Public Release Summary
on

Evaluation of the new active

ACIBENZOLAR-S-METHYL

in the product

BION PLANT ACTIVATOR SEED TREATMENT

Australian Pesticides and Veterinary Medicines Authority

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FOREWORD

The Australian Pesticides and Veterinary Medicines Authority (APVMA) is an independent statutory authority 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), Department of Environment and Water Resources (Risk Assessment and Policy Section) and State departments of primary industries and environment.

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 all products containing new active ingredients and for all proposed extensions of use for existing products.

The information and technical data required by the APVMA to assess the safety of new chemical products and the methods of assessment must be undertaken according to accepted scientific principles. Details are outlined in the APVMA's publication *The Manual of Requirements and Guidelines - MORAG for Agricultural and Veterinary Chemicals [AgMORAG & Vet MORAG]*.

This Public Release Summary is intended as a brief overview of the assessment that has been completed by the APVMA and 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.

More detailed technical assessment reports on all aspects of the evaluation of this chemical can be obtained by completing the order form in the back of this publication and submitting with payment to the APVMA. Alternatively, the reports can be viewed at the APVMA Library, 18 Wormald St, Symonston, ACT 2609.

The APVMA welcomes comment on the usefulness of this publication and suggestions for further improvement. Comments should be submitted to the Program Manager, Pesticides Program, Australian Pesticides and Veterinary Medicines Authority, PO Box E240, Kingston ACT 2604.

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CONTENTS

LIST OF ABBREVIATIONS AND ACRONYMS

AC	active constituent
ACR	Acute to chronic ratio
ADI	Acceptable Daily Intake (for humans)
AHMAC	Australian Health Ministers Advisory Council
ai	active ingredient
ARfD	Acute Reference Dose (for humans)
BBA	Biologische Bundesanalstalt fur Land – und forstwirschaft
bw	bodyweight
CRP	Chemistry and Residues Program
d	day
DAT	Days After Treatment
DM	Dry Matter
DT_{50}	Time taken for 50% of the concentration to dissipate
DT_{90}	Time taken for 90% of the concentration to dissipate
EA	Environment Australia
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
EC	Emulsifiable Concentrate
EEC	Estimated Environmental Concentration
E_rC_{50}	concentration at which the rate of growth of 50% of the test population is impacted
ESI	Export Slaughter Interval
EUP	End Use Product
FAO	Food and Agriculture Organisation of the United Nations
Fo	original parent generation
FW	Fresh Weight
g	gram
GAP	Good Agricultural Practice
GC/MS	gas chromatography/mass spectroscopy
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
GVP	Good Veterinary Practice
h	hour
ha	hectare
Hct	Heamatocrit
HDPE	High-density polyethylene
Hg	Haemoglobin
HPLC	High Pressure Liquid Chromatography *or* High Performance Liquid Chromatography
HPLC-UV	High Performance Liquid Chromatography with Ultra-Violet Detector
HR	Highest Residue
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
JMPR	Joint FAO/WHO Meeting on Pesticide Residues
kg	kilogram
K_{oc}	Organic carbon partitioning coefficient
L	Litre
LC_{50}	concentration that kills 50% of the test population of organisms
LD_{50}	dosage of chemical that kills 50% of the test population of organisms
LC-MS/MS	liquid chromatography, mass spectroscopy

LOEC	Lowest Observable Effect Concentration
LOEL	Lowest Observable Effect Level
LOD	Limit of Detection – level at which residues can be detected
LOQ	Limit of Quantitation – level at which residues can be dquantified
mg	milligram
mL	millilitre
MRL	Maximum Residue Limit
MSDS	Material Safety Data Sheet
MSMS	mass spectroscopy/mass spectroscopy
NOAEC	No Observable Adverse Effect Concentration
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
NOEC/NOEL	No Observable Effect Concentration/Level
OC	Organic Carbon
OM	Organic Matter
PHED	Pesticide Handlers Exposure Database
PHI	Pre-harvest interval
po	oral
POEM	Predictive Operator Exposure Model (UK)
ppb	parts per billion
PPE	Personal Protective Equipment
ppm	parts per million
Q-value	Quotient-value
RBC	Red Blood Cell Count
s	second
sc	subcutaneous
SC	Suspension Concentrate
STMR	Supervised Trials Median Residue
SUSDP	Standard for the Uniform Scheduling of Drugs and Poisons
TGA	Therapeutic Goods Administration
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
vmd	volume median diameter
WG	Water Dispersible Granule
WHO	World Health Organisation
WHP	Withholding Period

