitrogen	8.78 mg N/L
Ammonium	0.19 mg N/L
Nitrite	<0.10 mg NO_2/L
Nitrate	38.9 mg NO_3/L
Total phosphorus	0.20 mg P/L
Dissolved orthophosphate	0.21 mg P/L

Aliquots (5 L) of the surface water were added to four holding vessels (5 L) and two of these were dosed with S-abscisic acid (5 and 10 µg L^{-1}), one was dosed with aniline (10 µg L^{-1}) and one vessel was an untreated control. Aliquots (100 mL) of each treatment group were then dispensed into sets of Wheaton vials (160 mL) and the vials sealed. On each sampling occasion five replicates for each group were prepared (a total of 45 vials per group). The vials were placed horizontally on an enclosed reciprocating shaker and shaken in darkness at nominal temperature range of 20 to 25ºC for up to 48 hours.

Sampling

Samples were taken for analysis at time 0 hours and thereafter at approximately six hourly intervals (6, 12, 18, 24, 30, 36, 42 and 48 hours). At each sampling point, 1 mL of a sodium azide solution (1 g L^{-1}) was added to each replicate in order to stop the biochemical reactions and preserve the samples.

Description of analytical method

The test concentrations of S-abscisic acid and aniline were measured using an ultra-performance liquid chromatography with tandem mass spectrometric detection (UPLC-MS/MS) and gas chromatography with mass spectrometric detection (GC-MS) methods. On each occasion, five replicates were analysed following treatment with sodium azide solution. As aniline was shown not to be stable despite sodium azide treatment, the samples taken at 12, 18, 36 and 42 hours were extracted and refrigerated until analysis could be carried out. Control and test samples taken at these time points were also refrigerated until analysis could be carried out.

Evaluation of data

The DT_{50} values, lag period and first order rate constant of the test and reference substance were calculated. The data was also analysed using ModelMaker (version 4.0, ModelKinetix, Oxford, UK) employing procedures recommended in Sanco/10058/2005, version 2.0, June 2006 (Guidance Document on Estimating Persistence and degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration).

Results

Analysis of test concentrations

Preliminary assessments

An initial screen of the surface water for S-abscisic acid taken from the sampling point showed levels of S-abscisic acid ranging between 0.116 and 0.160 µg L^{-1}. At the start of the preliminary primary degradation assessment test, S-abscisic acid was measured in the control samples at concentrations ranging from 0.0133 to 0.0289 µg L^{-1}, however these results were below the limit of detection for the analytical method. S-abscisic acid was not measured, above the limit of detection for the analysis; in control samples in the second preliminary test.

Definitive test

At the start of the definitive test, the measured levels of S-abscisic acid at a nominal concentration of 5 µg L^{-1} ranged from 4.62 to 4.93 µg L^{-1} (92% to 99% of its nominal value). At 10 µg L^{-1} the measured levels ranged between 9.45 and 10.3 µg L^{-1} (95% to 103% of its nominal value) (Table B.8.4.3.2-02).

S-abscisic acid was not detected (limit of detection, <0.25 µg/L) in the control samples during the definitive test. Measured levels of aniline at the start of the definitive test ranged from 8.69 to 9.35 µg L^{-1} (87% to 94% of its nominal value) (Table 4.1.4.2-2).

Table 4.1.4.2-2 Measured S-abscisic acid and aniline concentrations

Time (hours)	S-abscisic acid concentration									
	R_A		R_B		R_C		R_D		R_E	
	µg/L	%N	µg/L	%N	µg/L	%N	µg/L	%N	µg/L	%N
Measured S-abscisic acid concentrations (5 µg/L)										
0	4.69	94	4.93	99	4.82	96	4.62	92	4.65	93
6	5.33	107	4.95	99	5.59	112	5.24	105	4.90	98
12	3.90	78	3.43	69	3.37	67	4.12	82	3.82	76
18	3.69	74	5.02	104	3.14	62	4.99	100	3.06	61
24	4.51	90	4.56	91	4.63	93	4.65	93	4.48	90
30	3.24	65	4.03	81	4.03	81	2.13	43	2.41	48
36	2.18	44	2.21	44	2.47	49	2.15	43	2.55	51
42	0.971	19	0.862	17	1.06	21	0.936	19	0.917	18
48	<LOQ	-	nd	-	nd	-	nd	-	<LOQ	-
Measured S-abscisic acid concentrations (10 µg L^{-1})										
0	9.75	98	10.2	102	9.45	95	9.90	99	10.3	103
6	10.8	108	10.6	106	11.7	117	11.0	110	10.6	106
12	9.84	98	9.18	92	10.7	107	8.65	87	10.1	101
18	9.10	91	9.14	91	7.66	77	9.64	96	8.50	85
24	9.74	97	10.1	101	9.71	97	9.74	97	9.49	95
30	4.47	45	6.98	70	8.72	87	8.90	89	8.36	84
36	5.98	60	6.47	65	5.69	57	6.19	62	5.66	57
42	1.93	19	1.82	18	1.64	16	2.85	29	2.61	26
48	nd	-	<LOQ	-	nd	-	nd	-	<LOQ	-
Measured aniline concentrations (10 µg L^{-1})										
0	9.31	93	9.17	92	9.35	94	9.20	92	8.69	87
6	8.71	87	8.27	83	8.23	82	8.95	90	7.21	72
12	9.46	95	10.2	102	9.66	97	8.52	85	10.6	106
18	8.71	87	8.08	81	8.76	88	8.90	89	8.97	90
24	7.42	74	7.44	74	6.65	67	6.84	68	8.06	81
30	5.71	57	4.84	48	6.44	64	4.70	47	5.71	57
36	7.69	77	7.88	79	6.32	63	7.90	79	6.57	66
42	nd	-	1.88	19	1.92	19	1.65	17	1.45	15
48	nd	-	nd	-	nd	-	nd	-	nd	-

nd: not detected (LOD <0.25 µg/l for S-abscisic acid) and (LOD <0.5 µg l^{-1} for aniline)

<LOQ: less than the limit of quantitation (0.5 µg l^{-1} for S-abscisic acid).

R_x: replicate identification.

%N: percent of nominal.

Degradation rate

At 5 µg L^{-1}, the log-linear data for S-abscisic acid showed a mean lag period of approximately 26 hours and a mean DT_{50} (from the end of the lag period) of 4.3 hours. At 10 µg L^{-1}, the mean lag period was approximately 30 hours with a mean DT_{50} of 2.6 hours. The rate constant k, was calculated to be 0.165 and 0.265, respectively (Table B.8.4.3.2-03).

For the linear-linear data S-abscisic acid, at 5 µg L^{-1}, showed a mean lag period of 27 hours and a mean DT_{50} of 6.9 hours. At 10 µg L^{-1}, S-abscisic acid had a mean lag period of 30 hours and a mean DT_{50} of 5.6 hours. The rate constant (ln2/k) was calculated to be 0.108 and 0.141 respectively (Table B.8.4.3.2.-03).

These results show at 5 and 10 µg L^{-1} the log-linear (k) value was similar. This indicates that the rate constant is independent of concentrations, therefore, the degradation of S-abscisic acid can be considered to follow first order kinetics. The linear-linear (ln2/k) was also considered similar.

At 10 µg L^{-1}, the log-linear data for aniline showed an approximate lag period of 30 hours. The DT_{50} (from the end of the lag period, break point) was 1.5 to 3.3 hours (mean 2.8 hours). For the linear-linear data, the lag period was approximately 35 to 36 hours and the DT_{50} 1.6 to 3.5 hours (mean 2.7 hours). The rate constant (k) ranged from 0.211 to 0.456 (mean 0.268) and ln2/k ranged from 0.200 to 0.433 (mean 0.272). As the DT_{50} of aniline was within the test period, these results show that the microorganisms present in the sample of water were viable and the test was valid.

Table 4.1.4.2-3 S-abscisic acid primary degradation summary

S-abscisic acid (5 µg L^{-1})								
Replicate	Log – linear plot (Excel)				Linear-linear plot (ModelMaker)			
	DT_{50} (hours)	Lag period (hours)*	R^2	k	DT_{50} (hours)	Lag period (hours)	r^2	ln2/k
A	4.9	24	0.9397	0.142	7.0	27	0.9192	0.099
B	4.5	24	0.9770	0.155	5.8	29	0.9299	0.120
C	4.5	24	0.9481	0.153	4.4	33	0.8255	0.156
D	3.9	30	0.9831	0.179	7.8	23	0.9494	0.089
E	3.6	30	0.9953	0.194	9.3	24	0.8515	0.075
Arithmetic mean	4.3	26	-	0.165	6.9	27	-	0.108
S-abscisic acid (10 µg L^{-1})								
Replicate	Log – linear plot (Excel)				Linear-linear plot (ModelMaker)			
	DT_{50} (hours)	Lag period (hours)*	r^2	k	DT_{50} (hours)	Lag period (hours)	r^2	ln2/k
A	2.6	30	0.9732	0.265	8.8	23	0.9137	0.079
B	2.6	30	0.9841	0.271	7.5	27	0.9269	0.092
C	2.7	30	0.9863	0.260	3.2	34	0.9201	0.215
D	2.6	30	0.9183	0.267	4.3	33	0.9616	0.161
E	2.7	30	0.9220	0.260	4.4	33	0.9542	0.159
Arithmetic mean	2.6	30	-	0.265	5.6	30	-	0.141

DT_{50} : primary degradation half life

* estimated from the graphical plot

Conclusions

At 5 µg L^{-1}, the log-linear data for S-abscisic acid showed a mean lag period of approximately 26 hours and a mean DT_{50} (from the end of the lag period) of 4.3 hours. At 10 µg L^{-1}, the mean lag period was approximately 30 hours with a mean DT_{50} of 2.6 hours. The rate constant (k), was calculated to be 0.165 and 0.265, respectively for the log-linear plots.

For the linear-linear data S-abscisic acid, at 5 µg L^{-1}, showed a mean lag period of 27 hours and a mean DT_{50} of 6.9 hours. At 10 µg L^{-1}, S-abscisic acid had a mean lag period of 30 hours and a mean DT_{50} of 5.6 hours. The rate constant (ln2/k) was calculated to be 0.108 and 0.141 respectively.

These results show at 5 and 10 µg L^{-1} the log-linear (k) value was similar. This indicates that the rate constant is independent of concentrations, therefore, the degradation of S-abscisic acid can be considered to follow first order kinetics. The linear-linear (ln2/k) was also considered similar.

Remarks

The OECD guideline 309 prescribes to use two concentrations with a factor five to ten difference. The tested concentrations only showed only a difference factor two from each other. Additionally, the criterion that the lowest concentration should not exceed 10 µg L^{-1} was met. But the conclusion that the rate constant is independent of the concentration cannot be obtained from the results presented in the report. The rate constant showed that the degradation in water was fast, arithmetic mean DT_{50} of 3.5 hours for log-linear plot (best fit), after the lag-phase of 30 hours.

No labelled S-abscisic acid was used in the test. Therefore, no metabolites could be identified and no indication of the route of degradation could be obtained via this study. A water/sediment study is mainly performed with labelled test substance in order to see the distribution of the radioactivity over the water and sediment and follow metabolite formation. The justification for performing the surface water study instead of the water/sediment study did not cover the metabolite formation aspect. Formation of hydrophobic metabolites that partition in the sediment is not observed using the surface water test. This is expected because in the soil degradation test (B.8.1.1.1) one third of the radioactivity was not extractable and remained on the soil. Additionally, mineralization of the test substance is also not measured when using unlabelled test-substance. Despite these remarks it is agreed with the other arguments to use the water/sediment study for this compound.

The overall conclusion is that the study is acceptable. A fast degradation of S-abscisic acid in water was observed.

4.1.4.3 STUDY 3 - aerobic soil degradation

Study reference: STUDY IIA, 7.1.1/001

Characteristics

year of execution	:	2010	study type	:	aerobic soil degradation
GLP statement	:	yes	incubation time	:	120 days
guideline	:	incl. OECD 307 (2002)	nominal concentration	:	1.67 mg/kg
test substance	:	^{14}C-S-abscisic acid	temperature	:	20°C
purity	:	97%	DT50	:	2.2, 0.44, 0.98 and 0.80

soils	:	sandy loam, sandy clay and 2 clay loam soils	metabolites acceptability	: :	days no metabolites acceptable

Study design

The route and rate of degradation of ^{14}C-S-abscisic acid 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}). Prior to study initiation, the soil moisture content and the moisture content equivalent to pF2 were measured (soil properties see Table 4.1.4.3-1).

Portions of soil, (50 g dry weight equivalent) were pre-conditioned in soil incubation chambers, under a continuous humid air supply, in the dark for 2 weeks at 20 ± 2°C prior to test substance application. The moisture content of the soil was adjusted to pF2, which was maintained for the duration of the study.

^{14}C-S-abscisic acid dissolved in acetonitrile was isotopically diluted by the addition of non-radiolabelled S-abscisic acid. An aliquot from this fortified solution was then applied to each vessel. Following application, the solvent was allowed to evaporate and the samples were then gently mixed. Water was added as necessary to maintain the moisture content at the pF2 level. Triplicate aliquots were taken from the fortified solution and the radioactivity determined by LSC.

Soil vessels were then incubated under a continuous humid air supply, in the dark at 20 ± 2ºC for up to 120 days. Incubation chambers were connected to 1 trap containing ethyl digol and 2 traps containing aqueous potassium hydroxide.

In addition, 5 vessels for each soil type containing 500 g of soil (dry weight equivalent) were established for the determination of biomass. Two vessels of each soil type were treated with an equivalent volume of solvent to that used to treat the vessels for the main experiment and the remaining 3 microbial biomass vessels were not treated. The moisture content of the soil was maintained at the pF2 level for the duration of the study.

Table 4.1.4.3-1 Properties of study soil

Soil characteristics	Bromsgrove soil	Elmton soil	Fladbury soil	Empingham soil
Particle size distribution:				
% sand (0.063 - 2 mm)	67	42	51	32
% silt (0.002 - 0.063 mm)	19	32	17	38
% clay (<0.002 mm)	14	26	32	30
Textural classification[a]	Sandy loam	Clay loam	Sandy clay	Clay loam
% sand (0.05 – 2 mm)	68	44	54	34
% silt (0.002 - 0.05 mm)	18	31	15	38
% clay (<0.002 mm)	14	26	32	30
Textural classification[b]	Sandy loam	Loam	Sandy clay loam	Clay loam
pH (water)	4.9	7.2	6.5	7.2
pH (0.01 M $CaCl_2$)	4.3	7.1	6.1	7.2
Organic carbon (%)	1.4	4.2	3.5	5.6
Soil density g/l	1190	1011	1091	1134
Cation exchange capacity (meq/100 g)	10.7	26.6	34.6	37.8

Water content at pF2 (%)	22.0	40.0	44.9	38.8
Microbial biomass				
Microbial biomass at start of incubation:				
% of organic carbon	1.74	8.89	5.54	4.83
Microbial biomass at end of incubation:				
Solvent treated (% of organic carbon)	1.17	6.63	5.44	4.61
Non-treated (% of organic carbon)	0.83	5.26	4.87	3.91

[a]: UK classification system.

[b]: USDA classification system.

Sampling

Duplicate samples of each soil type were taken for analysis immediately after application and after 1, 2, 3, 7, 14, 59 and 120 days of incubation. Trapping media were taken for analysis when the associated samples were taken for analysis. Additionally, all remaining media were taken for analysis and replaced with fresh media as necessary at 7, 14, 28, 42, 59, 70, 84, 98 and 120 days after application.

Microbial biomass was determined at the time of test substance application and at about the end of the incubation period.

Description of analytical method

The volume of each trapping solution was measured and duplicate aliquots were radio assayed by LSC to determine the amount of radioactivity in the traps.

Soil samples were extracted up to 5 times by shaking at room temperature for 30 minutes with acetonitrile, acetonitrile/water 1:1 v/v and water. Where necessary, the soils were further extracted by reflux for between 4 – 12 hours using 0.1 M NaOH (refluxing with acetonitrile/water and 0.1 M acid were also used to extract some Day 1 samples). Duplicate aliquots of the extracts were removed to determine the levels of radioactivity. The neutral extracts were pooled, concentrated by rotary film evaporation and analysed by HPLC and TLC.

Soil residues were air-dried. For day 0 and 1, non-extractable radioactivity was determined directly by combustion and radio assay of sub samples. For Day 2 – 120 samples, the non-extractable radioactivity was quantified by refluxing a subsample with ethanolamine. The non-extractable residue was then determined from the summation of radioactivity in the ethanolamine extract (LSC) and the resulting slurried solid (combustion). Non-extractable radioactivity in up to 2 samples >10% AR of each soil type was characterised into fulvic acid, humic acid and humin by acid/base fractionation.

Results

The achieved application rate was 1.63 mg/kg dry soil which was equivalent to a use rate of 1223 g a.s. ha^{-1}. Microbial activity of the test soil was confirmed at the start, during and at the end of aerobic incubation (see table 4.1.4.3-1). Results for the extraction, distribution and identification of radioactivity are given in Table 4.1.4.3-2 to 4.1.4.3-9. Recoveries of applied radioactivity in all four soils were in the range 87.7 – 108.9% (Table 4.1.4.3-2– 4.1.4.3-8).

Table 4.1.4.3-2 Distribution and recovery of radioactivity in Bromsgrove soil treated with ^{14}C-S-abscisic acid

Sampling occasion (days)	**0**		**1**		**2**		**3**	
Vessel number	**A01**	**A02**	**A03**	**A04**	**A05**	**A06**	**A07**	**A08**
Neutral Extracts	104.8	103.6	78.2	88.7	65.3	55.8	24.5	45.0
Base Extract	-	-	2.3	3.8	11.5	10.2	20.1	15.6

Total Extractable	104.8	103.6	80.5	92.5	76.8	66.0	44.6	60.6
Non-extractable Residue	1.0	1.0	7.3	5.2	16.7	14.9	29.1	22.9
Ethyl Digol Traps	ns	ns	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
KOH traps	ns	ns	3.2	1.9	6.8	6.8	19.2	15.9
Total volatiles	ns	ns	3.2	1.9	6.8	6.8	19.2	15.9
Total Recovery	105.8	104.6	91.0	99.6	100.3	87.7	92.9	99.4
Sampling occasion (days)	**7**		**14**		**59**		**120**	
Vessel number	**A09**	**A10**	**A11**	**A12**	**A13**	**A14**	**A15**	**A16**
Neutral Extracts	12.7	12.3	9.3	9.1	5.2	6.5	4.8	4.5
Base Extract	18.6	20.7	24.0	22.4	13.0	17.8	11.0	17.5
Total Extractable	31.3	33.0	33.3	31.5	18.2	24.3	15.8	22.0
Non-extractable Residue	31.6	32.8	26.9	24.8	35.4	28.7	15.0	14.0
Ethyl Digol Traps	<0.1	<0.1	<0.1	<0.1	0.2	<0.1	<0.1	<0.1
KOH traps	33.6	32.2	35.2	37.8	55.1	47.3	58.9	58.2
Total volatiles	33.6	32.2	35.2	37.8	55.3	47.3	58.9	58.2
Total Recovery	96.5	98.0	95.4	94.1	108.9	100.3	89.7	94.2

Results are expressed as % applied radioactivity.
ns: no sample

Table 4.1.4.3-3 Proportions of radioactive components in Bromsgrove soil treated with ^{14}C-S-abscisic acid

