Australian Government

Australian Pesticides and Veterinary Medicines Authority

PUBLIC RELEASE SUMMARY

on the Evaluation of the New Active Ametoctradin in the Product Zampro® Fungicide

APVMA Product Number P63651

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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 (OCS), Department of Sustainability, Environment, Water, Population and Communities (DSEWPaC), 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 consistent with accepted scientific principles and processes. 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 stakeholders 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 **Zampro® Fungicide** 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 matters include aspects of **public health, occupational health and safety, chemistry and manufacture, residues in food, environmental safety, trade,** and **efficacy and target crop or animal safety**. Submissions should state the grounds on which they are based. Comments received that address issues outside the relevant matters cannot be considered by the APVMA.

Submissions must be received by the APVMA by close of business on **31 July 2012** 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)
- Email or postal address (if available)
- The date you made the submission.

All personal information, and confidential information judged by the APVMA to be ***confidential commercial information (CCI)***[1] contained in submissions will be treated confidentially.

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:

Contact Officer
Pesticides Program
Australian Pesticides and Veterinary Medicines Authority
PO Box 6182
Kingston ACT 2604
Phone: 02 6210 4748
Fax: 02 6210 4776
Email: registration@apvma.gov.au

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

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: www.apvma.gov.au

1 INTRODUCTION

1.1 Applicant

BASF Australia Ltd

1.2 Details of Product

It is proposed to register Zampro® Fungicide containing a new active constituent ametoctradin (300 g/L) and an existing registered active constituent dimethomorph (225 g/L) as a suspension concentrate formulation. The product is intended for preventative use for the control of downy mildew (*Plasmopara viticola*) of grapevines. Zampro® Fungicide is intended to be used at a rate of 80 mL product/100 L water (dilute application rate), or by concentrate application methods in grapevines.

Ametoctradin is a new active constituent to the Australian market. It is a fungicide which belongs to the triazolopyrimidylamines chemical group and represents the first commercial development from this group. The Fungicidal Mode of Action has not been fully characterised however ametoctradin is a strong inhibitor of mitochondrial respiration in complex III (cytochrome bc1) of fungi belonging to the Class of Oomycetes. The exact binding site at complex III is not yet known. Ametoctradin is in the new Group 45 for fungicides resistance management. The existing active constituent dimethomorph is known to inhibit phospholipid biosynthesis and interfere with normal cell wall deposition, resulting in cell wall lysis and subsequent death of the fungal cell. Dimethomorph is in Group 40 (carboxylic acid amides) for fungicides resistance management. The new combination of active constituents will offer an additional resistance management option to growers currently using dimethomorph in grapevines.

Ametoctradin is currently registered for use in Argentina, Austria, Chile, Colombia, Ecuador, Estonia, France, Germany, Hungary, Korea, Latvia, Lithuania, Macedonia, Netherlands, Romania, Turkey and United Kingdom.

Zampro® Fungicide is new to the Australian market. The active ametoctradin as well as the end-use product will be manufactured overseas and imported into Australia.

This publication provides a summary of the data reviewed and an outline of the regulatory considerations for the proposed registration of **Zampro® Fungicide**, and approval of the new active constituent, **ametoctradin**.

This submission has been assessed under a Global Joint Review (GJR)/ workshare arrangement where registrations for the same formulation and end-use have been submitted concurrently in Australia, Canada and USA.

2 CHEMISTRY AND MANUFACTURE

2.1 Active constituent

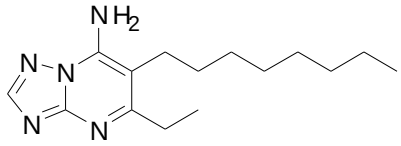
Ametoctradin is a new active constituent to be used as a fungicide in grapes for the control of downy mildew.

Manufacturing site

The active constituent ametoctradin is manufactured by Hikal Limited in Maharashtra State, India on behalf of BASF Corporation.

Chemical Characteristics of the Active Constituent

The chemical active constituent ametoctradin has the following properties:

COMMON NAME (ISO):	Ametoctradin
IUPAC NAME:	5-ethyl-6-octyl[1,2,4]triazolo[1,5-a]pyrimidin-7-amine
CAS NAME:	[1,2,4]triazolo[1,5-a]pyrimidin-7-amine, 5-ethyl-6-octyl-
CAS REGISTRY NUMBER:	865318-97-4
MANUFACTURER'S CODE	BAS Reg. No. 499353; BAS 650F
MOLECULAR FORMULA:	$C_{15}H_{25}N_5$
MOLECULAR WEIGHT:	275.40
STRUCTURE:	
CHEMICAL FAMILY:	Triazolopyrimidylamine

APVMA Active Constituent Standard for AMETOCTRADIN

Constituent	Specification	Purity Level
Ametoctradin	White solid	980 g/kg minimum

Physical and Chemical Properties of Pure Active Constituent

PHYSICAL FORM:	Solid
COLOUR:	White
ODOUR:	odourless
MELTING POINT:	197.7 – 198.7 °C
DENSITY:	1.12 g/cm^3 (at 20 ºC)
UV ABSORPTION:	ε (L mol^{-1} cm^{-1}]) in methanol: 16611 at 221 nm; 13113 at 295 nm
PARTITION COEFFICIENT (KOW):	4.40 (neutral, 20 °C); 4.24 (pH 4.0, 20 °C); 4.18 (pH 9.0, 20 °C)
VAPOUR PRESSURE:	2.1 x 10^{-10} Pa at 20°C; 6.0 x 10^{-10} Pa at 25°C
SOLUBILITY:	The solubility is low in most organic solvents. Solubility in water is as follows: deionized, 0.14 mg/L; buffer pH 4, 0.23 mg/L; buffer pH 7, 0.15 mg/L; buffer pH 9, 0.20 mg/L.
STABILITY (TEMPERATURE, METALS AND METAL IONS):	Stable in the presence of metal and metal ions at normal and elevated temperature.

2.2 Product

DISTINGUISHING NAME::	Zampro® Fungicide
FORMULATION TYPE:	Suspension Concentrate (SC)
ACTIVE CONSTITUENT CONCENTRATION:	Ametoctradin (300 g/L) Dimethomorph (225 g/L)

Physical and Chemical Properties of the Product

PHYSICAL FORM:	Liquid
COLOUR:	White
ODOUR:	Faint aromatic
SPECIFIC GRAVITY:	1.114 g/mL
PH (1% SOLUTION):	6.9 – 7.1
VISCOSITY:	81 mPa.s @ 23 °C
EXPLOSIVITY:	Not explosive
OXIDISING PROPERTIES:	Not oxidising
FLAMMABILITY:	Not flammable.
STORAGE STABILITY:	Stability data provided by the applicant indicates that the product is expected to remain within specification for at least two years when stored under normal conditions in HDPE containers.
LOW TEMPERATURE STABILITY:	No visible separation in the product was observed after 7 days storage at 0°C.

Recommendation

Based on a review of the chemistry and manufacturing details provided by the applicant, registration of Zampro® Fungicide is supported.

3 TOXICOLOGICAL ASSESSMENT

3.1 Summary

BASF Australia Ltd together with their parent company BASF international have submitted a comprehensive toxicology and public health dataset for registration of the active constituent ametoctradin and associated product in the United States of America, Canada and Australia as part of a Global Joint Review. The toxicology assessment of ametoctradin was conducted jointly by scientists from Canada (PMRA), the United States (USEPA) and Australia (OCS).

Ametoctradin is new to the Australian market, and belongs to a new class of chemicals triazolopyrimidylamines. The product Zampro® Fungicide is a suspension concentrate formulation containing 300 g/L ametoctradin and 225 g/L dimethomorph (an existing active ingredient), and is intended for the control of downy mildew in grapevines. The product will be available in 2.5 L or 5 L high-density polyethylene (HDPE) containers with twist caps.

Application rates are a maximum of 1.02 L product per hectare which may be used in a dilute spray volume of 80 mL product / 100 L water, or in a concentrate spray volume of a minimum 250 L/ha. The application may be made using ground-airblast or aerial application methods. Zampro® Fungicide is intended to be applied a maximum of four times per season at 7 to 14 day intervals, with a maximum of two consecutive applications before switching to a different fungicide product with a different mode of action.

The submitted studies on the active constituent ametoctradin in rats showed that following ingestion ametoctradin was rapidly absorbed with a moderate bioavailability, widely distributed into organs and tissues, and extensively metabolised and completely eliminated with no evidence of accumulation in the body. In contrast, dermal absorption of ametoctradin was poor (< 6.5 %) in rats.

In animal studies, both ametoctradin and Zampro® Fungicide were of low acute oral, dermal and inhalational toxicity in rats, and neither was an irritant to the skin and eyes of rabbits, or a skin sensitiser in guinea pigs and mice.

Ametoctradin was of low toxicity in repeat oral dose studies in rats, mice and dogs, with no adverse treatment-related effects seen at doses close to or exceeding the limit dose of 1000 mg/kg bw/d. Ametoctradin was not carcinogenic in the rat or mouse and was not mutagenic or genotoxic with and without metabolic activation in vitro, and was not genotoxic in vivo. Additionally, ametoctradin was not a reproductive toxicant in rats, a developmental toxicant in rats and rabbits, neurotoxic or immunotoxic in rats.

Toxicological studies on three major metabolites (M650 F02, M650 F03 and M650 F04) indicated they were not mutagenic or genotoxic with and without metabolic activation in vitro, genotoxic in vivo and/or no adverse treatment-related effects were seen at doses close to or exceeding the limit dose of 1000 mg/kg bw/d in repeat dose oral studies in rats.

3.2 Summary of the Evaluation of Toxicological Studies

The toxicological database for ametoctradin, which consists primarily of toxicity studies conducted in rats, mice, rabbits and dogs, is considered sufficient to determine the toxicology profile of ametoctradin and characterise the risk to humans. 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-Adverse-Effect-Level (NOAEL) 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 ametoctradin was conducted as part of a Global Joint Review (GJR) by scientists from the United States Environmental Protection Agency (US EPA), Health Canada Pest Management Regulatory Agency (PMRA) and the Office of Chemical Safety (OCS) within the Department of Health and Ageing. Since the assessment report relies significantly on international assessment collaboration between the agency partners, the OCS has adopted the no observed adverse effect level (NOAEL) and lowest observed adverse effect level (LOAEL) approach with scientific justification for the adoption of these NOAEL/LOAEL positions.

3.3 Chemical class

Ametoctradin belongs to a new class of chemicals, triazolopyrimidylamines. It controls major plant pathogens from the Oomycete class of fungi, specifically downy mildews and Phytophthora spp. on vine, vegetable crops and ornamentals. Ametoctradin prevents disease by inhibition of the infectious stages of the pathogen, through direct effects on the germinations of sporangia. It has been shown to be active against zoospores and zoosporangia by inhibiting mitochondrial respiration in complex III, and, thus, preventing zoospore formation, release and motility.

3.4 Toxicokinetics and Metabolism

Ametoctradin was rapidly absorbed when administered as a single dose in rats. A sex independent, sub-linear correlation between increasing administered dose and internal exposure was identified, probably due to saturation effects. Absorbed ametoctradin was rapidly and widely distributed to organs and tissues following a single oral dose to rats, with peak levels in each organ or tissue appearing within 1-2 hours post dosing. The highest tissue levels appeared in the liver, kidneys, thyroid, pancreas, as well as adipose tissue (low dose males), uterus (low dose females), adrenal glands, bone marrow and carcass (all at high dose). Excretion of administered ametoctradin was mostly via faeces (≥ 73% of the dose), and less in urine (≤ 22% of the dose). Only a negligible amount was excreted as CO_2 in exhaled air. The majority of the dose (≥ 85%)

was eliminated from the body within 48 hours post dosing in a gender independent excretion pattern. There was no evidence of significant tissue accumulation even following repeated high doses.

Bile cannulation studies in rats indicated biliary excretion of administered ametoctradin was higher in males than in females, with a total of 22.5% and 12.4% of the dose respectively at 50 mg/kg bw, and 10.9% and 3.2% of the dose at 500 mg/kg bw. Bioavailability was determined to be 36% and 42% of the dose at 50 mg/kg bw in males and females respectively, with corresponding bioavailability values of 23% and 16% of the dose at 500 mg/kg bw.

Following a single oral dose in rats, a considerable amount of the radioactivity (approximately 43 – 79% in males and 65 – 92% in females) was excreted as the parent compound in the faeces. Metabolites identified and quantified were M650F06, M650F01 and M650F05 in the urine, M650F06, M650F01 and the parent compound in the faeces, M650F06, M650F10 (males only), M650F11, M650F12, M650F01, M650F05 and M650F09 in the bile, and M650F06 and M650F09 in the liver, kidneys and plasma.

The metabolic processing of ametoctradin involves terminal oxidation of the octyl side chain to the respective carboxylic acid (M650F09). Subsequent degradation of the carboxylic side chain (loss of C2-units or C1-unit) comparable to the ß- and α-oxidation of fatty acids leads to the metabolites M650F06, M650F05 and M650F01. In addition, conjugates of the oxidized metabolites with taurine (M650F10, M650F12) or with glucuronic acid (M650F11 or isomer) have been identified.

3.5 Acute Studies

Ametoctradin is of low acute toxicity by the oral (LD_{50} > 2000 mg/kg bw), dermal (LD_{50} > 2000 mg/kg bw) and inhalational route (4 hr LC_{50} > 5500 mg/m3) in rats. Ametoctradin was non-irritating to the skin and eye of rabbits, and non-sensitising in a maximisation test in guinea pigs and a LLNA test in mice.

The product Zampro® Fungicide is of low acute toxicity by the oral (500 < LD_{50} < 2000 mg/kg bw), dermal (LD_{50} > 5000 mg/kg bw) and inhalational route (4 hr LC_{50}> 5100 mg/m3) in rats. Ametoctradin was non-irritating to the skin and eye of rabbits, and non-sensitising in a Buehler test in guinea pig and a LLNA test in mice.

3.6 Systemic effects

The overall repeat-dose toxicity of ametoctradin was low, with 4- and/or 13-week oral toxicity studies in rats, mice and dogs indicating no adverse treatment-related effects at doses close to or exceeding the limit dose of 1000 mg/kg bw/day. A 4-week dermal toxicity study in rats also indicated no adverse treatment-related effects at the limit dose of 1000 mg/kg bw/day, and a 1-year dog dietary study reporting no adverse treatment-related effects at the top dose tested (848/936 mg/kg bw/day for males/females).

Carcinogenicity & Genotoxicity

Chronic/carcinogenicity studies in rodents and dogs indicated an overall lack of treatment-related adverse findings at close to or above the limit dose. Ametoctradin was not carcinogenic in the rat or mouse. Ametoctradin did not show mutagenicity and/or genotoxicity activity in a battery of in vitro studies with and

without metabolic activation (Ames test, chromosome aberration test, gene mutation in mammalian cells) and in vivo studies (Micronucleous test in rats, micronucleous test in mice, unscheduled DNA synthesis). It is therefore concluded that ametoctradin has no mutagenic or genotoxic potential.

Reproductive & Developmental toxicity

Ametoctradin was not a reproductive toxicant in an oral rat 2-generation reproduction study, and was not a developmental toxicant in oral studies in rats and rabbits, at the limit dose of 1000 mg/kg bw/day.

Neurotoxicity

Ametoctradin was not a neurotoxicant in acute and sub-chronic oral studies in the rat at dose levels up to and including the limit dose.

Immunotoxicity

Ametoctradin was not immunotoxic in a short-term rat study at dose levels exceeding the limit dose.

Toxicological studies on metabolites

Toxicological studies were available on three major metabolites, M650F02, M650F03 and M650F04.

Metabolite M650 F02 was not mutagenic in vitro with and without metabolic activation or genotoxic in vivo.

Metabolite M650F03 was not mutagenic or genotoxic in vitro with and without metabolic activation or genotoxic in vivo. Additionally, repeat-dose toxicity was low, with a 13-week oral toxicity study in rats indicating no adverse treatment-related effects at a dose level close to the limit dose of 1000 mg/kg bw/day.

Metabolite M650F04 was not mutagenic or genotoxic in vitro with and without metabolic activation or genotoxic in vivo. Additionally, repeat-dose toxicity was low, with a 13-week oral toxicity study in rats indicating no adverse treatment-related effects at a dose level exceeding the limit dose of 1000 mg/kg bw/day.

PUBLIC HEALTH STANDARDS

3.7 Poisons Scheduling

The delegate to the Secretary of the Department of Health and Ageing sought advice from the Advisory Committee on Chemical Scheduling (ACCS) on the scheduling of ametoctradin.

Ametoctradin was discussed at the October 2011 meeting of the ACCS. The delegate noted and agreed with the ACCS recommendation to create an Appendix B entry for ametoctradin within the Standard for the Uniform Scheduling of Medicines and Poisons (SUSMP). This was the interim decision of the delegate. The delegate's final decision made on 1st February 2012 confirmed that ametoctradin be included in Appendix B of the SUSMP, along with an implementation date of 1 May 2012.