INTRODUCTION

This publication provides a summary of the data reviewed and an outline of the regulatory considerations for the proposed registration of *BION PLANT ACTIVATOR SEED TREATMENT,* which contains the new active constituent acibenzolar-S-methyl. The product is proposed to be used for the protection of cotton in supressing fusarium wilt *(Fusarium oxysporum* f.sp. *vasinfectum)* and black root rot *(Thielaviopsis basicola)*, by activating the plant's natural resistance mechanisms, when applied as a seed treatment and used as a component of an integrated management strategy.

Responses to this Public Release Summary will be considered prior to registration of the product. They 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.

Copies of full technical evaluation reports on acibenzolar-S-methyl, covering toxicology, occupational health and safety aspects, residues in food and environmental aspects are available from the APVMA on request (see order form on last page). They can also be viewed at the APVMA library located at the APVMA offices, 18 Wormald St, Symonston, ACT 2609.

Written comments should be received by the APVMA by 1 May 2007. They should be addressed to:

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Applicant

Syngenta Crop Protection Pty Limited

Product Details

It is proposed to register *BION PLANT ACTIVATOR SEED TREATMENT,* containing acibenzolar-S-methyl at 500 g/L as a suspension concentrate formulation. *BION PLANT ACTIVATOR SEED TREATMENT* will be imported fully formulated and packaged in 1L, 2L or 5L containers.

BION PLANT ACTIVATOR SEED TREATMENT is a new seed treatment which stimulates the plant's natural defence mechanisms by inducing Systemic Acquired Resistance (SAR) in plants. An activity group for resistance management has not yet been assigned to acibenzolar-S-methyl. Acibenzolar-S-methyl has no fungicidal properties and its mode of action is via activation of the plant's natural defence pathways resulting in acquired resistance in treated and systemic tissues of the plant. The full mode of action of the plant's natural defence pathways is still being examined and determined as discussed in the international literature.

Application of *BION PLANT ACTIVATOR SEED TREATMENT* is via a seed treatment to protect cotton by supressing fusarium wilt *(Fusarium oxysporum* f.sp. *vasinfectum)* and black

root rot *(Thielaviopsis basicola)* when used as a component of an integrated management strategy.

Overseas registrations: Acibenzolar-S-methyl formulations are currently registered in many overseas countries including Europe and the Americas however none pertain to cotton or seed treatment application.

CHEMISTRY AND MANUFACTURE

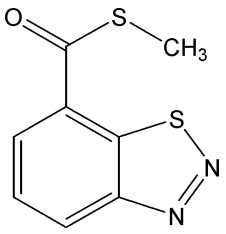
Active Constituent

The active constituent acibenzolar-*S*-methyl is manufactured in the UK by Syngenta Grimsby Limited, Pyewipe, Grimsby, North East Lincolnshire DN31 2SR, England and is approved by the APVMA (Approval Number: 54187).

The chemical active constituent has the following properties:

Common Name:	Acibenzolar-S-methyl
Chemical Name	
(IUPAC):	*S*-Methyl benzo[1,2,3]thiadiazole-7-carbothioate
(CA):	1,2,3-Benzothiadiazole-7-carbothioic acid *S*-methyl ester
CAS Registry Number:	135158-54-2
Empirical Formula:	$C_8H_6N_2OS_2$
Molecular Weight:	210.28
Physical state:#	Fine powder
Colour:#	Beige
Odour:#	Weak, burnt like
Melting Point:*	132.9°C
Density:*	1.54 g/cm^3 at 22°C
Octanol/water partition coefficient (log K_{OW}):*	3.1 at 25°C
Vapour pressure:*	2.2×10^{-4} Pa at 20 °C (extrapolated) 4.6×10^{-4} Pa at 25 °C (extrapolated)

Chemical Structure:



= technical material (97.9% w/w)
* = purified analytical standard (99.6% w/w)

Other characteristics of acibenzolar-S-methyl (toxicology, metabolism & kinetics and environmental fate) are covered in subsequent sections of this Public Release Summary.