Time (days)	**0**		**1**		**2**		**3**	
Vessel number	**A01**	**A02**	**A03**	**A04**	**A05**	**A06**	**A07**	**A08**
Component								
Extracts analysed	104.8	103.6	80.5	92.5	76.8	66.0	44.6	60.6
Abscisic acid	102.8	100.9	75.2	85.1	69.8	59.9	21.6	37.1
A	<0.1	<0.1	0.5	0.6	1.1	0.7	-	3.9
A1	-	-	-	-	-	-	5.5	-
A2	-	-	-	-	-	-	<0.1	-
A3	-	-	-	-	-	-	1.7	-
B	<0.1	<0.1	<0.3	<0.4	1.1	<0.5	4.7	3.8
C	<0.1	<0.1	<0.3	<0.4	<0.5	<0.5	2.1	0.9
D	<0.1	<0.1	1.6	2.1	2.3	1.7	4.5	6.2
E	1.8	2.4	3.3	3.9	2.5	2.6	4.5	5.9
Others	0.2	0.3	<0.3	0.7	<0.5	1.1	<0.7	2.7
Unanalysed fraction[a]	-	-	-	-	-	-	-	-
Total extractable	104.8	103.6	80.5	92.5	76.8	66.0	44.6	60.6
Total volatiles	ns	ns	3.2	1.9	6.8	6.8	19.2	15.9
Non-extractable residue	1.0	1.0	7.3	5.2	16.7	14.9	29.1	22.9
Total	105.8	104.6	91.0	99.6	100.3	87.7	92.9	99.4
Time (days)	**7**		**14**		**59**		**120**	
Vessel number	**A09**	**A10**	**A11**	**A12**	**A13**	**A14**	**A15**	**A16**
Component								
Extracts analysed	21.9	23.7	28.3	25.3	11.2	15.3	7.8	14.5
Abscisic acid	7.9	8.7	8.7	7.3	3.0	4.5	1.8	1.6
A	-	-	-	-	3.6	4.9	3.0	-
A1	2.6	2.1	3.4	1.5	-	-	-	2.3
A2	2.2	1.7	3.3	3.3	-	-	-	2.6
A3	1.6	2.3	2.2	3.2	-	-	-	4.3
B	2.5	2.5	3.2	2.8	1.1	1.4	1.0	1.9
C	0.7	0.9	1.4	1.2	0.5	0.8	0.2	0.3
D	2.0	2.3	2.7	2.8	1.4	2.0	1.0	0.9
E	2.2	2.7	2.7	2.1	1.0	1.4	0.9	0.7
Others	0.3	0.5	0.7	1.1	0.4	0.4	<0.2	<0.2
Unanalysed fraction[a]	9.4	9.3	5.0	6.2	7.0	9.0	8.0	7.5
Total extractable	31.3	33.0	33.3	31.5	18.2	24.3	15.8	22.0
Total volatiles	33.6	32.2	35.2	37.8	55.3	47.3	58.9	58.2

Non-extractable residue	31.6	32.8	26.9	24.8	35.4	28.7	15.0	14.0
Total	96.5	98.0	95.4	94.1	108.9	100.3	89.7	94.2

Results are expressed as % applied radioactivity.
Components quantified using HPLC method A except for A1 to A3 which were determined using TLC solvent system D.
Others: Accounts for areas on the chromatogram which sum above zero but which contain no discrete areas of radioactivity.
ns: no sample
[a]: acid insoluble fraction

Table 4.1.4.3-4 Distribution and recovery of radioactivity in Elmton soil treated with ^{14}C-S-abscisic acid

Sampling occasion (days)	**0**		**1**		**2**		**3**	
Vessel number	**B01**	**B02**	**B03**	**B04**	**B05**	**B06**	**B07**	**B08**
Neutral Extracts	105.8	101.2	21.7	17.1	8.6	10.2	9.1	10.4
Base Extract	-	-	5.9	6.9	13.3	13.5	14.2	14.0
Total Extractable	105.8	101.2	27.6	23.9	21.9	23.7	23.3	24.4
Non-extractable Residue	3.0	4.3	69.0	64.0	58.9	50.2	45.6	43.7
Ethyl Digol Traps	ns	ns	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
KOH traps	ns	ns	11.4	15.0	14.5	17.5	22.0	24.4
Total volatiles	ns	ns	11.4	15.0	14.5	17.5	22.0	24.4
Total Recovery	108.8	105.5	108.0	102.9	95.3	91.4	90.9	92.5
Sampling occasion (days)	**7**		**14**		**59**		**120**	
Vessel number	**B09**	**B10**	**B11**	**B12**	**B13**	**B14**	**B15**	**B16**
Neutral Extracts	10.3	9.5	8.4	9.3	15.5	10.6	7.5	7.3
Base Extract	11.5	10.4	15.9	16.2	10.9	12.8	15.5	11.9
Total Extractable	21.8	19.9	24.3	25.5	26.4	23.4	23.0	19.2
Non-extractable Residue	56.6	44.1	41.5	41.6	30.0	33.8	27.1	37.3
Ethyl Digol Traps	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
KOH traps	28.1	26.2	31.2	30.5	40.4	31.2	41.1	42.4
Total volatiles	28.1	26.2	31.2	30.5	40.4	31.2	41.1	42.4
Total Recovery	106.5	90.2	97.0	97.6	96.8	88.4	91.2	98.9

Results are expressed as % applied radioactivity.
ns: no sample

Table 4.1.4.3-5 Proportions of radioactive components in Elmton soil treated with ^{14}C-S-abscisic acid

Time (days)	**0**		**1**		**2**		**3**	
Vessel number	**B01**	**B02**	**B03**	**B04**	**B05**	**B06**	**B07**	**B08**
Component								
Extracts analysed	105.8	101.2	27.6	23.9	21.9	23.7	23.3	24.4
Abscisic acid	103.8	98.2	18.1	15.7	10.7	11.5	8.2	8.4
A	<0.1	<0.1	2.0	3.3	-	4.1	-	-
A1	-	-	-	-	4.3	-	2.2	1.9
A2	-	-	-	-	<0.1	-	2.1	2.4
A3	-	-	-	-	3.6	-	1.5	2.8
B	<0.1	<0.1	<0.4	1.7	0.6	2.8	2.7	2.6
C	<0.1	<0.1	1.2	0.6	0.5	<0.5	1.8	0.9
D	<0.1	<0.1	<0.4	1.1	2.6	2.8	2.4	2.4
E	2.0	2.6	6.3	1.5	2.0	2.5	2.3	2.2
Others	<0.1	0.4	<0.4	<0.5	<0.2	<0.5	<0.4	0.7
Unanalysed fraction[a]	-	-	1.2	1.5	-	-	-	-
Total extractable	105.8	101.2	27.6	23.9	21.9	23.7	23.3	24.4
Total volatiles	ns	ns	11.4	15.0	14.5	17.5	22.0	24.4
Non-extractable residue	3.0	4.3	69.0	64.0	58.9	50.2	45.6	43.7
Total	108.8	105.5	108.0	102.9	95.3	91.4	90.9	92.5
Time (days)	**7**		**14**		**59**		**120**	
Vessel number	**B09**	**B10**	**B11**	**B12**	**B13**	**B14**	**B15**	**B16**
Component								
Extracts analysed	16.5	15.5	21.5	22.3	21.0	16.9	12.5	7.7
Abscisic acid	3.7	2.6	4.1	4.5	3.2	2.7	1.5	0.9
A	-	-	-	-	-	-	-	4.3
A1	1.5	<0.1	2.1	0.6	2.1	1.6	0.9	-

A2	2.9	3.0	4.4	4.6	3.9	5.4	3.6	-
A3	2.2	4.4	2.9	4.7	2.8	2.1	2.3	-
B	2.3	2.6	2.9	2.9	2.5	2.3	1.9	1.3
C	0.7	0.7	1.3	1.4	1.0	0.8	0.8	0.5
D	1.4	1.0	1.8	1.7	1.6	1.1	0.8	0.4
E	1.4	1.1	1.5	1.5	1.3	0.9	0.8	0.4
Others	0.3	<0.2	0.4	0.2	2.8	<0.2	<0.1	<0.1
Unanalysed fraction[b]	5.3	4.4	2.8	3.2	5.4	6.5	10.5	11.5
Total extractable	21.8	19.9	24.3	25.5	26.4	23.4	23.0	19.2
Total volatiles	28.1	26.2	31.2	30.5	40.4	31.2	41.1	42.4
Non-extractable residue	56.6	44.1	41.9	41.5	30.0	33.8	27.1	37.3
Total	106.5	90.2	97.0	97.6	96.8	88.4	91.2	98.9

Results are expressed as % applied radioactivity.

Components quantified using HPLC method A except for A1 to A3 which were determined using TLC solvent system D.

Others: accounts for areas on the chromatogram which sum above zero but which contain no discrete areas of radioactivity.

ns: no sample

[a]: unanalysed acetonitile:water (1:1) reflux and/or 0.1M HCl reflux

[b]: acid insoluble fraction

Table 4.1.4.3-6 Distribution and recovery of radioactivity in Fladbury soil treated with ^{14}C-S-abscisic acid

Sampling occasion (days)	**0**		**1**		**2**		**3**	
Vessel number	**C01**	**C02**	**C03**	**C04**	**C05**	**C06**	**C07**	**C08**
Neutral Extracts	104.0	104.6	45.6	62.9	21.7	21.2	14.3	10.4
Base Extract	-	-	4.8	3.6	10.5	11.0	11.1	10.7
Total Extractable	104.0	104.6	50.4	66.6	32.2	32.2	25.4	21.1
Non-extractable Residue	1.7	1.6	33.7	26.9	41.8	40.4	48.7	57.7
Ethyl Digol Traps	ns	ns	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
KOH traps	ns	ns	11.5	7.9	18.9	19.1	17.6	23.8
Total volatiles	ns	ns	11.5	7.9	18.9	19.1	17.6	23.8
Total Recovery	105.7	106.2	95.6	101.4	92.9	91.7	91.7	102.6
Sampling occasion (days)	**7**		**14**		**59**		**120**	
Vessel number	**C09**	**C10**	**C11**	**C12**	**C13**	**C14**	**C15**	**C16**
Neutral Extracts	9.6	9.2	7.8	7.6	5.9	5.8	4.7	4.8
Base Extract	11.1	10.4	13.8	14.2	5.9	7.1	6.1	8.6
Total Extractable	20.7	19.6	21.6	21.9	11.8	12.9	10.9	13.3
Non-extractable Residue	33.5	34.4	28.8	33.0	38.3	37.9	30.2	33.2
Ethyl Digol Traps	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
KOH traps	39.6	35.0	40.6	34.9	48.8	46.4	52.0	49.3
Total volatiles	39.6	35.0	40.6	34.9	48.8	46.4	52.0	49.3
Total Recovery	93.8	89.0	91.0	89.8	98.9	97.2	93.1	95.8

Results are expressed as % applied radioactivity.
ns: no sample

Table4.1.4.3-7 Proportions of radioactive components in Flatbury soil treated with ^{14}C-S-abscisic acid

Time (days)	**0**		**1**		**2**		**3**	
Vessel number	**C01**	**C02**	**C03**	**C04**	**C05**	**C06**	**C07**	**C08**
Component								
Extracts analysed	104.0	104.6	50.4	66.6	32.2	32.2	25.4	21.1
Abscisic acid	102.2	102.8	42.5	61.1	22.0	21.7	10.2	9.4
A	<0.1	<0.1	2.0	0.7	2.5	2.4	4.3	3.4
A1	-	-	-	-	-	-	-	-
A2	-	-	-	-	-	-	-	-
A3	-	-	-	-	-	-	-	-

B	<0.1	<0.1	<0.4	<0.5	1.2	1.5	2.3	2.4
C	<0.1	<0.1	0.5	<0.5	0.5	0.4	1.2	<0.3
D	<0.1	<0.1	2.3	1.8	2.9	2.4	3.0	2.6
E	1.8	1.8	3.1	2.9	3.1	3.2	3.2	3.2
Others	<0.1	<0.1	<0.4	<0.5	<0.4	0.5	1.2	<0.3
Unanalysed fraction	-	-	-	-	-	-	-	-
Total extractable	104.0	104.6	50.4	66.6	32.2	32.2	25.4	21.1
Total volatiles	ns	ns	11.5	7.9	18.9	19.1	17.6	23.8
Non-extractable residue	1.7	1.6	33.7	26.9	41.8	40.4	48.7	57.7
Total	105.7	106.2	95.6	101.4	92.9	91.7	91.7	102.6
Time (days)	**7**		**14**		**59**		**120**	
Vessel number	**C09**	**C10**	**C11**	**C12**	**C13**	**C14**	**C15**	**C16**
Component								
Extracts analysed	16.9	14.8	17.8	17.5	9.7	11.8	7.3	6.4
Abscisic acid	3.7	3.5	3.9	3.7	1.7	2.6	1.0	0.8
A	-	-	-	-	4.4	4.6	3.6	3.1
A1	1.2	1.0	1.6	1.4	-	-	-	-
A2	2.9	2.6	4.1	3.6	-	-	-	-
A3	2.4	1.9	1.0	1.7	-	-	-	-
B	2.5	2.2	2.5	2.5	1.2	1.6	1.1	1.0
C	1.0	0.8	1.1	1.1	0.7	0.6	0.4	0.5
D	1.4	1.3	1.6	1.7	0.9	1.4	0.6	0.6
E	1.6	1.5	1.7	1.8	0.9	1.1	0.5	0.5
Others	<0.2	<0.1	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2
Unanalysed fraction[a]	3.8	4.8	3.8	4.4	2.1	1.1	3.6	6.9
Total extractable	20.7	19.6	21.6	21.9	11.8	12.9	10.9	13.3
Total volatiles	39.6	35.0	40.6	34.9	48.8	46.4	52.0	49.3
Non-extractable residue	33.5	34.4	28.8	33.0	38.3	37.9	30.2	33.2
Total	93.8	89.0	91.0	89.8	98.6	97.2	93.1	95.8

Results are expressed as % applied radioactivity.
Components quantified using HPLC method A except for A1 to A3 which were determined using TLC solvent system D.
Others: accounts for areas on the chromatogram which sum above zero but which contain no discrete areas of radioactivity.
ns: no sample
[a]: acid insoluble fraction

Table 4.1.4.3-8 Distribution and recovery of radioactivity in Empingham soil treated with ^{14}C-S-abscisic acid

Sampling occasion (days)	**0**		**1**		**2**		**3**	
Vessel number	**D01**	**D02**	**D03**	**D04**	**D05**	**D06**	**D07**	**D08**
Neutral Extracts	97.7	101.0	37.9	35.9	24.9	14.6	7.1	5.6
Base Extracts	-	-	9.7	14.2	11.7	12.2	11.9	11.7
Total Extractable	97.7	101.0	47.5	50.1	36.6	26.8	19.1	17.3
Non-extractable Residue	3.9	5.2	41.8	44.2	38.4	45.1	61.2	71.6
Ethyl Digol Traps	ns	ns	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
KOH traps	ns	ns	7.9	9.1	15.4	15.8	22.1	19.2
Total volatiles	ns	ns	7.9	9.1	15.4	15.8	22.1	19.2
Total Recovery	101.6	106.2	97.2	103.4	90.4	87.7	102.4	108.1
Sampling occasion (days)	**7**		**14**		**59**		**120**	
Vessel number	**D09**	**D10**	**D11**	**D12**	**D13**	**D14**	**D15**	**D16**
Neutral Extracts	8.9	9.1	7.3	6.7	7.6	8.4	6.7	6.3
Base Extract	11.6	9.8	19.4	17.0	8.0	9.9	10.7	12.0
Total Extractable	20.5	18.9	26.7	23.8	15.6	18.3	17.4	18.3
Non-extractable Residue	57.7	58.8	38.0	43.6	39.0	36.1	34.6	33.1
Ethyl Digol Traps	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
KOH traps	16.8	21.4	28.3	27.7	41.8	41.6	40.3	45.5
Total volatiles	16.8	21.4	28.3	27.7	41.8	41.6	40.3	45.5
Total Recovery	95.0	99.1	93.0	95.1	96.4	96.0	92.3	96.9

Results are expressed as % applied radioactivity.
ns: no sample

Table 4.1.4.3-9 Proportions of radioactive components in Empingham soil treated with ^{14}C-S-abscisic acid

Time (days)	**0**		**1**		**2**		**3**	
Vessel number	**D01**	**D02**	**D03**	**D04**	**D05**	**D06**	**D07**	**D08**
Component								
Extracts analysed	97.7	101.0	47.5	50.1	36.6	26.8	19.1	17.3
Abscisic acid	95.2	98.4	39.1	36.2	24.9	17.6	7.1	7.6
A	<0.2	<0.1	1.6	2.7	2.4	2.5	2.9	3.1
A1	-	-	-	-	-	-	-	-
A2	-	-	-	-	-	-	-	-
A3	-	-	-	-	-	-	-	-
B	<0.2	<0.1	0.7	2.2	1.4	1.0	2.3	2.1
C	<0.2	<0.1	0.5	1.3	1.4	<0.6	1.5	0.6
D	<0.2	0.6	2.5	3.3	3.0	2.6	2.9	1.7
E	2.3	1.7	3.1	4.4	3.0	3.2	2.3	2.2
Others	0.2	0.3	<0.5	<0.6	0.4	<0.6	<0.4	<0.6
Unanalysed fraction[a]	-	-	1.3	1.5	-	-	-	-
Total extractable	97.7	101.0	47.5	50.1	36.6	26.8	19.1	17.3
Total volatiles	Ns	ns	7.9	9.1	15.4	15.8	22.1	19.2
Non-extractable residue	3.9	5.2	41.8	44.2	38.4	45.1	61.2	71.6
Total	101.6	106.2	97.2	103.4	90.4	87.7	102.4	108.1
Time (days)	7		14		59		120	
Vessel number	D09	D10	D11	D12	D13	D14	D15	D16
Component								
Extracts analysed	15.5	16.3	26.5	22.5	15.6	16.0	12.9	11.5
Abscisic acid	3.7	3.6	8.7	9.0	3.1	3.3	1.9	2.0
A	-	-	-	-	-	-	-	-
A1	0.6	0.9	5.0	3.1	1.1	2.1	1.0	1.1
A2	2.5	2.9	3.1	2.1	4.0	3.0	4.6	2.7
A3	2.8	3.3	1.6	1.1	2.3	2.7	1.3	1.4
B	2.4	2.3	2.5	2.1	2.0	2.0	1.6	1.6
C	0.8	0.8	1.2	1.0	0.7	0.6	0.8	0.7
D	1.4	1.3	1.8	1.7	1.2	1.1	0.9	1.1
E	1.1	1.2	1.8	1.8	1.1	1.0	0.9	0.9
Others	0.2	<0.2	0.8	0.6	0.2	0.2	<0.2	<0.2
Unanalysed fraction[b]	5.0	2.6	0.2	1.3	-	2.3	4.5	6.8
Total extractable	20.5	18.9	26.7	23.8	15.6	18.3	17.4	18.3
Total volatiles	16.8	21.4	28.3	27.7	41.8	41.6	40.3	45.5
Non-extractable residue	57.7	58.8	38.0	43.6	39.0	36.1	34.6	33.1
Total	95.0	99.1	93.0	95.1	96.4	96.0	92.3	96.9

Results are expressed as % applied radioactivity.

Components quantified using HPLC method A except for A1 to A3 which were determined using TLC solvent system D.

Others: accounts for areas on the chromatogram which sum above zero but which contain no discrete areas of radioactivity.

ns: no sample

[a]: unanalysed acetonitile:water (1:1) reflux and/or 0.1M HCl reflux

[b]: acid insoluble fraction

Transformation of parent compound

S-abscisic acid was rapidly degraded in all soils and had decreased to 0.8 – 2.0% AR after 120 days (Table 4.1.4.3-3 – 4.1.4.3-9). There were seven discrete extractable products of S-abscisic acid degradation that

individually accounted for no more than 6.3% AR at any single interval and no metabolite was ever present above 4.5% for more than two consecutive intervals.

Unextractable residues became larger as S-abscisic acid disappeared. They too decreased with time as CO_2 levels increased to become the dominant product from soil metabolism of S-abscisic acid, accumulating to half of the applied radiocarbon by the end of the study. Since two to seven metabolites were present in varying low concentration throughout the study, a pattern of formation and decline of individual degradants could not be documented other than the accumulation of CO_2 from mineralization of S-abscisic acid. The various low level metabolites seem to be ultimately degraded to CO_2 about as fast as they were formed, thus not allowing any metabolite to accumulate to a level at which its structure could be identified.

Degradation rates

The decline of S-abscisic acid in aerobic soil with time was modelled using simple first order models. The kinetic data are summarised in Table4.1.4.3-10. The DT_{50} values were 2.2 days (Bromsgrove), 0.44 days (Elmton), 0.98 days (Fladbury) and 0.80 days (Empingham). DT_{90} values were 7.4 days (Bromsgrove), 1.5 days (Elmton), 3.3 days (Fladbury) and 2.7 days (Empingham).

Table 4.1.4.3-10 Kinetic data for the decline of S-abscisic acid in soil

Soil	Textural class	Organic carbon (%)	pH **(0.01M $CaCl_2$)**	**DT_{50} (days)**	**DT_{90} (days)**
Bromsgrove	sandy loam	1.4	4.3	2.2	7.4
Elmton	clay loam	4.2	7.1	0.44	1.5
Fladbury	sandy clay	3.5	6.1	0.98	3.3
Empingham	clay loam	5.6	7.2	0.80	2.7

Conclusions

S-abscisic acid was rapidly degraded in aerobic soil with DT_{50} values ranging from 0.44 to 2.2 days at 20°C. The degradation of S-abscisic acid resulted in the formation of carbon dioxide and the incorporation of residues into bound fractions. Low levels of some intermediate products were also detected although not identified.

Remarks

The study is acceptable and the endpoints reliable.

