Australian Government

Australian Pesticides and Veterinary Medicines Authority

# PUBLIC RELEASE SUMMARY

on the evaluation of the new active s-abscisic acid in the product ProTone SG Plant Growth Regulator Soluble Granule

APVMA Product Number 63314

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Website: This publication is available from the APVMA website: http://www.apvma.gov.au

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# PREFACE

The Australian Pesticides and Veterinary Medicines Authority (APVMA) is the Australian Government regulator with responsibility for assessing and approving agricultural and veterinary chemical products prior to their sale and use in Australia.

In undertaking this task, the APVMA works in close cooperation with advisory agencies, including the Department of Health and Ageing, Office of Chemical Safety and Environmental Health (OCSEH), Department of Environment, Water, Heritage and the Arts (DEWHA), and State Departments of Primary Industries.

The APVMA has a policy of encouraging openness and transparency in its activities and of seeking community involvement in decision making. Part of that process is the publication of public release summaries for products containing new active constituents.

The information and technical data required by the APVMA to assess the safety of new chemical products and the methods of assessment must be undertaken according to accepted scientific principles. Details are outlined in the APVMA's publications *Ag MORAG: Manual of Requirements and Guidelines* and *Vet MORAG: Manual of Requirements and Guidelines*.

This Public Release Summary is intended as a brief overview of the assessment that has been conducted by the APVMA and of the specialist advice received from its advisory agencies. It has been deliberately presented in a manner that is likely to be informative to the widest possible audience thereby encouraging public comment.

## About this document

This is a Public Release Summary.

It indicates that the Australian Pesticides and Veterinary Medicines Authority (APVMA) is considering an application for registration of an agricultural or veterinary chemical. It provides a summary of the APVMA's assessment, which may include details of:

- the toxicology of both the active constituent and product
- the residues and trade assessment
- occupational exposure aspects
- environmental fate, toxicity, potential exposure and hazard
- efficacy and target crop or animal safety.

Comment is sought from interested persons on the information contained within this document.

## Making a submission

In accordance with sections 12 and 13 of the Agvet Code, the APVMA invites any person to submit a relevant written submission as to whether the application for registration of **PROTONE SG PLANT GROWTH REGULATOR SOLUBLE GRANULE** should be granted. Submissions should relate only to matters that the APVMA is required by legislation to take into account in deciding whether to grant the application. These grounds are **public health aspects, occupational health and safety, chemistry and manufacture, residues in food, environmental safety, trade** and **efficacy**. Submissions should state the grounds on which they are based. Comments received outside these grounds cannot be considered by the APVMA.

Submissions must be received by the APVMA by close of business on **Tuesday 28/09/2010** and be directed to the contact listed below. All submissions to the APVMA will be acknowledged in writing via email or by post.

Relevant comments will be taken into account by the APVMA in deciding whether the product should be registered and in determining appropriate conditions of registration and product labelling.

When making a submission please include:

- Contact name
- Company or Group name (if relevant)
- Postal Address
- Email Address (if available)
- The date you made the submission.

All personal and ***confidential commercial information (CCI)***[1] material contained in submissions will be treated confidentially.

[1] A full definition of "confidential commercial information" is contained in the Agvet Code.

Written submissions on the APVMA's proposal to grant the application for registration that relate to the **grounds for registration** should be addressed in writing to:

Ag Products Coordinator
Pesticide Program
Australian Pesticides and Veterinary Medicines Authority
PO Box 6182
Kingston ACT 2604

**Phone:** (02) 6210 4700
**Fax:** (02) 6210 4776
**Email:** AgProductsCordinator@apvma.gov.au

## Further information

Further information can be obtained via the contact details provided above.

Copies of full technical evaluation reports covering toxicology, occupational health and safety aspects, residues in food and environmental aspects are available from the APVMA on request.

Further information on public release summaries can be found on the APVMA website: http://www.apvma.gov.au

# 1 INTRODUCTION

The Australian Pesticides and Veterinary Medicines Authority (APVMA) has before it an application from Valent BioSciences, a Division of Sumitomo Chemical Australia Pty Ltd for registration of a new product, ProTone SG Plant Growth Regulator Soluble Granule containing the new active constituent *S-abscisic acid*.

The slow coloration of berries and bunches in table grapes results in bunches not being harvested early enough for overseas markets. Also currently the harvest of table grapes requires multiple picks due to uneven coloration of grapes.

ProTone SG Plant Growth Regulator Soluble Granule is for use to accelerate or increase the coloration of berries and bunches of table grapes (*Vitis* spp.).

ProTone SG Plant Growth Regulator Soluble Granule contains the natural plant hormone *S-abscisic acid* that stimulates anthocyanin biosynthesis and accumulation in grapes. *S-abscisic acid* is one of the five major classes of naturally occurring plant hormones and besides the effect on anthocyanin production (a maturation response in grapes) *S-abscisic acid* is an important plant hormone, which is involved in many physiological effects in plants.

ProTone SG Plant Growth Regulator Soluble Granule will be packaged in containers between 250mL and 1L.

*S-abscisic acid* is currently contained in products registered in the U.S.A.

This publication provides a summary of the data reviewed and an outline of the regulatory considerations for the proposed registration of ProTone SG Plant Growth Regulator Soluble Granule, and approval of the new active constituent, *S- abscisic acid*.

This submission has been assessed under a joint review/ workshare arrangement where registrations for the same formulations and uses have been submitted concurrently in USA, and Australia.

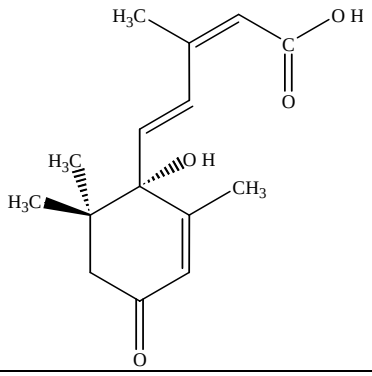
# 2 CHEMISTRY AND MANUFACTURE

## 2.1 ACTIVE CONSTITUENT

*S-abscisic acid* belongs to the pyrazolone family of compounds, and is a naturally occurring plant growth regulator. *S-abscisic acid* has a number of functions and hence a number of modes of action, including closure of stomata to reduce evapotranspiration by inhibition of H+/ATPases, maintenance of seed dormancy and ripening of some fruits. The mechanism for the latter two functions is not well understood.

Abscisic acid has an asymmetric or chiral carbon atom at the 1' ring position. This results in the molecule possessing optical activity with two possible enantiomers or isomers described by scientific convention as S-abscisic acid and R-abscisic acid. Only the S enantiomer is present in plant cells. Most of the endogenous *S-abscisic acid* is the 2-*cis*,4-*trans* isomer. The 2-*trans*,4-*trans* form (B) occurs in small amounts.

The chemical active constituent *S-abscisic acid* has the following properties:

| | |
|---|---|
| **COMMON NAME (ISO):** | *S*-abscisic acid |
| **CHEMICAL NAME:** | (*S*)-(2*Z*,4*E*)-5-(1-Hydroxy-2,6,6-trimethyl-4-oxo-cyclohex-2-en-1-yl)-3-methylpenta-2,4-dienoic acid |
| **PRODUCT NAME:** | ProTone SG Plant Growth Regulator Soluble Granule |
| **CAS REGISTRY NUMBER:** | 21293-29-8 |
| **EMPIRICAL FORMULA:** | $C_{15}H_{20}O_4$ |
| **MOLECULAR WEIGHT:** | 264.3 $gmol^{-1}$ |
| **PHYSICAL FORM:** | Powder |
| **COLOUR:** | White |
| **ODOUR:** | Odourless |
| **MELTING POINT:** | Does not melt, decomposes at 159-162 °C |
| **DENSITY:** | 1.21 $g/cm^3$ |
| **LOG OCTANOL/WATER PARTITION:** | Un-ionised form: 1.8; ionised form: 0.94 |
| **VAPOUR PRESSURE AT 25°C:** | $<2 \times 10^{-6}$ Pa (25 °C) |
| **STRUCTURAL FORMULA:** |  |

The Chemistry Section of the APVMA has evaluated the chemistry aspects of *S-abscisic acid* (physico-chemical properties, spectral data, stability, manufacturing process, quality control procedures, batch analysis results and analytical methods).

*S-abscisic acid* is a new active constituent and approval is pending. On the basis of the data provided, it is proposed to establish the following Active Constituent Standard for *S-abscisic acid*:

| Constituent | Specification | Level of Purity |
|---|---|---|
| *S*-abscisic acid | *S*-abscisic acid | Not less than 950 g/kg |

Based on a review of the data provided by the applicant, the APVMA is satisfied that the chemistry and manufacturing details of *S-abscisic acid* are acceptable.

Other characteristics of *S-abscisic acid* (toxicology, environmental fate etc) are covered in subsequent sections of this Public Release Summary.

## 2.2 FORMULATED PRODUCT

The Chemistry Section has evaluated the chemistry aspects of the product, ProTone SG Plant Growth Regulator Soluble Granule (physico-chemical properties, formulation process, quality control procedures, batch analysis results, stability, analytical methods and packaging).

ProTone SG Plant Growth Regulator Soluble Granule has the following properties:

| | |
|---|---|
| Appearance: | White granular solid with an odour described as sweet, resembling dirt and menthol-like |
| Formulation type: | Soluble granule |
| Active constituent concentration: | 200 g/kg |
| Bulk density | 0.5313 g/cm$^3$ |
| pH (1% dilution): | 3.17 |
| Safety properties: | Not corrosive, flammable or explosive |

The product will be formulated in the USA using *S-abscisic acid* manufactured in China. The manufacturing and quality control procedures, including compliance with the release specifications, are acceptable.

The applicant provided the results of real time and accelerated stability testing conducted using samples stored in high-density polyethylene containers (the proposed commercial container type). Testing of all of the important parameters for soluble granule formulations was conducted. The results indicate that the formulated product is expected to be stable for at least two years when stored under normal conditions in the proposed commercial packaging.

## Recommendation

Based on a review of the data provided by the applicant, the APVMA is satisfied that the chemistry and manufacturing details of ProTone SG Plant Growth Regulator Soluble Granule are acceptable.

# 3 TOXICOLOGICAL ASSESSMENT

## 3.1 Summary

*S-abscisic acid*, which naturally occurs in plants, is a new agricultural chemical to the Australian market. The product, ProTone SG Plant Growth Regulator Soluble Granule, contains 200 g/kg of S-*abscisic acid*. ProTone SG Plant Growth Regulator Soluble Granule will be used to accelerate or increase the red colouration in grape berries and bunches. *S-abscisic acid* is a monocyclic sesquiterpene plant hormone that is involved in many major processes during plant growth and development, and also plays a role in the development of pigments in fruits including grapes.

Since *S-abscisic acid* naturally occurs in plants and is therefore part of the normal human diet, an evaluation of its toxicokinetic profile was not considered necessary in this instance.

*S-abscisic acid* was of low acute oral, dermal and inhalational toxicity in rats. It was not a skin irritant in rabbits or a skin sensitiser in guinea pigs. It was a slight eye irritant in rabbits. The formulated product, containing 200 g/kg of *S-abscisic acid* was of low acute oral and dermal toxicity in rats, and it is estimated that it will have low acute inhalation toxicity. It was a slight skin and eye irritant in rabbits, and was not a skin sensitiser in guinea pigs.