Formulated Product

Distinguishing name:	Bion® Plant Activator Seed Treatment
Formulation type:	Suspension concentrate for seed treatment (FS)
Active constituent concentration:	500g/L acibenzolar-S-methyl

Physical and Chemical Properties of the Product

Physical state:	Liquid
Colour:	Yellow to brown
Odour:	Mouldy chalky
Acidity, alkalinity or pH value:	7.1 (1% in deionised water)
Density (at 20^{o}C):	1.212 g/cm^{3}
Flash point:	>100^{o}C
Auto ignition:	295^{o}C
Explosive properties:	Not explosive
Storage stability:	Stability data provided by the applicant supports a storage life of 2 years when stored under normal conditions in high density polyethylene containers.

Summary of the APVMA's Evaluation of Bion Plant Activator Seed Treatment

The Chemistry and Residues Program (CRP) has evaluated the chemistry aspects of Bion® Plant Activator Seed Treatment (manufacturing process, quality control procedures, batch analysis results, analytical methods, storage stability, and specifications for containers for the product) and found them to be acceptable.

TOXICOLOGICAL ASSESSMENT

Evaluation of Toxicology

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

Toxicokinetics and Metabolism

In rats, radio-labelled acibenzolar-S-methyl was rapidly absorbed after oral dosing, with peak blood levels reached at 15 to 30 minutes (0.5 mg/kg bw) or 4 to 8 h (100 mg/kg bw). At least 90% of an oral dose was absorbed. Blood and tissue levels rapidly declined, with highest levels in the liver and kidneys. Extensive absorption occurred after dermal dosing, with around 44% of a 0.5 mg/kg bw dose absorbed after 8 h. Around 6% of an applied dermal dose of 4.5 mg/kg bw was absorbed over 8 h. Metabolism was extensive, with no unchanged acibenzolar-S-methyl excreted except following oral doses of 100 mg/kg bw, when a small amount of parent compound was found in the faeces. Acibenzolar-S-methyl was readily hydrolysed *in vitro* by rat and human liver and skin homogenates, but less readily reacted with plasma or serum. The metabolism of acibenzolar-S-methyl proceeds via cleavage of the S-methyl group, to give the main metabolite, benzo[1,2,3]thiadiazole-7-carboxylic acid. This is followed by conjugation with glycine to form the minor metabolite, [(benzo[1,2,3] thiadiazole-7-carbonyl)amino] acetic acid. The S-methyl group is rapidly oxidised, and either excreted as carbon dioxide via expired air and sulfur or sulfates via the urine or it may be incorporated in serine, methionine, choline or creatine in the liver. Excretion was mainly via the urine (>90%), and was virtually complete within 24 h after a low oral dose or 48 h after a high oral dose. Faecal excretion accounted for around 5% of the administered dose, with virtually no excretion occurring via expired air.

Acute Studies

Acibenzolar-S-methyl was of low acute oral toxicity in mice (LD_{50} >6000 mg/kg bw in males and 5148 mg/kg bw in females) and rats (LD_{50}>2000 mg/kg bw). It was of low dermal and inhalation toxicity in rats (LD_{50}>2000 mg/kg bw and LC_{50}>5000 mg/m^3 respectively). It was not a skin irritant in rabbits, but was a slight eye irritant. Acibenzolar-S-methyl was a skin sensitiser in guinea pigs in a maximisation test.

BION® Plant Activator Seed Treatment was of low oral, dermal and inhalation toxicity in the rat (oral LD_{50} >3000 mg/kg bw, dermal LD_{50}>4000 mg/kg bw, inhalation LC_{50}>1379 mg/m^3). It was not a skin irritant in rabbits or a skin sensitiser in guinea pigs, but was a slight eye irritant in rabbits.