3.8 NOEL/ADI/ARfD

ADI

The Acceptable Daily Intake (ADI) is that quantity of an agricultural or veterinary chemical which can safely be consumed on a daily basis for a lifetime and is based on the lowest NOAEL obtained in the most sensitive species. This NOAEL 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.

No appreciable treatment-related adverse effects were observed in repeat-dose toxicity studies with ametoctradin at doses close to or exceeding the limit dose of 1000 mg/kg bw/day. Thus, OCS considers it appropriate to use the limit dose NOAEL value of 1000 mg/kg bw/d to set an ADI.

A 100-fold safety factor, consisting of factors of 10 for intraspecies and interspecies variation, was considered appropriate. The toxicological database for ametoctradin included several long-term oral studies and carcinogenicity studies in the mouse and rat, and was considered complete. Since no sensitive population groups were identified during the course of this evaluation no additional safety factor is required at this time. A safety factor of 100-fold was therefore applied to the most sensitive NOAEL for the determination of an ADI level. Considering the overall lack of adverse toxicological effects of ametoctradin across the repeat-dose toxicology dataset at close to or above the limit-dose of 1000 mg/kg bw/day, an ADI of 10 mg/kg bw/day is recommended, using a 100-fold safety factor.

ARfD

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 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.

An acute reference dose (ARfD) was not established or necessary since ametoctradin was considered unlikely to present an acute hazard to humans after single dose administration. Acute oral toxicity studies indicated ametoctradin was a low acute toxicant, with no clinical signs of toxicity reported. Acute neurotoxicity studies indicated no treatment-related changes in neurological function and behaviour, while reproductive and developmental toxicity was not observed at limit-dose concentrations in either maternal dams or foetal pups.

4 RESIDUES ASSESSMENT

4.1 Introduction

Zampro® Fungicide is a suspension concentrate formulation which contains the new active constituent ametoctradin as well as dimethomorph. It is intended for the control of downy mildew in wine and table grapes. As part of the residues assessment for ametoctradin, plant and animal metabolism studies, analytical methods and storage stability, supervised residue trials, crop rotation studies, processing studies and trade aspects were considered and details are provided below. An assessment of Overseas Trade Aspects of Residues in Food is considered elsewhere in this PRS.

Products containing dimethomorph are currently registered for use on grapes at a maximum rate of 18 g ai/100L, which is equal to the proposed rate of dimethomorph in Zampro Fungicide. The registered use of dimethomorph involves a maximum of 6 applications with a 7 to 14 day re-application interval and a 28 day withholding period whereas the proposed use is for a maximum of 4 applications at 7-14 day intervals and with a 28 day withholding period. The restraint 'DO NOT use in crops intended for drying' is associated with the registered use of dimethomorph. The potential for dimethomorph residues associated with the proposed use is not greater than that associated with currently registered products and therefore further consideration to the proposed use of dimethomorph is not required, provided the restriction on use on grapes for dried fruit production is maintained. Therefore the use of dimethomorph is not discussed further.

4.2 Metabolism

Metabolism data for ^{14}C-labelled ametoctradin (^{14}CBAS 650) in lettuce, tomato and potato, and spring wheat, lettuce and white radish as confined rotational crops, lactating goats and laying hens, were provided.

Plant metabolism

In lettuce, three foliar applications were made at 21, 31 and 39 days after planting with a mixture of [2,7-^{14}C]BAS 650 F and [2,5,7-^{13}C]BAS 650 F (formulated as 650 SC) at a rate of 223 g ai/ha (total rate 0.669 kg ai/ha). The plants were sampled 7 days after the last application. All samples were homogenized and total radioactive residues were determined by combustion/ LSC. Each sample was extracted three times using methanol and twice using water. Radioactivity in Post Extraction Solids (PES) was quantified by combustion/ LSC. The extracts were combined per solvent and radio-assayed (LSC). The identity of parent BAS 650 F in peak isolates from the methanol extract was confirmed by HPLC-ESI-MS and HPLC-ESI-MS/MS. Extractability was 99.3% TRR, predominantly using methanol (98.9% TRR). BAS 650 F parent ametoctradin was the only compound present in the methanol extract (98.9% TRR, 8.39 mg/kg).

In tomato, three foliar applications were made at 47, 54 and 61 days after planting with a mixture of [2,7-^{14}C]BAS 650 F and [2,5,7-^{13}C]BAS 650 F (formulated as SC) at a rate of 299.7 g ai/ha (total rate 0.90 kg ai/ha). Ripe tomato plants were sampled 1 day after the last application and separated into leaves and fruits. Leaf and fruit samples were homogenized and total radioactive residues were determined by combustion/LSC. Each sample was extracted three times using methanol and twice using water. Radioactivity in PES was quantified by combustion/LSC. The extracts were combined per solvent and radio-

assayed (LSC). Extractability was 99.3 - 99.4% TRR. Parent BAS 650 F was the only compound present (in leaf, 98.6% TRR, 9.035 mg/kg; in fruit, 99.1% TRR, 0.357 mg/kg).

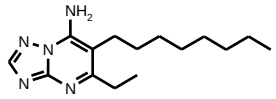
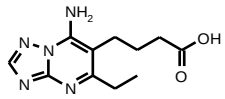
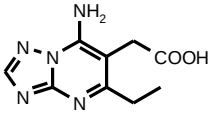
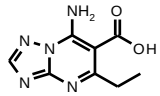
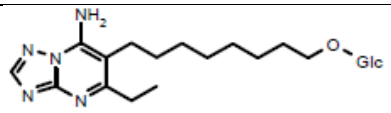
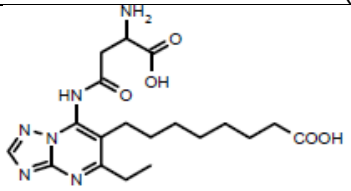
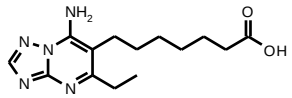
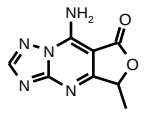
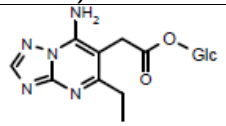
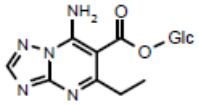
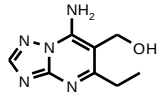
In potato, three foliar applications were made to the potato plants at 35, 21 and 7 days prior to harvest with a mixture of [2,7-^{14}C]BAS 650 F and [2,5,7-^{13}C]BAS 650 F (formulated as SC) at a rate of 440.8 g ai/ha (total rate 1.3224 kg ai/ha). Plant samples were taken directly after the second application (14 days before the last application, unripe plants, GS 43/44) and 7 days after the last treatment (7 days after last treatment, mature potatoes, GS 93). In both cases plant samples were separated into leaves and tubers. All samples were homogenized and total radioactive residues were determined by combustion/ LSC. Each sample was extracted three times using methanol and twice using water. Radioactivity in PES was quantified by combustion/ LSC. The extracts were combined per solvent and radio-assayed (LSC). The combined methanol and (leaves only) water extracts were concentrated and analysed by HPLC (2 methods) with identification using co-chromatography of reference standards. The identity of BAS 650 F and metabolites in peak isolates from the methanol extract was confirmed by HPLC-MS and HPLC-MS/MS. In immature and mature leaves, respectively, the TRR was 22 and 45 mg/kg, of which 99% was extractable, predominantly using methanol (98% TRR). Parent BAS 650 F was the main compound present in the extracts (95 and 85% TRR in immature and mature leaves respectively). All identified metabolites (M65F01and/or M650F04, M650F03, M650F18 (or isomer), M650F013 (or isomer) and M650F014 (or isomer), and M650F028 (or isomer)), were each ≤0.81 mg/kg (≤1.9% TRR). In immature and mature tubers, 89-92% TRR was extractable, predominantly using methanol (81 - 88% TRR). Parent BAS 650 F was the main compound in immature tubers (67% TRR, 0.017 mg/kg), but represented only 3.6% TRR (0.001 mg/kg) in mature tubers. Identified metabolites were M650F03 (in immature and mature tubers, respectively, 0.003 and 0.016 mg/kg, 13 and 40% TRR) and M650F04 (in mature tubers only, 0.011 mg/kg and 27% TRR).

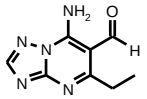
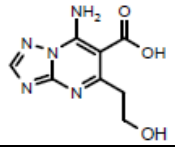
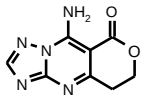
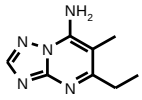
No metabolic pathway was proposed for plants since parent BAS 650 F was the only metabolite found in treated leaves and fruits. Since low levels of M650F03 and M650M04 are found in tubers and not in directly treated leaves and fruits.

A confined rotational crop study was conducted. Bare soil was sprayed with a mixture of non-radiolabeled BAS 650 F, [2, 7-^{14}C]BAS 650 F and [2, 5, 7-^{13}C]BAS 650 F at a rate of 1440 g ai/ha. Spring wheat, lettuce and white radish were sown or (in the case of lettuce) planted in the treated soil 30, 120 and 365 days after treatment (DAT). Parent BAS 650 F was only detected in ripe lettuce leaves at 30 DAT (0.009 mg/kg), wheat forage at 120 DAT (0.005 mg/kg) and wheat straw at 30 DAT (0.044 mg/kg) and at 120 DAT (0.029 mg/kg). The main components of residue were M650F03 and M650F04. In primary solvent extracts, M650F03 and M650F04, respectively, were up to 0.030 mg/kg (30 DAT) and 0.027 mg/kg (120 DAT) in lettuce, up to 2.4 mg/kg (30 DAT) and 0.008 mg/kg (120 DAT) in radish tops, up to 0.64 mg/kg (30 DAT) and 0.011 mg/kg (30 DAT) in radish roots, up to 0.34 mg/kg (120 DAT) and 0.87 mg/kg (120 DAT) in wheat forage, up to 2.6 mg/kg (30 DAT) and 2.0 mg/kg (120 DAT) in wheat straw, up to 0.55 and 3.6 mg/kg (both 30 DAT) in wheat chaff and up to 0.19 mg/kg and 1.3 mg/kg (both 30 DAT) in wheat grain.

Besides the above metabolites, ^{14}C-sugars (glucose, fructose, sucrose) were identified in primary solvent extracts from all samples. Other identified metabolites were found at varying levels but always ≤4.6% TRR and ≤0.021 mg/kg in crops for human consumption and ≤8.4% TRR and ≤0.34 mg/kg in crops for animal feed.

List of identified compounds in plants or rotational crops

Code	Chemical name	Formula
BAS 650 F	5-ethyl-6-octyl [1,2,4]triazolo [1,5-a]pyrimidin-7-amine	
M650F01	4-(7-amino-5-ethyl [1,2,4]triazolo [1,5-a]pyrimidin-6-yl) butanoic acid	
M650F03	(7-amino-5-ethyl [1,2,4]triazolo [1,5-a]pyrimidin-6-yl) acetic acid	
M650F04	7-amino-5-ethyl [1,2,4]triazolo [1,5-a]pyrimidine-6-carboxylic acid	
M650F13	Glucoside of 8-(7-amino-5-ethyl [1,2,4]triazolo [1,5-*a*]pyrimidin-6-yl)octan-1-ol	 (or isomer)
M650F14	8-[7-(β-aspartylamino)-5-ethyl[1,2,4]triazolo [1,5-*a*]pyrimidin-6-yl]octanoic acid	 (or isomer)
M650F18	7-(7-amino-5-ethyl [1,2,4]triazolo [1,5-a]pyrimidin-6-yl) heptanoic acid	 (or isomer)
M650F28	8-amino-5-methyl-5*H*,7*H*-furo[3,4-*d*] [1,2,4]triazolo [1,5-*a*]pyrimidin-7-one	 (or isomer)
M650F29	Glucoside of (7-amino-5-ethyl [1,2,4]triazolo[1,5-a]pyrimidin-6-yl)acetic acid	 (or N-glucoside)
M650F30	Glucoside of 7-amino-5-ethyl [1,2,4]triazolo [1,5-a]pyrimidine-6-carboxylic acid	 (or N-glucoside)
M650F32	(7-amino-5-ethyl [1,2,4]triazolo [1,5-*a*]pyrimidin-6-yl) methanol	

M650F33	7-amino-5-ethyl [1,2,4]triazolo [1,5-*a*]pyrimidine-6-carbaldehyde	
M650F37	7-amino-5-(2-hydroxyethyl)[1,2,4]triazolo[1,5-*a*]pyrimidine-6-carboxylic acid	
M650F38	9-amino-5,6-dihydro-8*H*-pyrano[4,3-*d*] [1,2,4]triazolo [1,5-*a*]pyrimidin-8-one	
M650F39	5-ethyl-6-methyl [1,2,4]triazolo [1,5-*a*]pyrimidin-7-amine	

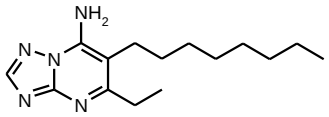
Animal metabolism

For 10 consecutive days, 9 laying hens received daily oral doses of a mixture of non-radiolabeled BAS 650 F, [2,7-^{14}C]BAS 650 F and [2,5,7-^{13}C]BAS 650 F, at a rate of 0.81 mg/kg bw/day equivalent to 11.5 ppm in the feed. The animals were kept individually in metabolism cages during the 6-day acclimatization and 10-day test period. Eggs were collected twice daily and excreta once daily. Hens were sacrificed 23 hours after administration of the last dose. Muscle, liver, adipose tissue, GI tract, GI tract contents and blood were collected. Samples were homogenized prior to quantification of radioactivity by LSC or combustion/LSC. Overall recovery was 93% Administered Radioactivity (AR), of which eggs and prepared tissues contained together <0.8% AR. Radioactivite concentrations in eggs reached a plateau value of about 0.040 mg/kg within 7 days. The highest radioactivite concentrations in edible tissues were found in liver (0.11 mg/kg), followed by muscle (0.026 mg/kg) and fat (0.014 mg/kg). The following compounds were identified; parent BAS 650 F (37% TRR), M650F01 (36% TRR), M650F06 (2.7% TRR) and M650F09 (1.1% TRR) were identified in excreta; parent BAS 650 F was identified in eggs (21.8% TRR, 0.008 mg/kg) and fat (10.7%, 0.001 mg/kg). M650F01 was identified in liver (8.7% TRR, 0.009 mg/kg), muscle (1.9% TRR, 0.000 mg/kg) and fat (28.1% TRR, 0.002 mg/kg) and M650F06 was identified in liver (1.3% TRR, 0.001 mg/kg) and muscle (1.1% TRR, 0.000 mg/kg).

For 10 consecutive days, 2 lactating goats received daily oral doses of a mixture of non-radiolabeled BAS 650 F, [2,7-^{14}C]BAS 650 F and [2,5,7-^{13}C]BAS 650 F, at a rate of 0.49 - 0.51 mg/kg bw/day equivalent to 11.99 - 12.69 mg/kg dry feed. Milk was collected twice daily, urine and faeces daily and a cage wash was performed at study termination. The animals were sacrificed 23 hours after administration of the last dose. Blood, liver, bile, gut and stomach, gut contents, stomach contents, urine bladder contents, kidney, kidney fat, intra-peritoneal fat and muscle were collected. The samples of edible tissues from both animals were combined. Samples were homogenized prior to quantification of radioactivity by LSC or combustion/LSC. The overall recoveries were 64% AR and 88% AR, of which radioactivity in urine and faeces represented 24 - 26% AR and 36 -58% AR respectively. Milk and edible tissues and organs contained together 0.20 - 0.24% AR. Radioactivite concentrations in milk reached a plateau value within 5 - 8 days. The highest radioactivite concentrations in edible products were found in liver (0.100 mg/kg), followed by milk (0.097 mg/kg), kidney (0.036 mg/kg), fat (0.016 mg/kg) and muscle (0.010 mg/kg). Parent BAS 650 F was only identified in faeces (64% TRR). M650F01, M650F06 and M650F09 were the other identified compounds in faeces (12%, 11% and 16% TRR, respectively) and were also detected in urine (31%, 47% and 17% TRR, respectively). In

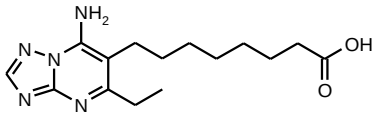
milk, liver, kidney and fat, M650F01 represented 14 - 26% TRR (0.003 - 0.014 mg/kg) and M650F06 22 - 47% TRR (0.006 - 0.021 mg/kg). In milk, kidney and fat M650F09 represented 7.7 - 9.4% TRR (0.002 - 0.003 mg/kg). The TRR in muscle (0.003 mg/kg) was low and only characterized. Non-identified but characterized peaks and fractions in milk and edible tissues/ organs from initial solvent extractions individually did not exceed 10.3% TRR and 0.005 mg/kg. The final residue in organs/ tissues (in case of liver and kidney after protease treatment) was up to 28% TRR, but of low concentration (≤0.009 mg/kg).