The labelled molecule used was ^{14}C-labelled on a methyl group. According to the OECD 307 guideline the molecule should be labelled on the most stable C-atom. The methyl C-atom seems not the most stable C-atom. The metabolites formed and measured were all below 10% or at two consecutive sampling events below 5.0%. The question raises if a more stable C-atom was labelled, metabolites would have been formed that were above 10% of the applied radioactivity. However, the scientific literature showed a large number of research articles have been written on the metabolism of S-abscisic acid. The metabolic pathways have been worked out in great detail. The 8'-carbon atom (position of the radiolabel) is never cleaved off at any early stage in the metabolic scheme (Figure 4.1.4.3-1). Therefore, there is no reason to require additional studies for S-abscisic acid with a different radiolabelled atom.

4.1.4.4 STUDY 4 – Photolytic degradation

Study reference: STUDY IIA, 7.6/001

Characteristics

reference	:	Kane, T.	incubation time	:	72 hours – 8 days
study type	:	aqueous photolysis	nominal concentration	:	1.5 mg L^{-1}
year of execution	:	2011	pH/temperature	:	pH 4,7,9 / temp 25°C
GLP statement	:	yes	quantum yield	:	0.015-0.018
guideline	:	OECD 316	conclusion	:	DT_{50} 1.2 – 2.5 days
test substance	:	^{14}C-S-abscisic acid	acceptability	:	acceptable
purity	:	> 97%			

Study Design

Solutions of S-abscisic acid 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. The initial concentration of S-abscisic acid used (1.5 mg/litre) was less than one half of its reported aqueous solubility. The light source had a similar spectral energy distribution to that of natural sunlight. Control solutions were incubated in darkness to determine the stability of S-abscisic acid by non-photolytic processes (i.e. hydrolysis). The photolytic degradation of S-abscisic acid was monitored using high performance liquid chromatography (HPLC) and thin-layer chromatography (TLC).

Prior to the main experiments described above, preliminary experiments were performed. This was carried out to assess the likely duration of irradiation required in the main experiment (and therefore determine the sampling intervals) and to assess the production of any volatile radioactivity.

The quantum yield of the photolysis of S-abscisic acid in aqueous buffer at each pH was determined.

Test System

Irradiation source

The study was conducted using a Suntest accelerated exposure unit (Heraeus Equipment Ltd, Brentwood, Essex, UK) fitted with a xenon arc light source. A system of mirrors and filters prevented ultra-violet radiation with a wavelength of less than 290 nm from reaching the test vessels. Hence the emission spectrum produced was similar to that of natural sunlight.

Irradiance measurements

The spectral energy distributions of the lamp source and natural sunlight were measured using a Spectrad system (model SR-2100-01P) incorporating a 1680B double spectrometer (250 - 800 nm) with a fibre linked integrating sphere (Glen Spectra Ltd, Stanmore, Middlesex, UK). The spectrophotometer incorporated a 1200 g/mm grating blazed at 330 nm, fixed entrance and exit slits, an R955 PMT detector and an automated filter wheel with order blocking filters (280 nm and 475 nm).

Measurements of light intensity (irradiance) were made at five representative positions in the Suntest apparatus at the beginning and end of each of the irradiation periods. Measurements were made at 2.5 nm intervals over the wavelength range 250 - 800 nm. The integrating sphere was positioned at the same

distance under the light source as the surface of the solutions during irradiation. The incident light was measured through the top part of a test vessel identical to those used in the study.

Test vessels and apparatus

Solutions for irradiation were contained in cylindrical vessels of internal diameter 2.5 cm and height 8.5 cm and constructed of borosilicate glass, with a quartz window at one end which transmits a wavelength spectrum similar to that of natural sunlight. Where the collection of volatile radioactivity was required, vessels were equipped with a double side-arm which acted as inlet and outlet ports for the collection otherwise vessels equipped with a single side arm were used. The vessels were irradiated from above through the end of the cylinder with the quartz window. An extra irradiated vessel was fitted with an in-dwelling thermocouple probe for temperature monitoring. For irradiation the vessels were placed in sockets in a water-cooled block within the photolysis apparatus. The cooling block also incorporated magnetic field switching devices to drive Teflon-coated magnetic stirrer bars in the test solutions.

The non-irradiated (dark control) test solutions were incubated in sealed borosilicate glass vessels. These vessels were placed on an oscillating shaker in darkness in a temperature-controlled room.

Where required the test vessels were incorporated into air flow-through systems to trap volatile radioactivity. A stream of humidified air was drawn through the test vessels using a pump, at a flow rate of approximately 5 mL minute^{-1}, and then through a series of trapping solutions as follows:

Empty vessel

Trap 1: Ethyl digol

Trap 2: 1 M aqueous potassium hydroxide solution with phenolphthalein indicator

Trap 3: 1 M aqueous potassium hydroxide solution with phenolphthalein indicator

Microbiological filters (0.2 µm, Sartorius AG, Epsom, UK) were incorporated in to each air flow-through system on either side of the test vessels in order to ensure sterility of the test solutions.

Preparation of buffers

pH 4 buffer (0.01 M)

Sodium dihydrogen orthophosphate dihydrate (1.5604 g) was dissolved in ca 900 ml water and the pH adjusted to pH 4.0 by the addition of aqueous HCl solution. The solution was made up to a volume of 1 litre with water. The pH of the final solution was checked.

pH 7 buffer (0.01 M)

Potassium dihydrogen orthophosphate (1.3603 g) was dissolved in ca 900 ml water and the pH adjusted to pH 7.0 by the addition of aqueous NaOH solution. The solution was made up to a volume of 1 litre with water. The pH of the final solution was checked.

pH 9 buffer (0.01 M)

Boric acid (0.6182 g) was dissolved in ca 900 ml water and the pH adjusted to pH 9.0 by the addition of aqueous NaOH solution. The solution was made up to a volume of 1 litre with water. The pH of the final solution was checked.

Sterilisation of buffers and glassware

The buffer, test vessels and glassware required for the preparation of the test solutions were autoclaved at 121°C for 15 minutes prior to use. Aseptic techniques were used throughout for the preparation of the test solutions. These procedures were carried out in a laminar flow cabinet. Samples of test solutions were taken for microbiological examination at the last sampling interval to confirm that sterile conditions had been maintained.

Test Methods

Preparation and application of test substance

A stock solution was prepared by combining an aliquot of a solution of ^{14}C-S-abscisic acid (1.4 mL, 0.18 mg S-abscisic acid mL^{-1}) with an aliquot of a solution of non-radiolabelled S-abscisic acid (0.82 mL, 0.92 mg S-abscisic acid mL^{-1}) and diluted to a known volume (5 mL) with ethanol such that the concentration of S-abscisic acid was nominally 0.2 mg mL^{-1}. The actual concentration of S-abscisic acid in the stock solution was determined by taking triplicate aliquots (10 µL) directly for radio assay by liquid scintillation counting (LSC).

Portions of the buffer solutions (600 mL) were treated with the stock solution to give a nominal concentration of S-abscisic acid of 1.5 mg L^{-1}. The amount of organic solvent present in the treated buffer solutions was less than 1% v/v. Triplicate aliquots (1 mL) of the solutions were taken for LSC. A portion (20 mL) of each treated solution was transferred to a pre-weighed test vessel which was then reweighed and incorporated into the test apparatus.

Irradiation and incubation of test solutions

Irradiated test solutions were maintained at 25 ± 2°C and were stirred continuously with Teflon-coated magnetic stirrer bars. The irradiation was continuous except for short periods when vessels were being removed for analysis. The non-irradiated vessels were maintained in darkness at 25 ± 2°C and were shaken continuously.

Sampling intervals

In the pH 4 experiment duplicate irradiated and non-irradiated samples were taken for analysis at approximately 1, 6, 12, 48 and 72 hours after the start of irradiation or incubation. In the pH 7 and 9 experiments duplicate irradiated and non-irradiated samples were taken for analysis at approximately 1 hour, then 1, 2, 5, and 8 days after the start of irradiation or incubation. Additional duplicate vessels were taken for analysis immediately after test substance application and provided a zero-time analysis for both irradiated and dark control experiments.

One irradiated and one non-irradiated sample from each buffer solution type were used for pH determination and sterility testing after either 72 hours (pH 4 experiment) or 8 days (pH 7 and 9 experiments) of irradiation/incubation.

Sample analysis

Each sampled test vessel was weighed and duplicate aliquots (1 mL by weight) of the solution were taken for radio assay. Aliquots of each solution were analysed directly by HPLC with radio detection.

For the vessels taken at the final sampling interval the associated trapping solutions were taken for analysis. The total volume of each solution was measured and duplicate aliquots (1 mL) taken for radio assay.

Analysis of test solutions was started on the day of sampling. Solutions not undergoing analysis were stored at <-15°C.

Quantum Yield Determination

Preparation and irradiation/incubation of the chemical actinometer

An aliquot (1 mL) of a 2 mg mL^{-1} solution of *p*-nitroacetophenone (PNAP) in acetonitrile was diluted up to 100 mL of a 4 g/L (0.05 M) solution of pyridine in sterile water. The concentration of PNAP in the actinometer solution was 21.44 µg mL^{-1} (pH 4 experiment), 45.22 µg/mL (pH 7 experiment) and 52.67 µg/mL (pH 9 experiment) as determined from a calibration line generated by analysing standard solutions of PNAP of various concentrations by HPLC and measuring the area of the peak in the absorbance chromatogram.

Portions (*ca* 20 mL) of actinometer solution were aseptically dispensed into borosilicate glass tubes, as used for the irradiated buffer solutions but with single side arms, which were then sealed.

Duplicate actinometer solutions were irradiated/incubated alongside the treated buffer solutions.

Sampling and analysis of actinometer solutions

Aliquots (ca 0.2 mL) of the irradiated and non-irradiated actinometer solutions were aseptically removed for analysis at the same sampling times (for each experiment). The remaining solutions were returned to the Suntest apparatus or dark control incubation.

Aliquots of each sampled actinometer solution were analysed by HPLC. The concentration of PNAP in each sample was determined by measurement of the peak area in the absorbance chromatogram in conjunction with the calibration line.

Solutions not undergoing analysis were stored at <-15°C.

Analytical Methods

Measurement of pH

Measurements of the pH of the test solutions were made using a pH meter that had been calibrated prior to each measurement using standard buffer solutions of the appropriate pH.

Microbiological examination of test solutions

Aliquots (1 mL) of test solution were used to prepare Tryptone Soya Agar (Oxoid CMO 131), tempered to 45°C, and pour-plates (in duplicate) were prepared. The plates were incubated at 30 - 35°C for at least five days. After incubation the plates were examined for bacterial or fungal growth and the results recorded.

Measurement of radioactivity

Radioactivity was measured by liquid scintillation counting (LSC), using either LKB-Wallac model 1219 Rackbeta Spectral or Wallac model 1409 and 1410 liquid scintillation counters with automatic quench correction (Wallac Oy, Turku, Finland). Radioactivity in gross amounts of less than twice the background level was considered to be below the limit of accurate determination.

Aliquots of liquid samples were mixed with Ultima Gold™ scintillator (PerkinElmer Life & Analytical Sciences, Boston, USA) for measurement of radioactivity.

High performance liquid chromatography (HPLC)

HPLC was carried out with UV and/or radioactivity detection using the following conditions (Table 4.1.4.4-1 and Table 4.1.4.4-2).

Table 4.1.4.4-1 HPLC method 1 used for radiochemical purity measurements and sample analysis

Column:	Luna 5µ (25 cm × 4.6 mm)		
Column temperature:	Ambient		
Mobile phase:	A:Water containing acetic acid at 0.2% (v/v) B: Methanol containing acetic acid at 0.2% (v/v)		
Gradient:	Time (minutes)	%A	%B
	0	100	0
	20	0	100
	30	0	100
Flow rate:	1 mL/minute		
Radioactivity detector:	Flow-through system using a liquid scintillant cell		
UV detection wavelength:	254 nm		

Table 4.1.4.4-2 HPLC method 2 used for actinometer analysis

Column:	Hichrom RPB 5µ (15 cm × 4.6 mm)
Column temperature:	Ambient
Mobile phase:	Acetonitrile/water/acetic acid 60/40/1 (v/v/v)
Flow rate:	2 mL/minute
UV detection wavelength:	280 nm
Software:	Laura, version 1.4a (LabLogic Systems Ltd, Sheffield, UK)

Assessment of chromatographic correspondence of S-abscisic acid and reference substances to radioactive chromatogram components was made after co-injection of the sample and a solution of non-radiolabelled S-abscisic acid. Correspondence was assessed by examination of the radioactivity and UV absorbance chromatograms (taking into account the time delay between the radioactivity and absorbance detectors) and comparison of the UV chromatogram with that obtained from the sample alone.

Thin-layer chromatography (TLC)

Normal phase TLC was carried out on pre-layered, glass-backed Merck silica gel 60 F_{254} plates, of layer thickness 0.25 mm. The developing solvent system was chloroform/methanol (9/1, by volume).

Two-dimensional chromatograms of the developed plates were obtained using a Fujifilm FLA-5000 Bio Image Analyser (Fuji Photo Film Co). Linear scaled radio chromatograms were generated using TINA software (version 2.09, Raytest Isotopenmessgerate GmbH).

Non-radiolabelled S-abscisic acid and reference substances were detected by the quenching of the UV fluorescent indicator on the TLC plate.

For establishing chromatographic correspondence of S-abscisic acid and reference substances to radioactive chromatogram components, a solution of the test substance was applied to the plate as a 2 cm wide band. The sample solution was also applied as a 2 cm wide band which overlapped with the test substance solution. Following development of the TLC plate, chromatographic correspondence was assessed by visual inspection of the plate under UV light and its associated radio chromatogram.

Calculations

Kinetic analysis

The decline of S-abscisic acid in solution was calculated by applying first order reaction kinetics.

First order kinetics are described by the equation:

Equation B.8.4.2-01 First order kinetics

$$M = M_0 \times e^{-kt}$$

where M is the amount of substance at time t,

M_0 is the amount of substance at zero-time and k is the rate constant.

DT_{50}, DT_{75} and DT_{90} values are:

Equation B.8.4.2-02 DT50, DT75 and DT_{90} values

$$DT_{50} = \ln 2/k \qquad DT_{75} = \ln 4/k \qquad DT_{90} = \ln 10/k$$

Calculation of equivalent days natural sunlight

The calculation of the equivalent days of natural sunlight is shown below.

Calculation of the equivalent duration of natural sunlight Latitude 40ºN, summer sunlight

Light in the wavelength range 290 – 400 nm was chosen for comparative purposes. To relate the light intensity of the xenon arc source to natural sunlight (summer sunlight at latitude 40°N) various corrections were necessary. Sunlight measurements were made on 03 June 2010 at the research laboratory (latitude 52°N). Published data indicate that the measured intensity on this day would be about 84% of the midday midsummer intensity at latitude 40°N. Additionally it was assumed that the average daily radiation intensity from the sun is about 75% of the peak intensity over a 12-hour period, whereas the radiation in the Suntest was of constant intensity. The equivalent days of latitude 40°N summer sunlight received by each sample was calculated from the formula:

Equation B.8.4.2-03 Equation to calculate the equivalent days

$$\text{Equivalent days} = \frac{h \times r \times 0.84}{0.75 \times 12}$$

where h is the duration of irradiation in the Suntest apparatus in hours;

r is the ratio of intensity of Suntest irradiation to sunlight (at latitude 52°N);

0.84 is the factor to correct for the difference in latitude (40°N/52°N) and the date difference between the sunlight measurement and midsummer;

0.75 is the factor to correct for the diurnal variation of natural sunlight;

12 is the factor to convert hours to days.

Example: pH 7 experiment

Samples irradiated for 120 hours (nominal 5 days).

The mean Suntest irradiance over the range 290 – 400 nm was 37.2 Wm^{-2}

The measured sunlight irradiance over the range 290 – 400 nm was 48.3 Wm^{-2}

Therefore, equivalent days of latitude 40°N summer sunlight received by this layer

$$= \frac{120 \times (37.2/48.3) \times 0.84}{0.75 \times 12} = 8.63 \text{ days}$$

Calculation of quantum yield

Determination of the quantum yield for the photo degradation of S-abscisic acid. The example data shown is for the pH 7 experiment. The following parameters were measured/calculated:

The average molar extinction coefficients ($\varepsilon\lambda$) for the wavelength intervals for the test substance ($\varepsilon\lambda^c$) and actinometer ($\varepsilon\lambda^a$). At any wavelength where $\varepsilon\lambda$ was less than 10, $\varepsilon\lambda$ was not calculated.

Determination of the average irradiance ($L\lambda$) of the artificial light source for each wavelength interval

Calculation of $\sum\varepsilon\lambda^a L\lambda$ and $\sum\varepsilon\lambda^c L\lambda$ at each wavelength interval where $\varepsilon\lambda$ is greater than 10.

Calculation of the quantum yield of the actinometer (Φ^a). For the actinometer *p*-nitroacetophenone/ pyridine, the quantum yield is calculated according to the following equation:

Equation B.8.4.2-04 Quantum yield of the actinometer

$$\Phi^a = 0.0169[\text{pyridine}]$$

where [pyridine] is the initial molar incubation of pyridine in the actinometer solution, *i.e.* 0.05 M.

Therefore $\Phi^a = 0.0169 \times 0.05 = 0.000845$

Determination of the rate constants for degradation of the actinometer (kp^a) and test substance (kp^c) from the photolysis experiment (using pH 7 buffer). The rate constants are corrected for any losses which occurred in the non-irradiated samples.

Equation B.8.4.2-05 Rate constant correction

$$kp\ (corrected) = kp\ (irradiated) - kp\ (control)$$

The quantum yield of the test substance (Φ^c) is given by the following equation:

Equation B.8.4.2-06 Quantum yield

$$\Phi^c = \frac{kp^c \times \sum \varepsilon\lambda^a L\lambda \times \Phi^a}{kp^a \times \sum \varepsilon\lambda^c L\lambda}$$

Calculation of theoretical lifetime determination

The theoretical lifetime of S-abscisic acid at the surface of aqueous systems was calculated from the quantum yield by use of the computer modelling software GCSOLAR. GCSOLAR is made available by the US EPA and downloaded from its official website.

Results

Preliminary experiment

The results of the preliminary experiments are shown below. The total recoveries of applied radioactivity (AR) were in the range 95.3 to.100.6% AR (Table 4.1.4.4-3). The radioactivity detected in the trapping solutions at 3 hours accounted for up to 0.1% AR. After three hours of irradiation, S-abscisic acid accounted for 47.8% (pH 4), 46.6% (pH 7) and 47.2% (pH 9; 4.1.4.4-4).

Table 4.1.4.4-3 Preliminary Experiment - Recoveries of radioactivity from irradiated test solutions

Sampling interval (hours)	In solution	Volatiles		Total recovery
pH 4 buffer		**Organic**	**$^{14}CO_2$**	
0	95.3	na	na	95.3
1	100.1	nd	nd	100.1
3	99.5	nd	nd	99.5
Sampling interval (hours)	**In solution**	**Volatiles**		**Total recovery**
pH 7 buffer		**Organic**	**$^{14}CO_2$**	
0	100.1	na	na	100.1
1	100.0	nd	nd	100.0
3	100.0	0.1	0.1	100.2
Sampling interval (hours)	**In solution**	**Volatiles**		**Total recovery**
pH 9 buffer		**Organic**	**$^{14}CO_2$**	
0	99.6	na	na	99.6
1	99.5	nd	nd	99.5
3	100.6	nd	nd	100.6

Results expressed as % applied radioactivity
na not applicable
nd not detected

Table 4.1.4.4-4 Preliminary Experiment - Proportions of radioactive components in irradiated test solutions

Component	Approximate retention time (minutes)	Sampling interval (hours) pH 4 buffer		
		0	1	3
1	18.6	0.5	2.2	2.4
2	19.0	nd	41.4	34.3
S-abscisic acid	19.6	92.5	47.4	47.8
4	20.0	nd	1.8	4.3
5	20.5	nd	nd	1.6
6	23.5	1.5	4.9	7.3
Others [a]	-	0.8	2.3	1.9
Component	**Approximate retention time (minutes)**	**Sampling interval (hours) pH 7 buffer**		
		0	**1**	**3**
1	18.6	nd	nd	0.8
2	19.0	nd	37.7	45.8
S-abscisic acid	19.6	96.7	52.0	46.6
4	20.0	0.2	0.2	1.7
5	20.5	nd	nd	nd
6	23.5	2.1	3.4	3.7
Others [a]	-	1.1	4.4	1.4
Component	**Approximate retention time (minutes)**	**Sampling interval (hours) pH 9 buffer**		
		0	**1**	**3**
1	18.6	0.4	nd	nd
2	19.0	nd	40.0	45.8
S-abscisic acid	19.6	95.9	57.1	47.2
4	20.0	nd	nd	1.1
5	20.5	nd	0.3	nd
6	23.5	2.3	1.9	3.4
Others [a]	-	1.0	0.2	1.9

Proportions are expressed as % applied radioactivity

nd not detected

[a] radioactivity not associated with designated components

Based on this preliminary work, the main pH 4 experiment was conducted for 72 hours irradiation/incubation whilst the main pH 7 and pH 9 experiments were conducted for 8 days irradiation/incubation. As only a small amount of volatile radioactivity was detected, the provision for collecting volatiles was applied only to samples taken at the final sampling occasions.