Short-Term Studies

Rats were dosed orally with acibenzolar-S-methyl at 0, 10, 100 or 800 mg/kg bw/day for 28 days. There were no deaths during the study. A hunched posture, piloerection and diarrhoea seen in one female at 800 mg/kg bw/day was considered possibly treatment related. Decreased bodyweight gain was seen in females at 100 and 800 mg/kg bw/day. Food consumption was decreased at 800 mg/kg bw/day. Decreased haemoglobin and mean cell haemoglobin concentration and an increased red cell distribution width were seen in both sexes at 800 mg/kg bw/day. Decreased erythrocyte count, haematocrit, mean cell volume and mean cell haemoglobin were also seen in females at 800 mg/kg bw/day. Decreased leucocyte count and an increased prothrombin time was seen in males at 100 and 800 mg/kg bw/day. Bilirubin levels were increased and protein and globulin levels decreased at 800 mg/kg bw. The liver and spleen weights were increased and thymus weights decreased at 800 mg/kg bw/day. One male at 800 mg/kg bw/day had small testes and testicular tubular atrophy. One female at 800 mg/kg bw/day was emaciated, with thymic atrophy and bone marrow hypocellularity. In 3 females at 800 mg/kg bw/day, hepatocyte necrosis and decreased splenic extramedullary haematopoiesis was seen.

Rats received skin applications of acibenzolar-S-methyl at 0, 10, 100 or 1000 mg/kg bw/day, 5 days/week for 4 weeks. Prothrombin time was increased in females at 1000 mg/kg bw/day. Adrenal weights were decreased in females at 1000 mg/kg bw/day, however this was not associated with any histopathological change.

Long-Term Studies

Mice were fed acibenzolar-S-methyl in the diet at 0, 200, 1000 or 4000 ppm for 3 months. Bodyweight gain was decreased in males at 1000 and 4000 ppm. Erythrocyte count, haemoglobin level and haematocrit were decreased at 4000 ppm, and haematocrit was decreased at 1000 ppm. Anisochromia was seen at 4000 ppm. Clinical chemistry analysis and urinalysis were not done. Spleen weight was increased in all groups of treated females and in males at 1000 and 4000 ppm. Spleens were enlarged at 1000 and 4000 ppm. Extramedullary haematopoiesis was increased in severity in treated mice. Splenic haemosiderosis and white pulp atrophy were seen at 1000 and 4000 ppm. No NOEL could be established in this study. The LOEL was 200 ppm, equal to 30.6 mg/kg bw/day in males and 47.4 mg/kg bw/day in females.

Rats were fed diets containing acibenzolar-S-methyl at 0, 40, 400, 2000 or 8000 ppm for 13 weeks. Bodyweight gain and food consumption was decreased and water consumption was increased at 8000 ppm, and food consumption was decreased at 2000 ppm. Haemoglobin and MCHC levels were decreased and the total leucocyte count and the percentage of neutrophils were increased in males at 8000 ppm. Increased creatinine levels were seen at 8000 ppm. Liver, kidney, spleen and adrenal weights were increased at 8000 ppm. Splenic haemosiderosis and glycogen deposition in hepatocytes were seen at 8000 ppm. Observed changes showed recovery during the recovery phase. The NOEL was 400 ppm, equal to 24.63 mg/kg bw/day in males and 26.29 mg/kg bw/day in females.

Dogs were dosed with acibenzolar-S-methyl at 0, 10, 50 or 200 mg/kg bw/day orally by gelatine capsule for 3 months. Bodyweight gain was decreased at 50 and 200 mg/kg bw/day. Decreased erythrocyte count, haemoglobin, mean cell haemoglobin concentration, haematocrit and basophils, and increased mean cell volume and reticulocyte count were seen at 200 mg/kg bw/day. Increased cholesterol, triglyceride and phospholipid levels, and decreased total protein, albumin and globulin were seen at 200 mg/kg bw/day. The myeloid:erythroid ratio in the bone marrow was decreased at 200 mg/kg bw/day. The liver weights were increased at 50 and 200 mg/kg bw/day, and spleen weights increased in females at 200 mg/kg bw/day. Splenic congestion and haemosiderosis, bone marrow hypercellularity and pigment deposition in the Kupffer cells of the liver were seen at 200 mg/kg bw/day. With the exception of pigment deposition in the Kupffer cells of the liver, all observed changes were reversible during the recovery phase. The NOEL was 10 mg/kg bw/day.