A metabolism scheme for animals is proposed in which degradation proceeds via oxidation of the aliphatic side chain to the respective terminal carboxylic acid and subsequent stepwise shortening of the aliphatic side chain.Proposed metabolic pathway in animals.



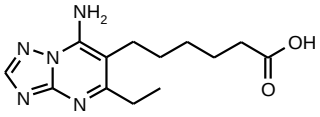
BAS 650 F

oxidation



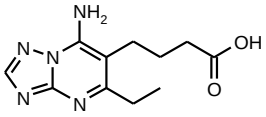
M650F09

ß-oxidation



M650F06

ß-oxidation



M650F01

Based on the available plant and animal metabolism data, it is concluded that:

Parent ametoctradin BAS 650 F was the only metabolite found in treated leaves and fruits. A residue definition of parent compound is proposed for ametoctradin in commodities of plant origin, for both MRL enforcement and dietary risk assessment.

The hen metabolism study shows that ametoctradin was the only identified compound in eggs. The goat metabolism study showed that the metabolites M650F01 and M650F06 were the major identified compounds in goat milk, liver, kidney and fat. Higher amounts of M650F06 than M650F01 were observed. A dairy cow feeding study showed that when finite residues of M650F01 were observed (in liver and kidney), higher residues of M650F06 were also observed. In addition finite residues of M650F06 were observed in liver at a lower feeding level. A residue definition of the sum of residues of ametoctradin and the metabolite M650F06 (6-(7-amino-5-ethyl [1,2,4] triazolo [1,5-a]pyrimidin-6-yl) hexanoic acid) is proposed for ametoctradin in commodities of animal origin, for both MRL enforcement and dietary risk assessment.

4.3 Analytical methods

Determination of ametoctradin residues in plant commodities

Two HPLC-MS/ MS methods were developed and validated for analysis of ametoctradin (BASF Method no. L0117) or ametoctradin and its metabolites M650F03 and M650F04 (BASF Method no. L0078), in various plant commodities. Samples were extracted with methanol/ water. For Method L0117, clean-up was achieved after centrifugation by partitioning against dichloromethane. For Method L0078, clean-up was achieved after centrifugation by solid phase extraction. Analyses were conducted using HPLC-MS/MS. Both methods were validated with a limit of quantitation (LOQ) of 0.01 mg/kg for all analytes. Mean recoveries at the quantitation transition for BAS 650 F, M650F03 and M650F04 after fortification at the LOQ were 72-108%, 72-106% and 75-99% respectively for Method L0078 and 76-107% for BAS 650 F for Method L0117. The relative standard deviations (RSD, %) for all commodities and all fortification levels were well below 20%.

Method no. L0117 proved to be suitable to determine residues of BAS 650 F in plant matrices such as wheat grain, potato, lettuce, tomato, grape, orange, onion and sunflower seed. Method no. L0078 proved to be suitable to determine residues of BAS 650 F and its metabolites M650F03 and M650F04 in plant matrices such as wheat grain, potato (including cooked potato, fried potato and potato flakes), lettuce, tomato (including canned tomatoes, raw tomato juice, tomato paste and tomato puree), grape (including wet pomace, rose wine, yeast deposit and raisins), orange, onion, sunflower seed and processed cucumber (gherkins).

Determination of residues of ametoctradin in animal tissues

A method was presented for the determination of ametoctradin and its metabolites M650F01 and M650F06 in animal tissues, milk and eggs. Samples were extracted using methanol/water, followed by solid phase extraction on a strong cation mixed-mode column. Analyses were conducted using HPLC-MS/MS. Mean recoveries were between 70 and 110%. The relative standard deviations (RSD, %) for all commodities and all fortification levels were well below 20%. The LOQ is 0.01 mg/kg for all analytes. The method proved to

be suitable to determine residues of BAS 650 F, M650F01 and M650F06 in animal matrices such as cow liver, kidney, muscle, fat, milk, cream and hen egg.

The methods are suitable for the proposed purposes and are acceptable.

4.4 Residue definition

The following residue definitions are recommended for ametoctradin for the purposes of dietary exposure assessment and for compliance and monitoring:

Table 3

COMPOUND	RESIDUE
ADD:	
Ametoctradin	
	Commodities of plant origin: Ametoctradin
	Commodities of animal origin: Sum of ametoctradin and 6-(7-amino-5-ethyl [1,2,4] triazolo [1,5-a]pyrimidin-6-yl) hexanoic acid

4.5 Storage stability

In a storage stability study, ametoctradin (BAS 650 F) was found to be stable in wheat forage, grain and straw and dried pea subjected to frozen storage for 752 days. A degradation of ametoctradin residues was observed in potato and lettuce. In grapes and tomatoes, ametoctradin residues were stable in samples stored for up to 365 days, but degradation occurred after that time point and the stability tests were not continued after 490 - 497 days.

In the Australian grape study, the US grape study and the German grape processing study, samples were stored for a maximum duration of 186, 301 and 283 days respectively under freezer conditions (≤ -15^{o}C, -20^{o}C and -18^{o}C respectively). Ametoctradin residues are stable in grape fruit for 365 days of frozen storage. In the animal feeding study, samples were stored frozen for a maximum of 34 days (for milk) and a maximum of 19 days for cream, skim milk, muscle, liver, kidney and fat. A storage stability study was investigated in milk only for 41 days for ametoctradin and M650F01 and 34 days for M650F06, which showed residues were stable for that duration of storage.

4.6 Residue trials

The proposed use involves a maximum of 4 applications with a 7 - 14 day re-application interval, by dilute spraying at a rate of 80 ml product / 100L (24 g ai/100L) or by concentrate spraying. The proposed withholding period is 4 weeks (28 days). It is proposed that restraints be included on the product label to prevent application by aircraft and application to crops intended for dry fruit production.

In support of the proposed grape use, the applicant provided details of 6 Australian residues trials (including two processing studies), 13 US residues trials and 4 German processing studies.

The Australian trials and the US trials which involved sampling at 28 days were considered relevant to the proposed use pattern.

In the Australian trials addressing the proposed GAP, including 3 foliar applications at 1x the proposed application rate, residues observed in grapes at 28 days after the last application were 0.25, 0.26, 0.44, 0.70 and 1.49 mg/kg.

A package of 13 decline residue trials conducted in USA was provided, in which application was by both dilute and concentrate spraying. Four applications were made at a re-treatment interval of 6-8 days. Residues observed in grapes at 28 days after the last application by dilute spraying were 0.18, 0.20, 0.21, 0.74, 0.79, 0.87, 0.92, 0.97, 1.07, 1.09, 1.34, 1.38 and 1.74 mg/kg while residues observed in grapes at 28 days after the last application by concentrate spraying were 0.07, 0.31, 0.32, 0.33, 0.38, 0.38, 0.43, 0.57, 0.59, 0.62, 0.93, 1.37 and 1.53 mg/kg.

Observations from these relevant Australian and U.S.A. trials gave an STMR of 0.62 mg/kg and an HR of 1.74 mg/kg. On the basis of these data an MRL of 3 mg/kg is proposed for ametoctradin in grapes.

4.7 Processing studies

Processing studies were conducted in Australia and Germany. The following processing factors were determined:

Processing factors (Australian trials)

Commodity	Processing factor
Wine	<0.02, <0.04, <1
Wet Pomace	3.1, 3.7
Dry Pomace	24, 29

Processing factors (German trials)

Commodity	Processing factors
Wine	<0.001, <0.002, 0.006, 0.009, 0.01, 0.02, 0.027, 0.032, <1
Wet Pomace	2.5, 2.7, 2.9, 3.9, 4.2, 4.8, 5.1, 5.2
Dry Pomace	16, 18, 19, 26, 28, 32, 34, 35
Raisins	1.9, 2.0, 4.8, 6.3

The available processing studies indicate that ametoctradin residues do not concentrate in wine during processing, with the highest processing factor for wine being <0.04× the residue observed in the RAC (grapes). Ametoctradin residue levels however do concentrate during processing into wet pomace, dry pomace and raisins, with the highest processing factor for wet pomace, dry pomace and raisins being 5.2, 35 and 6.3× the residue observed in grapes, respectively.

As the available processing studies indicate that ametoctradin residue levels do not concentrate during processing into wine, residues in wine that may occur as a result of the proposed use will be covered by the MRL recommended for grapes. The highest processing factor for dry pomace was determined to be 35× the

residue observed in grapes. The STMR and HR associated with the proposed use are 0.62 and 1.74 mg/kg respectively and therefore the STMR-P and HR-P are determined to be 21.7 and 60.9 mg/kg respectively. Based on the HR-P of 60.9 mg/kg for dry grape pomace, it is proposed that an MRL for AB 0269 Grape pomace, dry be established at 70 mg/kg.

The highest processing factor for raisins was determined to be 6.3× the residue observed in the RAC (grapes) and therefore the STMR-P and HR-P are determined to be 3.9 and 11.0 mg/kg respectively. The proposed label for Zampro® Fungicide includes the restraint “DO NOT use in crops intended for drying”, which is also present on the labels for dimethomorph products registered for use on grapes. As the use of Zampro® Fungicide should not occur on grapes intended for dried fruit production, an ametoctradin MRL for DF 0269 Dried grapes is not required at this time.

Animal feeds

Evaluation of the processing studies for grapes showed that ametoctradin residues could concentrate in grape pomace (see the above discussion on processing). The following entry in Table 4 of the MRL Standard was proposed: grape pomace (dry) - 70 mg/kg.

4.8 Crop rotation

Details of two rotational crop residues studies have been provided by the applicant. In one rotational crop study conducted in the United States (Georgia and California), soil was treated with three applications of ametoctradin at a rate of 300 g ai/ha. The rotational crops, radish, leaf lettuce and wheat, were each grown at plantback intervals of 30, 60, 90 and 120 days after treatment. The levels of ametoctradin (BAS 650 F) and its metabolites M650F03 and M650F04 in the rotational crops were determined. No quantifiable residues of parent BAS 650 F were observed in any rotational crops planted up to 4 months after the last application. Quantifiable residues of the metabolite M650F03 were observed in all rotational crops planted up to 4 months after the last application, with the exception of radish root, where M650F03 residues were last observed at 0.01 mg/kg at the 3-month plantback interval, and lettuce leaves, in which M650F03 residues were non-quantifiable throughout the study. Quantifiable residues of the metabolite M650F04 were also observed in all rotational crops planted up to 4 months after the last application, with the exception of radish root and tops and lettuce leaves, in which M650F04 residues were non-quantifiable throughout the study.

In the other rotational crop study, which was conducted in Europe (Germany, The Netherlands, Southern France and Italy), soil was treated once with ametoctradin at a rate of 960 g ai/ha. Crops of wheat, carrots, cauliflower and lettuce were planted at plantback intervals of 30 ±1, 120 ±1 and 365 ± 1 days after treatment. The levels of ametoctradin (BAS 650 F) and its metabolites M650F03 and M650F04, in the rotational crops were determined. Residues of the parent compound BAS 650F were observed twice. On the plot planted after 30 days, one sample of wheat straw contained BAS 650 F at 0.038 mg/kg while a residue of 0.020 mg/kg was observed in one sample of cauliflower inflorescence. No residues of BAS 650 F above the limit of quantitation were observed in samples obtained from the plots re-planted after 120 and 365 days. Metabolites M650F03 and M650F04 were observed in the rotational crops, with the occurrence of the two metabolites depending on the crop species and the replanting interval. The level of the two metabolites was greatest in wheat plants from the plot replanted 30 days after application. In each commodity, the level of the two metabolites declined as the replanting interval increased.

The proposed use of Zampro® Fungicide involves a maximum of 4 applications to grapevines only, by dilute spraying at a rate of 80 ml product / 100L (24 g ai/100L) or by concentrate spraying. The proposed residue definition for ametoctradin for commodities of plant origin is 'ametoctradin'.

In the two rotational crop studies, residues of parent ametoctradin (BAS 650 F) were only observed in two samples from the European study. The European study involved one application at a rate of 960 g ai/ha, in a spray volume of 300 L/ha, which is equivalent to a rate of 320 g ai/100L (13.3× the maximum rate proposed on a g ai/100L basis). The highest level of ametoctradin residue which was observed in the European study was 0.038 mg/kg in wheat straw, planted in soil 30 days after treatment. Based on the available crop rotation studies, it is concluded that the proposed use should not result in ametoctradin residues above LOQ in rotational crops, if rotational crops were planted on treated soil. It is noted that the only use of ametoctradin in Australia that is currently proposed is for grapevines, which are a permanent crop. The proposed use of Zampro® Fungicide should not result in rotational crop concerns.

4.9 Animal commodity MRLs

The applicant has provided an animal feeding study designed to determine the magnitude of the residues of BAS 650 F (ametoctradin) and its metabolites that may result in mammalian tissues and milk following oral administration.

The test item, BAS 650 F was administered orally to lactating dairy cows once daily for 28 days. The target dose levels of BAS 650 F were 2.5 mg/kg feed (1×), 7.5 mg/kg (3×) and 25 mg/kg (10×). Based on the average daily food consumption for each group during the treatment period, the mean BAS 650 F intakes in the diet over the 4 week dosing period were 0, 3.1, 7.8 and 30.3 mg/kg feed.

Milk samples from each animal were collected twice daily and combined as one pooled sample. On day 21, milk was also separated into cream and skim milk. Animals were sacrificed within 25 hours after the final dosing (day 28) and tissue samples were taken, except for two cows of the 10× group which were sacrificed 2 and 7 days after the final dose to monitor the decline of residue levels post dosing. All samples were stored frozen and remained frozen (≤ -20^{o}C) until analysis. Residues of BAS 650 F and the metabolites M650F01 and M650F06 in milk, skim milk, cream, muscle, liver, kidney and fat were determined according to the validated HPLC-MS-MS based method.

In milk, skim milk and cream, levels of BAS 650 F, M650F01 and M650F06 residue were <LOQ (0.01 mg/kg) for each treatment group. In muscle and fat sampled after 28 days of treatment, levels of BAS 650 F, M650F01 and M650F06 residue were <LOQ (0.01 mg/kg) for the 10× treatment group. The muscle and fat samples from the other dose levels were therefore not analysed. Levels of BAS 650 F residue in liver sampled after 28 days of treatment were below LOQ (0.01 mg/kg) for each treatment group. Levels of metabolite M650F01 of 0.04 mg/kg were detected in liver from the 10× treatment group, while levels were below LOQ (0.01 mg/kg) for treatment at 0, 3.3 and 7.8 mg/kg feed / day respectively. Levels of metabolite M650F06 of 0.015 and 0.047 mg/kg were detected from the 3× and 10× treatments respectively, while levels were below LOQ (0.01 mg/kg) for treatment at 0 + 1x. In kidney sampled after 28 days of treatment, levels of BAS 650 F, M650F01 and M650F06 residue were <LOQ (0.01 mg/kg), 0.011 and 0.027 mg/kg respectively for the 10× treatment group. Levels of BAS 650 F, M650F01 and M650F06 were <LOQ (0.01 mg/kg) in kidney for the 3× treatment group. The kidney samples from 0 + 1x doses were therefore not analysed.

In liver and kidney sampled after 28 days of treatment and 7 days of withdrawal, levels of BAS 650 F, M650F01 and M650F06 residue were <LOQ (0.01 mg/kg) for the 10× treatment group (30.3 mg/kg feed / day). This result indicates that M650F01 and M650F06 have a half-life of ≤ 3 and ≤ 5 days respectively in liver and kidney.

The dietary intake of ametoctradin by cattle consuming treated grape pomace is estimated below:

Dairy Cattle - 500 kg, 20 kg DM/day

Commodity	% in diet	Feed intake (kg DM)	Residue, mg/kg	% DM	Livestock dietary exposure		
					mg/animal	ppm	mg/kg bw
Grape pomace, dry	20	4	21.7 (STMR-P)	100	86.8	4.34	0.174

The potential exposure of ametoctradin to dairy cattle associated with consumption of dry grape pomace is calculated to be 4.34 ppm in the feed.

The maximum residues (BAS650F + M650F06) obtained from the ametoctradin feeding study, after 28 days of feeding at 30.3 ppm (6.98x the maximum animal feeding burden), were as follows:

Milk <0.02 mg/kg; Muscle <0.02 mg/kg; Liver, <0.057 mg/kg; Kidney <0.037 mg/kg and Fat <0.02 mg/kg. No concentration of residues was observed in cream at Day-21.

Predicted residues based on feeding at 4.34 ppm are Milk <0.0029 mg/kg; Muscle <0.0029 mg/kg; Liver, <0.0082 mg/kg; Kidney <0.0053 mg/kg and Fat <0.0029 mg/kg.