Given that *S-abscisic acid* is a naturally occurring chemical present in food, exposure to *S-abscisic acid* at food comparable levels is not expected to cause toxicological effects. Consistent with its nature as a food component, there were no clear signs of toxicity in a number of dietary studies with *S-abscisic acid* (active constituent) in rats: following a 4-week and a 13-week dietary study in rats, no adverse toxicological effects were seen at intakes of up to 20,000 ppm (around 1,500 mg/kg bw/d). There was no evidence of systemic toxicity in a repeat dose dermal study in rats with doses up to and including 1000 mg/kg bw/day. No chronic toxicity studies with *S-abscisic acid* were available for evaluation.

*S-abscisic acid* was negative in an *in vitro* reverse mutation assay in bacteria, an *in vitro* chromosome aberration assay in Chinese hamster ovarian cells and an *in vivo* mouse micronucleus assay. *S-abscisic acid* was not a developmental toxicant in rats. No reproductive studies with *S-abscisic acid* were available for evaluation, though no effects on reproductive organs were seen in studies in rats.

In a non-GLP reporter gene assay, *S-abscisic acid* did not demonstrate any estrogenic, anti-estrogenic, androgenic or anti-androgenic effects.

Workers may be exposed to the product when opening containers, mixing/loading, application and cleaning up spills and equipment. The main route of exposure to the spray will be dermal and inhalational, with potential accidental ocular exposure. Given the short term intermittent use pattern (i.e. prior to harvest, with up to six applications though it is more likely only one or two applications will occur) and lack of systemic toxicity in a 28-day dermal toxicity in rats (i.e. the 28 day dermal NOEL is 1000 mg/kg bw/d the highest dose tested), together with *S-abscisic acid*'s absence of treatment related systemic toxicity in a subchronic test, a quantitative occupational health and safety risk assessment is not required.

Based on a health risk assessment, First Aid Instructions, Warning Statements and Safety Directions have been recommended and shown on the product label.

Based on an assessment of the toxicology, it was considered that there should be no adverse effects on human health from the use of ProTone SG Plant Growth Regulator Soluble Granule when used in accordance with the label directions.

## 3.2 EVALUATION OF TOXICOLOGY

The toxicological database for *S-abscisic acid*, which consists primarily of toxicity tests conducted in laboratory animals, is quite extensive. In interpreting the data, it should be noted that toxicity tests generally use doses that are high compared with likely human exposures. The use of high doses increases the likelihood that potentially significant toxic effects will be identified. Findings of adverse effects in any one species do not necessarily indicate such effects might be generated in humans. From a conservative risk assessment perspective however, adverse findings in animal species are assumed to represent potential effects in humans, unless convincing evidence of species specificity is available. Where possible, considerations of the species specific mechanisms of adverse reactions weigh heavily in the extrapolation of animal data to likely human hazard. Equally, consideration of the risks to human health must take into account the likely human exposure levels compared with those, usually many times higher, which produce effects in animal studies. Toxicity tests should also indicate dose levels at which the specific toxic effects are unlikely to occur. Such dose levels as the No-Observable-Effect-Level (NOEL) are used to develop acceptable limits for dietary or other intakes (ADI and ARfD) at which no adverse health effects in humans would be expected.

The toxicology assessment of *S-abscisic acid* was conducted jointly by scientists from the United States (EPA) and Australia (OCSEH).

### Toxicokinetics and Metabolism

*S-abscisic acid* is naturally found in all plants thus far analysed, and is a natural constituent of plant growth regulation, is synthesized and metabolized by plants as a natural part of their physiology, and therefore has a long history as a natural part of the diet of all animals that eat plants. Therefore, its physiological intermediates, metabolites and catabolites are natural constituents and components an animal diet, assuming the diet contains plants. Consequently, no absorption, distribution, metabolism or elimination studies were submitted, as it was not considered necessary to undertake an evaluation of S-abscisic acid's toxicokinetic profile.

### Acute toxicity studies

*S-abscisic acid* was of low oral, dermal and inhalational toxicity in rats ($LD_{50}$ > 5000 mg/kg bw, LD50 > 5000 mg/kg and 4-hr $LC_{50}$ > 5130 mg/$m^3$ respectively). It was a slight eye irritant in rabbits, but was not a skin irritant in rabbits or a skin sensitiser in guinea pigs. The formulated product, ProTone SG Plant Growth Regulator Soluble Granule has low acute oral and dermal toxicity in rats ($LD_{50}$ > 5000 mg/kg bw and $LD_{50}$ > 5000 mg/kg bw respectively) and it is estimated that it will have low acute inhalation toxicity (4-hr $LC_{50}$ >5000 mg/$m^3$). It was a slight skin irritant and slight eye irritant in rabbits. It was not a skin sensitiser in guinea pigs.

## Short term and subchronic toxicity studies

*S-abscisic acid* is a monocyclic sesquiterpene plant hormone, which is involved in many major processes during plant growth and development. It also plays a role in the development of pigments in fruits including grapes through the stimulation of anthocyanin biosynthesis and accumulation. Given that *S-abscisic acid* is a naturally occurring chemical present in food, exposure to *S-abscisic acid* at food comparable levels is not expected to cause toxicological effects.

Consistent with its nature as a food component, there were no clear signs of toxicity in a number of dietary studies in rats up to subchronic length. Repeat-dose dietary studies were conducted at up to 20000 ppm (~1500 mg/kg bw/d) for 4 and 13 weeks. At the higher doses, there was a reduction in body weight gain, though this is considered to be due to a decrease in food intake due to reduced palatability. There was no evidence of treatment related systemic toxicity during a 3 week dermal study in rats with doses up to 1000 mg/kg bw/d.

## Chronic toxicity and carcinogenicity studies

No chronic or carcinogenicity studies were available, though exposure to *S-abscisic acid* at food comparable levels is not expected to cause toxicological effects, and no systemic toxicity was at seen at high dose levels seen in dietary studies in rats up to subchronic length.

## Reproduction and Developmental Studies

No reproductive tests were available for evaluation, though no effects on reproductive organs were seen in studies in rats up to subchronic length. In a rat developmental study (gavage) there were no developmental effects up to and including 1000 mg/kg bw/d. The presence of dried yellow and red material around the anogenital area of dams was observed at 750 and 1000 mg/kg bw/d, and 1000 mg/kg bw/d respectively. Wet and dried red material was also observed around the mouth of dams at 500 mg/kg bw/d and above, along with dried clear material at 750 mg/kg bw/d and above. However, these findings were only seen in a small number of animals. Furthermore, the findings around the mouth of dams lacked a dose response for both total occurrence and number of females the finding was observed in. Noting the above comments and absence of any other effects attributed to treatment, the observance of wet and dry material in dams is regarded as test-article related but not of biological significance. Consequently, no maternal toxicity was observed up to and including 1000 mg/kg bw/d.

In a non-GLP reporter gene assay, *S-abscisic acid* did not demonstrate any estrogenic, anti-estrogenic, androgenic or anti-androgenic effects.

## Genotoxicity Studies

Three genotoxicity studies were submitted, an *in vitro* reverse mutation assay using *Salmonella typhimurium* and *Escherichia coli* (+/- metabolic activation), an *in vitro* chromosome aberration assay using CHO cells (+/- metabolic activation), and an *in vivo* mouse micronucleus assay. *S-abscisic acid* was negative in all three studies. Thus, *S-abscisic acid* is not mutagenic/genotoxic *in vitro* or genotoxic *in vivo*.

### Neurotoxicity Studies

No neurotoxicity tests were available for evaluation.

## 3.3 PUBLIC HEALTH STANDARDS

### Poisons Scheduling

The National Drugs and Poisons Schedule Committee (NDPSC) considered the toxicity of the product and its active ingredients and assessed the necessary controls to be implemented under states' poisons regulations to prevent the occurrence of poisoning.

At its 57th meeting of October 2009, the NDPSC agreed that on the basis of the observed eye irritation, which was considered chemically induced eye damage and not simply physical damage, *S-abscisic acid* and all other stereoisomers of abscisic acid should be included in Schedule 5. Consequently, a general entry for abscisic acid was made in Schedule 5.

### NOEL/ADI /ARfD

The Acceptable Daily Intake (ADI) is that quantity of an agricultural compound, which can safely be consumed on a daily basis for a lifetime and is based on the lowest NOEL obtained in the most sensitive species. This NOEL is then divided by a safety factor, which reflects the quality of the toxicological database and takes into account the variability in responses between species and individuals.

The acute reference dose (ARfD) is the maximum quantity of an agricultural or veterinary chemical that can safely be consumed as a single, isolated event. The ARfD is derived from the lowest NOAEL (NOEL) as a single or short-term dose, which causes no effect in the most sensitive species of experimental animal tested, together with a safety factor, which reflects the quality of the toxicological database and takes into account the variability in responses between species and individuals.

The applicant has requested a Table 5 MRL entry for *S-abscisic acid* or an exemption from requiring an MRL to be established. Table 5 MRLs are for the uses of substances where maximum residue levels are not necessary. That is, situations where residues do not or should not occur in foods or animal feeds; or where the residues are identical to or indistinguishable from natural food components; or are otherwise of no toxicological significance. Data submitted showed that *S-abscisic acid* is present in a number of fruits, vegetables and grains and therefore consumed as part of a normal diet containing these items. Based on this data there are no objections on human health grounds to a Table 5 entry for *S-abscisic acid*. As a Table 5 MRL entry for *S-abscisic acid* is supported an ADI or ARfD are not considered necessary.

# 4 RESIDUES ASSESSMENT

## 4.1 Introduction

As part of the residues assessment for *S-abscisic acid*, plant and animal metabolism studies, supervised residue trials, crop rotation studies, processing studies, and trade aspects were considered. Details are provided below.