Characterisation of aqueous solutions

The sterile buffer solutions used in the main experiment were characterised and results given below (Table 4.1.4.4-5 and Table 4.1.4.4-6).

Table 4.1.4.4-5 Characterization of buffer solutions

Parameter pH 4 buffer	**Value**
Electrical condutivity	988 µs
pH	4.01
Dissolved oxygen	9%
Parameter pH 7 buffer	**Value**
Electrical condutivity	965 µs
pH	7.02
Dissolved oxygen	7%
Parameter pH 9 buffer	**Value**

Electrical condutivity	903 µs
pH	8.99
Dissolved oxygen	6%

Table 4.1.4.4-6 Sterility Data

Buffer	Irradiated	Dark control
pH 4[a]	No growth	No growth
pH 7[b]	No growth	No growth
pH 9[b]	No growth	No growth

[a] after 72 hours irradiation/incubation

[b] after 8 days irradiation/incubation

Radioactivity applied to the test solutions

The initial concentrations of radioactivity and S-abscisic acid in the test solutions were as shown below (Table 4.1.4.4-7).

Table 4.1.4.4-7 Initial concentrations of radioactivity and S-abscisic acid

Buffer	Radioactivity (dpm mL^{-1})	Specific activity (dpm μg^{-1})	S-abscisic acid (mg L^{-1})
pH 4	109643	69851	1.57
pH 7	107960	70661	1.53
pH 9	106725	68382	1.56

Irradiation and incubation conditions

The temperature of irradiated pH 4 solutions and all non-irradiated solutions remained within the range 25 ± 2°C except for short periods when samples were removed from the Suntest unit for analysis. For the irradiated pH 7 samples, the temperature rose above the upper limit for two periods; both for one hour between days 7 and 8 of irradiation. For the irradiated pH 9 samples, the temperature rose above the upper limit for one hour between 6 and 7 hours of irradiation due to an equipment failure. The samples were removed to dark refrigerated storage (ca 4°C) prior to re commencing the irradiation the following day. The results obtained indicate that these deviations did not affect the integrity of the data.

The duration of equivalent natural summer sunlight at latitude 40°N that each irradiated sample was exposed to was calculated by reference to a measurement of the spectral energy distribution of natural sunlight. The results are shown in Table 4.1.4.4-8.

Table 4.1.4.4-8 Equivalent times of natural sunlight received by the irradiated test solutions

Nominal sampling interval (hours) pH 4 40° N	Actual duration of irradiation (hours)	Equivalent duration of natural sunlight (days)
1	1	0.08
6	6	0.45
12	11.9	0.89
48	48	3.55
72	72	5.30
Nominal sampling interval (days) pH 7 40° N	**Actual duration of irradiation (hours)**	**Equivalent duration of natural sunlight (days)**
1[a]	1	0.07

1	24	1.73
2	48	3.45
5	120	8.63
8	192	13.8

Nominal sampling interval (days) pH 9 40° N	Actual duration of irradiation (hours)	Equivalent duration of natural sunlight (days)
1[a]	1	0.07
1	24	1.66
2	47.9	3.30
5	120	8.30
8	192	13.32

[a] 1 hour irradiation

Sterility and pH measurements

No evidence of contamination by micro-organisms was found in the irradiated or dark control buffer solutions taken for analysis taken at the end of the irradiation/incubation period.

The pH values of the buffer solutions following incubation/ irradiation were 4.64 – 4.70 (pH 4 experiment), 7.08 – 7.12 (pH 7 experiment) and 9.03 – 9.05 (pH 9 experiment).

Recovery of radioactivity

Recoveries of radioactivity from samples of irradiated buffer were in the range 93.4% AR to 101.8% AR (pH 4), 95.2% AR to 102.1% AR (pH 7) and 90.7% AR to 100.0% AR (pH 9).

For the non-irradiated samples recoveries of radioactivity were greater than 96.7% AR (range 96.7% - 103.9%AR) in all but one sample in which a recovery of 121.5%AR was measured (Table 4.1.4.4-9 to 4.1.4.4-11).

Table 4.1.4.4-9 Recovery of radioactivity in pH 4 buffer after application of ^{14}C-S-abscisic acid at 1.5 mg/L

Sampling interval (hours)	In solution	Volatiles irradiated		Total recovery
		Organic	$^{14}CO_2$	
1	97.1	na	na	97.1
1	99.0	na	na	99.0
6	97.2	na	na	97.2
6	97.3	na	na	97.3
12	93.4	na	na	93.4
12	96.5	na	na	96.5
48	95.2	na	na	95.2
48	95.5	na	na	95.5
72	94.4	0.3	2.5	97.2
72	100.0	0.1	1.7	101.8
Sampling interval (hours)	**In solution**	**Volatiles non-irradiated**		**Total recovery**
		Organic	**$^{14}CO_2$**	
0	97.8	na	na	97.8
0	98.1	na	na	98.1
1	97.5	na	na	97.5
1	98.0	na	na	98.0
6	97.6	na	na	97.6
6	97.6	na	na	97.6
12	97.5	na	na	97.5
12	97.7	na	na	97.7

48	97.3	na	na	97.3
48	97.5	na	na	97.5
72	102.3	nd	0.1	102.4
72	103.9	nd	nd	103.9

Results expressed as % applied radioactivity
na not applicable
nd not detected

Table 4.1.4.4-10 Recovery of radioactivity in pH 7 buffer after application of ^{14}C-S-abscisic acid at 1.5 mg/L

Sampling interval (days)	In solution	Volatiles irradiated		Total recovery
		Organic	$^{14}CO_2$	
1[a]	99.8	na	na	99.8
1[a]	97.8	na	na	97.8
1	101.6	na	na	101.6
1	102.1	na	na	102.1
2	98.4	na	na	98.4
2	95.3	na	na	95.3
5	96.2	na	na	96.2
5	96.6	na	na	96.6
8	91.9	0.2	3.8	95.9
8	91.8	0.2	3.2	95.2
Sampling interval (days)	**In solution**	**Volatiles non-irradiated**		**Total recovery**
		Organic	**$^{14}CO_2$**	
0	99.9	na	na	99.9
0	100.0	na	na	100.0
1[a]	99.7	na	na	99.7
1[a]	99.7	na	na	99.7
1	102.1	na	na	102.1
1	102.5	na	na	102.5
2	100.4	na	na	100.4
2	102.1	na	na	102.1
5	100.1	na	na	100.1
5	99.2	na	na	99.2
8	121.5	nd	<0.1	121.5
8	98.8	nd	0.1	98.9

Results expressed as % applied radioactivity
[a] sampling time in hours
na not applicable
nd not detected

Table 4.1.4.4-11 Recovery of radioactivity in pH 9 buffer after application of ^{14}C-S-abscisic acid at 1.5 mg/L

Sampling interval (days)	In solution	Volatiles irradiated		Total recovery
		Organic	$^{14}CO_2$	
1[a]	100.0	na	na	100.0
1[a]	99.7	na	na	99.7
1	98.5	na	na	98.5
1	99.1	na	na	99.1
2	96.9	na	na	96.9
2	98.3	na	na	98.3
5	98.5	na	na	98.5
5	93.8	na	na	93.8
8	88.5	0.2	2.0	90.7
8	92.6	0.7	2.9	96.2
Sampling interval (days)	**In solution**	**Volatiles non-irradiated**		**Total recovery**
		Organic	**$^{14}CO_2$**	
0	99.9	na	na	99.9
0	100.0	na	na	100.0

1 [a]	100.0	na	na	100.0
1 [a]	99.6	na	na	99.6
1	99.5	na	na	99.5
1	99.2	na	na	99.2
2	99.7	na	na	99.7
2	99.6	na	na	99.6
5	96.7	na	na	96.7
5	102.9	na	na	102.9
8	98.9	nd	nd	98.9
8	99.1	nd	<0.1	99.1

Results expressed as % applied radioactivity
[a] sampling time in hours
na not applicable
nd not detected

In the irradiated samples, volatile radioactivity in the potassium hydroxide traps reached a maximum of 0.8% AR after 8 days (192 hours) irradiation and this volatile radioactivity was shown to be associated with carbon dioxide. Volatile radioactivity was not detected in the non-irradiated samples.

Chromatographic analysis

Proportions of S-abscisic acid and photolysis products in test solutions were determined by reversed phase HPLC and normal phase TLC. Results are shown in Table 4.1.4.4-11 to 4.1.4.4-16.

The proportions of S-abscisic acid were quantified using HPLC methodology.

In the pH 4 experiment S-abscisic acid represented a mean of 95.0% AR at zero-time, declining to a mean value of 47.3% AR after 1 hour of irradiation and thereafter to a mean value of 6.6% AR at 48 hours irradiation. No S-abscisic acid was detected after 72 hours.

In the pH 7 experiment S-abscisic acid represented a mean of 97.8% AR at zero-time, declining to a mean value of 56.2% AR after 1 hour of irradiation and thereafter to a mean value of 5.5% AR at 5 days irradiation. No S-abscisic acid was detected after 8 days irradiation.

In the pH 9 experiment S-abscisic acid represented a mean of 97.8% AR at zero-time, declining to a mean value of 58.7% AR after 1 hour of irradiation and thereafter to a mean value of 8.0% AR at 5 days irradiation. No S-abscisic acid was detected after 8 days irradiation.

Three major photolysis products were detected in the irradiated samples (components 9A, 9B and 9C). Each had a similar retention time (ca 18.5 minutes) by HPLC, but was sufficiently resolved by TLC.

Component 9A

In the pH 7 and 9 samples taken after 1 hour of irradiation, only one component (9A) was resolved by HPLC (TLC analysis confirmed that the two other components, 9B and 9C, were not present) and accounted for means of 40.8% AR (pH 7) and 40.0% AR (pH 9). In the pH 4 samples taken after 1 hour of irradiation all 3 components were present and these together accounted for a mean of 43% AR (TLC analysis showed that components 9B and 9C accounted for ca 4 and 8% AR of this radioactivity, see Table 4.1.4.4-15). Component 9A showed co-chromatographic correspondence with (±) trans-trans abscisic acid (5-[1-hydroxy-2,6-trimethyl-4-oxocyclohex-2-en-1-yl]-3-methyl-[2E,4E]-pentadienoic acid).

Component 9B and 9C

The two other radioactive components, 9B and 9C accounted for maximums of 24.6% and 32.4% AR (pH 4) and 12.6% and 21.2% AR (pH 9), respectively throughout the irradiation period. Component 9B showed co-chromatographic correspondence with the reference material (Z,exo)-1',3',3'-trimethyl-2',5'-dioxobicyclo[4.1.0]heptan-7'-yl)but-3-enoic acid whilst component 9C showed co-chromatographic

correspondence with the reference material (Z,endo)-1',3',3'-trimethyl-2',5'-dioxobicyclo[4.1.0]heptan-7'-yl)but-3-enoic acid.

At least thirteen minor components were detected each accounting for no greater than 5.9% AR. Regions of diffusely eluting radioactivity not obviously associated with discrete components accounted for no greater than 11.6% AR.

No transformation of S-abscisic acid was observed in the non-irradiated samples.

Table 4.1.4.4-12 Proportions of radioactive components in irradiated pH 4 buffer after application of ^{14}C-S-abscisic acid at 1.5 mg L^{-1}

COMPONENT	RETENTION TIME (MINUTES)	Sampling interval (hours)									
		1	**1**	**6**	**6**	**12**	**12**	**48**	**48**	**72**	**72**
pH4/1	5.5	-	-	0.1	0.1	0.2	0.1	0.3	0.3	0.7	0.5
pH4/2	9	-	-	0.1	0.2	0.4	0.4	0.7	0.7	1.2	0.7
pH4/3	14.5	0.1	-	-	0.1	3.2	2.4	1.5	3.1	3.2	3.1
pH4/4	15.5	0.0	-	0.4	0.5	1.8	1.9	2.2	2.2	2.8	2.4
pH4/5	16.2	0.2	-	0.8	0.5	0.3	0.6	1.0	0.8	0.8	1.4
pH4/6	16.8	0.1	-	0.4	0.2	2.7	3.6	3.1	3.3	3.1	3.5
Region 7[c]	17 – 17.7	2.5	0.4	3.6	3.3	3.6	3.3	4.7	4.7	3.9	4.0
pH4/8	17.9	3.9	-	1.8	2.0	5.0	4.2	3.6	4.9	5.9	3.3
pH4/9	18.5	41.6[a]	44.3[a]	66.2[a]	64.3[a]	56.4[a]	55.3[a]	51.8[a]	53.0[a]	42.6[b]	50.8[b]
S-abscisic acid	19	42.3	51.3	17.3	21.0	6.4	7.7	7.9	5.3	-	-
Region 11[c]	19 – 19.8	4.8	1.4	3.5	2.5	2.7	3.4	2.5	2.7	4.2	2.8
pH4/12	20	-	0.1	0.2	-	1.6	2.2	1.5	1.7	2.9	2.8
pH4/13	20.7	0.1	-	0.1	-	0.7	1.6	0.9	0.6	1.3	2.3
pH4/14	21	0.1	-	0.1	0.3	1.9	3.0	3.1	2.8	3.3	4.2
Region 15[c]	21.3 - 24	0.5	0.6	1.7	1.3	3.1	3.2	5.3	4.2	4.2	4.2
Others[d]	-	1.0	1.0	0.9	1.0	3.5	3.6	5.1	5.3	14.3	14.0

Results are expressed as % applied radioactivity

Components resolved using HPLC method 1

- not detected

[a] contains trans,trans abscisic acid, (E,*exo*)-1',3',3'-trimethyl-2',5'-dioxobicyclo[4.1.0]heptan-7'-yl)but-3-enoic acid and (E,*endo*)-1',3',3'-trimethyl-2',5'-dioxobicyclo[4.1.0]heptan-7'-yl)but-3-enoic acid (resolved by TLC, see Table B.8.4.2-15)

[b] contains (E,*exo*)-1',3',3'-trimethyl-2',5'-dioxobicyclo[4.1.0]heptan-7'-yl)but-3-enoic acid and (E,*endo*)-1',3',3'-trimethyl-2',5'-dioxobicyclo[4.1.0]heptan-7'-yl)but-3-enoic acid only

[c] diffusely eluting radioactivity not associated with discrete components

[d] radioactivity not associated with specific components

Table 4.1.4.4-13 Proportions of radioactive components in irradiated pH 7 buffer after application of ^{14}C-S-abscisic acid at 1.5 mg L^{-1}

COMPONENT	RETENTION TIME (MINUTES)	Sampling interval (days)									
		1[a]	**1[a]**	**1**	**1**	**2**	**2**	**5**	**5**	**8**	**8**
pH7/1	5.5	-	-	0.4	0.3	0.4	0.6	2.0	1.1	1.7	1.7
pH7/2	14.5	-	-	-	-	0.2	0.4	1.7	0.7	1.6	1.7
Region 3[c]	14.8 – 15.5	0.1	0.1	0.2	0.5	0.4	0.4	0.6	0.4	0.5	1.0
pH7/4	15.8	-	-	0.8	0.5	1.6	1.6	1.7	2.4	1.8	2.1
Region 5[c]	15.9 – 16.7	0.1	-	1.3	1.0	2.4	2.7	4.4	4.5	4.0	3.7
pH7/6	16.9	0.1	-	0.7	0.9	2.6	1.7	3.4	4.3	4.7	4.1
pH7/7	17.2	-	-	0.9	0.6	0.6	0.8	2.6	2.8	2.2	2.6
pH7/8	17.6	-	0.2	0.9	1.3	1.6	2.4	3.4	2.3	3.9	2.5
pH7/9	18.5	36.7	44.8	52.6[b]	53.2[b]	51.0[b]	49.9[b]	38.0[b]	49.9[b]	43.7[b]	45.3[b]
S-abscisic acid	19	61.3	51.1	38.5	37.0	25.8	21.8	5.8	5.2	-	-
Region 11[c]	19.2 – 20.7	0.4	0.6	1.6	2.5	5.2	3.9	11.6	8.4	8.8	7.9
pH7/12	20.8	-	-	0.1	0.2	0.4	0.7	1.6	1.6	1.2	1.6
Region 13[c]	20.9 – 21.6	0.1	0.3	2.4	2.5	3.1	3.8	5.2	5.1	3.0	2.8
pH7/14	21.7	-	0.2	-	0.1	1.3	0.2	2.5	1.0	1.6	1.4
Region 15[c]	21.9 - 25	0.2	0.2	0.3	0.4	1.2	1.0	6.2	2.8	2.7	2.8
Others[d]	-	0.8	0.4	0.7	1.1	0.8	3.5	5.5	4.0	10.6	10.6

Results are expressed as % applied radioactivity

- not detected

[a] irradiation in hours

[b] May contains trans,trans abscisic acid (E,*exo*)-1',3',3'-trimethyl-2',5'-dioxobicyclo[4.1.0]heptan-7'-yl)but-3-enoic acid and (E,*endo*)-1',3',3'-trimethyl-2',5'-dioxobicyclo[4.1.0]heptan-7'-yl)but-3-enoic acid (components not generally resolved by TLC, see Table B.8.4.2-15)

[c] diffusely eluting radioactivity not associated with discrete components

[d] radioactivity not associated with specific components

Table B.8.4.2-14 Proportions of radioactive components in irradiated pH 9 buffer after application of ^{14}C-S-abscisic acid at 1.5 mg L^{-1}

COMPONENT	RETENTION TIME (MINUTES)	Sampling interval (days)									
		1[a]	**1[a]**	**1**	**1**	**2**	**2**	**5**	**5**	**8**	**8**
pH9/1	5.5	-	-	0.3	0.3	0.4	0.4	1.2	0.8	1.7	1.3
pH9/2	7.5	-	-	0.6	0.5	0.8	0.6	2.2	1.5	2.6	2.2
pH9/3	14.5	-	0.1	0.3	0.1	0.2	0.2	2.3	1.3	3.4	3.5
pH9/4	15.5	-	-	0.8	0.4	0.9	1.2	0.6	1.5	1.2	1.4

pH9/5	16	-	-	0.5	0.2	0.7	1.1	1.5	1.0	1.4	1.8
Region 6[e]	16.1 – 16.7	0.1	-	0.5	0.5	0.9	0.5	1.0	1.2	2.1	1.0
pH9/7	16.8	0.1	0.1	1.3	0.3	1.1	0.5	2.3	1.7	2.6	3.2
pH9/8	17.1	0.1	0.1	1.7	1.2	2.3	2.4	3.6	2.9	3.8	5.1
pH9/9	18.5	38.0[b]	41.0[b]	48.8[c]	46.8[c]	50.2[c]	48.1[c]	46.8[c]	47.9[c]	38.2[d]	42.7[d]
S-abscisic acid	19	60.3	57.0	28.8	40.7	25.6	28.2	5.7	10.3	-	-
pH9/11	19.5	0.1	-	1.4	1.5	1.3	1.1	1.8	1.5	1.2	1.4
pH9/12	20	-	-	0.3	0.4	0.4	0.6	3.2	1.6	1.3	2.6
Region 13[e]	20.1 – 21.5	0.1	-	2.9	0.2	2.4	2.6	5.9	5.0	6.5	6.3
pH9/14	21.6	-	-	2.9	0.9	3.1	2.4	3.1	3.2	2.4	2.5
Region 15[e]	21.8 - 25	-	0.1	1.9	0.4	0.5	1.2	5.4	3.1	2.7	2.2
Others[f]	-	1.2	1.3	5.8	4.8	6.3	7.5	12.1	9.2	17.3	15.4

Results are expressed as % applied radioactivity

- not detected

[a] irradiation in hours

[b] contains component 9A only

[c] contains trans,trans abscisic acid, (E,*exo*)-1',3',3'-trimethyl-2',5'-dioxobicyclo[4.1.0]heptan-7'-yl)but-3-enoic acid and (E,*endo*)-1',3',3'-trimethyl-2',5'-dioxobicyclo[4.1.0]heptan-7'-yl)but-3-enoic acid (resolved by TLC, see Table B.8.4.2-15)

[d] contains (E,*exo*)-1',3',3'-trimethyl-2',5'-dioxobicyclo[4.1.0]heptan-7'-yl)but-3-enoic acid and (E,*endo*)-1',3',3'-trimethyl-2',5'-dioxobicyclo[4.1.0]heptan-7'-yl)but-3-enoic acid only

[e] diffusely eluting radioactivity not associated with discrete components

[f] radioactivity not associated with specific components

Table 4.1.4.4-15 Proportions of radioactive components 9B and 9C in irradiated buffer solutions after application of ^{14}C-S-abscisic acid at 1.5 mg L^{-1}