The proposed residue definition for animal commmodities is ‘Ametoctradin + M650F06’ and based on the animal feeding study, ‘Ametoctradin + M650F06’ residue levels above LOQ (0.02 mg/kg) are not predicted in the milk, skim milk, cream, muscle, fat, liver and kidney of dairy cattle treated at 4.34 ppm in the feed.

It is therefore recommended that ametoctradin MRLs for MO 0105 Edible offal (mammalian), MM 0095 Meat [mammalian] and ML 0106 Milks be established at *0.02 mg/kg.

Dry grape pomace may contribute 20% to the diet of poultry.

The potential exposure to poultry fed dry grape pomace made from grapes treated with Zampro® Fungicide, based on the STMR-P of 21.7 mg/kg for dry grape pomace, is estimated below:

Poultry - 2 kg, 0.15 kg DM/day

Commodity	% in diet	Feed intake (kg DM)	Residue, mg/kg	% DM	Livestock dietary exposure		
					mg/animal	ppm	mg/kg bw
Grape pomace, dry	20	0.03	21.7 (STMR-P)	100	0.651	4.34	0.326

The potential exposure of ametoctradin to poultry associated with consumption of dry grape pomace is calculated to be 4.34 ppm in the feed. The proposed residue definition is ‘Ametoctradin + M650F06’. The laying hen metabolism study showed that after 10 daily dose administrations of BAS 650 F at 0.81 mg/kg

bw/day (equivalent to 11.5 ppm in the feed), ametoctradin residues were not detected in muscle and liver, but were detected at a level of 0.008 and 0.001 mg/kg respectively in eggs and fat. Metabolite M650F06 (0.001 mg/kg) was detected in liver.

As the feeding level of 11.5 ppm in the feed resulted in total 'ametoctradin + M650F06' equal to 0.008 in eggs and 0.001 mg/kg in liver and fat, it is calculated that the feeding level of 4.34 mg/kg may result in a residue level of 0.003 in eggs and 0.0004 mg/kg in liver and fat.

It is therefore recommended that ametoctradin MRLs for PE 0112 Eggs, PO 0111 Poultry, Edible offal of, and PM 0110 Poultry meat be established at *0.02 mg/kg.

It is appropriate to establish the following animal commodity MRLs (set at the LOQ) for amectoctradin: Edible offal *0.02 mg/kg; Eggs *0.02 mg/kg; Meat [mammalian] *0.02 mg/kg; Milks *0.02 mg/kg; Poultry, edible offal of *0.02 mg/kg and Poultry meat *0.02 mg/kg.

4.10 Spray drift

Application of Zampro® Fungicide using aircraft will not be permitted. Ground application of the product was modelled using AgDrift, which showed that the risk of drift from vineyard applications onto adjacent pasture resulting in detectable residues of ametoctradin in meat or dairy products is very low. The inclusion of a mandatory no-spray zone on the product label for Zampro® Fungicide is considered unnecessary.

4.11 Bioaccumulation potential

Ametoctradin has an octanol/ water partition coefficient ($log_{10}P_{OW}$) of 4.40 at pH 7, 4.24 at pH 4 and 4.18 at pH 9, indicating that it is fat soluble and has the potential for bioaccumulation.

In the animal feeding study conducted on lactating cattle, ametoctradin residue levels above LOQ (0.01 mg/kg) were not observed in the milk, skim milk, cream, muscle and fat of dairy cattle subject to any treatment regime. In the lactating goat metabolism study, ametoctradin was not observed in any sample including muscle or fat. These studies do not assist in determining if ametoctradin residues may concentrate in fat. The laying hen metabolism study however found that ametoctradin contributed to 10.7% of the TRR in fat (0.001 mg/kg), while it was not detected in muscle, which indicates that it may concentrate in the fat of poultry. The animal transfer study however, showed no evidence of preferential partitioning of residues into fat.

4.12 RISK ASSESSMENT CONCLUSIONS

Estimated dietary intake

The chronic dietary intake risk for ametoctradin has been assessed. The ADI for ametoctradin is 10 mg/kg bw/day, based upon a NOAEL of 1000 mg/kg bw/day and a 100-fold safety factor. The NEDI calculation is made in accordance with WHO Guidelines[2] and is a conservative estimate of dietary exposure to chemical residues in food. The NEDI for ametoctradin, is equivalent to 0.01% of the ADI. DIAMOND Modelling[3] of chronic dietary exposure is also performed on new chemicals. The DIAMOND model estimated the chronic dietary exposure of ametoctradin as 0.01% of the ADI for the general population.

The acute dietary exposure is estimated by the National Estimated Short term Intake (NESTI) calculation. The NESTI calculations are made in accordance with the deterministic method used by the JMPR[5] with 97.5th percentile food consumption data derived from the 1995 National Nutrition Survey of Australia. NESTI calculations are conservative estimates of short-term exposure (24 hour period) to chemical residues in food.

An ARfD has not been established and is not necessary for ametoctradin as it is not likely to present an acute hazard to humans. Hence the NESTI is not required.

It is concluded that the dietary exposure to ametoctradin is low and the risk from residues in food is acceptable when Zampro® Fungicide is used according to label directions.

Recommendations

The following amendments to the MRL Standard are recommended in relation to the proposed use of Zampro® Fungicide:

Table 1

COMPOUND		FOOD	MRL (mg/kg)
Ametoctradin			
ADD:			
MO	0105	Edible offal (mammalian)	*0.02
PE	0112	Eggs	*0.02
FB	0269	Grapes	3
MM	0095	Meat [mammalian]	*0.02

[2] Guidelines for predicting dietary intake of pesticide residues, WHO, 1997.
[3] DIAMOND: The DIamond Modelling Of Nutritional Data is a computer dietary modelling program based upon statistical software that is used by FSANZ.

COMPOUND		FOOD	MRL (mg/kg)
ML	0106	Milks	*0.02
PO	0111	Poultry, Edible offal of	*0.02
PM	0110	Poultry meat	*0.02

*MRL set at the limit of quantitation

Table 3

COMPOUND	RESIDUE
ADD:	
Ametoctradin	
	Commodities of plant origin: Ametoctradin
	Commodities of animal origin: Sum of ametoctradin and 6-(7-amino-5-ethyl [1,2,4] triazolo [1,5-a]pyrimidin-6-yl) hexanoic acid

Table 4

COMPOUND		ANIMAL FEED COMMODITY	MRL (mg/kg)
Ametoctradin			
ADD:			
AB	0269	Grape pomace, dry	70

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

HARVEST WITHHOLDING PERIOD

Grapes: Do not harvest for 4 weeks after application.

5 ASSESSMENT OF OVERSEAS TRADE ASPECTS OF RESIDUES IN FOOD

5.1 Commodities exported and main destinations

Some of the commodities of interest in connection with the proposed registration, namely grapes (including wine), mammalian and poultry meat and offal, eggs, and dairy produce are considered to be major Australian export commodities.

The major export markets for grapes and wine are tabulated below:

Australian Table Grape Exports in 2007/08 (Australian Bureau of Statistics)	
Destination	**Value, $ million**
Hong Kong	29.340
Indonesia	16.775
Thailand	12.587
Singapore	7.993
Malaysia	7.208
Vietnam	5.319
New Zealand	4.536
United Arab Emirates	3.667
Taiwan	3.325
Bangladesh	2.036
Sri Lanka	1.379
Other	6.346
TOTAL	100.511

Australian Wine Export value for each major export destination from 2005-06 until 2009-10.					
Destination	**Export wine value ($ million)**				
	2005-06	**2006-07**	**2007-08**	**2008-09**	**2009-10**
Canada	249.2	266.8	259.9	214.0	202.1
China	20.8	49.0	61.8	93.8	143.3
Germany	75.6	65.6	49.3	51.4	48.7
Hong Kong	25.1	26.4	33.8	43.1	42.0
Ireland	54.0	68.7	69.9	46.3	40.8
Japan	44.1	49.0	48.7	52.3	44.2
Netherlands	45.3	65.3	71.1	57.4	56.3
New Zealand	92.2	101.8	85.9	76.0	70.6
Singapore	37.6	42.9	46.0	41.3	40.9
Sweden	49.6	51.1	41.0	40.0	42.0
Switzerland	19.1	18.1	15.6	14.9	12.1
Thailand	7.8	10.4	13.3	12.0	12.3
United Kingdom	960.5	976.3	888.1	723.3	584.7
United States	901.3	956.1	745.5	741.2	629.4
Other	216.8	242.5	253.4	221.4	203.2
TOTAL	**2 799.0**	**2 989.9**	**2 683.2**	**2 428.4**	**2 172.5**
Source: Australian commodity statistics 2010, ABARES.					

Exports of dried vine fruit from Australia are of minor importance in comparison with wine and table grapes, with exports of 4000 tonnes in 2009/10 being worth $13 million. Zampro® Fungicide will not be used on grapes used for dried fruit production.

The significant export markets for animal commodities are listed in Part 5B of APVMA MoRaG.[4] Total exports of dairy products in 2009/10 were worth $2.0342 billion, with key export destinations being Japan, Singapore, China, the Philippines, Thailand and the USA. Total exports of beef and veal were worth $4.144 billion in 2009/10, with the major destinations being Japan, the USA, Korea, Indonesia and Taiwan. Total exports of lamb and mutton were worth $1.4555 billion in 2009/10, with the key destinations being the USA, the European Union, Japan, and the Middle East. Overseas MRLs are established or proposed in only some overseas markets.

5.2 Overseas registration status

The applicant indicated that ametoctradin products are registered for use in the following countries: Argentina, Austria, Chile, Colombia, Ecuador, Estonia, France, Germany, Hungary, Korea, Latvia, Lithuania, Macedonia, Netherlands, Romania, Turkey and United Kingdom (status October 2011).

Codex MRLs have not have been established for ametoctradin.

In the EU, the European Food Safety Authority established the following MRLs for ametoctradin:

Commodity	Tolerance (mg/kg)
Table grapes	5
Wine grapes	5
Cattle milk	*0.01
Poultry meat	*0.01
Poultry fat	*0.01
Poultry edible offal	*0.01
Bovine meat	*0.01
Bovine fat	*0.01
Bovine edible offal	*0.01
Chicken eggs	*0.01

The residue definition for the EU is ametoctradin.

The proposed residue definitions in Canada are ametoctradin for plant matrices and ametoctradin plus M650F06 for animal commodities.

[4] http://www.apvma.gov.au/morag_ag/vol_3/part_05b_trade.php

The following relevant residue MRLs/ tolerances for Canada have been proposed as part of the Global Joint Review:

Commodity	Tolerance (mg/kg)
Grapes (as part of Subgroup 13-07F)	4
Livestock commodities	*0.02

Applications have been received and are being considered by the US EPA to register pesticide products containing ametoctradin for use on brassica leafy vegetables, bulb vegetables, cucurbit vegetables, fruiting vegetables, leafy vegetables, tuberous and corn vegetables, grapes and hops.[5]

5.3 Potential risk to trade

An MRL of 3mg/kg is proposed for grapes. The proposed grape MRL is lower than the proposed grape MRL for Canada (4 mg/kg) and the established European Union MRL of 5 mg/kg.

Most major export destinations for table grapes do not currently have an MRL for grapes, while New Zealand accepts Australian MRLs under the Trans Tasman Mutual Recognition Agreement. Table grape exports are therefore at possible risk as a result of the proposed use of ametoctradin in Australian grapes.

A separate wine MRL is not required, as residues will not exceed the grape MRL. The highest observed processing factor for wine was 0.04x the residues observed in the RAC (grapes). The STMR and HR associated with the proposed use on grapes are 0.62 and 1.74 mg/kg respectively, so residues in wine are expected to be low, if detectable. As residues are expected to be low in wine, the risk to trade is considered to be low.

Detectable residues in animal commodities derived from animals fed on grape pomace through the use of amectoctradin in Zampro® Fungicide are unlikely. Hence the export of these livestock commodities should not affect trade between Australia and places outside Australia.

CONCLUSIONS

Grapes:

The available residues trial data show that grapes from vineyards treated with ametoctradin may contain residues when harvested. Processing studies indicated that ametoctradin residue levels do not concentrate during processing into wine, so residues in wine that may occur as a result of the proposed use, will be covered by the MRL recommended for grapes. A separate MRL for wine is therefore not required. The

[5] Federal Register Vol. 75, No.85 Tuesday May 4 2010 p. 23760, Vol. 75, No.96 Wednesday May 19 2010 p. 28012

proposed Australian MRL for grapes may potentially have an impact on the export of Australian table grapes and wine to the major importing countries. The Applicant has suggested the following label statement: "**PRECAUTION - Export of treated fruit or wine**. Growers should note that Maximum Residue Limits (MRLs) or import tolerances do not exist in all markets for fruit treated with ZAMPRO. Additionally, some export markets have established MRLs different to those in Australia. If you are growing fruit for export (either fresh or as wine), please check with Nufarm Australia Ltd or the Australian Wine Research Institute http://www.awri.com.au for the latest information on MRLs and import tolerances before using ZAMPRO."

The APVMA welcomes comment on whether ametoctradin residues will unduly prejudice Australian trade in table grapes or wine.

Animal commodities:

Metabolism data in lactating goats and estimation of the expected dietary burden in poultry and mammals feeding on commodities from crops treated with ametoctradin show that quantifiable residues are unlikely to be found in mammalian and poultry meat and offal, milk or eggs. MRLs are proposed for these commodities at the limit of quantitation. There is not expected to be any significant risk to Australian trade in meat, milk and eggs, however the APVMA welcomes comment on the proposed MRLs.

6 OCCUPATIONAL HEALTH AND SAFETY ASSESSMENT

6.1 Summary

Farmers and their employees will be the main users of Zampro® Fungicide. 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 product will be dermal with inhalation, although ocular exposure is also possible.

In the absence of exposure data for the proposed mode of application, the Pesticide Handler Exposure Database (PHED) Surrogate Exposure Guide (1998) was used to estimate exposure. Exposure to the product during mixing, loading and ground-airblast and aerial application were at an acceptable level without the use of personal protective equipment (PPE). Thus, PPE is not required during use (i.e. mixing, loading and application) of the product.

Based on the risk assessment, First Aid Instructions and Safety Directions have been recommended for inclusion 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 Zampro® Fungicide when used in accordance with the label directions.

6.2 Health hazards

Ametoctradin

Ametoctradin (CAS: 865318-97-4) is not listed on Safe Work Australia's (SWA) Hazardous Substances Information System (HSIS) Database (SWA, 2011). Based on the toxicological profile of ametoctradin, ametoctradin is not classified as a hazardous substance in accordance with the NOHSC Approved Criteria for Classifying Hazardous Substances (NOHSC, 2004).

Dimethomorph

Dimethomorph (CAS: 110488-70-5) is listed on Safe Work Australia's (SWA) Hazardous Substances Information System (HSIS) Database (SWA, 2011) with environmental risk phrases assigned. However, no human health-related risk phrases are assigned. This classification remains appropriate.

Zampro ® Fungicide

There are acute toxicity studies available on Zampro® Fungicide. Based on the product toxicology information, the OCS classified Zampro ® Fungicide as a hazardous substance in accordance with ASCC Approved Criteria for Classifying Hazardous Substances (NOHSC, 2004) with the following risk phrase:

R22	Harmful if swallowed

Formulation, packaging, transport, storage and retailing

The active constituent ametoctradin will be manufactured overseas. The product Zampro® Fungicide containing 300 g/L ametoctradin (and 225 g/L dimethomorph) will be formulated overseas and imported into Australia in 2.5 L or 5 L high-density polyethylene (HDPE) containers with twist caps.

Use pattern

Zampro ® Fungicide is a suspension concentrate formulation containing ametoctradin 300 g/L and dimethomorph 225 g/L, and is intended for the control of downy mildew in grapevines. Zampro® Fungicide is not intended for domestic use. The proposed maximum rate of application is 1.02 L product per hectare which may be used in a dilute spray volume of 80 mL product / 100 L water, or in a concentrate spray volume of a minimum 250 L/ha. The application may be made using ground-airblast or aerial application methods.

Zampro ® Fungicide is intended to be applied a maximum of four times per season at 7-14 day intervals, with a maximum of two consecutive applications before switching to a different fungicide product with a different mode of action.

Exposure during use

Farmers and their employees will be the main users of Zampro® Fungicide. 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 product will be dermal with inhalation, although ocular exposure is also possible.

In the absence of exposure data for the proposed mode of application, the Pesticide Handler Exposure Database (PHED) Surrogate Exposure Guide (1998) was used to estimate exposure. The toxic endpoint of concern and identified NOAEL is derived from repeat dose study in animals, and in this instance a margin of exposure (MOE) of 100 or above is acceptable.

The MOE takes into account both interspecies extrapolation, intraspecies variability and the seriousness of the critical health effect of concern.