## 4.2 Metabolism

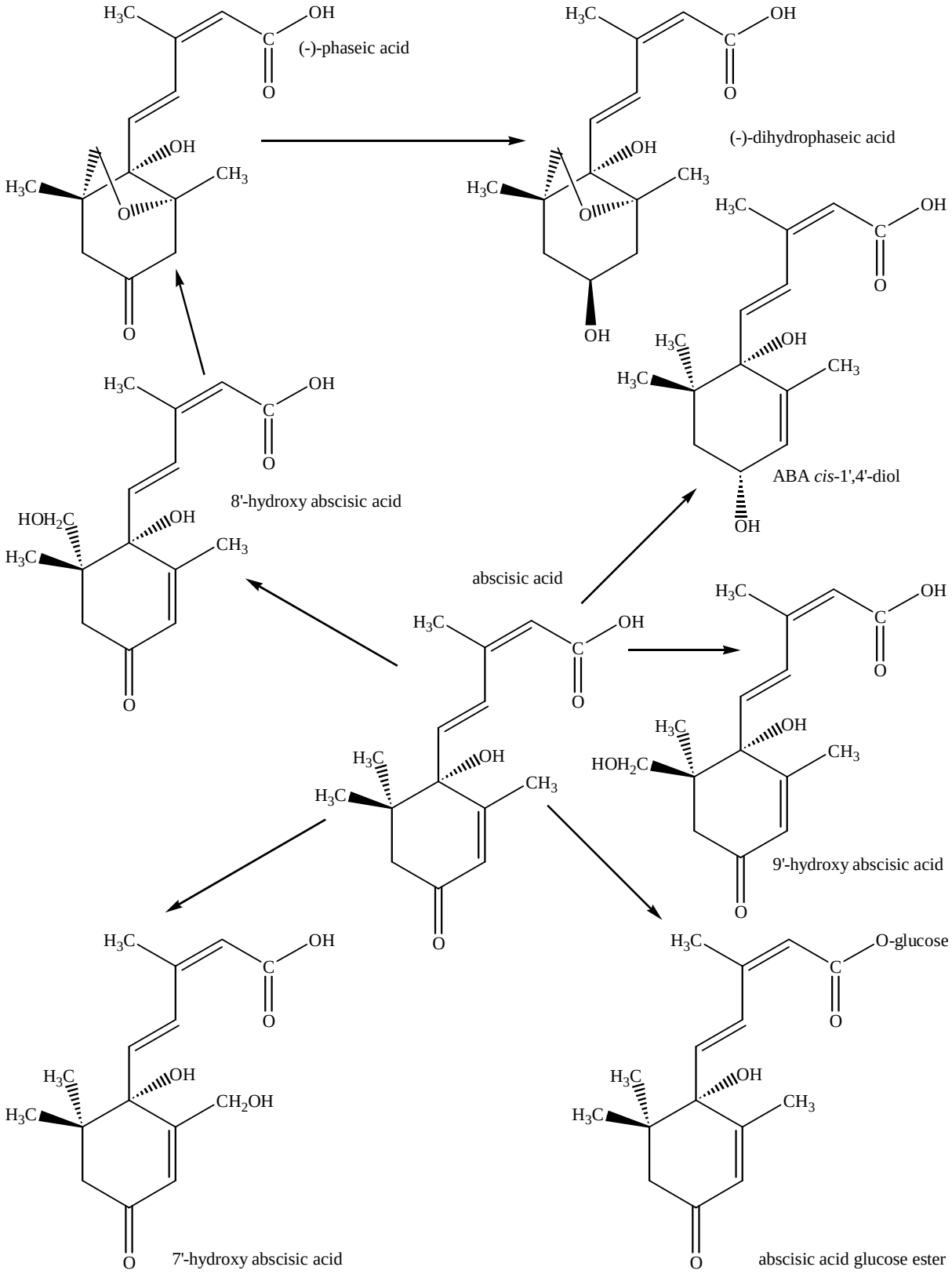
The applicant presented a literature review on the structure, functions, synthesis, occurrence and metabolism in plants of *S-abscisic acid*.

*S-abscisic acid* is a naturally occurring compound found at varying levels in all higher plants. It is involved in many important events in plant growth and development. It is responsible for closure of stomata (leaf guard cells) to reduce evapo-transpiration in response to water stress, it is responsible for inhibition of seed germination and maintenance of dormancy. It is a growth inhibitor in some plants, while in others increased levels are associated with the early stages of maturation.

*S-abscisic acid* is synthesised in plants from carotenoid compounds, with the first step dedicated to its production being the oxidative cleavage of 9-*cis*-neoxanthin, followed by opening of an epoxide linkage and dehydrogenation.

Metabolism of *S*-abscisic acid has been studied by a number of authors in various plant species. The metabolism is summarised in the diagram below. The main metabolic pathways identified are:

- Hydroxylation to give 8'-hydroxy abscisic acid followed by epoxidation and hydrogenation to give dihydrophaseic acid;
- Hydroxylation to give 9'-hydroxy abscisic acid;
- Hydroxylation to give 7'-hydroxy abscisic acid;
- Glucose conjugation; and
- Reduction of the ketone functionality to a hydroxyl group, giving abscisic acid *cis*-1',4'-diol.




S-ABSCISIC ACID METABOLISM IN PLANTS

## 4.3 Analytical methods

### Determination of S-abscisic acid residues in plant commodities

A reverse-phase isocratic HPLC-UV method has been developed to quantify the *S-abscisic acid* content of grape commodities. Samples were extracted using methanol/water/acetic acid and cleaned up by solid phase extraction. Quantitation was achieved by linear regression external standard calibration (99.7% purity reference standard). Sample raw data allowed calculations to be reproduced. Recoveries at fortifications ranging from 5 to 200 mg/kg were 75-107%, within the generally acceptable range of 70-120%. The limit of quantification is generally 5 mg/kg, although this depends on naturally occurring levels of *S*-abscisic acid in the sample.

The analytical method has been appropriately validated for analysis of *S-abscisic acid*, demonstrates excellent linearity, precision and accuracy and shows no evidence of any co-eluting interferents. It is therefore suitable for the determination of *S-abscisic acid* in grapes.

### Determination of residues of S-abscisic acid in animal tissues

An analytical method for *S-abscisic acid* in animal commodities was not provided.

Overall, the methods appear to be suitable for the proposed purposes and are acceptable.

## 4.4 Residue definition

*S-abscisic acid* used in ProTone SG Plant Regulator Soluble Granule will be manufactured by a fungal fermentation process and is indistinguishable from the compound occurring naturally in all higher plants. As MRLs will not be established for *S-abscisic acid*, a residue definition is not required and will not be established.

## 4.5 Residue trials

The applicant reviewed the literature and provided details of the levels of *S-abscisic acid* found naturally in various food crops (see table below).

**RANGE OF CONCENTRATION OF FREE (I.E. UNCONJUGATED) ABSCISIC ACID IN VARIOUS CROPS**

| Crop | Tissue | Abscisic acid | Reference |
|---|---|---|---|
| Apple | Developing and mature flesh | 0.2 and 0.06 mg/kg | Rock and Zeevart 1990 |
| Avocado | Mature flesh | Up to 10 mg/kg | Milborrow 1974 |
| Barley | Developing and mature seed | 0.2 and 0.04 mg/kg | Goldback and Michael 1976 |
| Citrus | Fruit peel | Up to 1.25 mg/kg | Goldschmidt et al 1973 |
| Maize | Mature kernel | Up to 0.33 mg/kg | Cheikh and Jones 1994 |

| | | | |
|---|---|---|---|
| Grape | Mature fruit skin<br>Mature fruit seed | Up to 3 mg/kg<br>Up to 2.5 mg/kg | Kondo and Kawai 1998 |
| Peach | Developing buds | Up to 1.2 mg/kg (dry weight basis) | Reeder and Bowen 1978 |
| Sweet cherry | Mature flesh | Up to 3 mg/kg | Kondo and Tomiyama 1998 |
| Tomato | Leaves | Up to 0.41 mg/kg | Rasmussen 1976 |
| Wheat | Developing and mature seed | 3.25 and 0.6 mg/kg | Walker-Simmons and Sessig 1990 |

## Grape Data

Further information on background levels of *S-abscisic acid* in untreated grapes was provided with the analytical method details and is tabulated below:

**LEVELS OF *S*-ABSCISIC ACID IN UNTREATED GRAPE SAMPLES**

| Sample number | Result (mg/kg) | | |
|---|---|---|---|
| | Set 1 | Set 2 | Set 3 |
| Replicate 1 | 5.4 | 0.15 | 1.3 |
| Replicate 2 | 4.2 | 0.23 | 2.7 |
| Replicate 3 | 4.4 | 0.09 | 2.5 |
| Replicate 4 | 3.6 | 0.08 | 2.7 |
| Replicate 5 | 3.6 | 0.05 | 4.0 |
| Mean result | 4.2 | 0.12 | 2.6 |
| Standard deviation | 0.7 | 0.07 | 1.0 |
| RSD | 17.5% | 59.5% | 36.3% |

Residue trials were conducted at the Nurioopta Research Station of the South Australian Research and Development Institute (SARDI). Plots of Cabernet and Shiraz grapevines were sprayed to run-off with an aqueous solution of technical *S*-abscisic acid at 20 g/100 L. 0.05% of sorbitan monolaurate (20) ethoxylate was included in the spray tank as a wetter. Some plots were regularly drip irrigated, while others were under the regulated deficient irrigation (RDI) system where irrigation is reduced to induce plant stress and initiate fruit maturation. In these trials the vines grown under RDI received no irrigation until before the spray treatments; after treatment, the RDI vines received the same irrigation as the other vines. Untreated control plots were set aside for both grape varieties and both irrigation systems; these plots received only the wetter. Treatment was timed so as the total soluble solids content was as close as possible to 18° Brix.

Grapes and leaves were collected prior to treatment on the day of application, and 1, 3, 7 and 14 days after spraying. Samples were placed on dry ice immediately after collection and transported to the laboratory within 2 hours. Samples were stored deep frozen (-40 °C) until analysis. Some leaf and berry samples were washed prior to freezing to study the effect of washing on residue levels.

For the Shiraz grapes, residues in both untreated control plots and the treated plot of restricted irrigation grapes were indistinguishable from each other by 14 DAA, and were back at levels seen before treatment (mean residues of around 0.4 mg/kg). Levels remained slightly elevated in the treated normally irrigated berries, with mean residues of around 1.5 mg/kg. It should be noted however that this result is not significantly different from that observed 1 DAA in untreated control grapes given restricted irrigation. In Cabernet Sauvignon grapes, mean *S-abscisic acid* residues at 14 DAA were around 0.8 mg/kg for the treated berries under both irrigation regimes, and around 0.2 mg/kg for the untreated berries under both irrigation regimes. There was a statistically significant difference between the untreated and treated berries, however it should be noted that even the treated Cabernet berry residues were about the same as residues in untreated restricted irrigation berries on 1 DAA.

On 1 DAA, washing treated Shiraz grapes reduce residues to those observed in control fruit, showing that *S*-abscisic acid was mostly present on the surface of the berries. On 14 DAA, the effect of washing was less significant, although where elevated levels were still present, a slight reduction was noted. This shows that most of the *S-abscisic acid* was present in the fruit pulp rather than on the surface by 14 DAA.

## Processing studies

A processing study was conducted as part of the residue trial work. Shiraz grapes that had received a single application of *S-abscisic acid* at a rate of 20 g ai/100 L were vinified either at the School of Wine and Food Sciences, Charles Sturt University, Wagga Wagga, or at the Hinginbotham winery at the University of Adelaide.

In Shiraz wine produced at the Charles Sturt University winery in Wagga Wagga, there was no statistically significant difference in levels of free *S-abscisic acid* or the glucose ester between wine from untreated grapes and wine from treated grapes. However, for the Shiraz wine produced in South Australia, a small but statistically significant increase in the residues of both the acid and the ester were observed for wine produced using treated grapes when compared with the wine made with untreated grapes. However, it should be noted that the wine from the treated grapes, containing around 0.7 mg/kg *S-abscisic acid*, still has well under the *S-abscisic acid* content of some untreated grapes (levels of up to 5.4 mg/kg have been measured, see tables above). Therefore, levels of *S-abscisic acid* in wine made with treated grapes are still well within the range of naturally occurring *S-abscisic acid* levels.

## Animal feeds

Residues of *S-abscisic acid* in pomace made from treated grapes are not expected to exceed levels found in pomace from untreated grapes. An MRL for *S-abscisic acid* in grape pomace is not necessary and will not be established.

## 4.6 Crop rotation

No rotational crop studies were provided with the application. Grapes are not a rotational crop, and further, since *S*-abscisic acid is naturally present in grapes and other fruits, it is ubiquitous in the environment already. Therefore rotational crop residue and metabolism studies are not required for registration of the use of *S-abscisic acid* on grapes.