Mice were fed acibenzolar-S-methyl in the diet at 0, 10, 100, 2000 or 6000 ppm for 18 months. Bodyweight gain was decreased at 6000 ppm. Erythrocyte count was decreased in females at 2000 and 6000 ppm, and the haemoglobin and haematocrit were decreased in females at 100, 2000 and 6000 ppm. Red cell distribution weight was increased at 6000 ppm. Spleen weight was increased at 2000 ppm in females and at 6000 ppm in both sexes. Enlarged spleens with haemosiderosis and extramedullary haematopoiesis were seen at 2000 and 6000 ppm. Haemosiderosis was also seen in the bone marrow and liver at 2000 and 6000 ppm. Hyperplasia of the exocrine pancreas was seen in males at 2000 and 6000 ppm. Tumour incidences were unaffected. The NOEL was 10 ppm, equal to 1.14 mg/kg bw/day in both sexes.

Rats were fed acibenzolar-S-methyl in the diet at 0, 20, 200, 2500 or 7500 ppm for 2 years. Mortality was decreased in males at 2500 and 7500 ppm. Hair loss and hunched posture were seen and bodyweight gain was decreased at 7500 ppm. Food consumption was decreased in females at 7500 ppm and water consumption was increased in both sexes at 7500 ppm and in females at 2500 ppm. Decreased erythrocyte count, haemoglobin, haematocrit, and an increased reticulocyte count were seen at 7500 ppm. Decreased protein and globulin levels and increased total bilirubin and potassium were seen at 7500 ppm. Increased bilirubinuria was seen in males at 2500 and 7500 ppm. Increased spleen weights were seen at 7500 ppm. The incidence of marked haemosiderosis n the spleen was increased at 2500 and 7500 ppm. Haemosiderosis in the liver and alveolar foam cells in the lung were increased in females at 7500 ppm. The incidence of mammary gland fibroadenoma, mammary duct dilatation and secretory activity in the mammary acinus was decreased in females at 7500 ppm. Fatty change in the liver was also decreased at 7500 ppm, and the incidence of chronic progressive nephropathy in the kidney was decreased in males at 7500 ppm. Tumour incidences were unaffected. The NOEL was 200 ppm, equal to 7.77 mg/kg bw/day in males and 9.08 mg/kg bw/day in females.

Dogs were dosed with acibenzolar-S-methyl at 0, 5, 25 or 200 mg/kg bw/day by gelatine capsule for 12 months. There were no treatment related deaths or abnormal clinical signs. Bodyweight gain was decreased in females at 200 mg/kg bw/day. In females, the erythrocyte count, haemoglobin, haematocrit and basophil count was decreased and reticulocyte increased at 200 mg/kg bw/day, while in males the erythrocyte count, haemoglobin and haematocrit were decreased at all doses and the MCHC and basophil count were decreased and the reticulocyte count and platelet count increased at 200 mg/kg bw/day. Decreased total protein and albumin and increased cholesterol and triglyceride levels were seen at 200 mg/kg bw/day. Liver weights were increased at 200 mg/kg bw/day and spleen weights were decreased in males at 200 mg/kg bw/day. Haemosiderosis was seen in the bone marrow, spleen and liver and extramedullary haematopoiesis was seen in the spleen and intrahepatic cholestasis in the liver at 200 mg/kg bw/day. No NOEL could be established; the LOEL is 5 mg/kg bw/day based on haematological effects seen at this dose.

Reproduction and Developmental Studies

Rats were fed acibenzolar-S-methyl in the diet at 0, 20, 200, 2000 or 4000 ppm for 2 generations. Bodyweight gain was decreased in F1 males and F0 and F1 females at 4000 ppm. Food consumption was decreased in F0 females at 4000 ppm and in F1 females at 4000 ppm during lactation. There were no treatment-related effects on any reproductive parameters, or on pup survival. Pup bodyweight gain was decreased at 2000 and 4000 ppm. Eye opening was slightly delayed in F1a pups. Spleen weights were increased in adult rats at 2000 and 4000 ppm. Splenic congestion was increased at 4000 ppm, and the severity of splenic haemosiderosis was increased at 2000 and 4000 ppm. The NOEL was 200 ppm, equal to 13.1 mg/kg bw/day in males and 16.2 mg/kg bw/day in females.