The MOEs for mixing and loading and application by ground-airblast and aerial application are at an acceptable level (i.e. > 100) without the use of personal protective equipment (PPE). Thus, PPE is not required during use (i.e. mixing, loading and application) of the product.

Exposure during re-entry

No re-entry statement is required.

Recommendations for safe use

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

6.3 Conclusion

The registration of Zampro® Fungicide containing ametoctradin 300 g/L and dimethomorph 225 g/L (an existing active ingredient) for the control of downy mildew in grapevines is supported.

Zampro® Fungicide 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 Material Safety Data Sheet.

7 ENVIRONMENTAL ASSESSMENT

7.1 Environmental Fate

Route and Rate of Degradation in Soil

Aerobic degradation in laboratory soils

The transformation of ametoctradin in aerobic soils was examined in three laboratory studies encompassing a variety of sand and loam soil types from Europe and North America at temperatures ranging from 10–25°C. Based on these studies, it was concluded that the transformation of the active constituent in aerobic soils is governed by microbial decomposition, which proceeds primarily by means of oxidative cleavage of the n-octyl side chain. The four major transformation products are carboxylic acid metabolites which have successively shorter side chains (M650F01–4). With sufficient microbial exposure, the pyrimidine-carboxylic acid ring structure may be broken leading to mineralization of the active constituent and formation of bound residues.

The rate of transformation of ametoctradin in the dark at 20°C was determined on a sandy loam soil from a fallow agricultural field in Germany in a 360 day study. The rate of primary biodegradation of ametoctradin on this microbially active soil was rapid and the average concentration of the parent compound decreased from 96.1% applied radioactivity (AR) at day 0 to below 10% AR by day 10 and to 0.6–0.8% AR on day 269–360. The decrease in concentration of ametoctradin with time was well described by the double-first-order in parallel (DFOP) bi-exponential kinetic model. Based on this kinetic analysis, the bi-exponential DT_{50} for primary biodegradation of ametoctradin on sandy loam soil at 20°C is 1.3 days. The DT_{90} was calculated to be 7.3 days.

The initial steps in the degradation of the active constituent in this soil involved microbial oxidation of the octyl side chain to the four major carboxylic acid metabolites, M650F01–4. All four of these oxidative transformation products were detected at >10% AR over the course of the incubation, although only M650F04 was present at substantial levels at the end of the incubation period (13.3% AR at day 360). After 360 days, the majority of the applied active constituent had been mineralised (42.1% AR as CO_2) or converted into non-extractable bound residues (28.5% AR).

The rates of biotransformation of ametoctradin on a further three soils from Germany were investigated in a separate study which included an evaluation of the role of temperature in the aerobic soil degradation of the active constituent. In this second study, a sandy loam and two loamy sand soils were treated with the active constituent and incubated for 120 days at 20°C (and at 10°C for one loamy sand soil). The rates of primary biodegradation of ametoctradin in these three aerobic soils from Germany were again rapid at 20°C. The decrease in concentration of ametoctradin with time in each soil was again well described by the DFOP model, with bi-exponential DT_{50} values ranging from 1.5–3.2 days and corresponding DT_{90} values of 7.6–12.8 days for primary biodegradation at 20ºC. The transformation rate was slower at 10ºC, with bi-exponential DT_{50} and DT_{90} values of 6.4 days and 24.9 days, respectively. The four major carboxylic acid metabolites, M650F01–4, were identified in each soil and the overall transformation pathway for ametoctradin in these three soils was again consistent with degradation of the active constituent by microbial oxidation of the octyl side chain.

A final laboratory study on the aerobic soil transformation of ametoctradin was conducted with four soils from the United States with textures ranging from loams to loamy sands. Although these soils were microbially active throughout the 365-day incubation period, there were substantial changes in microbial biomass (both increases and decreases) in three out of the four test soils and significant differences in the amount of $^{14}CO_2$ that was evolved both between soils and between replicates. This variability in microbial activity coupled with some technical deficiencies with the sampling regime employed gave results that are not considered as reliable as the preceding two studies conducted on German soils. Nevertheless, for all four soils, it was clear that ametoctradin underwent relative rapid primary biodegradation to the same set of four major carboxylic acid metabolites as observed on other soils. The decay in the concentration of ametoctradin with time for each soil was adequately described either by DFOP or single first-order (SFO) kinetic models.

The primary biodegradation of ametoctradin on microbially active aerobic soils is rapid with typical DT_{50}s <4 days (at 20°C). This active constituent is therefore not classified as persistent in aerobic soils according to the criteria adopted in Australia.

The degradation rates of the ametoctradin aerobic soil transformation products, M650F03 and M650F04, were each determined in a single study conducted at 20°C in four aerobic soils (three German and one U.S. soil) ranging in texture from sand to loam. M650F03 was not persistent under the conditions of the study, with DFOP and SFO DT_{50}s ranging between 29 and 68 days and DT_{90}s between 96 and 249 days. The only major transformation product, M650F04, continued to accumulate to the end of the study period (120 DAT), which is consistent with the studies on the parent substance.

M650F04 transformed slowly in the four study soils, with DFOP and SFO DT_{50} values of 106–289 days in the sandy loam and loamy sands and a DFOP DT_{50} of 28 days in loam. The faster transformation rate in the loam was attributed, in part, to a higher microbial population in this soil. No transformation product (except CO_2) was identified. However, the presence of significant amounts of non-extractable radioactivity (mean values of 20.0–45.9% AR) and total evolved CO_2 (mean values of 5.1–29.9% AR) at study termination, indicate that M650F04 will ultimately be bound to the soil matrix and/or mineralized. M650F04 can be classified as persistent on some aerobic soil types. The SFO and DFOP DT_{90} values for M650F04 were in the range 139–1020 days.

Anaerobic degradation in laboratory soils

The rate of biotransformation of doubly radiolabelled ametoctradin on sandy loam soil under anaerobic conditions was evaluated according to a Guideline method in a single study lasting 118 days. The active constituent degraded to approximately 45% AR during the initial 2-day period of aerobic incubation on soil. However, the rate of primary degradation decreased rapidly once the soil had been flooded and anaerobic conditions were established. Indeed, under strongly reducing conditions in flooded soil, ametoctradin is persistent with a single first-order DT_{50} of 182 days and a DT_{90} of 606 days. The slow removal of the active constituent under these conditions is primarily through formation of bound residues. The transformation products formed during the initial aerobic phase (M650F01, M650F02, M650F03, and other unidentified minor products) were stable under anaerobic conditions.

Photolysis

The photolysis of ametoctradin on soil was investigated in a single study conducted on a sandy loam soil that was also used to evaluate the rate of aerobic soil biotransformation of the active constituent. Radio-labelled active constituent was applied to sieved dry soil and irradiated continuously with artificial light for 15 days at 22°C. The light intensity was adjusted to simulate a clear summer day at 48°N latitude and filtered to remove short wavelength UV radiation (λ <290 nm). The soil moisture levels were adjusted daily to maintain 40% of the maximum water holding capacity (MWHC).

Ametoctradin was found to degrade in soil with and without the presence of light. In this system, the rate of transformation was slower under irradiation than in the dark control, indicating that irradiation does not enhance transformation of ametoctradin in soil. After 15 days of continuous irradiation, the quantity of parent substance in the dark controls had declined to 26.6% AR whereas 67.8% AR remained in the irradiated samples. The four major aerobic soil microbial transformation products of ametoctradin were detected in both the irradiated samples and darks controls, except for M650F04 which was not detected in the irradiated samples. There was only a minor amount of CO_2 evolved (≤2.7% AR).

The decrease in concentration of ametoctradin with time in the irradiated soil sample was adequately described by an SFO kinetic model. Based on this kinetic analysis, the single first-order DT_{50} and DT_{90} values for continuous irradiation of ametoctradin on soil are 22.6 days and 75.0 days, respectively. The decay of the active constituent in the dark controls was well described by an SFO model. For the dark controls, the first-order DT_{50} = 7.4 days and DT_{90} = 24.6 days. A photo-transformation half-life for ametoctradin could not be determined in this case because the rate of transformation of the active constituent was faster in the dark controls.

Dissipation under field conditions

Two terrestrial field dissipation studies were conducted at five locations in North America (Ontario, Illinois, Florida, California and Washington) using the active constituent as parent substance. One terrestrial field dissipation study each was also carried out on bare soil in Europe for the two stable soil metabolites, M650F03 and M650F04. European field studies were not performed with ametoctradin due to its rapid transformation rates observed in laboratory aerobic soil studies. For the European studies, M650F03 and M650F04 were applied as parent material to three locations in Northern Europe (Denmark, UK, and Germany) and two in Southern Europe (Italy and Spain). The observational period was 360 days for each study.

Ametoctradin was applied at either 3 × 300 g a.c./ha (Ontario, Illinois and Florida) or 4 × 300 g a.c./ha (California and Washington). The five trial sites encompassed soils with sand, sandy loam and loam textures. The active constituent was not persistent under bare soil conditions. The SFO, first-order multi compartment (FOMC), and double first-order in series (DFOS) DT_{50} values derived from kinetic analyses of soil dissipation of the active constituent ranged from 0.4–5.0 days and corresponding DT_{90}s ranged from 3.2–16.5 days.

Three major transformation products, M650F01, M650F02 and M650F03, were similarly not persistent, with DT_{50}s ranging from 1.3–13.3 days, with corresponding DT_{90}s of 4.5–144 days. However, M650F03 was more persistent in California sandy loam with a DT_{50} and DT_{90} of 101 and 336 days, respectively. Dissipation of the fourth major transformation product, M650F04, was more variable between trial sites. M650F04 was

persistent in Ontario (estimated DT_{50} >365 days). This transformation product was less persistent in Illinois and California soils (DT_{50} = 177 and 113 days, respectively), and even less so in Washington soils (DT_{50} = 36.1 days). M650F04 was not found in significant quantities at the Florida study site.

The major route of dissipation of ametoctradin in bare soil was biotransformation. Among all sites, the maximum levels of the transformation products relative to the initial measured amount of the parent following the final application were: 14–29% for M650F01, 4–23% for M650F02, 38–171% for M650F03 (except Florida), and 37–77% for M650F04 (except Florida). At the site in Florida, M650F03 and M650F04 were observed at lower amounts than other sites (4–12% of the applied parent active constituent). However, transformation pathways were consistent with the other sites.

Ametoctradin was not detected below the 15–30 cm segment at any site (LOQ = 0.01 mg/kg). Soil moisture conditions were favourable for leaching during and/or shortly after the application period at most sites, indicating the active constituent has low inherent susceptibility for leaching. Ametoctradin is not volatile, and therefore this route of dissipation was not evaluated in the field.

Ametoctradin did not carry over to the following growing season at any of the trial sites. Of the four transformation products monitored in the field, only M650F04 was present at the beginning of the following growing season in both Ontario and Illinois test plots (279–280 days after first application), at 23–43% of the amount of applied parent material.

A single spring application of M650F03 was made at a nominal rate of 150 g M650F03/ha to bare soil at five different sites in Europe. DT_{50} values ranged from 6.9–19.8 days based on a variety of kinetic models including SFO, FOMC and DFOP. There were no remarkable differences in the derived DT_{50} values between regions. DT_{90} values ranged from 48.8–65.8 days in Northern Europe, but 250–420 days in Southern Europe, with the longer dissipation period in the Southern region attributed to reduced soil moisture levels. M650F04 was the only major transformation product (up to 30.6% of applied parent), and was mobile in the soil column, reaching depths of 80–90 cm. Dissipation times were not determined for M650F04. M650F03 is not considered persistent in soil.

A single spring application of M650F04 was made at a nominal rate of 100 g M650F04/ha to bare soil at five different sites in Europe. M650F04 migrated downwards in the soil column, reaching depths of up to 70 cm within one year following application, which is consistent with the study conducted with M650F03. Different kinetic models were used to evaluate the dissipation of this transformation product depending on the soil. These included SFO, DFOP and FOMC. The DT_{50} values derived from these various kinetic analyses ranged from 25.1–186.5 days and DT_{90} values were 168– >656 days, with no significant differences between regions. M650F04 is considered to meet the criteria for persistence in soil under some conditions.

Adsorption, desorption and mobility in soil

The sorption of ametoctradin onto sterilised soil was investigated using the radiolabelled active constituent in a single standard laboratory batch equilibrium study. The study was conducted with eight different soils from loams to loamy sands at a soil to solution ratio of 1:50. The sorption of ametoctradin in the eight test soils showed only a weak relationship to organic carbon content and no relationship existed to clay content and cation exchange capacity (CEC). The sorption also showed a weak negative correlation to pH (i.e., higher sorption at lower pH). This trend is consistent with the expectation that this weakly basic active constituent

(pK_b = 11.22 est.) will not form significant concentrations of the protonated ametoctradin cation except in acid aqueous environments (pH <5).

The Freundlich adsorption coefficient normalized for organic carbon content, K_{Foc}, determined for ametoctradin on these soils ranged from 1580 L/kg to 6620 L/kg. Based on these K_{Foc} values the mobility of ametoctradin in soil is classified as low to immobile.

The mobilities in soil of the four carboxylic acid transformation products (M650F01–4) were also evaluated. The conjugate base anions of each of these acids will predominate in aqueous environments at near neutral to alkaline conditions (pH 6–9). These organic anions are expected to adsorb less strongly to soil than the corresponding acids and the partitioning of each transformation product between the soil and water compartments could therefore be expected to decrease as the pH of the aqueous phase increases. This was partially confirmed in laboratory adsorption studies which showed that the adsorption of some transformation products to soil was lower when the pH of the solution phase was greater than 5.

The soil mobilities of M650F01 and M650F02 were evaluated on the same set of soils as those used for the tests with ametoctradin (1 study each). These tests were also conducted as sorption only studies by means of standard laboratory batch equilibrium methods. The sorption of both transformation products was not correlated with soil organic carbon content, clay content or CEC. There was only a weak negative correlation between sorption and solution pH for M650F01 and no correlation for M650F02. The mobility of M650F01 in soil is medium to very high based on the range of K_{Foc} values determined for this transformation product. The mobility of M650F02 in soil is high to very high based on the narrower range of K_{Foc} values determined for this transformation product.

The soil sorption properties of M650F03 and M650F04 were both evaluated by the same batch equilibrium method used for the two other transformation products and for ametoctradin (2 studies each). Desorption isotherms for both transformation products were also evaluated in these studies to establish the reversibility of the adsorption of these substances on soil. A separate study for each transformation product was conducted to more directly evaluate the effect of pH on adsorption to soil.

The sorption of M650F03 to soil was not correlated with soil organic carbon content, clay content or CEC. In one study, there was weak negative correlation between pH and sorption when a trend analysis of the full set of results was conducted. However, adsorption of M650F03 on the most acid soil (sand: pH 4.1 in 0.01 M $CaCl_2$) was significantly greater than that observed for less acid soils (K_{Foc} = 199 L/kg *vs*. K_{Foc} ≤33 L/kg). The Freundlich desorption coefficients were comparable to the adsorption coefficients in both studies which indicates that adsorption of M650F03 to soil is reversible.

The K_{Foc} values determined for M650F03 span a range of soil mobility classifications. However, in both studies there was a tendency for stronger adsorption to acid soils. The highest accepted value is 199 L/kg on acid sandy soil which indicates that this transformation product should be categorised as having medium mobility in acid soils. The remaining K_{Foc} values determined for M650F03 on less acid soils fall in the range 11–63 L/kg which categorises the soil mobility of this transformation product as high to very high.

The soil adsorption of M650F04 was evaluated on the same soils as those used for evaluating the mobility of M650F03. The overall soil adsorption properties of M650F04 (including dependence on soil characteristics and reversibility) all followed similar trends as those observed for M650F03. The adsorption of M650F04 was

again significantly higher on an acid sandy soil compared with less acid soils (K_{Foc} = 118 L/kg *vs*. K_{Foc} ≤47.0 L/kg). On the basis of these results, M650F04 is categorised as having high mobility in acid soils and very high mobility in other soils.

No soil column leaching studies, lysimeter studies, field leaching studies, nor laboratory soil volatility studies were submitted. These studies were not required in this case as reliable adsorption values for ametoctradin and its main soil transformation products were determined by batch equilibrium experiments conducted according to a Guideline method. The soil mobility of the active constituents and its main metabolites was also evaluated indirectly in multiple field dissipation studies. Ametoctradin is not likely to volatilize from moist soils based on the low Henry's law constant for this active constituent.

Fate and Behaviour in Water

Hydrolysis

A single screening level test of the hydrolytic stability of ametoctradin was conducted under aseptic conditions according to a Guideline method. In this test, radiolabelled active constituent was incubated in water at pH 4, 5, 7 and 9 for 7 days at 50°C. No significant hydrolysis of the active constituent occurred in any of the test solutions. According to the guideline, the extrapolated half-life for hydrolysis of ametoctradin at 25°C is >1 year. This active constituent is therefore considered to be hydrolytically stable.