## 4.7 Animal commodity MRLs

Animals already consume significant amounts of *S-abscisic acid* that is present naturally in livestock feeds. Animals have adapted through evolution to consume it. Given that the use pattern is unlikely to result in *S-abscisic acid* residues distinguishable from that present naturally in plants, animal commodity MRLs are not required.

## 4.8 Spray drift

For the reasons discussed in the previous paragraph, transfer of *S-abscisic acid* residues to meat or dairy products through ingestion by animals is not of concern. Further, only ground based (airblast sprayer) application is proposed, reducing any potential spray drift. Spray drift modelling was therefore not conducted.

## 4.9 Bioaccumulation potential

*S*-abscisic acid has an octanol/water partition coefficient ($\log_{10}P_{OW}$) of 1.8 at 25 °C. It is not therefore considered to be fat soluble.

## 4.10 Estimated dietary intake

The toxicological aspects of *S-abscisic acid* have been evaluated. Given the natural occurrence of *S*-abscisic acid in all higher plants, and given that levels in treated grapes are unlikely to differ significantly from those found in untreated grapes, an Acceptable Daily Intake (ADI) and Acute Reference Dose (ARfD) are not required. Therefore, dietary intake calculations are not required and were not undertaken.

It is concluded that the dietary exposure to *S-abscisic acid* is low and the risk from residues in food is acceptable when ProTone SG Plant Regulator Soluble Granule is used according to label directions.

## 4.11 Recommendations

The following amendments to the MRL Standard are recommended in relation to the proposed use of ProTone SG Plant Regulator Soluble Granule:

### MRL STANDARD – TABLE 5 AMENDMENTS

| COMPOUND | USE |
|---|---|
| ADD: | |
| *S*-Abscisic acid | For use on grapevines to accelerate or increase the colouration of berries. |

The following withholding period statement is required in conjunction with the above entry:

## HARVEST WITHHOLDING PERIOD

Grapes: Withholding period not required when used as directed.

# 5 ASSESSMENT OF OVERSEAS TRADE ASPECTS OF RESIDUES IN FOOD

## 5.1 Overseas registration status

The applicant has also applied to register products containing *S-abscisic acid* for accelerating or increasing the colouration of grape berries in the USA. The USA is proposing a similar regulatory control measure to the APVMA Table 5 entry. The APVMA is not aware of any MRLs for *S-abscisic acid* in overseas jurisdictions.

### Potential risk to trade

Given that residues of *S-abscisic acid* in treated grapes are not expected to be distinguishable from levels present naturally in grapes, the risk to export trade in grapes, wine, or meat or dairy products from animals that have consumed treated grapes is low.

## 5.2 CONCLUSIONS

***Grapes*:** An MRL for *S-abscisic acid* in grapes will not be established, as residues in treated grapes are not likely to differ significantly from natural levels of *S-abscisic acid*. A Table 5 entry will be established. ***There is not expected to be any risk to Australian trade in grapes, dried grapes or wine.***

***Animal commodities*:** MRLs will not be established in animal commodities. Residues in meat or dairy products produced by animals consuming grape pomace from grapes treated with *S*-abscisic acid will not differ from animals not exposed to *S-abscisic acid*. ***There is not expected to be any risk to Australian trade in meat, milk and eggs.***

# 6 OCCUPATIONAL HEALTH AND SAFETY ASSESSMENT

## 6.1 Health hazards

*S-abscisic acid* has low acute oral, dermal and inhalational toxicity in rats. It was not a skin irritant in rabbits or a skin sensitiser in guinea pigs. It was a slight eye irritant in rabbits. *S-abscisic acid* is not listed on the Safe Work Australia's (SWA) Hazardous Substances Information System (HSIS) Database (SWA, 2009). With the available toxicology information, S-*abscisic acid* was not classified as a hazardous substance according to NOHSC *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004).

The formulated product, containing 200 g/kg of *S-abscisic acid* was of low acute oral and dermal toxicity in rats, and it is estimated that it will have low acute inhalation toxicity. It was a slight skin and eye irritant in rabbits, and was not a skin sensitiser in guinea pigs.

Based on the product toxicology information and concentrations of S-*abscisic acid* and other ingredients in the product, ProTone SG Plant Growth Regulator Soluble Granule is not classified as a hazardous substance according to NOHSC *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004).

## 6.2 Formulation, packaging, transport, storage and retailing

ProTone SG Plant Growth Regulator Soluble Granule will be manufactured overseas and imported into Australia as a soluble granule in high-density polyethylene (HDPE) bottles. It will be available in 0.5 L to 1 L HDPE containers. Transport workers and store persons will handle the packaged products and could only become contaminated if packaging were breached.

## 6.3 Use pattern

ProTone SG Plant Growth Regulator Soluble Granule is a new agricultural product, which will be applied to grapes to improve colouration prior to harvest. It contains 200 g/kg of S-*abscisic acid*, and the formulation is a soluble granule product. There may be up to six applications over a 12-week period, however, it is more likely that only one or two applications will occur.

## 6.4 Exposure during use

Farmers and their employees will be the main users of the products. The users may be exposed to the product when opening containers, mixing/loading, application and cleaning up spills and equipment. The main route of exposure to the undiluted product will be dermal although ocular exposure is also possible.

There are no worker exposure studies on S-*abscisic acid* or the product (ProTone SG Plant Growth Regulator Soluble Granule) available for assessment. In the absence of worker exposure data, the OCSEH generally uses the Pesticide Handler Exposure Database (PHED) Surrogate Exposure Guide (1998) to estimate the worker exposure during mixing/loading and application based on the maximum product use rate according to the Australian use pattern. However, a quantitative OH&S assessment, including post

application exposure assessment, is not required for ProTone SG Plant Growth Regulator Soluble Granule due its toxicological profile and lack of toxic effects observed in a 28-day dermal rat study.

The toxicology data demonstrates that:

- Provided that workers wear elbow-length chemical resistant gloves, no further PPE is required for potential repeat exposure effects when using *ProTone SG Plant Growth Regulator Soluble Granule* to accelerate or increase the red colouration in grape berries and bunches prior to harvest.

## 6.5 Exposure during re-entry

There is no risk associated with re-handling this product.

## 6.6 Recommendations for safe use

Users should follow the First Aid Instructions and Safety Directions on the product label.

SAFETY DIRECTIONS: Will irritate the eyes and skin. Avoid contact with eyes and skin. When preparing spray wear elbow-length chemical resistant gloves. Wash hands after use. After each day's use wash gloves.

FIRST AID: If poisoning occurs, contact a doctor or Poisons Information Centre (ph: 13 11 26).

## 6.7 Conclusion

The registration of *ProTone SG Plant Growth Regulator Soluble Granule* containing 200 g/kg of *S-abscisic acid*, to accelerate or increase the red colouration in grape berries and bunches, is supported.

*ProTone SG Plant Growth Regulator Soluble Granule* can be used safely if handled in accordance with the instructions on the product label and any other control measures described above. Additional information is available on the product MSDS.

# 7 ENVIRONMENTAL ASSESSMENT

## 7.1 Environmental fate summary

*S-abscisic acid* is readily soluble in water, very slightly volatile and, based on the Henry's Law constant, unlikely to volatilise from water or moist soil. Its low octanol water coefficient indicates S-*abscisic acid* is unlikely to bioaccumulate.

Environmental fate data submitted by Valent BioSciences included laboratory study reports and summaries of published scientific information that supported the natural occurrence of *S-abscisic acid*, relevant biosynthetic pathways and metabolism in plants and microorganisms, and published information on the physical and chemical degradation of *S-abscisic acid*. Also included were discussions of the known biochemical and physiological effects of *S-abscisic acid* within plants. DEWHA's assessment of the environmental fate and physicochemical degradation of *S-abscisic acid* made use of this information as well additional data obtained via the scientific literature.

Abscisic acid has been shown to be present in a variety of soils with the diversity of the soils in which it has been found, both alone and with associated plants, suggesting that the presence of abscisic acid in soil is not uncommon. Levels of abscisic acid in soil have been reported in the literature as ranging from 0.05 to 110 µg abscisic acid/kg soil dry weight, with the highest levels being in the rhizosphere. The proposed maximum use rate of 1000 g *S-abscisic acid*/ha equates to a worst case concentration of ~670 µg abscisic acid/kg soil, dry weight in a 10 cm soil profile. This value is, at a minimum, approximately 6 times greater than the maximum value reported for natural occurrences of abscisic acid in the soil (110 µg abscisic acid/kg soil, dry weight) and, based on the lowest determined soil concentration of 0.05 µg abscisic acid/kg soil, dry weight, some 13,000 times greater. Thus exogenous levels of *S-abscisic acid* in the soil arising from the proposed use pattern could be far greater than natural levels present in untreated soils.

When an 80% foliar interception of the applied *S-abscisic acid* is allowed for, the predicted environmental concentration in the soil is ~130 µg abscisic acid/kg soil, dry weight. This value is approximately equivalent to the maximum value reported for natural occurrences of abscisic acid in the soil (110 µg abscisic acid/kg soil, dry weight in the rhizosphere), but, based on the lowest determined soil concentration of 0.05 µg abscisic acid/kg soil, dry weight, is still some 2700 times greater. These estimated soil concentrations show that under realistic use, levels of *S-abscisic acid* in the soil following the proposed Australian use pattern on grape vines are likely to be at best similar to naturally found levels in the rhizosphere and at worst, several thousand-fold greater.

The high levels of *S-abscisic acid* in grape vine leaves following treatment at the proposed 1000 g *S-abscisic acid*/ha (see below) has shown increases from ~250 to 1000 ng *S-abscisic acid*/g before spraying to ~15,000 to ~40,000 ng/g a day after spraying reported, which confirms that levels on vegetation present as a result of the proposed treatment of grapevines can be far greater than those occurring naturally.

The hydrolysis of *S-abscisic acid* was studied using sterile aqueous buffer solutions at pH 4, 7 and 9 incubated at 25, 40, and 50°C. *S-abscisic acid* hydrolysed slowly (less than 10% breakdown) in pH 7 and 9 buffer solutions during preliminary studies conducted at 50°C for 5 days. Hydrolysis of *S-abscisic acid* exceeded 10% in a pH 4 buffer preliminary study and, as a result, the hydrolysis of *S-abscisic acid* in pH 4

buffer solution was repeated for the definitive study at 25 and 40°C for up to 32 days. The half-lives of *S-abscisic acid* in the definitive study were determined to be 791.6 and 161.9 days for the 25 and 40°C data sets, respectively, using first-order kinetics. In a summary provided for a less reliable study, buffer solutions of pH 5.0, 7.0 and 9.0 were prepared and the hydrolysis at either 25 or 50°C over 72 days determined. The amounts of hydrolysis at both temperatures were similar for all pH values, at 25°C; there was 32 to 36% hydrolysis and at 50°C, 35 to 44%. Half lives at 25°C ranged from 100 to 126 days, and at 50°C, 91 to 108 days. The reason for this is unclear and identities of the degradates were not determined. *S-abscisic acid* is identified as undergoing, at best, slight hydrolysis, and therefore this mode of degradation is not indicated as a significant route of degradation at environmental pHs and temperatures.