Rats were dosed with acibenzolar-S-methyl at 0, 10, 50, 200 and 400 mg/kg bw/day from days 6 to 15 of gestation inclusive. A haemorrhagic discharge was seen in the perineal area of rats from all treatment groups, and a clear discharge was seen in rats at 50, 200 and 400 mg/kg bw/day. Decreased bodyweight gain and food consumption was seen at 400 mg/kg bw/day, with decreased food consumption seen at 200 mg/kg bw/day. An increase in early resorptions and decrease in the number of viable foetuses was seen at 400 mg/kg bw/day, with surviving foetuses having a lower bodyweight than controls. A range of visceral abnormalities, including omphalocoele, internal hydrocephalus, fusion of the lung lobes and splenic hypoplasia and aplasia were seen at 400 mg/kg bw/day. Skeletal abnormalities consistent with delayed development were seen at 200 and 400 mg/kg bw/day. No NOEL for maternal effects could be established due to the haemorrhagic discharge at all doses. The NOEL for developmental effects was 50 mg/kg bw/day and the NOEL for embryotoxic effects was 200 mg/kg bw/day.

In a range-finding study, rats were dosed with acibenzolar-S-methyl at 400 mg/kg bw/day on consecutive days of gestation inclusive (days 6 & 7, 8 & 9, 10 & 11, 12 & 13 or 14 & 15). One female dosed on days 6 and 7 died on day 20 after a haemorrhagic vaginal discharge was seen. Four rats dosed on days 8 and 9 had a bloody vaginal discharge from day 16 and at the end of the study had total resorption of foetuses. There was a decrease in the number of viable foetuses seen in the groups dosed on days 6 and 7 and on days 8 and 9, associated with an increase in the number of early resorptions. The foetal bodyweight was decreased in the offspring of rats treated on days 6 and 7, 8 and 9 and 10 and 11, but not in the other groups. There were no treatment related external findings in the foetuses. In this study, acibenzolar-S-methyl was considered toxic to dams at 400 mg/kg bw/day, and embryotoxic when given on gestational days 6 to 9 inclusive.

In a range-finding study, rats were dosed with acibenzolar-S-methyl at 300 mg/kg bw/day either on gestation days 6 to 15 inclusive or on two consecutive days between gestation days 6 and 15 (days 6 & 7, 8 & 9, 10 & 11, 12 & 13 or 14 & 15). A haemorrhagic vaginal discharge was seen in all treatment groups except rats treated on gestation days 14 and 15. In one female this was associated with total resorption of foetuses, however other rats produced viable foetuses. Bodyweight gain was decreased in rats treated from gestational day 6 to 15 inclusive. The number of early resorptions was increased in rats treated from gestation day 6 to 15 inclusive, with a consequent decrease in the number of foetuses. Early resorptions were slightly increased in rats treated on days 8 & 9 and 12 & 13. Foetal bodyweight was decreased in rats treated on gestation days 6 to 15 inclusive. There were no maternal abnormalities or treatment-related external abnormalities in the foetuses on post-mortem examination. Acibenzolar-S-methyl was considered toxic to dams and embryos when given at 300 mg/kg bw/day.

Acibenzolar-S-methyl was applied to the skin of rats at 0, 10, 100 or 500 mg/kg bw/day from gestation day 6 to 15 inclusive. There were no deaths, abnormal clinical signs or effects on bodyweight gain or food consumption. No developmental parameters were affected, and there were no treatment-related increases in foetal abnormalities.

Rabbits were treated with acibenzolar-S-methyl orally at 0, 10, 50, 300 or 600 mg/kg bw/day on gestation days 7 to 19 inclusive. Deaths were seen at 300 and 600 mg/kg bw/day. Some rabbits showed a bloody perineal discharge, diarrhoea and decreased activity prior to death. Bodyweight gain and food consumption were decreased in these rabbits, but were not otherwise affected. Stomach haemorrhages were seen in some decedents. There was a slight increase in skeletal abnormalities at 600 mg/kg bw/day, but no increase in resorptions. The NOEL for maternal effects was 50 mg/kg bw/day and for foetal effects was 300 mg/kg bw/day.

Genotoxicity

Acibenzolar-S-methyl was not mutagenic in an Ames test or with Chinese Hamster cells. It was not clastogenic *in vitro* in CHO cells, or *in vivo* in a mouse micronucleus study. It did not induce DNA repair in rat hepatocytes *in vitro* or following oral dosing. Overall, acibenzolar-S-methyl was not considered genotoxic.