Aqueous photolysis

The rate of photo-degradation of ametoctradin was determined in sterile buffered water at pH 7 and 22°C. The radiolabelled active constituent degraded slowly under continuous irradiation (3 mW/cm^2; λ >290 nm), with approximately 70% AR remaining after 15 days. No major transformation products were observed, although low amounts of oxidation products (hydroxylation and keto-formation at the octyl side-chain) were detected. A small amount of CO_2 (≤4.2% AR) was evolved. The half-life of ametoctradin under continuous irradiation was 38.4 days based on a single first-order kinetic model. Assuming 12 hours of daylight, the estimated single first-order DT_{50} for aqueous photolysis of ametoctradin is 76.8 days on a clear summer day at 50º N. The calculated quantum yield for aqueous photolysis of ametoctradin is 3.0×10^{-5} mole/Einstein.

The rate of photo-degradation of M650F03 was determined in sterile buffered water (phosphate buffer; pH 7) and also in sterilized natural water taken from a small stream in a German forest (1 study). The continuous irradiation conditions were similar to those used to evaluate the aqueous photolysis of ametoctradin and both were chosen to simulate a clear summer day at 50°N. Photo-transformation of M650F03 proceeded faster in sterilized natural waters than buffered solutions, with approximately 30% AR remaining after 15 days continuous irradiation in natural water and 60% in buffered solution. Three major transformation products (i.e., >10% AR) were speculated to be M650F32, M650F39, and M650F51. Multiple minor transformation products were identified, including M650F04, with more occurring in the natural water system. In addition, a small amount of CO_2 (≤2.3% AR) was evolved.

The single first-order DT_{50} for aqueous photolysis of M650F03 under continuous irradiation in sterile buffer solution and sterile natural water systems was 17.8 days and 5.8 days, respectively. Assuming 12 hours of daylight, the estimated single first-order DT_{50} for aqueous photolysis of M650F03 in environmental waters is in the range between 11.6 days and 35.6 days on a clear summer day at 50ºN. Based on the DT_{50}s

determined under continuous irradiation, M650F03 is categorised as fairly to moderately degradable by aqueous photolysis in water.

Ready biodegradability

The ready biodegradability of ametoctradin was evaluated in a single standard 28-day CO_2 evolution test using activated sewage sludge from a municipal waste water plant in Germany. Ametoctradin was not toxic to the inoculum and biodegradation of the active constituent occurred slowly but continuously under the conditions of this test. However, after 28 days only 18–24% of the active constituent had degraded. Ametoctradin is therefore not ready biodegradable.

Dissipation in aerobic water/sediment systems

The behaviour of ametoctradin in an aerobic water/silt loam and an aerobic water/sand system was evaluated under dark and irradiated conditions (Xenon light; 13/11 hour light/dark regime) in a single study. The biologically active water and sediment samples used for these tests were all taken from environmental water bodies surrounded by forest. The radio-labelled active constituent was spiked to the surface of the water-sediment test systems to give a nominal initial concentration of 40 µg a.c./L. The degradation and partitioning of the active constituent between water and sediments under dark conditions was monitored for up to 100 days at 20°C.

Under dark conditions, ametoctradin transformed and partitioned rapidly from the water phase of both water/sediment systems, declining to ≤3.4% AR within 4 days. In the sediments, consequently, ametoctradin increased to maximum levels of 24–34% AR after 1 day followed by gradual declines to 1% AR at the end of incubation. The rate of dissipation of ametoctradin from the water and sediment compartments was evaluated with an SFO kinetic model in both water/sediment systems. The whole system dissipation was evaluated with a DFOP kinetic model. The single first-order DT_{50}s for dissipation of ametoctradin from the water compartment in these water/sediment systems were 0.69 and 0.89 days. For the sediment compartment, the single first-order DT_{50}s for dissipation were 2.07 and 2.17 days for the two systems. The bi-phasic whole system dissipation DT_{50}s for ametoctradin were 1.72 and 1.51 days. The short lifetimes for ametoctradin derived in these studies shows that this active constituent is not persistent in the water or sediment compartments of biologically-active aerobic water/sediment systems.

The long aliphatic side-chain of the parent was shortened to form metabolites found at >10% AR in the water phase of these aerobic water/sediment systems. The four major biotransformation products identified were: M650F01 (max. 21.3% AR), M650F02 (max. 10.2% AR), M650F03 (max. 55.3% AR) and M650F04 (max. 14.4% AR). The non-extractable fraction in sediment increased to a maximum of 19–23% AR, and CO_2 increased to 1.2–1.3% AR. In the sediment of dark systems, M650F03 (max. 19.6% AR) was the only metabolite found at >10% AR. The other major metabolites detected in the water phase appeared also in the sediment, however in lower amounts. No other compound exceeded 2% AR at any sampling time or in any system. The distribution of transformation products between water and sediment phases indicates that the products were more water soluble than the parent compound.

The rates of dissipation of M650F03 and M650F04 were significantly slower than the other main metabolites and both were present at significant levels at the end of the incubation: M650F03 (total system: 58.9% AR; water phase: 43.6% AR) and M650F04 (total system: 16.4% AR; water phase: 12.4% AR). There were

insufficient degradation data points to estimate the DT_{50}s for M650F04, however, this metabolite persisted at relatively stable levels to the end of the incubation. The DT_{50} and DT_{90} values for M650F03 in the two systems could be estimated and they were found to be 328 and 1090 days in water (SFO), 208 and 692 days in sediment (SFO), and 479 and 1590 days in the total system (SFO). The slow rates of dissipation of M650F03 determined in these studies indicate that this metabolite is persistent in both the water and sediment compartments of microbially-active aerobic water/sediment systems.

The total system DT_{50} values for the degradation of ametoctradin under irradiated conditions were in the same order of magnitude as under dark conditions. However, as the purpose of laboratory aerobic biotransformation study is to determine biological degradation pathways and rates, the results from the irradiated system have not been used.

Dissipation in anaerobic water/sediment systems

The behaviour of ametoctradin in an anaerobic aquatic environment was studied in a single pond water/sandy sediment system from White Lake, South Dakota, USA for 365 days under a blanket of nitrogen in the dark at 25 ± 2°C. The water surface of the biologically active anaerobic water/sediment system was spiked with radio-labelled ametoctradin to give a nominal initial concentration of 80 µg a.c./L. The water layer and sediment layer both maintained slightly reducing to reducing conditions based on measurements of the redox potential and dissolved oxygen content of these compartments.

The active constituent bio-transformed and dissipated rapidly from the water layer to the sediment and reached a level of <1% AR in the water phase within 30 days. In the sediment, the concentration of the active constituent increased to a maximum of 33% AR by day 7 and declined to <1% AR within 120 days. The rate of dissipation of ametoctradin from the water and sediment compartments was evaluated with the indeterminant-order rate equation (IORE). The whole system dissipation was evaluated with a DFOP kinetic model. The IORE DT_{50} for dissipation of ametoctradin from the water compartment was 1.6 days, and for the sediment compartment the IORE DT_{50} was 13.8 days. The bi-phasic whole system dissipation DT_{50} for ametoctradin was 7.4 days. The short lifetimes for ametoctradin derived in this study indicates that this active constituent is not persistent in the water or sediment compartments of biologically active anaerobic active water/sediment systems.

In this anaerobic sediment/water system, most of the parent compound was bio-transformed to M650F03, reaching a total of 81% AR (72% AR in water and 9% AR in sediment) by the end of the study (365 DAT). The levels of M650F03 continue to accumulate until the end of the test which indicates that this metabolite is also persistent in biologically active anaerobic active water/sediment systems. The levels of M650F04 although low at the end of the study (total system: 5.4% AR; water phase: 4.9% AR) were also stable from DAT 64 to DAT 365 which indicates that it is also persistent under these conditions.

M650F01 was a transient major bio-transformation product, appearing only in the water layer and decreasing from a maximum of 38% AR on day 7 to <0.5% AR by day 90. Only a small amount of radioactivity was detected in the non-extractable fraction in sediment, with a maximum of <9% AR occurring on day 90. Minimum amounts of CO_2 were evolved (<0.6% AR).

Fate and Behaviour in Air

The overall rate constant for oxidation of ametoctradin in the troposphere by the hydroxyl radical at 298 K is 39.4×10^{-12} cm^3/molecule-sec as calculated with AOPWIN v1.92. The principal contributors to the overall photochemical oxidation of ametoctradin are predicted to be hydrogen abstraction and addition to fused rings by hydroxyl radicals. The overall rate constant for oxidation is equivalent to a troposphere half-life for ametoctradin of 3.3 hours when adjustments are made for average diurnal and average annual concentrations of hydroxyl radicals. Based on this relatively short estimated half-life, ametoctradin is not categorised as persistent in the air compartment.

7.2 Environmental Effects

Effects on Birds

The effects of ametoctradin on avian wildlife were evaluated in seven studies conducted according to Guideline methods. On the basis of these studies it is concluded that ametoctradin has low acute and chronic toxicity to birds. For example, the acute effects of the active constituent on birds were studied in single-dose oral (gavage) tests with bobwhite quail, mallard duck and zebra finch at nominal concentrations up to 2000 mg a.c./kg bw. No mortality, clinical signs of toxicity or treatment related effects were observed during the study with any of the bird species. Hence, the median lethal dose (LD_{50}) and the no-observed effect level (NOEL) for all three species are >2000 and 2000 mg a.c./kg bw, respectively. Ametoctradin is therefore categorised as practically non-toxic to birds by acute oral exposure.

In sub-acute dietary toxicity studies with bobwhite quail and mallard duck, no treatment-related mortality was observed after a 5-day dietary exposure period when treated at rates of 5000 mg a.c./kg diet for up to 3 days. A statistically significant decrease in body weight (15%) at the highest dose (i.e., 5000 mg a.c./kg diet) compared to controls was observed for mallard duck at the end of the 3 day recovery period. This was considered to be treatment related and the no-observed effect concentration (NOEC) was set equal to the next highest test concentration of 2000 mg a.c./kg diet. As the sub-acute dietary median lethal effect concentration in feed for both bird species is >5000 mg a.c./kg diet, ametoctradin is categorised as practically non-toxic to birds in short term dietary exposures.

The reproductive effects of ametoctradin on birds were studied in 21 week dietary dose-response studies with bobwhite quail and mallard duck at concentrations up to 1400 mg a.c./kg diet. There were no treatment-related effects on any reproductive parameter at all dose levels and no adverse effects on the parental generation. Hence, the NOEC for reproductive effects of ametoctradin in birds is taken to be 1400 mg a.c./kg diet.

Effects on Aquatic Organisms

Fish

The acute toxicity of ametoctradin to four species of freshwater fish and one species of marine fish (sheepshead minnow) was investigated by standard laboratory test methods in 5 separate studies. The aquatic effects testing of ametoctradin is complicated by the low solubility of this active constituent in water

(<1 mg a.c./L). All of the fish toxicity tests were conducted under flow-through conditions using dilutions of saturated solutions of the active constituent to achieve the required series of exposure concentrations up to the saturation concentration of ametoctradin in the respective dilution waters. The actual exposure concentrations of ametoctradin in each test were analytically determined and found to be acceptably stable across the 96-hour exposure period used for each test. The maximum exposure concentration of ametoctradin achieved was 0.129 mg a.c./L in the test with bluegill sunfish (*Lepomis macrochirus*).

In the acute toxicity tests with common carp, bluegill sunfish, fathead minnow, and sheepshead minnow, there were no dose-dependent mortalities and no evidence of sub-lethal effects. Hence, ametoctradin is not considered to be acutely toxic to these species of fish up to its functional water solubility limit. However, for rainbow trout on the last day of exposure, there were signs of sub-lethal effects (tottering) and 10% mortality in fish exposed to the highest test concentration. Hence, the 96-hour NOEC for sub-lethal effects of ametoctradin on rainbow trout is taken to be the next lowest test concentration (0.0366 mg a.c./L).

The chronic toxicity of ametoctradin to fish was investigated with newly fertilised eggs of fathead minnow in a standard 33-day early life stage toxicity test conducted under flow-through conditions. The only adverse treatment related effect was 100% mortality in larvae exposed to the saturation concentration of ametoctradin in the dilution water (0.124 mg a.c./L). The NOEC for fathead minnow based on day 0–7, day 7–33, and overall day 0–33 larval and fry survival is taken to be the next lowest test concentration (0.0480 mg a.c./L). No abnormal behaviour, signs of toxicity or morphological abnormalities were recorded for surviving fish. Based on these chronic lethal effects on the early life stage of fathead minnow, ametoctradin is categorised as moderately toxic to fish.

The bioconcentration potential of ametoctradin in fish was evaluated with bluegill sunfish in a standard test involving a 28-day uptake phase followed by a 16-day depuration phase. The test was conducted under flow-through conditions at two concentrations of the active constituent which were both well below the saturation concentration of the active constituent in dilution water (0.1 and 1.0 µg a.c./L). The doubly radiolabelled test substance, [2,7-^{14}C]BAS 650 F, was used both to quantify the partitioning of ametoctradin between water and fish and the partitioning of the active constituent (and its metabolites) between the edible and inedible portions of the fish. In the test, the majority of radioactive residues were found to be concentrated in the inedible portions of the fish. The residues in both the edible and inedible portions of exposed fish reached a plateau in the first 7 to 14 days of the uptake phase at both exposure concentrations. The depuration of radioactive residues from these fish was rapid and, for example, declined to less than 2% of the steady state concentration in inedible tissue after 4 days. The depuration half-life for whole fish based on total radioactive residues was in the range 0.29–0.46 days.

Ametoctradin was metabolised in bluegill sunfish by side-chain oxidation processes, which afforded polar degradants containing shortened alkyl side chains with terminal carboxylic acids functions. The parent substance was concentrated in fish in the edible tissues where it constituted 15.5–24.4% of total radioactive residues during the uptake phase at the highest test concentration. However, as ametoctradin was not detected in the inedible portions of fish, the steady state bioconcentration factor, BCF_{SS}, for the parent substance in whole fish is low (0.41 L/kg at Day 28).

The steady state bioconcentration factor and the kinetic bioconcentration factor, BCF_K, determined in this study were greater when these parameters were calculated based on total radioactive residues. However, the extensive metabolic transformation of ametoctradin to metabolites of higher polarity after uptake in

bluegill sunfish, and the rapid elimination of radioactive residues indicate that this active constituent has a low potential to bioconcentrate in fish.

The acute toxicity of the two carboxylic acid transformation products of ametoctradin, M650F03 and M650F04, were each evaluated with rainbow trout in standard limit tests (1 study each). As both transformation products are significantly more soluble in dilution water than the active constituent, these tests could be adequately conducted under static non-renewal exposure conditions. There were no mortalities or clinical signs of sub-lethal toxicity observed during the 96-hour exposure period of either test. The median lethal effect concentration for both transformation products is therefore greater than the analytically confirmed test concentration for M650F03 (82.6 mg/L) and M650F04 (95.8 mg/L). Based on these results, M650F03 and M650F04 are, at most, slightly toxic to fish.

Aquatic invertebrates

The effects of ametoctradin on aquatic invertebrates were investigated with three standard test species which include the freshwater crustacean, *Daphnia magna*, the estuarine crustacean, *Americamysis bahia*, and the marine mollusc, *Crassostrea virginica*. The effects on all three species were evaluated by means of standard test methods. One acute study was conducted for each species and one additional chronic toxicity test was conducted with *D. magna*.

The acute toxicity test with *D. magna* was conducted as a static test and no mortality/immobility was observed after 48 hours of exposure to ametoctradin at a measured saturation concentration of 0.155 mg a.c./L. Hence, ametoctradin has no acute toxic effects on *Daphnia* up to its functional water solubility limit.

Although ametoctradin does not appear to have toxic effects on *D. magna* in short-term exposures, low concentrations of this active constituent do have significant adverse effects on this species in long-term exposures. This was determined in a standard 21-day chronic reproductive toxicity test conducted under semi-static exposure conditions (renewals every 2–3 days) using a co-solvent to increase the solubility limit of ametoctradin in dilution water to *ca*. 0.8 mg a.c./L. In this test, a significant reduction in the growth of adult *Daphnia* was observed at a mean measured exposure concentration of 0.180 mg a.c./L. A significant reduction in the fecundity (offspring per living female) of *Daphnia* was recorded at and above a mean measured exposure concentration of 0.088 mg a.c./L. The 21-day chronic NOEC for ametoctradin was therefore taken to be equal to the next lowest exposure concentration (0.044 mg a.c./L), which categorises this active constituent as moderately toxic to freshwater invertebrates.