COMPONENT (RF)	**Sampling interval (hours) pH 4**									
	1	**1**	**6**	**6**	**12**	**12**	**48**	**48**	**72**	**72**
9B (0.17)	3.7	4.1	9.9	9.4	19.4	19.4	24.6	24.2	10.6	20.1
9C (0.23)	8.3	-	12.6	12.3	20.1	23.4	24.0	28.7	25.0	32.4
COMPONENT (RF)	**Sampling interval (days) pH 7**									
	1[a]	**1[a]**	**1**	**1**	**2**	**2**	**5**	**5**	**8**	**8**
9B (0.17)	nr	nr	31.8	8.9	nr	nr	nr	nr	nr	nr
9C (0.23)	nr	nr	10.6	10.1	nr	nr	nr	nr	nr	nr
COMPONENT (RF)	**Sampling interval (days) pH 9**									
	1[a]	**1[a]**	**1**	**1**	**2**	**2**	**5**	**5**	**8**	**8**
9B (0.17)	-	-	12.6	6.9	12.5	12.2	11.4	11.5	9.0	12.6
9C (0.23)	-	-	13.4	6.8	13.0	11.4	21.2	13.6	19.8	17.5

results are expressed as % applied radioactivity

components resolved by TLC (chloroform/methanol, 9/1 by volume)

component 9B Identified as (E,*exo*)-1',3',3'-trimethyl-2',5'-dioxobicyclo[4.1.0] heptan-7'-yl)but-3-enoic acid

component 9C Identified as (E,*endo*)-1',3',3'-trimethyl-2',5'-dioxobicyclo[4.1.0] heptan-7'-yl)but-3-enoic acid

- not detected

[a] hours irradiation

nr components not resolved

Table 4.1.4.4-16 Proportions of radioactive components in non-irradiated buffer after application of ^{14}C-S-abscisic acid at 1.5 mg L^{-1}

COMPONENT	Sampling interval (hours) pH 4											
	0	**0**	**1**	**1**	**6**	**6**	**12**	**12**	**48**	**48**	**72**	**72**
S-abscisic acid	95.1	94.8	95.5	96.0	95.2	95.4	95.5	95.6	95.0	95.6	99.9	101.3
Others[b]	2.8	3.4	2.1	2.0	2.5	2.3	2.1	2.1	2.4	2.0	2.3	2.6
COMPONENT	Sampling interval (days) pH 7											
	0	**0**	**1[a]**	**1[a]**	**1**	**1**	**2**	**2**	**5**	**5**	**8**	**8**
S-abscisic acid	97.6	97.9	97.4	97.3	99.5	100.2	97.8	100.1	97.3	96.7	97.8	96.4
Others[b]	2.3	2.1	2.3	2.4	2.5	2.2	2.6	2.0	2.8	2.5	2.2	2.4
COMPONENT	Sampling interval (days) pH 9											
	0	**0**	**1[a]**	**1[a]**	**1**	**1**	**2**	**2**	**5**	**5**	**8**	**8**
S-abscisic acid	97.8	97.7	97.5	97.5	95.9	96.6	97.1	97.0	93.9	100.4	96.5	97.2
Others[b]	2.1	2.3	2.5	2.1	3.6	3.4	2.6	2.6	2.8	2.4	2.4	1.9

Results are expressed as % applied radioactivity

- not detected

[a] incubation time in hours

[b] radioactivity not associated with specific components

Transformation pathway

In irradiated buffer solutions at pH 4, 7 and 9 S-abscisic acid underwent rapid photo isomerisation an equilibrium mixture of the active ingredient with a near-equal amount of the trans-trans abscisic acid isomer. Thereafter, intramolecular migration resulted in the formation of bi-cyclic isomers (tentatively identified as (Z,exo)-1',3',3'-trimethyl-2',5'-dioxobicyclo[4.1.0]heptan-7'-yl)but-3-enoic acid and (Z,endo)-1',3',3'-trimethyl-2',5'-dioxobicyclo[4.1.0]heptan-7'-yl)but-3-enoic acid. Other minor components and carbon dioxide were also formed. The pathway is shown in Figure 4.1.4.4-1.

Kinetic analysis

Kinetic data for the photo transformation of S-abscisic acid in buffer at three pH values are shown in Table B.8.4.2-17. At latitude 40°N summer sunlight the DT_{50} and DT_{90} in pH 4 buffer were 1.2 and 3.9 equivalent days, 2.3 and 7.6 equivalent days (pH 7) and 2.5 and 8.5 equivalent days (pH 9), respectively. The lines of best fit are shown in Figure 4.1.4.4-2 to 4.1.4.4-4. S-abscisic acid did not degrade in non-irradiated solutions.

Table4.1.4.4-17 Kinetic parameters for the photo degradation of ^{14}C-S-abscisic acid

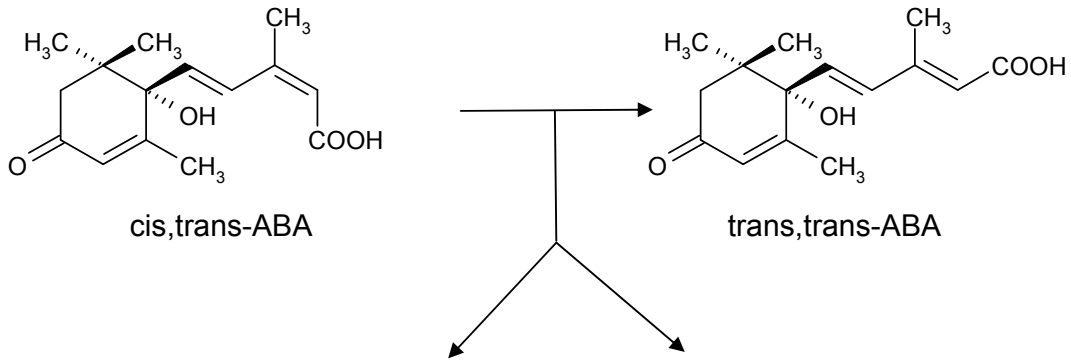
	pH 4	pH 7	pH 9
k (hours^{-1})[a]	0.585262	0.301001	0.271892
k (hours^{-1})[b]	0.043131	0.519427	0.451184
DT_{50} (days)[a]	1.2	2.3	2.5
DT_{50} (days)[b]	0.7	1.3	1.5
DT_{90} (days)[a]	3.9	7.6	8.5
DT_{90} (days)[b]	2.2	4.4	5.1
r^2	0.53	0.96	0.91
Φ (molecules degraded photon^{-1})	0.018	0.015	0.016

[a] equivalent days of natural summer sunlight at latitude 40°N
[b] hours of Suntest irradiation
Φ quantum yield

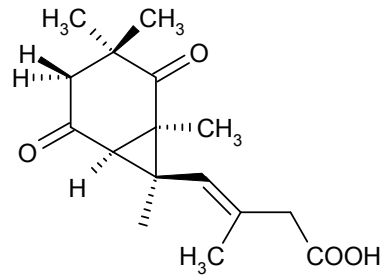
Quantum yield

The quantum yield (Φ) for S-abscisic acid in buffer was 0.018 (pH 4), 0.015 (pH 7) and 0.016 (pH 9).

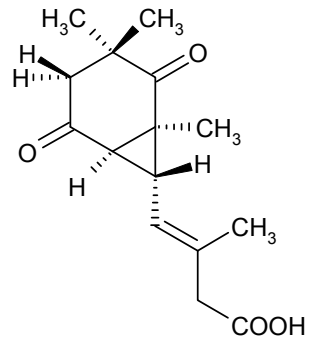
Figure 4.1.4.4-1 Proposed pathway for the photo degradation of in aqueous buffer solutions



O_2 and minor metabolites



(Z,*exo*)-1',3',3'-trimethyl-2',5'-dioxobicyclo[4.1.0]heptan-7'-yl)but-3-enoic acid (component 9B)



(Z,*endo*)-1',3',3'-trimethyl-2',5'-dioxobicyclo[4.1.0]heptan-7'-yl)but-3-enoic acid (component 9C)

Figure 4.1.4.4-2 **Proportions of S-abscisic acid in irradiated pH 4 buffer showing the line of best fit (summer sunlight at latitude 40ºN)**

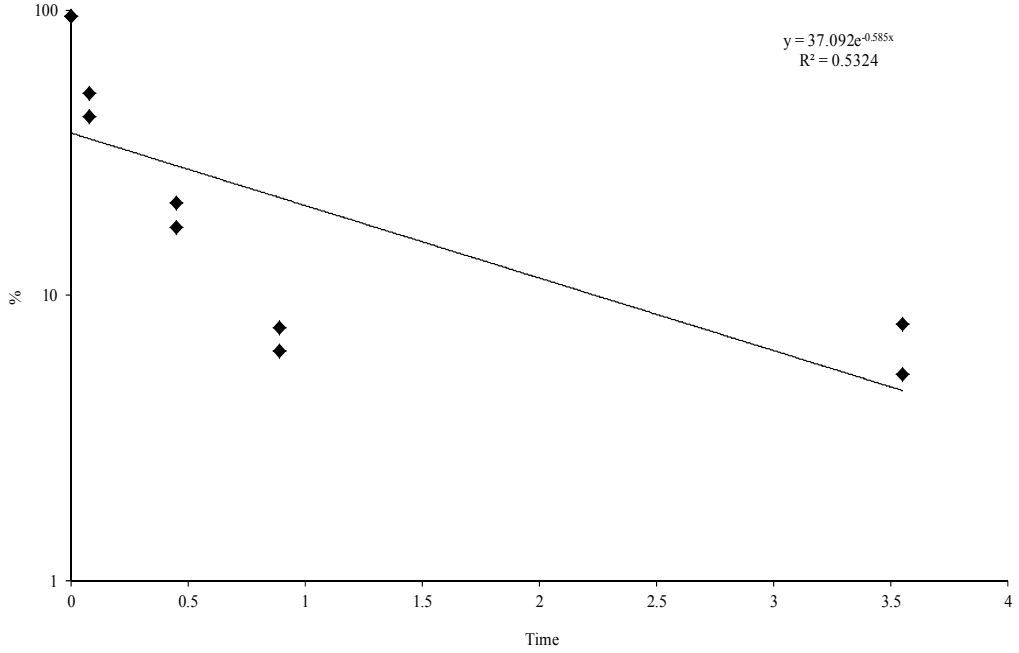


Figure 4.1.4.4-3 **Proportions of S-abscisic acid in irradiated pH 7 buffer showing the line of best fit (summer sunlight at latitude 40ºN)**

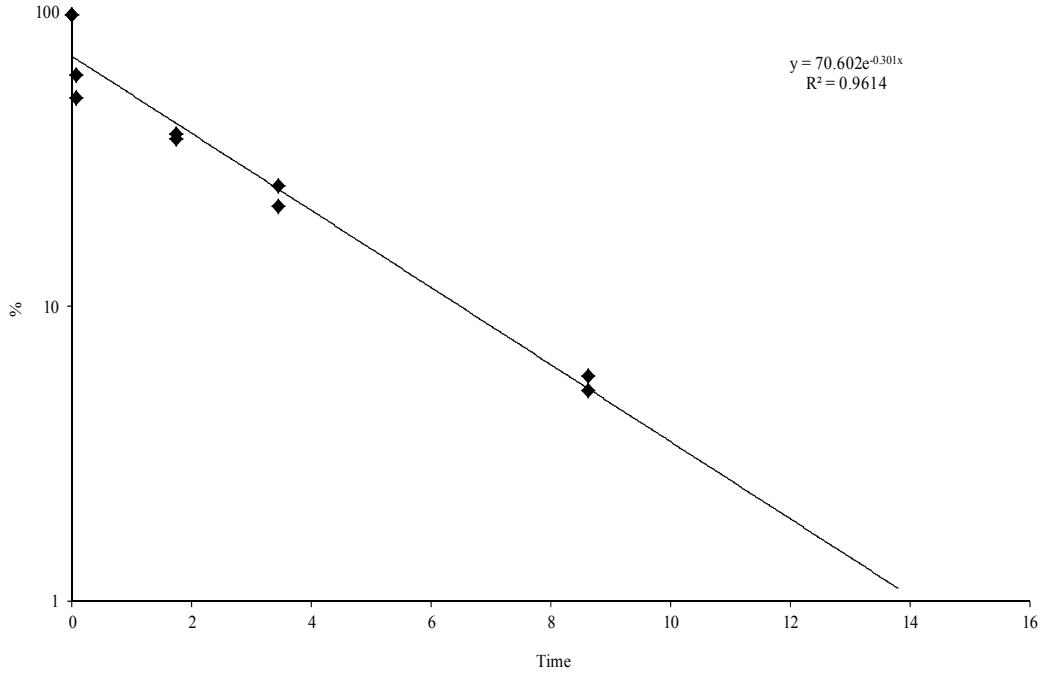
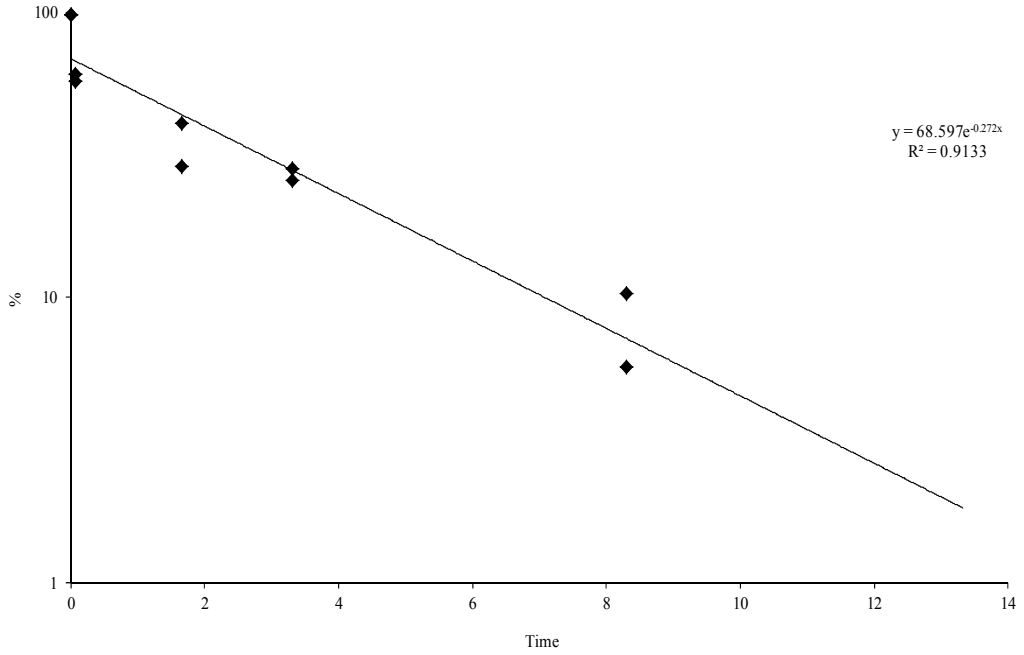


Figure 4.1.4.4-4 Proportions of S-abscisic acid in irradiated pH 9 buffer showing the line of best fit (summer sunlight at latitude 40ºN)



Theoretical lifetime

From the quantum yield the theoretical lifetime was determined for each season at 10° longitude and 40°N latitude and close to the surface of water (Table 4.1.4.4-18). Values ranged from between 0.93 – 1.09 days in summer to between 3.92 – 4.61 days in winter.

Table 4.1.4.4-18 Estimation of theoretical lifetime for S-abscisic acid

Theoretical lifetime at the surface of water (days)	pH	Spring	Summer	Autumn	Winter
Latitude 40°N	4	1.19	0.93	2.11	3.92
	7	1.40	1.09	2.49	4.61
	9	1.35	1.05	2.39	4.44

GCSOLAR conditions: Longitude 10°, terrestrial atmosphere, typical ephemeride and ozone values.

Conclusion

S-abscisic acid was photolytically unstable in buffer solution at three pH values. DT_{50} values were between 1.2 and 2.5 equivalents days of latitude 40°N summer sunlight.

In irradiated buffer solutions at pH 4, 7 and 9 S-abscisic acid underwent rapid photo isomerisation to an equilibrium mixture of the active ingredient with a near-equal amount of the trans-trans abscisic acid isomer. Thereafter intramolecular migration resulted in the formation of bi-cyclic isomers (identified as (Z,exo)-1',3',3'-trimethyl-2',5'-dioxobicyclo[4.1.0]heptan-7'-yl)but-3-enoic acid and (Z,endo)-1',3',3'-trimethyl-2',5'-dioxobicyclo[4.1.0]heptan-7'-yl)but-3-enoic acid. Other minor components and carbon dioxide were also formed.

The quantum yield (Φ) for S-abscisic acid in buffer was between 0.015 and 0.018. These values indicate that photolysis is likely to be a significant route of transformation for S-abscisic acid. The theoretical lifetime ranged from 0.93 – 1.09 days in summer to between 3.92 – 4.61 days in winter.

Remarks

The photolytic degradation study is acceptable and performed under sterile conditions. The obtained endpoints are reliable and acceptable. The substance is rapidly isomerised to trans-trans abscisic acid (component 9A) and two major components formed during photolysis. The components 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).

4.1.4.5 STUDY 5 – Soil photolysis

Study reference: STUDY IIA, 7.1.3/002

Characteristics

reference	:	Ponte, M.	study type	:	soil photolysis
year of execution	:	2012	incubation time	:	up to 9 d
GLP statement	:	yes	nominal concentration	:	0.079 mg/g
guideline	:	OPPTS 835-2410 (2008) SETAC (1995) OECD guideline for soil photolysis	temperature	:	20°C
test substance	:	^{14}C-S-abscisic acid			
purity	:	100 %	conclusion	:	study results reliable
soil	:	sandy clay loam	acceptability	:	acceptable

Executive summary

The photo degradation of S-abscisic acid was investigated in a sandy clay loam soil with continuous exposure to artificial sunlight for periods up to nine days (216 hours). Two-mm-thick layers of soil were treated with ^{14}C-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 on the soil surface when exposed to artificial light, accounting for an average of 50.3% AR after 40 hours of irradiation and 7.6% AR after 216 hours of irradiation. Photo induced isomerization of S-Abscisic acid to *trans*-ABA resulted in a maximum average of 37.5% AR *trans*-ABA after 40 hours of irradiation before declining to an average of 8.8% AR after 216 hours irradiation. After the first 72 hours of exposure, multiple minor metabolites were observed in the irradiated soil samples; none represented in average > 6.1% AR at any time. Mineralisation to CO_2 and non-extractable radioactivity accounted for an average of 27.1% AR and 21.6% AR after 216 hours, respectively.

Degradation of S-Abscisic acid was slow in the dark control samples for the first 72-hours of incubation, representing an average of 94.0% AR. After this time, S-Abscisic acid showed an increase in the degradation rate and at the end of 216 hours of incubation accounted for an average of 10.6% AR. No significant isomerization to *trans*-ABA was observed throughout the incubation (<1%). There were only minor

metabolites present in the dark control samples; none represented in average > 2.8% AR at any time. Mineralisation to CO_2 and non-extractable radioactivity accounted for an average of 46.6% AR and 28.9% AR after 216 hours, respectively.

S-abscisic acid degraded rapidly in irradiated soil with a DT_{50} of 2.3 OECD solar days. S-abscisic acid underwent photo-induced isomerization to *trans*-ABA in irradiated samples. The DT_{50} of *trans*-ABA was calculated as 2.6 OECD solar days.

S-abscisic acid degraded extensively in the dark control samples with a DT_{50} of 5.2 days. No isomerization was observed in the dark control samples.