A summary of an aquatic photolysis study reported that the aquatic half-life of aqueous solutions of *S-abscisic acid* exposed to simulated sunlight is 52.5 minutes, assuming first order kinetics. *S-abscisic acid* is therefore classed as very readily degraded by phototransformation in water (half-life less than 1 day); this route of degradation is expected to be of importance in the environment. The scientific literature shows that photolysis at 254 nm (ultraviolet light) resulted in rapid isomerisation (*cis, trans* to *trans, trans*) and further photolysis, i.e. degradation of the *S-abscisic acid* or its photoisomers to unknown degradates. In contrast, photolysis at sunlight wavelengths results essentially in the *cis, trans* and *trans, trans* interconversion. Consequently, *S-abscisic acid* in natural waters can be expected to undergo photoisomerisation to form an approximately 50:50 mixture of *cis, trans* and *trans, trans* isomers. The optimum wavelength for photoisomerisation appeared to be in the UV-A range (320-400 nm). The significant result of conversion to the *trans, trans* isomer is loss of activity in plants (presumably with respect to all physiological responses due to *S-abscisic acid* as the *cis, trans* conformation is required).

No soil photolysis studies were submitted. However, it is expected that most of the applied *S-abscisic acid* will be intercepted by the treated foliage (up to 80% of the applied *S-abscisic acid* can be expected to be retained as a result of this foliar interception), with only a small amount expected to reach the soil as drip, direct spray deposition and possibly also as rain wash-off. The foliar *S-abscisic acid* is expected to be principally taken up by the treated grapes and grapevines where it will integrate with the vine's normal biochemical processes. *S-abscisic acid* which deposits on the ground is expected to undergo isomerization to the less active trans, trans isomer through photodegradation and also to readily undergo aerobic soil degradation. Because the majority of *S-abscisic acid*, which reaches the soil, is expected to be readily inactivated by conversion to the *trans, trans* isomer and fast degradation in the soil, the role of soil photolysis in degradation of *S-abscisic acid* may not be significant when compared to other dissipation/degradation processes.

Modelled atmospheric oxidation and ozone oxidation of *S-abscisic acid* in the upper atmosphere at 25°C gave a half-life (hydroxyl radical) of 0.07 days and an ozone half-life of ~0.18 days. Such values indicate *S-abscisic acid* would not persist in the upper atmosphere.

A summary of an aerobic soil degradation study reported that when *S-abscisic acid* was added to three soils and incubated at 25°C under aerobic conditions, the *S-abscisic acid* readily degraded with half-lives between 2 to 3 days. Because *S-abscisic acid* has a slow hydrolysis half-life, it was considered reasonable to speculate that soil microbes caused the degradation of *S-abscisic acid* in the soils, especially as the degradation half-life for *S-abscisic acid* in a sterilised sample of one of the soils was some 25 times longer than in the unsterilised soil. *S-abscisic acid* is expected to be readily degraded in soils with, from literature results, formation of two metabolites, phaseic acid and dihydrophaseic acid.

Studies on aquatic degradation of *S-abscisic acid* were not presented. However, the fate of *S-abscisic acid* in aerobic water/sediment systems is of importance as it reflects an actual environmental exposure route (spray drift to an aerobic water body, entry into the water column and then possible movement to the sediment). Based on the demonstrated lack of hydrolysis of *S-abscisic acid* at environmental pHs and its ready water solubility, it can be expected that any *S-abscisic acid* which enters a waterbody will stay in the water column and not move to the sediment. Aquatic photolysis may occur in clear waters but the effectiveness of this degradation route would be reduced in turbid waters associated with many Australian streams and rivers. While the route of degradation and ultimate fate of *S-abscisic acid* in natural waterbodies is unclear, photolysis is considered likely to occur (with at least photo-isomerisation to the less active isomer occurring) with an associated lowering of the concentration of the *S-abscisic acid* in the water column.

Adsorption/desorption, column and lysimeter studies have not been presented. Modelling of the abscisic acid molecule's structure gave a calculated soil Koc value of 10. Such a value is indicative of very high mobility in the soil. This is consistent with the high water solubility of *S-abscisic acid* in distilled water, 3192 mg/L and the pKa (dissociation constant) of 4.61 in distilled water. The latter result indicates that at most environmental pHs, abscisic acid is expected to be 100% ionized, i.e. in the carboxylate anion form, which is consistent with the observed high water solubility and again suggests very high mobility in soils.

The methodology used for analysis of *S-abscisic acid* in water and soil used HPLC with UV detection and was satisfactory. Average recoveries of 88.4% to 93.6% at fortification levels of 0.05 to 1.00 mg/kg were reported with a limit of detection of 0.01 mg/kg in soil and 0.005 mg/kg in water.

## 7.2 Environmental toxicity summary

When male and female northern bobwhite quail were administered a single oral dose of 2250 mg *S-abscisic acid*/kg body weight, there were no mortalities over a 14 day post-treatment period in the control group or treatment group. All control birds were normal in appearance and behaviour throughout the test as were all the birds in the 2250 mg/kg treatment group. When compared to the control group, there were no apparent treatment-related effects on body weight or feed consumption among males or females in the 2250 mg/kg treatment group. The acute oral LD50 value for northern bobwhite exposed to *S-abscisic acid* as a single oral dose was determined to be greater than 2250 mg *S-abscisic acid*/kg body weight, the highest dosage tested. The no-mortality level and the no-observed-effect level were 2250 mg/kg body weight. *S-abscisic acid* is practically non-toxic to the bobwhite quail with respect to acute oral toxicity.

Male and female Japanese quail were given single doses of *S-abscisic acid* of 0 to 200 mg *S-abscisic acid*/quail and the birds were observed for the following 96 hours (a non-standard test period). Some quail treated with 200 mg *S-abscisic acid*/quail (i.e. 2000 mg *S-abscisic acid*/kg body weight based on a 100 g bird body weight) showed ruffled feathers and anorexia symptoms at the beginning of treatment. These symptoms had disappeared at 24 hours after treatment. Quail tested with the other doses of ABA grew normally. Therefore the LD50 for *S-abscisic acid* to quail was determined as >1800 mg/kg body weight. The shortness of the observation period (cf. the normal 14 days) and the absence of details on the control birds reduce the reliability of this study.

No sub-acute avian dietary toxicity or chronic/reproductive studies were made available.

Rainbow trout (*Oncorhynchus mykiss*) were exposed for 96 hours under static-renewal conditions to *S-abscisic acid* at a mean measured limit concentration of 121 mg *S-abscisic acid*/L. Test solutions were renewed at approximately 48 hours. Mean measured test concentrations were determined and observations of mortality and other signs of toxicity were made approximately 5, 24, 48, 72 and 96 hours after test initiation. There were no mortalities or treatment-related effects among fish in the control or treatment groups. The 96-hour LC50 value was determined to be >121 mg *S-abscisic acid*/L, the single limit test concentration. The no-mortality concentration and the NOEC were both 121 mg *S-abscisic acid*/L and *S-abscisic acid* is rated as practically non-toxic to fish.

When zebra fish (*Brachydanio rerio*) were exposed to 0 (control) and to 804 to 2000 mg *S-abscisic acid*/L for 96 hours, there was 10 to 50% mortality after 48 hours, 10 to 80% after 72 hours and 10 to 100% mortality at 96 hours, with the percentage mortality shown to be dose dependent. At 96 hours, deaths occurred in all test concentrations (10% mortality at 804 mg/L increasing to 100% mortality at 2000 mg/L). Zebra fish in each treatment had un-adaptive (i.e. adverse) reactions to *S-abscisic acid* at the beginning of the experiment, i.e. fish swam rapidly and breathed at the water surface. While fish exposed to *S-abscisic acid* concentrations above 1157 mg/L were most sensitive, these effects disappeared after several hours. The LC50 values for zebra fish at 48 hours was 1582 mg/L, at 72 hours was 1318 mg/L and at 96 hours was 1180 mg/L (results adjusted for the 90% purity of the *S-abscisic acid* tested), and *S-abscisic acid* is indicated as practically non-toxic to fish.

Chronic fish toxicity data were not presented.

*Daphnia magna* were exposed to a single limit test concentration (120 mg *S-abscisic acid*/L) and a negative control (dilution water) for 48 hours under static-renewal conditions. Observations of mortality, immobility and other signs of toxicity were made approximately 6, 24 and 48 hours after test initiation. The no-mortality/immobility concentration was determined by visual interpretation of the mortality and immobility data. Test solutions were prepared daily during the test and all surviving daphnids were transferred from old to new solutions at approximately 24 hours. At test initiation and termination, all solutions appeared clear and colourless in the test chambers. Daphnids in the negative control group appeared normal throughout the test. No mortality or immobility was observed in daphnids in the *S-abscisic acid* treatment group throughout the test. However, 53% of the daphnids in the treatment replicates appeared lethargic at test termination with this effect not recorded at the 24 h observation period. The control daphnids showed no effects at any of the observation times. The 48 hour EC50 value for immobilisation was estimated to be ≥116 mg *S-abscisic acid*/L, the single limit test concentration. The no mortality/immobility concentration was 116 mg *S-abscisic acid*/L. The 48 h NOEC (absence of adverse effects [immobilisation and absence of lethargy]) was determined as <116 mg *S-abscisic acid*/L. While *S-abscisic acid* is rated as practically non-toxic to aquatic invertebrates on the basis of the 48 hour EC50 result, the high level of lethargy seen at test termination should be noted.

Data on the toxicity of *S-abscisic acid* to algae were not presented. However, because the proposed use pattern does not require use of bunch-directed application, and also because the levels likely to be present in the environment are expected to be far greater than naturally occurring levels for a finite time, algal toxicity has been modelled with 96 hour EC50 values for green algae calculated as being from 30 to 664 mg *S-abscisic acid*/L. Chronic values (taken as NOECs) were 11.5 to 216 mg *S-abscisic acid*/L. Such values indicate slight to practically no acute toxicity and very slight chronic toxicity.

Data on the effects of *S-abscisic acid* on aquatic plants such as duckweed (*Lemna* species) were not provided but literature references reported effects such as reduction in leaf and root growth when *Lemna minor* plants were exposed to concentrations of up to 10 mg *S-abscisic acid*/L, and that daughter fronds remained attached to the parent with more fronds per colony than controls. Other references stated *S-abscisic acid* regulated the growth of *Lemna* by inhibiting the frond multiplication rather than being toxic. A doctoral dissertation identified in the scientific literature examined the effects of abscisic acid on *Lemna minor*. The plants, at the 3-frond stage, were exposed to 0 (control), 50, 100, 200, 400, and 800 ppb ABA for a week and then transferred to growth medium free of abscisic acid. At the end of 14 days the plants were counted. Abscisic acid treated plants would seldom colonise at all past the three to four frond stage with the number of fronds developed shown to be a function of abscisic acid concentration. Another effect of the abscisic acid was the inhibition of the number of daughter fronds in relation to the life span of the mother fronds. A 14 day EC10 of 0.08 mg *S-abscisic acid*/L and an EC25 of 0.26 mg *S-abscisic acid*/L, based on frond number, were calculated. These provide an indication that this chemical might be expected to have toxic effects on duckweed and other aquatic plants.

The oral and contact toxicity of *S-abscisic acid* to the honeybee (*Apis mellifera* L.) was determined in a limit test in which the bees were exposed to the dose of 100 µg *S-abscisic acid*/bee by feeding and by topical application. In both control groups of the oral toxicity test and in the water treated group of the contact test no mortality occurred during the 48 hours test period. In the oral toxicity test the highest intake was 108.28 µg *S-abscisic acid*/bee. At this dose no mortality was observed during the 48 hours observation period. At the highest contact toxicity dose of 100 µg *S-abscisic acid*/bee, no mortality was observed after 48 hours. The 24 and 48 h LD50 values of *S-abscisic acid* in the oral toxicity test were both >108.3 µg *S-abscisic acid*/bee. The 24 and 48 h LD50 values in the contact toxicity test were both >100 µg *S-abscisic acid*/bee. Based on these oral and contact LD50 values, *S-abscisic acid* is identified as very slightly toxic to the honeybee.