The effects of ametoctradin on the two species of marine invertebrate were investigated in standard 96-hour dose-response toxicity tests. For both tests, exposure to ametoctradin was achieved using dilutions of a saturated solution of the active constituent in natural sea water delivered to the test organisms under flow-through conditions. In the test with the mysid shrimp, *A. bahia*, no dose-dependent mortalities or sub-lethal effects were observed after 96 hours exposure to ametoctradin at a series of concentrations up to the saturation concentration of this active constituent in natural seawater (0.094 mg a.c./L). Based on this result, ametoctradin is not considered acutely toxic to marine crustaceans up to its functional solubility limit in seawater.

For the acute toxicity test with the oyster, *C. virginica*, exposure to a saturated solution of ametoctradin with a mean measured concentration of 0.097 mg a.c./L for 96 hours resulted in a statistically significant reduction

in shell growth of 36% relative to controls. The median concentration for effects of ametoctradin on *C. virginica* was therefore taken to be greater than the saturation concentration of the active constituent in natural seawater. However, toxic effects are observed in this species at below saturation concentrations (96-hour NOEC = 0.037 mg a.c./L).

The effects of four transformation products of ametoctradin, M650F01–4, on aquatic invertebrates were each evaluated with *D. magna* by standard test methods. The acute tests for all four transformation products were each 48-hour static exposures in which exposure concentrations were confirmed analytically in each case. For all transformation products, except M650F03, no immobility was observed in *Daphnia* exposed to the maximum test concentration. The median effect concentration for these three compounds is therefore greater than the respective maximum exposure concentrations. As a result, M650F01 is categorised as practically non-toxic to aquatic invertebrates whereas both M650F02 and M650F04 are, at most, slightly toxic to aquatic invertebrates. For M650F03, 35% immobility of *Daphnia* was observed at the second highest test concentration of 56 mg M650F03/L, although the 48-hour median effect concentration for M650F03 is greater than the highest measured test concentration (82.6 mg M650F03/L). On the basis of this test result, M650F03 is categorised as slightly toxic to aquatic invertebrates.

A standard 21-day chronic reproductive toxicity test of M650F03 with *D. magna* was conducted under semi-static conditions with renewals every 2–3 days. The highest test concentration (83.5 mg M650F03/L) caused 70% mortality of parent *Daphnia*, which demonstrates comparable sensitivity of adults to the lethal effects of this transformation product as those found in the acute test. The extended exposure to M650F03 also demonstrated a reduction in fecundity (cumulative number of offspring per living female) and parental growth at the highest test concentration. No adverse effects were noted at all other test concentrations. The 21-day NOEC for both survival and fecundity was taken to be the second highest concentration of M650F03 used in this test (41.8 mg M650F03/L), which categorises this metabolite as very slightly toxic to aquatic invertebrates.

Algae

The effects of ametoctradin on algae were assessed with three species of freshwater algae and one species of marine algae in standard static toxicity tests (4 studies). The exposure period for each test species was extended from the normal 72-hour exposure period to 96 hours for all of these tests, although toxicity metrics were calculated for both time intervals. The 72- and 96-hour concentrations for 10% inhibition of specific growth rate (E_rC_{10}) for the green alga (*Pseudokirchneriella subcapitata*), the cyanobacterium (*Anabaena flos-aquae*) and the marine diatom (*Skeletonema costatum*) were equal to or above the average maximum exposure concentration of the active constituent in the respective nutrient medium used for these three test species. Hence, ametoctradin is not considered to be toxic to these algal species up to its functional solubility limit in media.

For the freshwater diatom, *Navicula pelliculosa*, the 72-hour E_rC_{50} is 20.7 µg a.c./L (95% CL: 15.5–31.3 µg a.c./L) which is comparable with the maximum geometric mean exposure concentration for ametoctradin in this test of 20.3 µg a.c./L over 96 hours. At this mean exposure concentration, there was 49.8% inhibition of the growth rate of *N. pelliculosa* after 72 hours which confirms that ametoctradin has significant adverse effects on the growth of this species of algae at below saturation concentrations. Based on the observed growth rate inhibition of this freshwater diatom species, ametoctradin is categorised as very highly toxic to

algae. The 96-hour E_rC_{10} was 3.2 µg a.c./L which categorises the active constituent as highly toxic to this species of diatom in extended exposures.

The effects of M650F03 and M650F04 on algae were also evaluated by means of standard 72-hour static toxicity tests with *P. subcapitata*. In the test with M650F03, no growth inhibition of yield and no inhibition of specific growth rate were recorded at any test concentration. The 72-hour E_rC_{50} for adverse effects on this species of green alga is therefore greater than the highest nominal exposure concentration of M650F03 (82.6 mg/L), which was corrected for purity of the test substance and confirmed analytically. In the test with M650F04, there was up to 8.9% inhibition in the growth rate of *P. subcapitata* after 72 hours exposure. However, the 72-hour E_rC_{10} (and hence the E_rC_{50}) is greater than the highest nominal exposure concentration of M650F04 (95.8 mg/L), which was again confirmed analytically. Both of these transformation products of ametoctradin are categorised as, at most, slightly toxic to algae.

Aquatic plants

The acute toxicity of ametoctradin to aquatic plants was investigated with the duckweed species, *Lemna gibba*, in a standard 7-day laboratory test. This test was conducted under semi-static conditions (1 renewal at day 3) with saturated solutions of ametoctradin diluted in standard nutrient solution to achieve a series of five exposure concentrations. The actual exposure concentrations were analytically determined and used to calculate the time-weighted mean exposure concentrations for ametoctradin over 7 days after one renewal of the test medium. The median concentration for effects on growth rate (E_rC_{50}) of duckweed after 7 days exposure to ametoctradin was calculated to be greater than the highest time-weighted mean exposure concentration (0.211 mg a.c./L). No morphological effects on duckweed were observed at any of the concentrations tested. On the basis of this result, ametoctradin is not considered to be toxic to duckweed up to its functional solubility limit in media.

Effects on Bees

The acute toxicity of ametoctradin to the honey bee by oral and contact exposure routes was investigated in standard dose-response toxicity tests. No sub-lethal effects were observed at any dose level in the oral or contact toxicity test, except that in the contact test a few bees were apathetic or vomiting in those treatment groups where mortality occurred. The mortality observed in the contact study was not dose dependent and the median lethal dose for both exposure pathways is greater than the highest dose (>100 µg a.c./bee). Ametoctradin is therefore categorised as very slightly toxic to bees.

Effects on other Arthropod Species

The lethal and sub-lethal effects of ametoctradin (as a 200 g a.c./L SC formulation: BAS 650 00 F) on the three arthropod test species (*Aphidius rhopalosiphi*, *Typhlodromus pyri*, and *Chrysoperlea carnea*) were evaluated in standard laboratory tests with fresh dried residues of this formulation on treated glass plates (3 studies). The contact test with the predatory mite, *T. pyri*, revealed no statistically significant effect on mortality up to the highest nominal equivalent field application rate of ametoctradin (1843 g a.c./ha). For the green lacewing, *C. carnea*, no treatment related mortality and no effects on reproduction (fecundity and fertility) occurred up to the highest nominal field application rate (736 g a.c./ha). However, for the parasitic wasp, *A. rhopalosiphi*, a dose dependent effect on mortality was observed with more than 70% mortality

occurring after 48 hours exposure at the highest field application rate of 1843 g a.c./ha. The 48-hour median lethal application rate (LR_{50}) determined for *A. rhopalosiphi* is 234 g a.c./ha.

The lethal and sub-lethal effects of ametoctradin on *A. rhopalosiphi* were further investigated in an extended laboratory study involving dried residues of the end-use product on barley seedlings. In this test, no repellence of adult wasps from sprayed seedlings was observed and no statistically significant effect on mortality of adults was observed after 48 hours exposure to the dried residues. The 48-hour LR_{50} for adult wasps in this test was therefore taken to be greater than the highest tested field application rate (1962 g a.c./ha). There was a significant effect on fecundity (number of mummies per female) of adult wasps exposed to the two highest application rates. The NOEC for reproductive effects was therefore taken to be the next highest application rate (491 g a.c./ha). The ER_{50} for sub-lethal effects on this species is greater than the highest application rate.

The effects of ametoctradin (as BAS 650 00 F) on *T. pyri* population density in vineyards were evaluated in six separate field studies conducted in Germany and France to assess the short and long term effects of this active constituent (and end-use product) on predatory mites in vineyards. Four applications of 491 g a.c./ha were made at 10–15 day intervals. Nearly all mites identified at all six trial sites were *T. pyri*. Relative to untreated control areas, no adverse effects on predatory mite populations were observed in five of the six vineyards studied in Southern Germany and France after 4 applications (April–July) of 491 g ametoctradin/ha. In one vineyard in Southern France, slight and transient effects were seen, with a significant reduction in mite populations (24.2% reduction) nine days after the first application only. However, no significant differences between treated and control population levels occurred thereafter.

Effects on Earthworms

The acute toxicity of ametoctradin to earthworms was investigated in a standard 14-day dose-response test. The test was conducted on artificial soil with reduced organic content (5%). This acceptable procedural modification was employed to limit the possibility that strong adsorption of this hydrophobic active constituent on soil organic carbon would reduce the bioavailability of ametoctradin to earthworms. Under the conditions of this test, there was no more than one mortality (in 40 test subjects) at each test concentration and the median lethal effect concentration for ametoctradin was therefore greater than the highest test concentration (1000 mg a.c./kg soil d.w.). On the basis of this single acute toxicity test, ametoctradin is categorised as very slightly toxic to earthworms.

The acute and chronic toxicity of ametoctradin (as BAS 650 00 F) to earthworms was determined in standard 14-day and 56-day exposure tests (1 study for each end-point). As for the tests with the active constituent alone, the effects of the formulation of ametoctradin on earthworms were also conducted on artificial soil with reduced organic matter. In short term toxicity tests, there was no statistically significant mortality in earthworms exposed up to the maximum concentration tested (182 mg a.c./kg soil d.w.). In the chronic toxicity test, no statistically significant mortality or adverse effects on fecundity were found up to the highest test concentration (21 mg a.c./kg soil d.w.).

The acute toxicity of three carboxylic acid transformation products of ametoctradin (M650F01, M650F03, and M650F04) to earthworms was investigated in standard 14-day dose-response tests (1 study each). The short term toxicity tests on earthworms with these test substances were conducted in artificial soil with organic matter content of 10% because these relatively more polar substances are less likely to absorb to soil

organic carbon. For each test substance, the median lethal effect concentration exceeded the highest test concentration. In the case of M650F01, the highest test concentration was 817 mg/kg soil d.w. which indicates that this transformation product may be categorised as, at most, slightly toxic to earthworms. The highest test concentration for both M650F03 and M650F04 was 1000 mg/kg soil d.w. and both transformation products are therefore categorised as very slightly toxic to earthworms.

The potential sub-lethal effects of M650F03 and M650F04 on earthworms were evaluated in standard 56-day tests (1 study each). The tests were conducted on artificial soils with 10% organic matter content. For M650F03, no significant mortality or effects on fecundity (mean number of juveniles) of earthworms were observed up to the highest test concentration (83.5 mg M650F03/kg soil d.w.). Similarly for M650F04, no significant mortality or effects on fecundity were observed up to the highest test concentration which in this case was 95.8 mg M650F04/kg soil d.w.

Effects on other Soil Non-Target Macro-organisms

The chronic effects on soil arthropods of ametoctradin (as BAS 650 00 F), as well as the soil transformation products, M650F03 and M650F04, were each assessed in single studies with the parthenogenetic species of Collembola (springtails), *Folsomia candida*. The chronic effects of the two ametoctradin transformation products on the predatory soil mite species, *Hypoaspis aculeifer*, were also investigated. Survival and reproduction were assessed over 28 days for springtails and 14 days for soil mites.

The effects of ametoctradin on the reproductive output of the important soil detritivore species, *F. candida*, were conducted on artificial soil with reduced organic matter (5%). In this soil medium, there were no statistically significant effects on reproductive output (mean number of juveniles per replicate) or mortality of adult springtails following 28 days exposure at any nominal test concentration. Hence, the 28-day NOEC for chronic effects of ametoctradin on this species is taken to be equal to the maximum soil concentration tested (194.8 mg a.c./kg soil d.w.).

The effects of the two polar soil transformation products of ametoctradin on the reproductive output of *F. candida* were evaluated on artificial soil with 10% organic matter. For the test with M650F04, no statistically significant effects on reproductive output or mortality of adult springtails were observed up to the maximum nominal soil test concentration of 95.8 mg M650F04/kg soil d.w. However, survival and reproduction in springtails were significantly reduced following 28 days of exposure to the maximum nominal test concentration of M650F03 (100 mg/kg soil d.w). This maximum test concentration was taken to be the 28-day LOEC for chronic adverse effects on *F. candida* and the next highest test concentration (50 mg M650F03/kg soil d.w.) was taken to be the NOEC.

The effects of M650F03 and M650F04 on the reproductive output of *H. aculeifer* were conducted on artificial soil with 5% organic matter content which is the recommended level for this test species. In this soil matrix, there were no significant effects on the reproductive output (mean number of juveniles per replicate) or survival of adult mites after 14 days exposure to either transformation product up to the highest concentrations tested (100 mg M650F03/kg soil d.w. and 95.8 mg M650F04/kg soil d.w.). There were also no changes or abnormalities in the behaviour or the morphology of the mites observed in either test. Hence, the 14-day NOEC for the chronic effects of both transformation products on *H. aculeifer* was taken to be the maximum nominal soil concentration tested for each compound.

Effects on other Non-target Organisms (Flora and Fauna) believed to be at Risk

Soil micro-flora

The effects on microbially-mediated nitrogen and carbon mineralization processes in aerobic soils of ametoctradin (as BAS 650 00 F), and two of the more persistent transformation products of ametoctradin in soil, M650F03 and M650F04, were each evaluated by standard laboratory test methods. These tests were conducted over 28 days on homogenised sandy loam soils taken from the top 20 cm of agricultural fields that had not received organic or inorganic fertilizer for at least the previous 4 years.

Under the conditions of these tests, ametoctradin had no significant effect on nitrogen or carbon mineralization processes in soil at either of the two nominal soil concentrations tested (0.38 and 3.84 mg a.c./kg soil d.w.). Hence, the active constituent is assessed as having no long-term influence on carbon or nitrogen transformation in soil.

The effects of the two transformation products on nitrogen and carbon mineralization in soil were both evaluated in four separate studies (8 studies overall). No significant effects were observed on nitrogen or carbon mineralization processes in soil after 28 days exposure to maximum levels of M650F03 (6.70 mg/kg soil d.w.) or M650F04 (13.4 mg/kg soil d.w.) in any of these tests. Hence, both transformation products are assessed as having no long-term influence on carbon or nitrogen transformation in soil.

Terrestrial plants

The toxicity of ametoctradin (as BAS 650 00 F) to terrestrial plants was determined by means of a single standard 21-day vegetative vigour assay and a single seedling emergence assay conducted on a range of monocot and dicot crop species. These tests were conducted as limit tests in which emerged seedlings (vegetative vigour test) or seeds planted one day before treatment (seedling emergence test) were exposed to a single application of a prepared spray of this formulation of ametoctradin at an effective application rate of 570 g a.c./ha. No significant effects on growth end-points were found for any plant species in either the vegetative vigour or seedling emergence assays. There were also no signs of phytotoxicity in either assay. The effective application rate for ametoctradin used in these limit tests was therefore taken to be the 21-day NOER for vegetative vigour and seedling emergence.

The formulation of ametoctradin used in these assays is expected to have contained the phytotoxic impurity, amitrole. However, based on the specifications for technical ametoctradin, the maximum effective application rate for this impurity would have been <0.1 g amitrole/ha, which is well below the application rate at which phytotoxic effects from this substance would be expected to occur.

Effects on Biological Methods of Sewage Treatment

The effects of ametoctradin on sewage sludge micro-organisms were evaluated in a single standard 3-hour microbial respiration inhibition test. This test established that ametoctradin has no inhibitory effects on aerobic micro-organisms from a municipal waste water plant after 3 hours incubation with the highest of three nominal test concentrations (1000 mg a.c./L). On the basis of this study, ametoctradin is not considered to pose a significant hazard to aerobic sewage sludge micro-organisms.

7.3 Environmental Risk Assessment

Zampro® Fungicide will be applied up to four times per season to grapevines by airblast sprayer over a minimum interval of 28 days. This use pattern does have the potential to result in exposure to the environment of the product and the two active constituents (ametoctradin and dimethomorph). The main routes of environmental exposure are likely to involve spray drift during airblast spraying of the product diluted in water or through transport of the two active constituents in run-off water and/or eroded soil following a high rainfall event. The two active constituents both have a low potential for transport in the vapour phase and wider exposure to the environment through atmospheric transport is therefore not expected to be a significant factor in the environmental risk profile of this product.

The risk assessments for various non-target organisms that may be exposed to ametoctradin present in combination with dimethomorph in the product, Zampro® Fungicide, as a result of spray drift or run-off have shown that there are no unacceptable risks of adverse effects on any non-target organisms, except aquatic organisms.