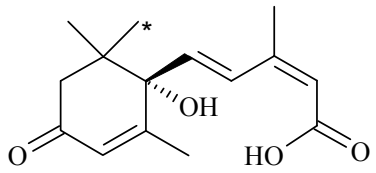
MATERIALS AND METHODS

A. Materials

Test material

Common name:	Abscisic acid (ABA, S-ABA, *cis-trans*-ABA)
Chemical name (IUPAC):	(2*Z*,4*E*)-5-[(1*S*)-Hydroxy-2,6,6-trimethyl-4-oxo-2-cyclohexen-1-yl]-3-methyl-2,4-pentadienoic acid
CAS no.:	21293-29-8
Lot/batch no.:	030806D1
Purity:	99.6%
Description:	Off-white powder
Common name:	(±)-trans, trans-Abscisic acid
Chemical name (IUPAC):	(5-[1-Hydroxy-2,6,6-trimethyl-4-oxocyclohexen-2-ene-1-yl]-3-methyl-2E,4E-pentadienoic acid
Lot/batch no.:	C1014
Purity:	>99%
Description:	Off-white crystalline powder
Common name:	(±)-trans, trans-Abscisic acid

Radiolabelled material:



* denotes position of radiolabel

Name:	^{14}C-S-abscisic acid
Lot/batch no.:	V0451-030 Run 2, fraction 9
Radiochemical purity:	100% (nominal)
Specific activity:	61 mCi/mmol
Description:	Solid

Table 4.1.4.5-1 Characteristics of the test soil

Origin	Iowa, USA (Lat. 42.1094712; Lon. 94.2963634)
Particle size distribution (USDA):	
Sand	53
Silt	25
Clay	22

USDA Textural Classification FAO Textural Classification	Sandy clay loam Medium
pH in 1:1 soil:water ratio pH in 0.01M CaCl2 (1:2) Moisture holding capacity at 1/10 Bara Organic matter (%) Cation exchange capacity (meq/100g)	6.7 6.3 28.0% 4.4 17.4
Biological activity: Biomass prior to application (µg C/g soil) Aerobic bacteria at application, Set 1b (cfu/g) Aerobic bacteria at end of incubation, Set 1b (cfu/g) Aerobic bacteria at application, Set 2c (cfu/g) Aerobic bacteria at end of incubation, Set 2c (cfu/g)	 539 5.1 x 106 5.1 x 106 (irradiated) 5.45 x 106 (dark control) 2.26 x 106 1.99 x 106 (irradiated) 1.09 x 106 (dark control)

a: Moisture content used in the study

b: Samples for 0, 6, 17, 40 and 72 hours

c: Samples for 5 and 9 days

cfu: Colony forming units

Soil Sandy clay loam
Light intensity 41.2 W/m^2 (300 – 400 nm range)

B. STUDY DESIGN AND METHODS

Experimental conditions

3.1 g of 2-mm sieved soil (dry weight equivalent) were weighed into 50 mm quartz and pyrex soil dishes, equipped with Teflon®-lined silicon septum screw caps. Samples to be irradiated were prepared in quartz vessels and dark control samples were prepared in Pyrex vessels. Deionized water was added to the soil to create slurry. The 2-mm thick soil layers were then dried with ambient air prior to use. A 75-µl aliquot of a solution of ^{14}C-Abscisic acid in ethanol : water was evenly applied to each layer to give a nominal dose rate of 79 µg/g equivalent to a field rate of approximately 1250 g/ha.

The fortified soil layers (except time zero samples) were connected to their respective traps (one empty trap to prevent backflow of trapping media, one ethylene glycol trap to collect organic volatiles, another empty trap and two 10% aqueous sodium hydroxide traps to collect CO_2) and placed either in a Heraeus Suntest CPS+ unit containing a xenon arc lamp, equipped with a quartz glass filter for irradiation or wrapped in aluminium foil. Air was first humidified before being drawn over the soil surface and through the trapping solutions. The irradiated samples were placed in a water bath maintained at 20 ± 2°C. The dark control samples were placed in a controlled temperature walk-in chamber maintained 20 ± 2°C. In both the temperature was monitored continuously.

Both irradiated and dark control samples were maintained at a moisture content equivalent to pF 2.0 throughout the study, with the addition of deionized water as necessary.

The microbial biomass of the test soil was measured prior to the experimental start by the Anderson and Domsch (1978) method. In addition six samples were established to monitor the microbial viability at the study start and end; these were prepared in the same way as the irradiated and dark control.

Sampling

Duplicate soil samples were taken for analysis immediately after application. Duplicate irradiated and duplicate non-irradiated soil samples were taken for analysis after 6, 17, 40, 72 hours and 5 and 9 days of continuous irradiation or incubation. Trapping solutions were sampled and replaced with fresh solutions at each sampling interval.

The microbial biomass of the test soil was measured prior to the experimental start and the microbial viability was measured prior to application and at the end of the incubation.

Description of analytical method

Soil samples were extracted twice with acetonitrile: water (1 : 1, v/v) and once with water by shaking at ambient temperature for 30 minutes. Extraction solutions and soil solids were separated by centrifugation. All three extracts were combined, the volume measured and aliquots radio assayed (LSC). For each sample, aliquots of the pooled extract were analysed directly by HPLC, with confirmatory two-dimensional thin layer chromatography (2D TLC). The remaining soil after extraction was combusted and radio assayed by LSC. Non-extractable radioactivity in a representative sample (216-hour irradiated and dark control) was characterised by a humic acids/fulvic acids partition.

The volumes of the trapping solutions were measured and aliquots of each solution were radio assayed (LSC). The radiocarbon trapped in the NaOH solution was verified as $^{14}CO_2$ by the addition of saturated aqueous $BaCl_2$ to selected traps and ascertaining the formation of the precipitate $Ba^{14}CO_3$ by LSC of the supernatant.

RESULTS AND DISCUSSION

Soil biological activity

The soil remained viable throughout the incubation period (Table 4.1.4.5-1).

Experimental conditions

Recorded temperatures for the irradiated and control samples lay within the range 20 ± 2°C.

Mass balance

All total recoveries of radioactivity from irradiated and control samples lay in the range 91.4 - 109% AR (Table 4.1.4.5-2 and 4.1.4.5-3).

Extractable and non-extractable radioactivity

The extractability in the irradiated soils decreased throughout the study from an average of 104.2% at zero-time to an average of 43.2% AR (irradiated soils) and 18.6% AR (control soils) after 216 hours (Table 4.1.4.5-2 and 4.1.4.5-3). Non-extractable radioactivity increased during the study reaching an average of 21.6% AR in irradiated soils and 28.9% AR in the control soils after 216 hours (Table 4.1.4.5-2 and 4.1.4.5-3).

The non-extractable radioactivity was characterised in the 216-hour irradiated and dark control samples. After the humic/fulvic partition, the insoluble humin fraction represented 3.9% AR, while the humic acid and the fulvic acid fractions corresponded to 4.8% AR and 14.5% AR respectively, in irradiated samples. For the dark control samples, the insoluble humin fraction was the most abundant (16.4% AR), and the humic and fulvic acids represented 2.9% and 9.4% of the applied radiocarbon, respectively.

Volatilisation

The amount of volatile radioactivity in the sodium hydroxide traps (characterised as $^{14}CO_2$) increased with time, to an average of 27.1% and 46.6% AR in the irradiated and dark control soils, respectively (Table

4.1.4.5-2 and 4.1.4.5-3). Volatile radioactivity in the ethylene glycol trap was minimal for both irradiated and dark control soils (0.2% AR in irradiated soils and 0.0% in dark control soils) (Table 4.1.4.5-2 and 4.1.4.5-3).

Table 4.1.4.5-2 Distribution and recovery of radioactivity in irradiated soil (including zero-time) treated with ^{14}C-S-abscisic acid

Sample ID	Time (hours)	% Applied radioactivity (AR)				
		Soil extract	Bound residue	Volatiles	CO_2	Total recovery
Replicate A	0	103.6	1.1	-	-	104.7
Replicate B		104.8	1.2	-	-	106.0
Mean		**104.2**	**1.2**	**-**	**-**	**105.4**
Replicate A	6	103.1	1.9	0.0	0.2	105.2
Replicate B		101.6	1.9	0.0	0.2	103.7
Mean		**102.4**	**1.9**	**0.0**	**0.2**	**104.5**
Replicate A	17	103.3	3.1	0.0	0.8	107.2
Replicate B		97.4	3.1	0.0	1.4	101.9
Mean		**100.4**	**3.1**	**0.0**	**1.1**	**104.6**
Replicate A	40	93.2	5.8	0.1	3.4	102.5
Replicate B		95.0	6.9	0.2	2.8	104.9
Mean		**94.1**	**6.4**	**0.2**	**3.1**	**103.7**
Replicate A	72	68.2	22.3	0.1	18.4	109.0
Replicate B		84.1	9.9	0.1	6.8	100.9
Mean		**76.2**	**16.1**	**0.1**	**12.6**	**105.0**
Replicate A	120	73.1	14.1	0.2	9.6	97.0
Replicate B		52.3	21.2	0.1	20.2	93.8
Mean		**62.7**	**17.7**	**0.2**	**14.9**	**95.4**
Replicate A	216	41.9	23.2	0.1	26.2	91.4
Replicate B		44.5	19.9	0.0	28.0	92.4
Mean		**43.2**	**21.6**	**0.1**	**27.1**	**91.9**

Extracts 1 – 2: acetonitrile:water (1:1, v/v), ambient

Extract 3: water, ambient

-:not applicable

Table 4.1.4.5-3 Distribution and recovery of radioactivity in dark control soil (not including zero-time) treated with ^{14}C-S-abscisic acid

Sample ID	Time (hours)	% Applied radioactivity (AR)				
		Soil extract	Bound residue	Volatiles	CO_2	Total recovery
Replicate A	6	106.1	1.3	0.0	0.1	107.9
Replicate B		102.5	1.3	0.0	0.1	103.9
Mean		**104.3**	**1.3**	**0.0**	**0.1**	**105.7**
Replicate A	17	101.0	1.9	0.0	0.0	102.9
Replicate B		99.7	1.9	0.0	0.4	102.0
Mean		**100.4**	**1.9**	**0.0**	**0.2**	**102.5**
Replicate A	40	101.0	3.0	0.0	1.3	105.3
Replicate B		99.0	4.1	0.0	1.8	104.9
Mean		**100.0**	**3.6**	**0.0**	**1.6**	**105.1**
Replicate A	72	92.3	6.8	0.0	3.6	102.7
Replicate B		97.3	6.0	0.0	3.3	106.6

Mean		**94.8**	**6.4**	**0.0**	**3.5**	**104.7**
Replicate A	120	60.5	17.9	0.0	19.4	97.8
Replicate B		73.8	14.2	0.0	10.7	98.7
Mean		**67.2**	**16.1**	**0.0**	**15.1**	**98.3**
Replicate A	216	16.5	28.7	0.0	48.6	93.8
Replicate B		20.7	29.1	0.0	44.6	94.4
Mean		**18.6**	**28.9**	**0.0**	**46.6**	**94.1**

Extracts 1 – 2: acetonitrile:water (1:1, v/v), ambient

Extract 3: water, ambient

Table 4.1.4.5-3 Characterisation of non-extractable radioactivity

Incubation time (days)	Incubation conditions	% Applied radioactivity			
		Post extracted soil	Bound soil residue fraction		
			Fulvic acid	Humic acid	Humin
216	Irradiated	23.2	14.5	4.8	3.9
	Dark control	28.7	9.4	2.9	16.4

Transformation of parent compound

S-abscisic acid degraded rapidly on the soil surface when exposed to artificial light, accounting for an average of 50.3% AR after 40 hours of irradiation and 7.6% AR after 216 hours of irradiation (Table B.8.1.1.3-09). Photo induced isomerization of S-Abscisic acid to *trans*-ABA resulted in a maximum average of 37.5% AR *trans*-ABA after 40 hours of irradiation before declining to an average of 8.8% AR after 216 hours irradiation. After the first 72 hours of exposure, multiple minor metabolites were observed in the irradiated soil samples; none represented in average > 6.1% AR at any time.

Degradation of S-Abscisic acid was slow in the dark control samples for the first 72-hours of incubation, representing an average of 94.0% AR (Table 4.1.4.5-5). After this time, S-Abscisic acid showed an increase in the degradation rate and at the end of 216 hours of incubation accounted for an average of 10.6% AR. No significant isomerization to *trans*-ABA was observed throughout the incubation (<1%). There were only minor metabolites present in the dark control samples; none represented in average > 2.8% AR at any time.

The degradation rate of S-abscisic acid in irradiated and dark control soils were calculated using the FOCUS Approach with Kingui software version 1.1. Degradation of S-Abscisic acid in the irradiated samples was best described using the Double First Order in Parallel model (DFOP). Single First Order model (SFO) model was selected to determine DT_{50} and DT_{90} values for the dark controls and *trans*-ABA degradation. The models where good fit to the data (visually acceptable) and low chi^2 error results (<15%) were obtained. S-Abscisic acid and *trans*-ABA degraded rapidly in light exposed samples with a half-life of 2.3 and 3.1 OECD solar days, respectively. S-Abscisic acid degraded extensively in the dark control with a half-life of 5.2 days. Irradiated kinetic data are summarised in Table 4.1.4.5-5.

The proposed degradation pathway for the photo degradation of S-abscisic acid on soil is presented in Figure 4.1.4.5-1.

Table 4.1.4.5-4 Proportions of radioactive components in irradiated soil (including zero-time) treated with ^{14}C-S-abscisic acid

Sample ID	Time (hours)	% Applied radioactivity (AR)						
		S-ABA	*trans*-*ABA*	U-1	U-2	U-3	U-4	Others
Replicate A	0	103.6	0.0	0.0	0.0	0.0	0.0	0.0
Replicate B		104.8	0.0	0.0	0.0	0.0	0.0	0.0
Mean		**104.2**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
Replicate A	6	92.5	10.6	0.0	0.0	0.0	0.0	0.0

Replicate B		84.8	16.2	0.0	0.6	0.0	0.0	0.0
Mean		**88.7**	**13.4**	**0.0**	**0.3**	**0.0**	**0.0**	**0.0**
Replicate A		70.8	31.1	0.0	0.6	0.5	0.0	0.3
Replicate B	17	60.6	33.3	0.0	0.5	1.7	0.4	1.0
Mean		**65.7**	**32.2**	**0.0**	**0.6**	**1.1**	**0.2**	**0.7**
Replicate A		51.2	37.9	0.0	0.6	0.7	0.7	2.1
Replicate B	40	49.3	37.1	0.0	0.5	2.9	1.3	3.8
Mean		**50.3**	**37.5**	**0.0**	**0.6**	**1.8**	**1.0**	**3.0**
Replicate A		29.5	26.8	1.8	1.9	2.1	2.7	3.4
Replicate B	72	44.3	34.6	0.6	0.5	0.6	1.0	2.4
Mean		**36.9**	**30.7**	**1.2**	**1.2**	**1.4**	**1.9**	**2.9**
Replicate A		29.4	24.0	1.3	8.0	1.2	0.8	8.4
Replicate B	120	15.7	14.6	2.1	4.2	1.3	1.2	13.1
Mean		**22.6**	**19.3**	**1.7**	**6.1**	**1.3**	**1.0**	**10.8**
Replicate A		7.6	8.6	1.8	3.3	1.1	2.2	17.2
Replicate B	216	7.6	8.9	2.2	3.7	2.3	1.5	18.5
Mean		**7.6**	**8.8**	**2.0**	**3.5**	**1.7**	**1.9**	**17.9**

Table 4.1.4.5-5 Proportions of radioactive components in dark control soil (including zero-time) treated with ^{14}C-S-abscisic acid

Sample ID	Time (hours)	% Applied radioactivity (AR)						
		S-ABA	*trans-ABA*	U-1	U-2	U-3	U-4	Others
Replicate A		105.8	0.0	0.0	0.0	0.0	0.3	0.0
Replicate B	6	101.9	0.6	0.0	0.0	0.0	0.0	0.0
Mean		**103.9**	**0.3**	**0.0**	**0.0**	**0.0**	**0.2**	**0.0**
Replicate A		100.6	0.4	0.0	0.0	0.0	0.0	0.0
Replicate B	17	99.3	0.4	0.0	0.0	0.0	0.0	0.0
Mean		**100.0**	**0.4**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
Replicate A		99.3	1.1	0.0	0.0	0.0	0.0	0.6
Replicate B	40	98.4	0.6	0.0	0.0	0.0	0.0	0.0
Mean		**98.9**	**0.9**	**0.0**	**0.0**	**0.0**	**0.0**	**0.3**
Replicate A		91.6	0.5	0.0	0.0	0.0	0.0	0.3
Replicate B	72	96.3	0.5	0.0	0.0	0.0	0.0	0.0
Mean		**94.0**	**0.5**	**0.0**	**0.0**	**0.5**	**0.0**	**0.2**
Replicate A		57.7	0.0	1.3	0.7	0.3	0.0	0.7
Replicate B	120	71.8	0.0	0.9	1.1	0.0	0.0	0.0
Mean		**64.8**	**0.0**	**1.1**	**0.9**	**0.0**	**0.0**	**0.4**
Replicate A		9.0	0.3	2.8	0.4	0.2	0.6	3.2
Replicate B	216	12.2	0.4	2.7	0.5	0.5	0.8	3.7
Mean		**10.6**	**0.4**	**2.8**	**0.5**	**0.4**	**0.7**	**3.5**

Table 4.1.4.5-6 Kinetic data for the decline of S-Abscisic acid in soil

	Global summer day[a]	
	DT_{50} (days)	**DT_{90} (days)**
S-Abscisic acid	2.3	13.9
trans-ABA	2.6	8.7

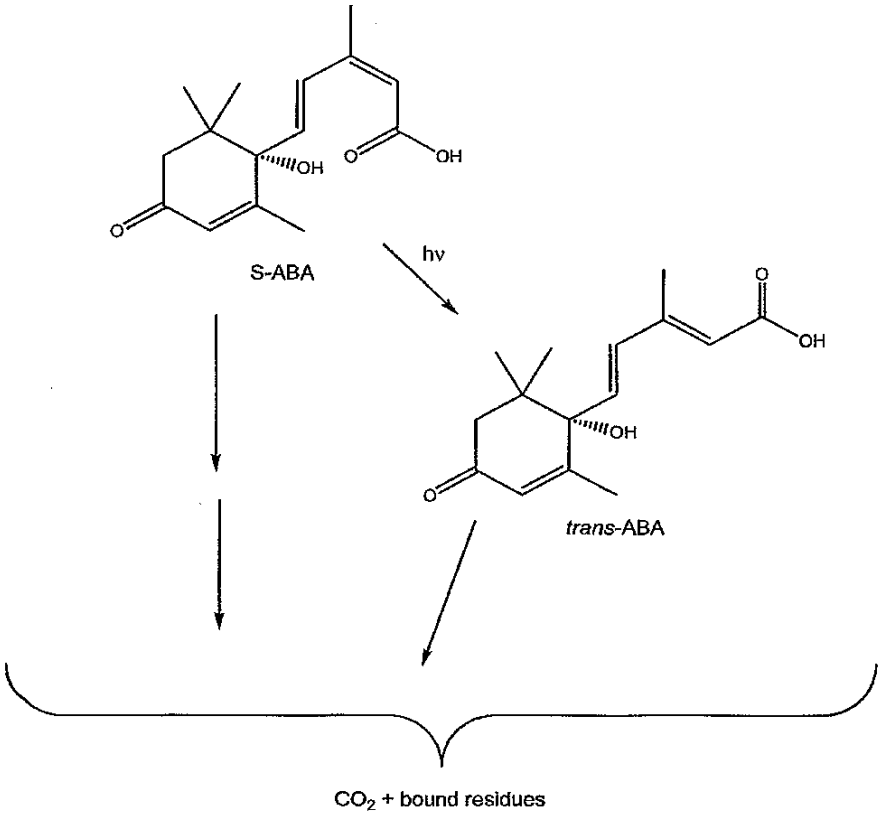
[a]: Summer day at 30 – 50 ºN Latitude (300 – 400 nm)

CONCLUSIONS

S-abscisic acid degraded rapidly in light exposed samples with a half-life of 2.3 OECD solar days and represented 7.6 % AR at the end of the 216 hours of exposure. S-abscisic acid underwent photo-induced isomerization to *trans*-ABA in irradiated samples. Both isomers of abscisic acid degraded extensively in the irradiated samples with CO_2 and bound residues as the major degradants. The half-life of *trans*-ABA was calculated as 2.6 OECD solar days.

S-abscisic acid degraded extensively in the dark control samples to CO_2 and bound residues, with a half-life of 5.2 days. No isomerization was observed in dark control samples during the incubation period.

Figure 4.1.4.5-1 The proposed degradation pathway for the photo degradation of S-abscisic acid on soil



Remarks

The soil photolysis study shows transformation during of the S-abscisic acid in both the irradiated system and dark control. The obtained endpoint is agreed and the study is considered reliable and acceptable.

4.1.4.6 STUDY 6 - Batch sorption

Study reference: Study IIA, 7.4.1/001

Characteristics

reference	:	Corden, M.T.	soils	:	2 x clay loam, 1 x sandy loam, 1 x loamy sand, 1 x sandy loam/sandy clay loam
year of execution	:	2010	concentrations	:	0.05 – 5 µg/mL
GLP statement	:	yes	temperature	:	20°C
guideline	:	Including OECD 106	$K_F$oc	:	2.78 – 77.0
test substance	:	^{14}C-R-Abscisic acid	1/n	:	0.65 – 1.38
purity	:	98.6%	acceptability	:	acceptable

Note concerning the use of the R enantiomer of the test substance

Enantiomers (stereoisomers that are mirror images of each other) have identical physical properties: melting points, boiling points, densities, refractive indices and any other physical constant one might measure except for the direction of rotation of plane polarized light. One isomer rotates plane-polarized light to the right and the other isomer rotates plane-polarized light equally to the left. An equal mixture of the two isomers (a racemic mixture) would cancel each other and plane-polarized light would not appear to rotate. Since enantiomers have identical physical properties, except for the direction of rotation of plane polarized light, S-abscisic acid and R-abscisic acid (the two enantiomers of abscisic acid) will have the same physical properties with regard to the adsorption and desorption to soil (a physical interaction with soil). Therefore, using ^{14}C-R-abscisic acid in this study, admixed with non-radiolabelled S-abscisic acid, will give the same results as using S-abscisic acid alone.