A summary report of an acute contact and feeding toxicity study with *S-abscisic acid* and honeybees was also supplied. In the feeding component of the study, the honeybees were fed honey solutions containing 0 (control) and 5 to 500 mg *S-abscisic acid*/L as a food source. For the contact study, bees were treated with 0 to 10 µg of *S-abscisic acid*/bee. The exposed bees were then observed over the following 96 hours. Results from the bees fed a honey/*S-abscisic acid* mixture showed that within 96 h, bees in the treatment with 500 mg/L ABA died due to rejecting eating and thus starving. Bees in treatment of 100 mg/L ABA or below grew normally and did not show any toxicity or death, indicative of very slight toxicity to the honeybee. In the contact toxicity test, there were no significant toxic symptoms in each treatment group of bees. Death numbers are also low except that a 30% mortality was seen in the high dose group. The reported contact LD50 at 96 hours was >10 µg *S-abscisic acid*/bee.

Standard tests on non-target arthropods such as the parasitoid wasp, *Aphidius rhopalosiphi*, and the predatory mite, *Typhlodromus pyri*, were not presented.

When earthworms (*Eisenia fetida*) were exposed to a single limit dose of 1000 mg/kg of *S-abscisic acid* in an artificial soil over a 14 day period, there were no mortalities in the control group or the treatment group during the 14-day test and all earthworms were normal in appearance and behaviour throughout the test period. Because mortality was less than 50% in the treatment group, the 14 day LC50 and NOEC were, respectively, >1000 and 1000 mg *S-abscisic acid*/kg soil (dry weight), the highest concentration tested. *S-abscisic acid* is rated as very slightly toxic to earthworms.

No specific studies on microbial soil metabolism and degradation, i.e. carbon mineralization or nitrogen transformation, were provided.

The phytotoxicity of *S-abscisic acid* was investigated by vegetative vigour and seedling emergence studies conducted to relative US EPA guidelines. The vegetative vigour study is a 14-28 day foliar spray study while the seedling emergence is a 14-21 day test. Both studies were conducted as limit studies with exposure conducted at one test concentration only, approximately 2000 mg *S-abscisic acid*/L (ppm), which was ten times the maximum rate of 200 ppm proposed for Australian use. The exposure phase in the vegetative vigour study was 28 days and, in the seedling emergence study, 21 days, during which time possible phytotoxic effects of the test substance on vegetative vigour, seedling emergence and growth of emerged seedlings were evaluated. Higher tier testing was not triggered because no adverse effects greater than 25% with respect to untreated controls were determined. However, adverse effects were seen in both studies. Plants used in the two studies were the monocotyledons corn (maize), ryegrass, oats and onions and the dicotyledons cabbage, lettuce, radish, soybean, sugarbeet and tomatoes. In the vegetative vigour study, the application of *S-abscisic acid* resulted, on some occasions, in adverse effects on seedling condition, height, survival, or dry weight of the plant species tested. Such effects were generally transient and decreased over time. There was 97 to 100% survival in the control plants and 100% in the *S-abscisic acid* exposed plants after 28 days and exposure to 2000 ppm *S-abscisic acid* appears to be without effect with respect to plant survival. The 28 day LC50 and EC25 for plant survival is >2000 ppm *S-abscisic acid*.

While plant height in the untreated controls was generally greater than that in the *S-abscisic acid* exposed plants, the height reduction seen in the exposed plants did not exceed 25% of the control plant heights.

The plant height EC25 and EC50 values for all time intervals and for all plants were the same, namely >2000 ppm *S-abscisic acid* (equivalent to 5000 g *S-abscisic acid*/ha with 2500 L of spray solution applied per hectare). However, eight of the ten *S-abscisic acid* exposed plants had statistically significantly lower heights at day 7 with the largest reduction seen in tomatoes and radish, which both showed height reductions of 21% compared to the respective controls. By day 28, only the ryegrass and lettuce heights were still significantly less than the equivalent controls (respectively 7 and 10% lower) and their day 28 plant height EC10s were, respectively, >2000 and 1988 mg *S-abscisic acid*/L.

Statistically significant differences between mean plant dry weights at day 28 were observed for two of the ten plant species, namely sugarbeet and tomatoes. In both cases, there was an 11% reduction in dry weight with respect to the respective controls at that time. The lowest 28 day EC10 result was 1819 ppm (equivalent to 4548 g *S-abscisic acid*/ha) for tomatoes.

With the exception of radish, noticeable signs of toxicity such as necrosis, chlorosis, and leaf curl were observed only in sugarbeet, cabbage and soybean. There they were limited to only one plant of 30 plants of each of these species, did not appear directly after treatment (but appeared on the day 14 or day 28 after treatment observations), and were generally categorized as a slight effect. Radish had very slight observable chlorosis and necrosis on day 7 through day 21 on about half of the 30 plants in the treatment group. The noticeable frequency and extent of the radish phytotoxicity decreased over the period of the test and by test termination (day 28) were not present on any plant. The vegetative vigour results show phytotoxicity effects caused by the proposed use pattern are expected to be limited and transient in nature.

In a seedling emergence study, a 2002 ppm mixture of *S-abscisic acid* in water was sprayed onto soil containing plant seeds. This resulted, on some occasions, in adverse effects on seedling emergence, survival, height, or condition after 21 days in some of the ten terrestrial plant species tested (the US EPA guideline followed in this study states that results are expressed as the percent of detrimental effect growth compared to the control after at least 14 days). Such effects were generally transient and decreased over time. Because none of these effects resulted in a reduction of greater than 25% with respect to the control plants, a higher tier test was not required.

However, there was a noticeable delay in emergence of eight of test species (onion, ryegrass, corn, sugarbeet, cabbage, soybean, lettuce and radish), which became less evident as the test progressed. Mean emergence at the termination of the trial did not differ by more than 25% from the corresponding control for any of the ten species tested. The greatest difference in emergence (numbers) at day 21 was a percentage reduction of 19% for radish. On day 7, there were percentage reductions of more than 10% in treatment group mean emergence relative to the control mean observed on eight species. Of these, onion exhibited a 50% reduction in percentage mean emergence from the corresponding controls, which was the maximum reduction reported for all plants over the 21 days of observation. After day 7 the number of emerged seedlings in the treatment group increased substantially, so that by day 14 the difference was 10% or less for five of the original eight species. For the other three species, the day 14 differences of greater than 10% were maintained through to day 21 at which time onions and sugarbeet still had a 15% reduction compared to the control heights while a 19% reduction remained in radish.

Survival of the emerged seedlings did not differ from the corresponding control groups by more than ±5% for any of the ten species tested. The 21 day LC50 for plant survival is >2002 ppm *S-abscisic acid*.

Six plant species showed a 1 to 7% reduction in mean plant height at termination of the test, while two plant species, oats and tomatoes, showed an increase in mean plant height (2% and 23%) at termination of the test. The mean heights of sugarbeet and lettuce in the treatment group were both reduced by approximately 16%, relative to the control means. The statistical analysis of the 21 day plant height showed that treated sugarbeet, lettuce and radish had statistically significantly different heights compared to the untreated controls. The lowest calculated day 21 plant height EC10 was 1183 mg *S-abscisic acid*/L or ~2960 g *S-abscisic acid*/ha seen in lettuce.

Although there were no apparent effects on dry weight of seven test species (with percentage increases of up to 25% and decreases of 18% with respect to control dry weights seen), decreases in onion, cabbage and lettuce exhibited noticeable reductions in mean dry weight of 35, 25, and 48%, respectively, relative to their corresponding control means at termination of the study. Since there were minimal adverse effects observed on the seedling emergence, survival, height, or observed condition of these three test species, the weight reductions were likely attributable to the delayed emergence and subsequently reduced period available for growth prior to test termination. The lowest calculated day 21 plant dry weight EC10 was 418 mg *S-abscisic acid*/L or ~1045 g *S-abscisic acid*/ha seen in lettuce.

*S-abscisic acid* did not result in toxicity effects on any of the species tested, but did result in regulation of the seed emergence and growth cycle of treated plants relative to the untreated controls. This regulatory effect was largely overcome by most of the tested species by test termination, but was still evident in the mean growth weight comparisons to controls for three species.

## 7.3 Risk Assessment

Because the application to grapevines is expected to be by ground application only and is dependent on good contact with the grapes, aerial application is not expected to be used. A restraint on aerial application is recommended.

*S-abscisic acid* has slight acute oral avian toxicity and, based on comparisons of the proposed use patterns and estimated dietary toxicity, exposure of birds to *S-abscisic acid* via feeding is not expected to be of concern. The proposed use pattern and expected relatively short environmental life of *S-abscisic acid* will result in limited chronic or reproductive risk to birds.

Acute risk to fish, aquatic invertebrates and algae from the proposed use pattern as a result of spraydrift onto a nearby waterbody is expected to be acceptable. Chronic risk to fish, aquatic invertebrates and alga is expected to be acceptable based on results from a worst-case spraydrift scenario. Risk to aquatic plants from a direct overspray was initially indicated as unacceptable. However, when a more realistic spraydrift was considered, the risk was shown to be acceptable.

Risk to aquatic species other than aquatic plants from runoff waters containing *S-abscisic acid* is expected to be acceptable. The expected ready degradation of *S-abscisic acid* in soil and dilution of the runoff water containing the *S-abscisic acid* with significant volumes of runoff water from untreated areas, means risk to aquatic plants should be acceptable.

Risk to honeybees from contact with spray solution containing *S-abscisic acid* at the maximum proposed use rate is acceptable. Acute and chronic risk to earthworms from the proposed use pattern of ProTone SG Plant Growth Regulator Soluble Granule on grapevines is also acceptable. As studies on the effect of *S-abscisic acid* on other non-target beneficial insects were not provided, it is not possible to know whether the proposed use pattern could have adverse effects on such species or on practices such as IPM. Since IPM is practised in vineyards, a label warning has been recommended. The proposed use pattern is not likely to adversely effect soil microflora mediated nitrogen transformation or carbon mineralisation.

Terrestrial non-target plants exposed to levels of *S-abscisic acid* at approximately ten-fold the proposed maximum use rate exhibited no significant or persistent adverse effects with respect to plant survival or phytotoxicity. There were decreased height and dry weights after 28 days in some plants with the differences between treated plants and their controls being statistically significant on occasion. A risk assessment using the most sensitive estimated plant height and dry weight day 28 EC10s showed that inadvertent spraying or spraydrift of the formulated material onto non-target vegetation has acceptable risk.