An assessment of the possible effects resulting from direct overspray of a water body with the product revealed an unacceptable risk of adverse effects on aquatic life based on the toxicity of ametoctradin to *N. pelliculosa*. Hence, direct overspray of a water body with Zampro® Fungicide must be avoided.

The results of a standard spray drift risk assessment for application of Zampro® Fungicide to grapevines by airblast sprayer demonstrated that a Downwind No-Spray Zone of 10 metres is required to protect the aquatic environment. There are no unacceptable risks to aquatic organisms from run-off of ametoctradin bound to eroded soil and dimethomorph dissolved in water based on the calculated concentrations of the two active constituents at the edge of a vineyard.

8 EFFICACY AND SAFETY ASSESSMENT

The applicant BASF Australia Ltd, seeks registration of the proposed new product Zampro® Fungicide, a suspension concentrate product containing 300g/L ametoctradin and 225 g/L dimethomorph, for control of downy mildew (*Plasmopara viticola*) of grapevines.

8.1 Proposed use pattern

Zampro® Fungicide is intended to be used as a protectant fungicide against downy mildew infection in grapevines. The product is to be applied at a rate of 80mL product/100L water (dilute application rate), or by concentrate methods at concentration factors not greater than 5X, at 7-10 day intervals when humid or wet conditions favour infection, but before disease is evident. Use is proposed in all Australian States and territories.

8.2 Summary of Evaluation of Efficacy and Crop Safety

Zampro® Fungicide is a suspension concentrate containing 300g/L ametoctradin and an existing registered active constituent dimethomorph (225 g/L). The product is intended for preventative use for the control of downy mildew (*Plasmopara viticola*) of grapevines. Zampro® Fungicide is intended to be used at a rate of 80 mL product/100 L water (dilute application rate), or by concentrate application methods in grapevines.

Ametoctradin is a new active constituent to the Australian market. It is a fungicide which belongs to the triazolopyrimidylamines chemical group and represents the first commercial development from this group. The Fungicidal Mode of Action has not been fully characterised however ametoctradin is a strong inhibitor of mitochondrial respiration in complex III (cytochrome bc1) of fungi belonging to the Class of Oomycetes. The exact binding site at complex III is not yet known. Ametoctradin is in the new Group 45 for fungicides resistance management. The existing active constituent dimethomorph is known to inhibit phospholipid biosynthesis and interfere with normal cell wall deposition, resulting in cell wall lysis and subsequent death of the fungal cell. Dimethomorph is in Group 40 (carboxylic acid amides) for fungicides resistance management. The new combination of active constituents will offer an additional resistance management option to growers currently using dimethomorph in grapevines.

A total of nine trials on grapevines were conducted in commercial vineyards over five years in three Australian States. Three grape cultivars were tested and in the trials, efficacy of either ametoctradin alone or Zampro® Fungicide as a downy mildew protectant fungicide, was compared with a total of three currently registered appropriate industry standards. The mildew infections present were either naturally occurring or resulting from inoculation. Where artificial inoculation was used it was either by spraying spores or attaching infected leaves, sourced elsewhere, to leaves in buffer rows. All trials were randomized complete blocks with 3-4 replicates, untreated plots, and plots treated with one or two industry standards. Efficacy was determined by assessing the percentage infected leaves and also the severity of oilspots or sporulating tissue on these at several observation dates. There were 2-10 applications of fungicides. All results were statistically analysed using ANOVA.

Ametoctradin in all three sets where it was evaluated gave control equivalent or superior to industry standards. Zampro (dimethomorph + ametoctradin) was compared with dimethomorph alone, and shown to give greater control in all data sets where it was tested. Similarly, Zampro in all data sets where it was evaluated gave control levels equivalent to or greater than that demonstrated by the industry standards. It is concluded that the efficacy of the new active constituent ametoctradin, and the new product, Zampro ® Fungicide, are adequately demonstrated under appropriate commercial conditions.

Phytotoxicity was monitored in all trials and no symptoms seen when Zampro was applied at above label rate and ametoctradin at four times label rate in up to 10 applications on three grape cultivars.

Label wording is appropriate including a recommendation to use the shorter application interval of 7 days when conditions favouring infection are creating a high risk. There is adequate warning that the product is a protectant and to use alternative products if disease is already established.

Assessment of study/trial data

Ametoctradin efficacy

Ametoctradin was used in two trials and in the three data sets extracted from them it gave statistically significant control of infection. Furthermore the control levels were equivalent or superior to that by industry standard fungicides.

There is also indirect evidence of the efficacy of ametoctradin obtained by comparing the efficacy of dimethomorph with that of Zampro® Fungicide (i.e. dimethomorph + ametoctradin) - thus any advantage of Zampro® Fungicide over dimethomorph could be attributed to the contribution by ametoctradin.

From four trials the eight representative data sets extracted show that dimethothorp + ametoctradin gave statistically superior control in four sets and was superior, though not statistically significant, in an additional four data sets to control achieved by dimethomorph alone.

Zampro efficacy

In the 15 data sets extracted from seven trials Zampro® Fungicide gave significant control of infection in all sets. Furthermore, control levels were equivalent or superior to control by industry standards.

Results in the grapevines trials indicate that when Zampro® Fungicide is applied as directed it is expected to provide equivalent or superior control of downy mildew compared with the three currently registered industry standards.

Crop safety

Observations were made for phytotoxicity in all nine trials where Zampro® Fungicide was applied at above label rate or ametoctradin at four times label rate in up to ten sequential sprays. The grape varieties trialled were Shiraz, Cabernet Sauvignon and Chardonnay, the three major wine grape varieties in Australia. No phytotoxicity symptoms were noted on any variety with any dose rate.

Resistance management

The new active constituent ametoctradin has been included in the new Group 45 (triazolopyrimidylamines) for fungicides resistance management. The Fungicidal Mode of Action has not been fully characterised however ametoctradin is a strong inhibitor of mitochondrial respiration in complex III (cytochrome bc1) of fungi belonging to the Class of Oomycetes. The exact binding site at complex III is not yet known. The existing active constituent dimethomorph is known to inhibit phospholipid biosynthesis and interfere with normal cell wall deposition, resulting in cell wall lysis and subsequent death of the fungal cell. Dimethomorph is in Group 40 (carboxylic acid amides) for fungicides resistance management. The new combination of active constituents will offer an additional resistance management option to growers currently using dimethomorph in grapevines. The label for Zampro® Fungicide, a Group 45 40 Fungicide, warns to apply a maximum of two consecutive sprays before changing to an alternative mode of action for at least one application, with a maximum number of applications of Zampro® Fungicide of four per season.

8.3 Conclusion

The claims on the proposed product label that the product Zampro® Fungicide provides acceptable control of downy mildew of grapevines when used as directed, are supported by the results from the Australian trials. Acceptable crop safety is also to be expected when the product is used as directed. The Directions for Use are appropriate and consistent with fungicide use in commercial agricultural production in this crop in Australia. The label Restraints, Spray Drift Restraints, Critical Comments, General Instructions, Withholding Period and Export Warning statements, Fungicides Resistance statements and Application Instructions are consistent with Australian Good Agricultural Practice (GAP) and other risk assessments conducted for the product.

The application by BASF Australia Ltd for the registration of Zampro® Fungicide is supported on efficacy and crop safety grounds when used in accordance with the proposed label instructions and Good Agricultural Practice (GAP).

9 LABELLING REQUIREMENTS

CAUTION

KEEP OUT OF REACH OF CHILDREN

READ SAFETY DIRECTIONS BEFORE OPENING OR USING

ZAMPRO® FUNGICIDE

ACTIVE CONSTITUENT: 300 g/L AMETOCTRADIN
225 g/L DIMETHOMORPH

GROUP **45 40** FUNGICIDE

For the control of downy mildew in grapes as specified in the Directions for Use Table.

CONTENTS: 1 – 20 L

BASF Australia Ltd ABN 62 008 437 867
Level 12, 28 Freshwater Place Southbank VICTORIA 3006
Customer Service Hotline: 1800 006 393

APVMA Approval No.:63651/XXXXX

STORAGE AND DISPOSAL
Store in the closed, original container in a cool, well-ventilated area. DO NOT store for prolonged periods in direct sunlight. Triple rinse containers before disposal. Add rinsings to the spray tank. D0 NOT dispose of undiluted chemicals on-site. If not recycling, break, crush or puncture and deliver empty packaging to an approved waste management facility. DO NOT burn empty containers or product.

SAFETY DIRECTIONS
Harmful if swallowed.

FIRST AID
If poisoning occurs, contact a doctor or Poisons Information Centre. Phone Australia 131126.

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

CONDITIONS OF SALE

All conditions and warranties rights and remedies implied by law or arising in contract or tort whether due to the negligence of BASF Australia Ltd or otherwise are hereby expressly excluded so far as the same may legally be done provided however that any rights of the Buyer pursuant to non- excludable conditions or warranties of the Trade Practices Act 1974 or any relevant legislation of any State are expressly preserved but the liability of BASF Australia Ltd or any intermediate Seller pursuant thereto shall be limited if so permitted by the said legislation to the replacement of the goods sold or the supply of equivalent goods and all liability for indirect or consequential loss or damage of whatsoever nature is expressly excluded. This product must be used or applied strictly in accordance with the instructions appearing hereon. This product is solely sold for use in Australia and must not be exported without the prior written consent of BASF Australia Ltd.

APVMA Approval No: 63651/XXXXX

Batch No:
Date of Manufacture:

BASF Australia Ltd
ABN 62 008 437 867
Level 12, 28 Freshwater Place
Southbank VICTORIA 3006

FOR SPECIALIST ADVICE IN AN EMERGENCY ONLY PHONE 1800 803 440 TOLL FREE-ALL HOURS-AUSTRALIA WIDE

CAUTION

KEEP OUT OF REACH OF CHILDREN

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ZAMPRO® FUNGICIDE

ACTIVE CONSTITUENT: 300 g/L AMETOCTRADIN
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CONTENTS: 1 – 20 L

BASF Australia Ltd ABN 62 008 437 867
Level 12, 28 Freshwater Place Southbank VICTORIA 3006

APVMA Approval No.:63651/XXXXX

DIRECTIONS FOR USE

RESTRAINTS

DO NOT apply with aircraft.

SPRAY DRIFT RESTRAINTS

Except when applying with orchard airblast equipment, DO NOT apply with spray droplets smaller than a MEDIUM spray droplet size category according to nozzle manufacturer specifications that refer to the ASAE S572 Standard or the BCPC Guideline.

DO NOT apply when wind speed is less than 3 or more than 20 kilometres per hour as measured at the application site.

DO NOT apply during surface temperature inversion conditions at the application site.

Users of this product MUST make an accurate written record of the details of each spray application within 24 hours following application and KEEP this record for a minimum of 2 years. The spray application details that must be recorded are: 1) date with start and finish times of application; 2) location address and paddock/s sprayed; 3) full name of this product; 4) amount of product used per hectare and number of hectares applied to; 5) crop/situation and weed/pest; 6) wind speed and direction during application; 7) air temperature and relative humidity during application; 8) nozzle brand, type, spray angle, nozzle capacity and spray system pressure measured during application; 9) name and address of person applying this product. (Additional record details may be required by the state or territory where this product is used.)

MANDATORY NO-SPRAY ZONES

DO NOT apply if there are aquatic and wetland areas including aquacultural ponds within **10 metres** downwind from the application area.

SITUATION	DISEASE	RATE	WHP	CRITICAL COMMENTS
Grapevines	Downy mildew (*Plasmopara viticola*)	**Dilute spraying** 80 mL/ 100 L **Concentrate spraying** Refer to the application section	4 weeks	DO NOT use in crops intended for drying. Also see CAUTION section re export commodities. Apply at 7 to 14 day intervals when humid or wet conditions favour infection but before disease is evident. Use the shorter interval when conditions favouring infection are creating a high risk. Apply a maximum of 2 consecutive sprays before changing to an alternative mode of action for at least one application. ZAMPRO should be used solely as a protectant fungicide. Products containing metalaxyl or metalaxyl-M (methoxam) are recommended if downy mildew infection may have already occurred. Apply using dilute or concentrate spraying equipment. Apply the same total amount of product to the target crop whether applying this product by dilute or concentrate spraying methods. DO NOT use in equipment that requires greater than 400 mL/ 100 L (5X). DO NOT apply more than 4 sprays of ZAMPRO per season, as a precaution against the development of disease resistance.

ABBREVIATIONS

ac	active constituent
ADI	Acceptable Daily Intake (for humans)
AHMAC	Australian Health Ministers Advisory Council
AC/ac	Active constituent
ACCS	Advisory Committee on Chemicals Scheduling
ANOVA	Analysis of Variance
ai	active ingredient
AR	Administered Radioactivity
ARfD	Acute Reference Dose
BBA	Biologische Bundesanalstalt fur Land – und forstwirschaft
BCF_K	Kinetic Bioconcentration Factor
BCF_{SS}	Steady State Bioconcentration Factor
bw	bodyweight
^oC	Degrees Centigrade
^{14}C	Carbon-14
CEC	Cation Exchange Capacity
d	day
DALT	Days after Last Treatment
DAT	Days after Treatment
DFOP	Double-First-Order in Parallel
DM	Dry Matter
DNA	Deoxyribonucleic acid
DSEWPaC	Department of Sustainability Environment Water Population and Communities
DT_{50}	Time taken for 50% of the concentration to dissipate
E_bC_{50}	concentration at which the biomass of 50% of the test population is impacted
EC_{50}	concentration at which 50% of the test population are immobilised
EEC	Estimated Environmental Concentration

E_rC_{50}	concentration at which the rate of growth of 50% of the test population is impacted
EI	Export Interval
EGI	Export Grazing Interval
ESI	Export Slaughter Interval
EUP	End Use Product
F_o	original parent generation
FOMC	First-order Multi-compartment
g	gram
GAP	Good Agricultural Practice
GCP	Good Clinical Practice
GI	Gastro-intestinal
GJR	Global Joint Review
GLP	Good Laboratory Practice
GS	Growth Stage
GVP	Good Veterinary Practice
h	hour
ha	hectare
Hct	Heamatocrit
Hg	Haemoglobin
HPLC	High Pressure Liquid Chromatography *or* High Performance Liquid Chromatography
HPLC-ESI-MS/MS	HPLC-Electrospray Ionisation-Mass Spectrometry
HR	Highest Residue
HR-P	HR-Processing
id	intradermal
im	intramuscular
ip	intraperitoneal
IPM	Integrated Pest Management
iv	intravenous
in vitro	outside the living body and in an artificial environment

in vivo	inside the living body of a plant or animal
K	Kelvin
kg	kilogram
K_{Foc}	Freundlich adsorption coefficient normalised for organic carbon content
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
LLNA	Local Lymph Node Assay
LOAEL	Lowest Observable Adverse Effect Level
LOD	Limit of Detection – level at which residues can be detected
$Log_{10}PoW$	Partition Co-efficient Octanol/Water
LOQ	Limit of Quantitation – level at which residues can be quantified
LR_{50}	Median Lethal Application Rate
LSC	Liquid Scintillation Counting
mg	milligram
mL	millilitre
MRL	Maximum Residue Limit
MSDS	Material Safety Data Sheet
MWHC	Maximum Water Holding Capacity
NDPSC	National Drugs and Poisons Schedule Committee
NEDI	National Estimated Daily Intake
NESTI	National Estimated Short Term Intake
ng	nanogram
NHMRC	National Health and Medical Research Council
NOAEL	No Observable Adverse Effect Level
NOEC/NOEL	No Observable Effect Concentration/Level
OC	Organic Carbon
OCS	Office of Chemical Safety in the Department of Health and Ageing

OM	Organic Matter
PES	Post Extraction Solids
po	oral
ppb	parts per billion
PPE	Personal Protective Equipment
ppm	parts per million
PRS	Public Release Summary
Q-value	Quotient-value
RAC	Raw Agricultural Commodity
RBC	Red Blood Cell Count
RSD	Relative Standard Deviations
s	second
SFO	single-first-order
sc	subcutaneous
SC	Suspension Concentrate
STMR	Supervised Trial Median Residue
STMR-P	STMR-Processing
SUSMP	Standard for the Uniform Scheduling of Medicines and Poisons
TGA	Therapeutic Goods Administration
TGAC	Technical grade active constituent
TRR	Total Radioactive Residues
T-Value	A value used to determine the First Aid Instructions for chemical products that contain two or more poisons
µg	microgram
UV	Ultra-violet
vmd	volume median diameter
WG	Water Dispersible Granule
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 a material from or through 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	repels water
Leaching	Removal of a compound by use of a solvent
Log Pow	Log to base 10 of octanol water partitioning co-efficient, synonym KOW
Metabolism	The chemical processes that maintain living organisms
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

REFERENCES

Australian Pesticides and Veterinary Medicines Authority 2008, *Ag MORAG: Manual of Requirements and Guidelines*, APVMA, Canberra.

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