Study design

A batch equilibrium adsorption study with five soils (Table 4.1.4.6-1) was conducted on abscisic acid.

Table 4.1.4.6-1 Physical and chemical properties of the test soils

	Bromsgrove	**Elmton**	**Evesham 3**	**Warsop**	**Hodnet**
Origin	Warwick-shire, UK	Worcester shire, UK	Cambridge-shire, UK	Nottingham-shire, UK	Warwick-shire, UK
Particle size distribution (%) (BS):					
<2 µm (clay)	14	28	33	6	18
2 µm - 63 µm (silt)	17	30	31	8	28
63 µm - 2 mm (sand)	69	42	36	86	54
Textural classification (BS)	sandy loam	clay loam	clay loam	loamy sand	sandy loam or sandy clay loam
Particle size distribution (%) (USDA):					
<2 µm (clay)	14	26	40	nm	18
2 µm – 50 µm (silt)	15	28	32	nm	26
50 µm - 2 mm (sand)	71	46	28	nm	56
Textural classification (USDA)	sandy loam	loam	clay/clay loam	-	sandy loam
pH (water)	5.5	7.7	8.0	4.9	6.4
pH (0.01 M $CaCl_2$)	5.0	6.9	7.3	3.9	5.9
Organic carbon (%)	1.7	4.3	1.6	0.6	1.9
Organic matter (%)	2.9	7.4	2.8	1.0	3.3
Total nitrogen (%)	nm	nm	nm	0.04	0.05
Cation exchange capacity (meq/100 g)	10.1	21.6	17.4	4.1	13.6[a]

BS: British Standard
UDSA: United States Department of Agriculture
nm: not measured
A: result expressed as cmol/kg

Preliminary experiments

Preliminary experiments were conducted with ^{14}C-abscisic acid to determine the optimal soil-to-solution ratio, the time required to achieve an equilibrium between the test substance in solution and that adsorbed to soil and any binding of the test substance to the glass vessels.

Experimental conditions

In the main experiment aliquots of 0.01 M calcium chloride were added to duplicate portions of each soil type to give a soil-to-solution ratio of 1 : 1 (w/v). The vessels were shaken in the dark at 20°C for at least 18 hours. At the end of this period, aliquots of ^{14}C-abscisic acid fortification solutions in acetonitrile:water (1:1, v/v) at 25, 50, 125, 500 or 2500 µg/l were added to the appropriate vessels to achieve final test solution concentrations of approximately 0.05, 0.10, 0.25, 1.0 or 5.0 mg/l (final co-solvent volume <0.1% v/v). A third replicate vessel of each soil type was prepared at the highest concentration (5.0 mg/l) to assess any degradation of the test substance in the aqueous solution and soil during the adsorption process. Degradation of the test substance in the aqueous solution and soil during the desorption phase was determined in the other two vessels of each soil type at the highest concentration.

The fortified test vessels were shaken in darkness at 20°C for the adsorption equilibrium time of 24 hours. The soil/solution mixtures were separated by centrifugation. The supernatant was removed, the volume recorded and aliquots were taken for LSC to determine the concentration of radioactivity in solution after adsorption.

Following adsorption, the volume of supernatant removed was replaced by an equal volume of 0.01 M calcium chloride solution (except for one vessel of each soil type at the highest concentration). The test vessels were shaken in darkness at 20°C for the desorption equilibrium time of 24 hours. The volume of each supernatant was recorded and aliquots taken for radio assay by LSC. Soil-less controls (at the highest concentration) were prepared to establish the extent of adsorption to the glass tubes.

The additional vessels of each soil type at the highest concentration were removed after 24 hours adsorption and the remaining two vessels at the highest concentration were removed after 24 hours desorption and analysed to determine the amount of abscisic acid in supernatants and soil extracts. A mass balance was obtained for these samples. The calcium chloride supernatant solutions were analysed by HPLC and TLC. The soil samples were extracted once with acetonitrile followed twice with acetonitrile:water (1:1 v/v) by shaking for 20 minutes. The volumes of the extracts were measured and aliquots of each extract were radio assayed (LSC). For each soil type, and separately for the adsorption and desorption phases, the extracts were pooled and analysed by HPLC and TLC. The remaining soil after extraction was air-dried, combusted and radio assayed by LSC.

Results and discussion

Preliminary tests

Preliminary tests showed that the optimum soil-to-solution ratio for all soils was 1 : 1 (w/v), with an adsorption equilibrium time of 24 hours. The same equilibrium time of 24 hours was used for the desorption phase. There was no significant adsorption of radioactivity to the glass tubes in the absence of soil.

Transformation of test compound

Abscisic acid represented 83.6 – 99.6% of the solution radioactivity and 52.1 – 98.7% of the sample radioactivity in soil extracts after the absorption phase, as measured in vessels treated at the highest concentration. After the desorption phase, abscisic acid represented 86.1 – 100% of the solution radioactivity and 11.3 – 96.9% of the sample radioactivity in soil extracts, again as measured in vessels treated at the highest concentration (Table 4.1.4.6-2). The supernatant and soil extract concentrations were adjusted for the amount of abscisic acid present before the calculation of Freundlich adsorption and desorption coefficients.

Table 4.1.4.6-2 Amount of abscisic acid in supernatants and soil extracts after adsorption and adsorption/desorption equilibrium

Soil	Abscisic acid (%)			
	Adsorption		Desorption	
	Supernatant	Soil extracts	Supernatant	Soil extracts
Bromsgrove	97.7	98.6	97.5	92.1
Elmton	83.6	52.1	98.1	11.3
Evesham 3	98.9	98.5	100	96.9
Warsop	99.6	98.7	99.4	96.5
Hodnet	86.4	82.1	86.1	85.8

Results

The Freundlich adsorption coefficients (K_f^{ads}) ranged from 0.042 - 3.31. Freundlich adsorption coefficients adjusted for soil organic carbon content (K_{Foc}^{ads}) ranged from 2.69 - 77.0 (Table 4.1.4.6-3). Abscisic acid was classified as having a high mobility (Elmton soil) or very high mobility (Bromsgrove, Evesham 3, Warsop and Hodnet soils) in soil.

For each soil type, the Freundlich desorption constants (K_{Foc}^{des}) were slightly higher than the corresponding values for the adsorption phase and were in the range 12.8 - 80.2. Freundlich adsorption exponents (1/n) are in the range 0.65 to 1.38.

Table 4.1.4.6-3 Adsorption and desorption characteristics of abscisic acid in five soils at 20°C

Soil	pH	Organic carbon (%)	Adsorption			Desorption		
			K_f^{ads}	$K_f^{ads}oc$	1/n	K_F^{des}	K_{Foc}^{des}	1/n
Bromsgrove	5.0	1.7	0.314	18.5	0.65	0.670	39.4	0.40
Elmton	6.9	4.3	3.31	77.0	0.73	3.45	80.2	0.96
Evesham 3	7.3	1.6	0.0417	2.69	1.32	0.324	20.3	1.43
Warsop	3.9	0.6	0.223	37.2	1.10	0.371	61.8	1.03
Hodnet	5.9	1.9	0.0529	2.78	1.38	0.243	12.8	1.13

K_F^{ads} Freundlich adsorption distribution coefficient

K_{Foc}^{ads} Coefficient of adsorption per unit organic carbon

K_F^{des} Freundlich desorption distribution coefficient

K_{Foc}^{des} Coefficient of desorption per unit organic carbon

1/n Exponent of the Freundlich desorption isotherm

Mass balance

The recoveries of radioactivity were within the range of 74.5 to 91.0% (Table 4.1.4.6-4). The low total recoveries were assumed to be a result of the degradation of abscisic acid to carbon dioxide, which was not collected in this study. Carbon dioxide is a known degradation product of abscisic acid in soil.

Table 4.1.4.6-4 Mass balances

Fraction	Soil type / % applied radioactivity				
	Bromsgrove	**Elmton**	**Evesham 3**	**Warsop**	**Hodnet**
Adsorption solution	45.5	10.3	51.7	53.9	49.5
Desorption solution	18.6	1.3	18.8	23.2	19.1
Soil extract[a]	8.3	4.9	13.7	12.3	15.3
Non-extractable[a]	2.1	58.6	1.8	1.6	2.7
Total recovery	74.5	75.1	86.0	91.0	86.6

[a]: includes only the solution separated from soil after centrifugation
[b]: after desorption

Conclusions

The Freundlich adsorption constant (K_{Foc}^{ads}) for abscisic acid ranged from 2.69 to 77.0 and the K_{Foc}^{des} ranged from 12.8 to 80.2. Abscisic acid was therefore classified as having a high mobility (one soil) or very high mobility (four soils) in soil.

Remarks

The study is acceptable and the endpoints reliable and acceptable. The fact that ^{14}C-R-abscisic acid was used instead of ^{14}C-S-abscisic acid has no influence on the obtained endpoints.

4.2 Bioaccumulation

4.2.1 Bioaccumulation test on fish

No data available.

4.2.2 Bioaccumulation test with other organisms

No data available.

4.2.3 Other studies

Study reference: Ponte, 2006b,

Test substance: S-abscisic acid (ABA, S-ABA, cis-trans-ABA, (+)-2-cis, 4 –trans-abscisic acid), lot/ batch number 030806D1, purity 99.7%

Methods:

The log Pow of S-abscisic acid (non-iodized) was estimated to be 2.38, based on its chemical structure, using KOWWIN version 1 67 software (EPA program in EPIWIN suite, 2000).

The n-octanol/water partition coefficient of S-abscisic acid purified ingredient was determined for the ionized and non-ionized forms of the compound by HPLC with a C18 reversed phase column. For the

ionized S-abscisic acid, the eluent used was methanol/ water, 3/1 v/v at pH 6.2, and for the non-ionized form the eluent was methanol/ 0.1% trifluoroacetic acid, 3/1, v/v at pH 2.5.

Results:

The log Pow of S-abscisic acid was determined as 0.94 and 1.8 for the ionized and non-ionized forms, respectively, based on triplicate measurements.

4.3 Acute toxicity

4.3.1 Short-term toxicity to fish

Study reference: STUDY IIA, 8.2.1/01

Characteristics

Type of study	:	Acute toxicity study	Species	:	Rainbow trout (*Oncorhynchus mykiss*)
Year of execution	:	2007	Exposure duration	:	96 hours
GLP statement	:	Yes	Nominal concn.	:	0 and 120 mg a.s./L
Guideline	:	OECD 203, OPPTS 850.1075	Dosing method	:	Semi-static; direct addition of test substance to dilution water
Test substance	:	VBC-30054, lot no. 124-164-W9	Acceptability	:	Acceptable
Purity	:	97%	96-h LC50	:	>121 mg a.s./L

Methods

A 96-hour acute toxicity limit test in rainbow trout (*Oncorhynchus mykiss*) (3 replicates of 10 fish, length range 40-49 mm, not fed for at least 2 days prior to test initiation) was conducted according to OECD 203 under semi-static conditions (renewal after 48 hours) with VBC-30054 at a test concentration of 120 mg a.s./L, with untreated control. Test solutions were prepared by direct addition of the test substance to the dilution water followed by mixing. Concentrations of VBC-30054 were analysed by HPLC/UV in centrifuged and non-centrifuged samples (diluted where necessary) of the test solutions taken at the start and the end of exposure and after 48 hours, prior to renewal.

Results

Measured concentrations of VBC-30054 in centrifuged and non-centrifuged samples ranged between 97% and 104% of nominal (mean measured concentration 101% of nominal). Test endpoints were based on the mean measured concentration (121 mg a.s./L), which is acceptable. Other validity criteria of OECD 203 were satisfied (mortality in control ≤10%, oxygen concentration at least 60% of saturation). Water quality parameters (pH, oxygen concentration and temperature) were in accordance with the OECD 203 guideline.

No mortality occurred in the treated and control replicates, and no clinical signs of intoxication were observed. The 96-hour LC50 is >121 mg a.s./L.

Conclusion

96-hour LC50 >121 mg a.s./L, based on mean measured concentrations.

Guidelines and limitations

The study is acceptable.

4.3.2 Short-term toxicity to aquatic invertebrates

Study reference: STUDY IIA, 8.3.1/01

Characteristics

Type of study	:	Acute toxicity study	Species	:	*Daphnia magna*
Year of execution	:	2008	Exposure duration	:	48 hours
GLP statement	:	Yes	Nominal conc.	:	0 and 120 mg a.s./L
Guideline	:	OECD 202, OPPTS 850.1010	Dosing method	:	Static; direct addition of test substance to dilution water
Test substance	:	VBC-30054, lot no. 124-164-W9	48h-EC50	:	>116 mg a.s./L
Purity	:	97%	Acceptability	:	Acceptable

Methods

A 48-hour acute toxicity limit test in *Daphnia magna* (three replicates of 10 daphnids (<24 hours old) per concentration) was conducted according to OECD 202 under semi-static conditions (renewal after 48 hours) with VBC-30054 at a test concentration of 120 mg a.s./L, with untreated control. Test solutions were prepared by direct addition of the test substance to the dilution water followed by sonication and mixing. Concentrations of VBC-30054 were analysed by HPLC/UV in samples of the test solutions (diluted where necessary) taken at the start and the end of exposure and after 24 hours, prior to renewal.

Results

Measured concentrations of VBC-30054 in test samples ranged between 93% and 101% of nominal (mean measured concentration 97% of nominal). Test endpoints were based on the mean measured concentration (116 mg a.s./L), which is acceptable. The results of the test satisfied the validity guideline requirements (OECD 202: immobilisation in the control group not more than 10%, dissolved oxygen concentration ≥3 mg/L). Water quality parameters were in accordance with the OECD 202 guideline. No immobility occurred in the treated and control replicates, hence the 48-hour EC50 is >116 mg a.s./L. However, 53% of the daphnids in the treated replicates appeared lethargic at test termination.

Conclusion

48-hour EC50 >116 mg a.s./L, based on mean measured concentrations.

Guidelines and limitations

The study is acceptable.

4.3.3 Algal growth inhibition tests

4.3.3.1 STUDY 1 – Algae growth inhibition

Study reference: STUDY IIA, 8.4/01

Characteristics

Type of study	:	Algae growth inhibition	Species	:	*Pseudokirchneriella subcapitata*
Year of execution	:	2010	Exposure duration	:	72 hours
GLP statement	:	Yes	Nominal concn.	:	1.0, 3.2, 10, 32 and 100 mg/L
Guideline	:	OECD 201 (2006)	Dosing method	:	Static; stock solution in test water
Test substance	:	VBC-30054, lot no. 183-912-W9-00	Acceptability	:	Acceptable
Purity	:	98.0%	72h-EbC50 & 72h-ErC50 & 72h-EyC50	:	>95.3 mg/L

Methods

A 72-hour growth inhibition test on green algae (*Pseudokirchneriella subcapitata*) (3 replicates per test concentration, each containing 1 x 10^4 cells/mL at the start) was conducted with VBC-30054 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. A stock solution of the test material was prepared in test water. The test concentrations were prepared by adding an aliquot of this stock solution to test water. Concentrations of VBC-30054 were analysed by LC-MS/MS (sufficiently validated) in samples of all test solutions taken at the start and the end of exposure.

Results

Measured concentrations of VBC-30054 in test samples ranged between 110% and 113% of nominal at test initiation and between 71% and 81% at test termination. Test endpoints were based on the geometric mean measured concentrations (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), which is acceptable. Water quality parameters (pH and temperature) were in accordance with the OECD 201 guideline. 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% (0-72 hours), which satisfies the validity criteria of OECD 201 (2006).

The biological results are summarised in the table below. The report presented only endpoints for yield and growth rate, the % inhibition values for biomass were calculated according to the procedures laid down in OECD 201 (1984). The 72-h EbC50, ErC50 and EyC50 was >95.3 mg/L. The reported 72-h NOErC and NOEyC was 29.3 mg/L, and although the data for biomass were not statistically analysed, the 72-h NOEbC may also be set at 29.3 mg/L, based on 34% reduction of biomass at the highest test concentration.

Table 4.3.3.1-1 Summary of biological results of algae growth inhibition test with VBC-30054

Geometric mean measured concentration (mg/L)	inhibition in 72 h (biomass, % of control)[(A)]	inhibition in 72 h (growth rate, % of control)	inhibition in 72 h (yield, % of control)
0.885	9	2	8
2.95	10	2	8

Geometric mean measured concentration (mg/L)	inhibition in 72 h (biomass, % of control)(A)	inhibition in 72 h (growth rate, % of control)	inhibition in 72 h (yield, % of control)
9.08	9	1	7
29.3	7	1	4
95.3	34	8*	35*

* Statistically significant difference from control at 5% level.

(A) Not statistically analysed.

Conclusion

72-h EbC50, ErC50 and EyC50 >95.3 mg/L; 72-h NOEbC, NOErC and NOEyC 29.3 mg/L.

Guidelines and limitations

The study was performed according to OECD 201 (2006) and is acceptable.

4.3.3.2 STUDY 2 – Algae growth inhibition

Study reference: IIA, 8.4/02

Characteristics

Type of study	:	Algae growth inhibition	Species	:	*Navicula pelliculosa*
Year of execution	:	2010	Exposure duration	:	72 hours
GLP statement	:	Yes	Nominal concn.	:	1.0, 3.2, 10, 32 and 100 mg/L
Guideline	:	OECD 201 (2006)	Dosing method	:	Static; stock solution in test water
Test substance	:	VBC-30054, lot no. 183-912-W9-00	Acceptability	:	Acceptable
Purity	:	98.0%	72h-EbC50 & 72h-ErC50 & 72h-EyC50	:	>90.1 mg/L

Methods

A 72-hour growth inhibition test on the freshwater diatom *Navicula pelliculosa* (3 replicates per test concentration, each containing 1 x 10^4 cells/mL at the start) was conducted with VBC-30054 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. A stock solution of the test material was prepared in test water. The test concentrations were prepared by adding an aliquot of this stock solution to test water. Concentrations of VBC-30054 were analysed by LC-MS/MS (sufficiently validated) in samples of all test solutions taken at the start and the end of exposure.

Results

Measured concentrations of VBC-30054 in test samples ranged between 100% and 109% of nominal at test initiation and between 75% and 81% at test termination. Test endpoints were based on the geometric mean measured concentrations (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), which is acceptable. Water quality parameters (pH and temperature) were in accordance with the OECD 201 guideline. 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), which satisfies the validity criteria of OECD 201 (2006).

The biological results are summarised in the table below. None of the differences between the cell density, growth rate and yield in the treated solutions after 72 hours were statistically significantly different from the control at the 95% certainty level. The report presented only endpoints for yield and growth rate. However, the results in the Table below clearly indicate that there was no effect on biomass up to the highest test concentration. The 72-h EbC50, ErC50 and EyC50 was >90.1 mg/L. The 72-h NOEbC, NOErC and NOEyC was 90.1 mg/L.

Table 4.3.3.2-1 Summary of biological results of algae growth inhibition test with VBC-30054

Geometric mean measured concentration (mg/L)	inhibition in 72 h (cell density, % of control)	inhibition in 72 h (growth rate, % of control)	inhibition in 72 h (yield, % of control)
0.880	-3	-1	-3
2.92	-6	-1	-6
9.02	2	0.5	2
28.7	0.3	0.01	0.3
90.1	-2	0.4	-2

Note: negative effects represent increases relative to the control, and are therefore not adverse effects.

Conclusion

72-h EbC50, ErC50 and EyC50 >90.1 mg/L; 72-h NOEbC, NOErC and NOEyC 90.1 mg/L.

Guidelines and limitations

The study was performed according to OECD 201 (2006) and is acceptable.

4.3.4 *Lemna* sp. growth inhibition test

Study reference: STUDY IIA, 8.6/1

Characteristics

type of study	:	Duckweed growth inhibition test	species	:	Duckweed (*Lemna gibba*)
year of execution	:	2010	duration	:	7 days
GLP statement	:	Yes	nominal conc.	:	Test 1: 0.01, 0.032, 0.1, 0.32 and 1.0 mg/L; Test 2: 0.001, 0.0032, 0.01, 0.032, 0.1 and 0.32 mg/L
guideline	:	OECD 221 (2006)	dosing method	:	Semi-static; stock solution in dilution water
test substance	:	S-abscisic acid, batch no. 183-912-W9-00	acceptability	:	Acceptable
Purity	:	98.0%	Lowest NOEC	:	0.0025 mg/L
			Lowest EC50	:	0.0240 mg/L

Methods

Two 7-day semi-static toxicity tests with S-abscisic acid (98.0% pure) on the growth of duckweed (*Lemna gibba*) were performed. The 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.