When seeds of terrestrial non-target plants (seedling emergence study) were exposed to *S-abscisic acid* at approximately five-fold the proposed maximum use rate, no significant effects were observed on seedling emergence, seedling survival or phytotoxicity. Statistically significant decreases in the day 21 plant heights were found in three of the ten plant species tested but risk assessment based on the most sensitive plant height day 21 EC10 showed risk would be acceptable. Comparison of the control and *S-abscisic acid* exposed seedlings day 21 dry weights showed that large differences were seen on occasion with the largest difference being for lettuce with a 48% reduction relative to the corresponding control mean. Risk assessment using the most sensitive 21-day plant dry weight EC10 and based on a worst-case 10% spraydrift event showed that risk with respect to seedling emergence would be acceptable. The seedling

emergence study showed that, while *S-abscisic acid* exposure did not result in any significant toxicity effects on any of the species tested, it did result in regulation of the seed emergence and growth cycle of treated plants relative to the untreated controls with a particularly noticeable delay in emergence of eight of the ten test species. This regulatory effect was largely overcome by most of the tested species by test termination, but was still evident in the mean growth weight comparisons to controls for three species.

The lack of use of a surfactant and the uncertainty as to whether the phytotoxicity tests were carried out long enough to show reproductive effects such as bud and fruit formation were considered in detail. The former is most significant in the vegetative vigour test where plants are sprayed directly rather than on soil as in the seedling emergence test, where there will be opportunity for the *S-abscisic acid* and the surfactant to separate. Spraydrift modelling showed that at 0 metres the risk quotient was below 0.02 and at 3 metres, below 0.01. Such results confirm that establishment of a protective no-spray zone is not required.

Provided the label is amended as recommended to include a prohibition on aerial application and include IPM and toxicity to aquatic plant statements, DEWHA recommends that the APVMA be satisfied that use of ProTone SG Plant Growth Regulator Soluble Granule as proposed would not be likely to have an unintended effect that is harmful to animals, plants or things or to the environment.

# 8 EFFICACY AND SAFETY ASSESSMENT

## 8.1 Proposed use pattern

The intended use of ProTone SG Plant Growth Regulator Soluble Granule is to accelerate or increase the coloration of berries and bunches of table grapes (*Vitis* spp.)

## 8.2 Summary of Evaluation of Efficacy and Crop safety

An appropriate evaluation program ran over 5 years, with 27 trials comprising of 21 replicated field experiments and 7 commercial scale trials conducted in four Australian States – NSW, Victoria, South Australia and Queensland and were applied to the table grape varieties Flame seedless, Red Globe, Crimson Seedless and Rallis Seedless and the wine grape variety Shiraz. While initial trials were conducted using technical grade active, the majority of trials used the proposed formulation (20% wettable granule) The objectives of the program were to determine the efficacy of ProTone SG Plant Growth Regulator Soluble Granule to a) accelerate or increase the coloration in berries and bunches of table grapes; b) application of ProTone SG Plant Growth Regulator Soluble Granule when grapes are covered or not covered with raincovers; c) the required addition of a surfactant and d) the required application water volumes. The replicated experiments were conducted and reported according to an appropriately standard of scientific investigation, including the use of statistical designs and analyses that were sufficiently robust to determine meaningful treatment effects. The commercial trials were statistically analysed using Chi Square to determine the effect of *ProTone SG Plant Growth Regulator Soluble Granule*.

The information available and the results from each set of trials were adequately and accurately presented and summarised by the applicant.

The data supported a label claim that applying *S-abscisic acid* as ProTone SG Plant Growth Regulator Soluble Granule will accelerate or increase colouration of berries in red table grape varieties when applied at a rate of 25-100 g/100 L water, applied in 1750 to 2500 L/ha water volumes, before or after plastic vine covers are applied, The data also supports a claim that use of a surfactant added to the tank mix may improve the spread of ProTone SG Plant Growth Regulator Soluble Granule on table grape berries. Likewise, it was demonstrated that ProTone SG Plant Growth Regulator Soluble Granule consistently produced no signs of crop injury to grape varieties used in the experiments.

Hence, in terms of the evidence for the efficacy of the product and its safety to target and non-target species, the application by Valent BioSciences, a Division of Sumitomo Chemical Australia Pty Ltd for the registration of ProTone SG Plant Growth Regulator Soluble Granule is supported.

### Assessment of study/trial data

The evaluation program submitted by the applicant demonstrated through the provision of 21 trials that *S-abscisic acid* accelerated or increased coloration of berries and bunches of grapes. Data supplied provided evidence for the need for separate application directions for short and long maturation varieties. In short maturation varieties application is from 1 week prior to veraison until veraison. In long maturation varieties

efficacy was demonstrated with application from 1 week prior to veraison until 2-3 weeks prior to harvest. Long maturation varieties may also require multiple applications, 2-3 weeks apart.

The applicant provided 4 replicated trials and a summary of field data demonstrating that the addition of the surfactant improved spray droplet coverage and provided more even coloration of grapes and bunches. The claim for application in water volumes of between 1750 and 2500 L/ha was supported through data from 6 replicated trials. Data from 2 replicated trails showed that application of ProTone SG Plant Growth Regulator Soluble Granule could be conducted while the grape vines had raincovers installed and achieve the same berry coverage to applications prior to installation of raincovers.

## Crop safety

The phytotoxicity of *S-abscisic acid* to grape bunches and berries were evaluated in all studies. Phytotoxicity was only observed were higher than label rates were used in either a single application or multiple applications.

# 9 CONCLUSION

The claims on the proposed product label that the product is capable of accelerating or increasing the coloration in berries and bunches of table grapes are supported by the results from the Australian efficacy experiments. The draft Directions for Use are clear. Appropriate advice is given on specific restraints, such as applying the product by air, apply product to plants under pest, nutritional or water stress and not applying product if rainfall or irrigation is likely to occur within 6 hours after spraying. The directions and recommendations are supported by the experimental results. Advice or critical comments on application techniques and withholding periods are appropriate. Warnings in regard to restricting annual application to less than 5 kg/ ha and protection statements with regards to wildlife of crops, native and non-target species are appropriate.

Therefore, in terms of the evidence for the efficacy of the product and its safety to target and non-target species, the application by Valent BioSciences, a Division of Sumitomo Chemical Australia Pty Ltd for the registration of ProTone SG Plant Growth Regulator Soluble Granule is supported when used in accordance with the proposed label instructions and Good Agricultural Practice (GAP).

# 10 LABELLING REQUIREMENTS

**CAUTION**
**KEEP OUT OF REACH OF CHILDREN**
**READ SAFETY DIRECTIONS BEFORE OPENING OR USING**

## ProTone SG™

**PLANT GROWTH REGULATOR**

**SOLUBLE GRANULE**

**ACTIVE CONSTITUENT: 200 g/kg S-ABSCISIC ACID**

To accelerate or increase the red colouration of berries and bunches of red table grapes varieties (*Vitis* spp.)

IMPORTANT: READ THE ATTACHED LEAFLET BEFORE USING THIS PRODUCT

250 g -1kg

VALENT BIOSCIENCES™
A division of Sumitomo Chemical Australia Pty Ltd
242 Beecroft Road,
Epping NSW 2121 Australia
Phone: 1800 060 671

**PROTECTION OF CROPS, NATIVE AND OTHER NON-TARGET PLANTS**
DO NOT apply under weather conditions, or from spraying equipment, that may cause spray to drift onto nearby susceptible plants/crops.

**PROTECTION OF WILDLIFE, FISH, CRUSTACEA AND ENVIRONMENT:**
S-abscisic acid is a plant growth regulator, which is toxic to aquatic plants.
DO NOT contaminate streams, rivers or waterways with chemical or used containers.

**STORAGE AND DISPOSAL:**
Store in the closed, original container in a well-ventilated area, as cool as possible. DO NOT store for prolonged periods in direct sunlight. Triple rinse containers before disposal. Add rinsings to spray tank. DO NOT dispose of undiluted chemicals on site. If not recycling, break, crush or puncture and deliver empty containers to an approved waste management facility. DO NOT burn empty containers or product.

**SAFETY DIRECTIONS:** Will irritate the eyes and skin. Avoid contact with eyes and skin. When preparing spray wear elbow-length chemical resistant gloves. Wash hands after use. After each day's use wash gloves.

**FIRST AID:** If poisoning occurs, contact a doctor or Poisons Information Centre (ph: 13 11 26).

**MATERIAL SAFETY DATA SHEET:** Additional information is listed in the Material Safety Data Sheet.

**EMERGENCY INFORMATION:** Contact Sumitomo Chemical Australia Pty Ltd, 242 Beecroft Road, NSW 2121, Australia. Telephone Number 1 800 060 671



APVMA Approval No. 53027/45309

D.O.M.
Batch No.

| **THIS PRODUCT IS NOT CONSIDERED TO BE A DANGEROUS GOOD UNDER THE AUSTRALIAN CODE FOR THE TRANSPORT OF DANGEROUS GOODS BY ROAD OR RAIL.** | |
|---|---|
| **In a Transport Emergency**<br>**Dial 000**<br>**Police or Fire Brigade** | **SPECIALIST ADVICE**<br>**IN EMERGENCY ONLY**<br>**ALL HOURS - AUSTRALIA WIDE**<br>**1800 024 973** |

**CAUTION**
**KEEP OUT OF REACH OF CHILDREN**
**READ SAFETY DIRECTIONS BEFORE OPENING OR USING**

# ProTone SG™

## PLANT GROWTH REGULATOR

## SOLUBLE GRANULE

**ACTIVE CONSTITUENT: 200 g/kg S-ABSCISIC ACID**

To accelerate or increase the red colouration of berries and bunches of red table grapes varieties (*Vitis* spp.)

**Important: Read this leaflet before use**

VALENT BIOSCIENCES™
A division of Sumitomo Chemical Australia Pty Ltd
ACN 081 096 255
Building B, Level 3, Suite 402
242 Beecroft Road,
Epping NSW 2121 Australia
Phone: (02) 8752 9000 Fax: (02) 8752 9099
Phone: 1800 060 671

## DIRECTIONS FOR USE

**Restraint:** DO NOT apply to plants under pest, nutritional or water stress.
DO NOT apply if rainfall or overhead irrigation use is likely within 6 hours of spraying.
DO NOT apply with aircraft.

| Crop | Use | Situation | Rate | Timing | Critical Comments |
|---|---|---|---|---|---|
| Table Grapes *(Vitis* spp.*)* | Accelerate or increase the red colouration in berries and bunches | Where the time between veraison and 1st pick is expected to be less than 3-4 weeks | 25-100 g/100 L | From 1 week prior to veraison until veraison | Typical for varieties such as Flame Seedless and Ralli Seedless, but also suitable for some blocks of other varieties such as Crimso Seedless and Red Globes where rapid maturation is typical.<br><br>Apply in 1750 to 2500 L water /ha with a surfactant Pomade™ Wetting Agent as detailed in Mixing Instructions.<br><br>Ensure even coverage of all bunches as uneven coverage may result in unevenly coloured bunches especially when using rates above 50 g/100 L.<br><br>Colour responses increase with increasing dose rate and it is recommended that growers initially spray small areas with lower dose rates (25-50 g/100L is the recommended starting point) in order to gain familiarity with ProTone SG effects on specific blocks as the effect will vary based on variety, canopy structure, crop loac rootstock and season. |
| | | Where the time between veraison and 1st pick is expected to be greater than 3-4 weeks | 25-100 g/100 L | From 1 week prior to veraison to 2-3 weeks prior to harvest | Typical for varieties such as Crimson Seedless and Red Globe, bu also suitable for some blocks of other long-season varieties. These types of varieties typically respond best to treatments initiated one week after veraison.<br><br>Apply in 1750 to 2500 L water /ha with Pomade™ Wetting Agenta surfactant as detailed in mixing instructionsMixing Instructions.<br><br>Ensure even coverage of all bunches as uneven coverage may result in unevenly coloured bunches especially when using rates above 50 g/100 L.<br><br>Colour responses increase with increasing dose rate and it is recommended that growers initially spray small areas with lower dose rates (25-50 g/100L is the recommended starting point) in order to gain familiarity with ProTone SG effects on specific blocks as the effect will vary based on variety, canopy structure, crop loac rootstock and season.<br><br>Multiple applications may be necessary in poor colouring blocks or where growers have used lower dose rates at veraison and the colour enhancement is still insufficient for marketing. Maximum effect of ProTone SG is achieved 2-3 weeks after application (although the grapes may continue to colour naturally) and growers should examine the sprayed blocks after 2-3 weeks in order to determine if further sprays are necessary.<br><br>In varieties with multiple and extended harvests, applications can be made to enhance the colour of later harvests by applying ProTone SG at 25 g/100 L 2-3 weeks prior to the next harvest.<br><br>When making multiple applications DO NOT apply more than 5kg/ha ProTone SG per season. |

**NOT TO BE USED FOR ANY PURPOSE OR IN ANY MANNER CONTRARY TO THIS LABEL, UNLESS AUTHORISED UNDER APPROPRIATE LEGISLATION.**

**WITHHOLDING PERIOD: NOT REQUIRED WHEN USED AS DIRECTED.**

## GENERAL INSTRUCTIONS:

### FRUIT QUALITY (GRAPES)

ProTone SG contains the natural plant hormone S-ABA (Abscisic Acid). S-ABA stimulates anthocyanin biosynthesis and accumulation. S-ABA mediates a number of responses in grapes (*Vitis* spp.) including switching on the anthocyanin genes. Anthocyanins are responsible for the red colour of the skins of the red varieties of table and wine grapes. This process occurs naturally in grapes and other plant species but the extent of the colouration can be reduced when the grapes are grown in hot conditions or when shaded (such as when grown under plastic 'rain covers'). Weather and seasonal conditions, rootstocks, soil types and other factors can all lead to grape colouration which may be insufficient for marketing.

Applications of ProTone SG can accelerate or increase red grape colouration. In most cases colour development resulting from ProTone SG application will be visible 5 to 7 days after application. Colour development initiated by ProTone SG may continue for 2 to 3 weeks.

ProTone SG has not been shown to accelerate or enhance sugar development in grapes and growers should be aware that even though the grapes may have sufficient colouration they may have insufficient sugar for some markets. This means that the grapes should be left on the vines until sugar accumulation has reached that expected by the market.

It is recommended that 'rain covers' are used to protect berries and bunches that may be prone to splitting as the surfactants used with ProTone SG may impact on berry turgor in conditions conducive to berry splitting resulting in increased splitting.

ProTone SG can be applied as a single application but for varieties where the time from veraison (10%-30% colour in the block) to harvest is extended (4-12 weeks) multiple applications may need to be considered.

When using low dose rates (25 g/100 L) there may be insufficient colouration from a single application and multiple applications may be necessary.

If multiple applications are being considered prior to any spray applications for the season then low rate applications of around 25 g/100 L should be used from veraison onwards, with resprays every 2-3 weeks until the desired colour is achieved.

Do not apply multiple applications of high rates (100g/100L water) in high water volumes (>2500 L water/ha). In some situations this may result in excessive colouring, softening or splitting or berry shatter in storage in cultivars susceptible to these problems – for more information please contact your Sumitomo Chemical Australia representative.

DO NOT apply more than 5kg/ha ProTone SG per season.

The possible effects of ProTone SG on integrated pest management (IPM) strategies have not been studied. However, based on available information, it cannot be ruled out that ProTone SG may have an adverse effect on non-target beneficial insects where IPM is practiced.

**MIXING**
Do not premix ProTone SG with water in a small container prior to adding to the spray vat. Fill spray vat with 70%-80% of required volume and agitate. Add required quantity of ProTone SG and surfactant while still agitating. Add the remaining water. Maintain good agitation while travelling to the block to be sprayed and during spraying.

A Pomade™ Wetting Agent must be added to the spray vat in order to aid the wetting of berry skins by ProTone SG. Pomade™ Wetting Agent should be used at 20-50 ml/100 L but to a maximum of 500 ml in a full spray vat. Please contact your Sumitomo Chemical Australia representative for more information.

DO NOT leave solutions in spray vat overnight.

**APPLICATION**
Timing:
Veraison is the initial stage of grape ripening and is marked by changes in colour and softening of the berry. At or near veraison, grape berries become responsive to ProTone SG.

For ProTone SG applications under Australian conditions, veraison is considered to be where 10%-30% colour occurs in the block to be treated.

Vines should not be under water, insect or other stress. Make sure vines are watered prior to application of this product. Apply under cool conditions as these are best for slow drying of applied material to ensure adequate absorption of product into the berry skins.

Apply in a sufficient amount of water to ensure thorough coverage of the berries and bunches as application to vine foliage has no effect on grape colouration. Water volume would normally range from 1750 to 2500 L water/ha depending on the canopy structure, age of vines, and density of foliage and type and condition of spray equipment. It is essential to ensure even coverage on all bunches and on both sides of the bunches. Fine or smaller droplets will assist in obtaining even coverage. It is recommended that growers setup and calibrate their sprayers in order to direct sprays at grape bunches and to achieve even coverage. Uneven coverage will result in Uneven fruit colouration

For spraying under rain covers, the top nozzles should be shut-off and the lower nozzles angled to give appropriate coverage to the targeted bunches. Water volume/ha should be maintained by adding more low-flow nozzles to the sprayer and not by increasing nozzle size. More information on appropriate sprayer set-up for ProTone SG is available from your local retail agronomist or from your Sumitomo Chemical Australia representative.

**COMPATIBILITY:** Do not mix with other products as the compatibility of ProTone SG with other products has not been investigated.

**PROTECTION OF CROPS, NATIVE AND OTHER NON-TARGET PLANTS**
S-Abscisic acid is a plant growth regulator, which is toxic to aquatic plants.
DO NOT apply under weather conditions, or from spraying equipment, that may cause spray to drift onto nearby susceptible plants/crops.

**PROTECTION OF LIVESTOCK, WILDLIFE, FISH, CRUSTACEA AND ENVIRONMENT:**
DO NOT contaminate streams, rivers or waterways with the chemical or used containers.

**STORAGE AND DISPOSAL:**
Store in the closed, original container in a well-ventilated area, as cool as possible. DO NOT store for prolonged periods in direct sunlight. Triple rinse containers before disposal. Add rinsings to spray tank. DO NOT dispose of undiluted chemicals on site. If not recycling, break, crush or puncture and deliver empty containers to an approved waste management facility. DO NOT burn empty containers or product.

**SAFETY DIRECTIONS:** Will irritate the eyes and skin. Avoid contact with eyes and skin. When preparing spray wear elbow-length chemical resistant gloves. Wash hands after use. After each day's use wash gloves.

**FIRST AID:** If poisoning occurs, contact a doctor or Poisons Information Centre (Ph. 13 11 26).

**MATERIAL SAFETY DATA SHEET:** Additional information is listed in the Material Safety Data Sheet.

**EMERGENCY INFORMATION:** Contact Sumitomo Chemical Australia Pty Ltd 242 Beecroft Rd, NSW 2121, Australia. Telephone Number 1 800 060 671

**EXCLUSION OF LIABILITY**

Unless otherwise expressly stated in writing neither Valent BioSciences, Sumitomo Chemical Australia Pty Ltd ("the Companies") nor the distributor has any knowledge or the particular use to which the buyer proposes to put this product. In purchasing this product the buyer must rely solely upon his own skill and judgement as to its suitability for the particular purpose for which it is required. Except to the extent that exclusion or denial of liability is prohibited under the Trade Practices Act or any relevant state legislation, the Companies and the distributor expressly exclude any warranty as to the quality or fitness of any goods sold for any purpose whatsoever and deny all responsibility in contract tort negligence or otherwise for any harm or damage resulting from the use of such goods or from acting on the advice or recommendations as to such use given in good faith by any representative of the Companies or the distributor. If these conditions are unacceptable to the buyer, the goods should be returned to Valent BioSciences Sumitomo Chemical Australia Pty Ltd unopened within seven (7) days for refund of purchase price.

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APVMA Approval No. 53027/45309

# ABBREVIATIONS

| | |
|---|---|
| ac | active constituent |
| ADI | Acceptable Daily Intake (for humans) |
| ai | active ingredient |
| ArfD | Acute Reference Dose |
| bw | bodyweight |
| d | day |
| DAA | Days After Application |
| $EC_{10}$ | concentration at which 10% of the test population are immobilised |
| $EC_{25}$ | concentration at which 25% of the test population are immobilised |
| $EC_{50}$ | concentration at which 50% of the test population are immobilised |
| g | gram |
| GAP | Good Agricultural Practice |
| h | hour |
| ha | hectare |
| HDPE | high density polyethylene |
| HPLC | High Pressure Liquid Chromatography *or* High Performance Liquid Chromatography |
| IPM | Integrated Pest Management |
| kg | kilogram |
| $K_{oc}$ | Organic carbon partitioning coefficient |
| L | Litre |
| $LC_{50}$ | concentration that kills 50% of the test population of organisms |
| $LD_{50}$ | dosage of chemical that kills 50% of the test population of organisms |
| mg | milligram |
| mL | millilitre |
| MRL | Maximum Residue Limit |
| MSDS | Material Safety Data Sheet |

| | |
|---|---|
| NDPSC | National Drugs and Poisons Schedule Committee |
| ng | nanogram |
| NHMRC | National Health and Medical Research Council |
| NOEC/NOEL | No Observable Effect Concentration / Level |
| OC | Organic Carbon |
| OM | Organic Matter |
| Pa | Pascals |
| $P_{ow}$ | octonol water partitioning co-efficient |
| PHED | Pesticide Handler Exposure Database |
| ppb | parts per billion |
| PPE | Personal Protective Equipment |
| ppm | parts per million |
| RDI | regulated deficient irrigation |
| s | second |
| SG | Soluble granule |
| SUSDP | Standard for the Uniform Scheduling of Drugs and Poisons |
| µg | microgram |
| UV | Ultra violet |
| vmd | volume median diameter |
| WHP | Withholding Period |

# GLOSSARY

| | |
|---|---|
| Active constituent | The substance that is primarily responsible for the effect produced by a chemical product |
| Acute | Having rapid onset and of short duration. |
| Carcinogenicity | The ability to cause cancer |
| Chronic | Of long duration |
| Codex MRL | Internationally published standard maximum residue limit |
| Desorption | Removal of an absorbed material from a surface |
| Efficacy | Production of the desired effect |
| Formulation | A combination of both active and inactive constituents to form the end use product |
| Genotoxicity | The ability to damage genetic material |
| Hydrophobic | Water repelling |
| Leaching | Removal of a compound by use of a solvent |
| Log Pow | Log to base 10 of octonol water partitioning co-efficient |
| Metabolism | The conversion of food into energy |
| Photodegradation | Breakdown of chemicals due to the action of light |
| Photolysis | Breakdown of chemicals due to the action of light |
| Subcutaneous | Under the skin |
| Toxicokinetics | The study of the movement of toxins through the body |
| Toxicology | The study of the nature and effects of poisons |

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