The tests employed 3 replicates per concentration, each containing at test initiation three plants with four fronds each (test 1) or four plants with 3 fronds each (test 2). The tests were conducted under semi-static conditions (Test 1: renewal on day 3 and 5; test 2: daily renewal). The nominal test concentrations of test 1 were 0.01, 0.032, 0.1, 0.32 and 1.0 mg/L, and those of test 2 were 0.001, 0.0032, 0.01, 0.032, 0.1 and 0.32 mg/L, with untreated control tested in 3 replicates. In each test, one additional replicate was set up to study recovery during a 7-day phase following the 7 day exposure phase (transfer to untreated medium, incubation under the same test conditions).

A stock solution of the test material was prepared in test water. The test concentrations were prepared by adding an aliquot of this stock solution to test water. Concentrations of S-abscisic acid were analysed by LC-MS/MS (sufficiently validated). In test 1, samples of all freshly prepared test solutions of day 0 and of all aged solutions of day 3 were analysed. In test 2, samples of all freshly prepared test solutions of day 0 and day 6 were analysed, and of 24-hour aged test solutions of day 1 and 7. On day 0, 2, 5 and 7, pH, frond number and any change in plant development were recorded. Frond dry weight per vessel was recorded on day 0 (sample of 200 fronds) and 7 (all replicates).

Results

Test 1.

The measured concentrations in fresh solutions ranged between 94% and 100% of nominal, whereas that in 3-day aged solutions of the highest concentration was 6% of nominal, and all others were below the LOQ. Although test concentrations were not maintained between 80% and 120% of nominal, reported endpoints were based on nominal concentrations. Reported endpoints for test 1 were not recalculated based on mean measured concentrations since mean measured concentrations cannot be accurately calculated from the reported results (concentrations <LOQ in 3-day aged solutions). However, test 2 was conducted at lower concentrations, with more frequent renewals, and with analytical results which allowed calculation of mean measured concentrations. Therefore the reported endpoints of test 1 will be included in this summary, but the ultimate endpoints from this study will be taken from test 2.

Water quality parameters (light intensity, pH and temperature) were in accordance with the OECD 221 guideline. The 7-day mean doubling time in the control was 1.41 days, hence the validity criterion of OECD 221 (2006) was satisfied (doubling time <2.5 days). Phytotoxic effects were observed at 0.032-1.0 mg/L nominal (curved fronds, short and coiled roots; at 1.0 mg/L necrosis). Other test results are summarised in the Table below.

Based on nominal concentrations (confirmed by initial measured concentrations), the lowest NOEC is <0.01 mg/L, the lowest EC50 is 0.048 mg/L.

Table 4.3.4-1 The toxicity of S-abscisic acid to *Lemna gibba* (test 1)

nominal conc. (mg/L)	frond number		biomass	
	% inhibition growth rate	% inhibition yield	% inhibition growth rate	% inhibition yield
0.01	6.59	20.8*	2.30	7.18
0.032	14.9*	41.5*	8.12*	24.5*
0.1	40.6*	77.7*	25.9*	59.5*
0.32	63.2*	91.6*	39.1*	76.9*
1	83.8*	97.4*	49.6*	83.1*
Reported EC50[(A)] (mg product/L)	0.191	0.048	>1.0 Note: this EC50 may be set at 1.0 mg/L, since there were sufficient data	0.081

			points with a good dose-response relationship, and approximately 50% inhibition (i.e. 49.6%) at the highest concentration of 1.0 mg/L.	
Reported NOEC(A) (mg product/L)	0.01	<0.01	0.01	0.01

* statistically significant difference from control at 5% level.

(A) Based on nominal concentrations, confirmed by initial measured concentrations.

Data on recovery are shown in the Table below. After 7 days of recovery, frond numbers had increased by at least 7-fold (representing the validity criterion of OECD 221) in all solutions. The results demonstrate that recovery had occurred. The number of fronds in treated solutions was lower than those in the control, but without a concentration-relationship over a 100X concentration range. Statistical analysis was not possible (only one replicate per concentration was used).

Table 4.3.4-2 Recovery of *Lemna gibba* after 7-day exposure (renewal on day 3 and 5) to S-abscisic acid (test 1)

Concentration (mg test item/L)	Frond Number Day 0	Frond Number Day 3	Frond Number Day 5	Frond Number Day 7	Fold Increase Day 0-Day 7
Control	12	56	174	385	32.1
0.01	12	53	136	308	25.7
0.032	12	53	122	288	24.0
0.1	12	46	100	214	17.8
0.32	12	50	120	300	25.0
1.0	12	53	109	250	20.8

Test 2.

The measured concentrations in fresh solutions ranged between 94% and 133% of nominal, whereas those in 1-day aged solutions were in the range 0-91% of nominal. 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. Although test concentrations were not maintained between 80% and 120% of nominal, reported endpoints were based on nominal concentrations. No justification was provided. The reported endpoints were in addition calculated based on the reported individual replicate values for frond number and frond dry weight, and using the mean measured concentrations. The procedure for determination of the EC50 values was linear regression analysis of the percentage inhibition values of specific growth rate and yield versus the log-transformed mean measured concentrations. Only the results for the 5 highest test concentrations (yield based on frond number) or 4 highest test concentrations (growth rate based on frond number, yield based on dry weight) were used for the regression analysis, in order to use only data points within the linear range.

Table 4.3.4-3 Measured concentrations of S-abscisic acid (test 2)

Nominal conc (mg/L)	meas conc (mg/L)				meas conc (% nominal)					mean meas conc (mg/L)
	day 0	day 1	day 6	day 7	day 0	day 1	day 6	day 7	overall	
0.001	0.00123	0.000554	0.00101	0.000716	123	55	101	72	88	0.00088
0.0032	0.00397	0.000702	0.00333	0.00207	124	22	104	65	79	0.0025
0.01	0.0133	0.00166	0.0103	0.00365	133	17	103	37	72	0.0072
0.032	0.0405	0.0166	0.0308	0.00217	127	52	96	7	70	0.023
0.1	0.13	0.0958	0.116	0	130	96	116	0	85	0.085
0.32	0.392	0.292	0.354	0.0101	123	91	111	3	82	0.26

Water quality parameters (light intensity, pH and temperature) were in accordance with the OECD 221 guideline. The 7-day mean doubling time in the control was 1.35 days, hence the validity criterion of OECD 221 (2006) was satisfied (doubling time <2.5 days). Phytotoxic effects were observed at 0.01- 0.32 mg/L nominal (curved fronds, smaller fronds, short roots, less abundant roots; at 0.32 mg/L chlorosis). Other test results are summarised in the Table below.

Based on mean measured concentrations, the lowest NOEC is 0.0025 mg/L, the lowest EC50 is 0.024 mg/L.

Table 4.3.4-4 The toxicity of S-abscisic acid to *Lemna gibba* (test 2)

nominal conc. (mg/L)	mean measured conc. (mg/L)	frond number		biomass	
		% inhibition growth rate	% inhibition yield	% inhibition growth rate	% inhibition yield
0.001	0.00088	1.74	5.86	-0.04#	-0.53#
0.0032	0.0025	3.18	10.9	-1.54#	-5.88#
0.01	0.0072	10.3*	31.6*	4.82	15.6
0.032	0.023	16.9*	46.8*	12.7*	36.8*
0.1	0.085	35.1*	73.6*	28.4*	64.8*
0.32	0.26	57.0*	89.5*	45.3*	81.9*
Reported EC50(A) (mg/L)		0.249	0.040	>0.32	0.060
Reported NOEC(A) (mg/L)		0.0032	0.0032	0.01	0.01
Recalculated EC50(B) (mg/L)		0.20	0.024	>0.26	0.044
Recalculated NOEC(B) (mg/L)		0.0025	0.0025	0.0072	0.0072

Negative effect, hence no adverse effect.

* statistically significant difference from control at 5% level.

(A) Based on nominal concentrations.

(B) Recalculated, based on mean measured concentrations.

Data on recovery are shown in the Table below. After 7 days in untreated medium, frond numbers had increased by at least 7-fold (representing the validity criterion of OECD 221) in all solutions. The number of fronds in treated solutions of the 3 highest concentrations was lower than those in the control, but without a dose-relationship. The reduced increase at the 3 highest concentrations is due to reduced growth during the first interval (day 0-3). During the remaining intervals, the growth factor in all treated solutions was comparable to that in the control. Therefore it is acceptable to conclude that recovery had occurred. Statistical analysis was not possible (only one replicate per concentration was used).

Table 4.3.4-5 Recovery of *Lemna gibba* after 7-day exposure (daily renewal) to S-abscisic acid (test 2)

Concentration (mg test item/L)	Frond Number Day 0	Frond Number Day 3	Frond Number Day 5	Frond Number Day 7	Fold Increase Day 0-Day 7
Control	12	62	159	358	29.8
0.001	12	63	165	402	33.5
0.0032	12	51	125	299	24.9
0.01	12	60	154	343	28.6
0.032	12	31	83	209	17.4
0.1	12	36	88	204	17.0
0.32	12	37	81	200	16.7

Conclusion

Semi-static test (daily renewal): lowest NOEC 0.0025 mg/L, lowest EC50 0.024 mg/L (all based on mean measured concentrations); recovery occurred within 7 days after transfer to untreated medium.

Guidelines and limitations

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.

4.4 Chronic toxicity

4.4.1 Fish early-life stage (FELS) toxicity test

No data available.

4.4.2 Fish short-term toxicity test on embryo and sac-fry stages

No data available.

4.4.3 Aquatic Toxicity – Fish, juvenile growth test

No data available.

4.4.4 Chronic toxicity to aquatic invertebrates

No data available.

4.4.5 Chronic toxicity to algae or aquatic plants

Study reference: STUDY IIA, 8.6.2/01

Characteristics

type of study	:	Duckweed growth inhibition in microcosms	species	:	Duckweed (*Lemna gibba*)
year of execution	:	2011	duration	:	14 days
GLP statement	:	Yes	nominal conc.	:	4.08, 20.4 and 102 µg/L
guideline	:	OECD 221 (2006), OPPTS 850.4400	dosing method	:	Static; stock solution in test water
test substance	:	S-abscisic acid, batch no. 185-473-W9	acceptability	:	Acceptable
Purity	:	98.2%	NOEC	:	<3.31 µg/L
			EAC	:	23.6 µg/L

Methods

The effects of S-abscisic acid on the growth of duckweed (*Lemna gibba*) was examined under laboratory conditions in small scale laboratory microcosms containing sediment, natural pond water, macrophytes and any invertebrates naturally found in the water or the sediment, under static conditions for a period of 14 days.

Twenty laboratory microcosms were established in 3 L beakers containing sediment (500 g), natural pond water containing the constituents of synthetic *Lemna* growth medium (1.8 L), macrophytes (*Elodea*, 3 lengths of 20 cm each) and any invertebrates naturally found in the water or the sediment. The sediment was collected from an outdoor microcosm and had pH 7.0, 1.10% organic carbon and CEC 29.2 meq/100 g. Following difficulties of culturing the *Lemna* in pond water, the test medium was changed from pond water to pond water containing the constituents of synthetic *Lemna* growth medium. Each beaker was loosely covered with a transparent plastic bag. Prior to treatment and addition of the test organisms, the beakers were incubated for 29 days at 22-26°C under continuous illumination provided by fluorescent overhead light at an intensity of 3000-5000 lux. The beakers were re-allocated to new random positions twice each week. *Elodea* plants grew substantially during this establishment phase and were cut back to standardise the amount of plant material in each beaker 2 days prior to the start of the definitive test. No information was provided on the condition of *Elodea* plants during exposure.

After the 29-day establishment phase, each microcosm was treated with 200 mL of a stock solution of the test substance in natural pond water containing the constituents of synthetic *Lemna* growth medium, followed by gentle stirring. The nominal test concentrations were 4.08, 20.4 and 102 µg/L. Five replicate vessels were prepared for the control and each treatment level, each containing 50 fronds. The test solutions were not renewed during the study. Concentrations of S-abscisic acid were determined by LC-MS/MS (sufficiently validated) in samples of all freshly prepared test solutions of day 0, and in all test solutions collected after 12, 24, 48 and 96 hours, and 8, 11 and 14 days (study end). Test vessels were maintained at 22-25°C under continuous illumination provided by fluorescent overhead light at an intensity of 3656-3836 lux. The beakers were re-allocated to new random positions 7 times during the study. During the test, the pH and dissolved oxygen were monitored on day 0, 4, 7, 12 and 14: the pH was in the range 9.7-10.6, dissolved oxygen in the range 134-225% of saturation. Hardness (as $CaCO_3$) was measured at the start of the test: 240-334 mg/L. On day 3, 5, 7, 9, 12 and 14, frond number and any change in plant development were recorded. Frond dry weight per vessel was recorded on day 0 (sample of 200 fronds) and 14 (all replicates).

Results

The measured concentrations in fresh solutions of individual replicates ranged between 81% and 118% of nominal, except for one replicate of 4.08 µg/L nominal (55% of nominal). However, this was not due to incorrect preparation, but due to lack of homogeneity, since the concentration in this replicate was 73% of nominal after 12 hours. 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.

Table 4.4.5-1 Measured concentrations of S-abscisic acid (% of nominal)

	Measured concentrations of S-abscisic acid (% of nominal)						
nom conc (µg/L)	0 hr	12 hr	24 hr	48 hr	96 hr	8 d	11 d
4.08	81	79	58	19	21	0	0
	90	81	78	35	23	0	0
	90	92	73	33	14	0	0
	90	92	64	37	20	0	0
	55	73	53	22	0	0	0
mean	*81*	*83*	*65*	*29*	*16*	*0*	*0*
20.4	117	97	85	43	23	0	0
	118	105	88	51	31	4	2
	114	94	68	42	20	0	0
	115	107	80	45	25	0	0
	115	90	77	29	20	0	0
mean	*116*	*99*	*80*	*42*	*24*	*1*	*0*
102	103	79	75	20	13	0.4	0
	111	106	96	33	38	3	1
	113	98	98	25	31	2	1
	102	98	85	25	24	1	0
	102	91	82	16	19	2	0
mean	*106*	*94*	*87*	*24*	*25*	*2*	*0*

The doubling time of the fronds in the control was 7 days at the end of the test, which corresponds to an about 4-fold increase in frond number.

Mean total frond and plant counts and dry weight measurements are shown in Table 4.4.5-2 below. These results show that after 14 days the number of fronds in all treated solutions was lower than that of the control, but this reduction appears to be due to the reduction in growth during the first (day 0-3) interval. This is confirmed by the results in Table 4.4.5-3, which shows the percent increase in frond number during each assessment interval: a reduction in percent increase during assessment intervals was only apparent for the first interval. Further confirmation is obtained when the data were expressed in terms of average specific growth rate for each assessment period (see Table 4.4.5-4): a reduction of growth rate based on frond numbers was only apparent during the first assessment interval.

Table 4.4.5-2 Mean total frond and plant counts and dry weight measurements

	frond number on day:								
mean meas conc (µg/L)	0	3	5	7	9	12	14	Bio-mass day 14, mg	Bio-mass per frond day 14, mg
control	50	115	133	147	170	188	201	34.2	0.17
3.31	50	96.2	115	124	142	160	172	31.5	0.183
23.6	50	87.6	108	122	144	163	172	29.1	0.169
108	50	69.8	80.6	97.8	135	169	181	27.5	0.152

Table 4.4.5-3 Percent increase in frond number during each assessment interval

Treatment groups (µg/L)	% increase in frond number between assessment Day					
	0 to 3	3 to 5	5 to 7	7 to 9	9 to 12	12 to 14
Control mean	130	15.9	10.3	16.0	11.0	6.8
4.08 [3.31] mean	92.4	19.8	7.4	15.0	12.2	7.7
20.4 [23.6] mean	75.2	23.2	12.7	18.4	13.3	5.4
102 [108] mean	39.6	15.1	21.5	39.0	25.7	7.3

[ ] : initial mean measured concentrations

Table 4.4.5-4 Effects on average specific growth rate during each assessment interval

meas conc (µg/L)	% inhibition of average specific growth rate during interval:					
	Day 0-3	day 3-5	day 5-7	day 7-9	day 9-12	day 12-14
3.31	21*	-23	27	6	-11	-13
23.6	32*	-42	-22	-14	-20	-20
108	60*	6	-98*	-121*	-118*	-7

Note: negative effects represent increases relative to the control and are not adverse effects.

* statistically significant difference from control at 5% level or below.

Visual observations made at 3.31 µg/L were comparable to those in the control and not attributable to treatment. At 23.6 µg/L, small fronds were observed from day 5 onwards, with about half of the fronds being small and gibbous on day 9. However, the majority of the fronds had recovered by days 12 and 14, with only a minority still affected; hence the potential for recovery was apparent. At 108 µg/L, small and gibbous fronds were observed in all vessels from day 5 onwards, and this was sustained for the remainder of the test. These observations were supported by the reduced final biomass per frond (by 11%, statistically significant, see Table4.4.5-5) at 108 µg/L.

The results for the standard test endpoints are summarized in the table below. The report stated that for the standard endpoints, those endpoints derived from frond number and total final biomass do not describe the re-growth of the plants or the lack of sustained effects, and that therefore, these data are not appropriate for expressing the test results in this type of study. This statement is acceptable. The lack of sustained effects on parameters based on frond numbers is however also apparent from the observation that in the Table below there are either no remarkable differences between the day 7 and 14 percent inhibition values (3.31 and 23.6 µg/L), or there is a clear reduction in effect after 14 days compared to after 7 days (108 µg/L).

Table 4.4.5-5 Effects on growth of *Lemna* (standard test endpoints)

evaluation day	parameter	% inhibition at meas conc (ug/L):		
		3.31 µg/L	23.6 µg/L	108 µg/L
7	frond count	16*	17*	33*
	frond count (AUC)	25*	34*	63*
	frond count (av. spec. growth rate)	16*	17*	38*
	frond count (yield)	24*	26*	51*
14	frond count	15*	14*	10*
	frond count (AUC)	15*	17*	26*
	frond count (av. spec. growth rate)	11*	11*	7*
	frond count (yield)	19*	19*	13*
	dry weigth (growth rate)	5	10	14*
	dry weigth (yield)	10	19*	20*
	dry weigth (final biomass)	8	17*	19*
	dry weigth (final biomass per frond)	-8	1	11*

Note: negative effects represent increases relative to the control and are not adverse effects.

* statistically significant difference from control at 5% level or below.

Evaluation

Treatment with S-abscisic acid reduced the growth of *Lemna gibba* during the first assessment interval (day 0-3). Thereafter the growth rate in the treated microcosms was comparable to or higher than that in the control. The reductions in frond number by about 10-25% in treated microcosms, observed at test termination, are associated with the reduced growth during day 0-3. Therefore it may be concluded that the NOEC for effects on frond number was <3.31 µg/L, but that the effects on frond number were slight and transient, with recovery within 14 days at all test concentrations. After 14 days, total biomass (dry weight) was unaffected at 3.31 µg/L, but there were reductions of 17-19% (statistically significant) at 23.6 µg/L. Since at 23.6 µg/L the biomass per frond was comparable to that in the control, these reductions are considered to be related to the reduced frond number at test termination (reduction of 14%), due to the reduced growth during day 0-3. Hence the reductions of total biomass at 23.6 µg/L are considered to be an indirect effect of the reduced growth during day 0-3. At test termination, at 108 µg/L frond counts were reduced by 10%, but total biomass showed reductions of up to 20%. These reductions of total biomass at 108 µg/L cannot be attributed only to the reduced growth during day 0-3, but they are also a consequence of 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).

Based on the above considerations, the EAC may be set at 23.6 µg/L, since at this test concentration (and at the lower concentration of 3.31 µg/L); effects observed were slight and transient, with full recovery within 14 days. Effects on frond number at 108 µg/L were also slight and transient, but effects on biomass were noted after 14 days, for which recovery could not be demonstrated, due to the test design.

Conclusion

Static test conditions, laboratory microcosms containing sediment, natural pond water containing the constituents of synthetic *Lemna* growth medium and macrophytes, exposure for 14 days: initial measured concentrations 3.31, 23.6 and 108 µg/L; NOEC <3.31 µg/L: EAC 23.6 µg/L (effects slight and transient, full recovery within 14 days); at 108 µg/L slight and transient effects on frond number, but effects on biomass, for which recovery could not be demonstrated.

Guidelines and limitations

The test is acceptable.

4.5 Acute and/or chronic toxicity to other aquatic organisms

No data available.