Special Studies

The metabolite

The metabolite (CGA 210007 - (benzo[1,2,3]thiadiazole-7-carboxylic acid)) was of low acute oral and dermal toxicity in rats (LD_{50}> 2000 mg/kg bw/day). It was not a skin irritant in rabbits or a skin sensitiser in guinea pigs, but was a moderate eye irritant in rabbits.

CGA 210007 was given by oral gavage to female rats at 0, 100, 400 or 800 mg/kg bw/day for 28 days. Deaths and abnormal clinical signs including piloerection, hypoactivity and hunched posture were seen at 400 and 800 mg/kg bw/day, with ataxia, dyspnoea and prostration seen at 800 mg/kg bw/day. All rats in the 800 mg/kg bw/day group died or were killed moribund within 2 weeks. Bodyweight gain and food consumption were decreased and water consumption increased at 400 and 800 mg/kg bw/day. Decreased erythrocyte and leucocyte counts, haemoglobin, haematocrit and basophil and monocyte counts were seen at 400 mg/kg bw/day. Prothrombin time was increased at 400 mg/kg bw/day. Blood glucose levels were elevated and urea, protein and globulin levels decreased at 400 mg/kg bw/day. The liver and spleen weights were increased and thymus and adrenal weights decreased at 400 mg/kg bw/day. Bone marrow hypocellularity, atrophy of the splenic white pulp, atrophy of the thymus, mucosal atrophy of the small intestine and oedema of the caecum were seen at 400 and 800 mg/kg bw/day. Necrosis of the ovarian granulosa theca was increased at 800 mg/kg bw/day, as were other intestinal changes including oedema and inflammatory cell infiltrate of the small intestine and caecal dilatation. There were no effects seen following oral dosing with CGA 210007 at 100 mg/kg bw/day.

In genotoxicity tests, CGA 210007 was weakly mutagenic in the Ames test in *S. typhimurium* strain TA98 when a 95.1% purity was tested. In a repeat test using CGA 210007 at 99.4%, no mutagenic activity was seen. It was not genotoxic in any tests in other strains of *S. typhimurium* or in *E. coli*, or in Chinese hamster cells. No clastogenicity was seen *in vitro* in Chinese hamster ovary cells or *in vivo* in a mouse micronucleus test. CGA 210007 did not induce DNA repair in rat hepatocytes. Overall, CGA 210007 was not genotoxic.

Neurotoxicity studies

In acute neurological tests in rats, there was possibly slight CNS excitation following a single oral dose of acibenzolar-S-methyl at 3500 mg/kg bw in a range-finding study. Following an oral dose of 2000 mg/kg bw, one death was seen, with a slight decrease in activity in the animal that subsequently died. There were no neuropathological changes associated with dosing.
In a 90-day study, acibenzolar-S-methyl was fed at 0, 400, 2000 or 8000 ppm in the diet. Decreased food consumption and bodyweight gain were seen at 8000 ppm, but there was no evidence of any neurological changes on the FOB or assessment of motor activity, and no neuropathological changes on macroscopic or microscopic examination of the tissues. Under the conditions of these tests, acibenzolar-S-methyl did not appear to be neurotoxic.

Mechanism studies

In *in vitro* tests to investigate the mechanism of acibenzolar-S-methyl associated haemolytic anaemia, no effect on haemolysis, osmotic fragility or erythrocyte glucose-6-phosphatase activity was seen with acibenzolar-S-methyl or its metabolites, CGA 210007 or methanethiol. Total erythrocyte glutathione content was reduced following treatment with acibenzolar-S-methyl or methanethiol, but not after treatment with CGA 210007. Spectral analysis of haemoglobin pigments indicated that both methaemoglobin and sulfhaemoglobin were present after incubation with acibenzolar-S-methyl or methanethiol, but not with CGA 210007. Erythrocytes also appeared to be under oxidative stress in these conditions. This may indicate that methanethiol, released when acibenzolar-S-methyl is metabolised to CGA 210007, may be responsible for many of the haematological effects observed.

Pharmacology studies

There was no evidence for the formation of antibodies to acibenzolar-S-methyl or its serum albumin conjugate in rats.

Acibenzolar-S-methyl at oral doses of 5000 mg/kg bw in mice produced decreased reactivity and spontaneous motor activity, along with a reduction in grip strength and body tone. As one mouse died at this dose, this may be a generalised toxic effect, rather than a specific effect on the neurological system. No effects were seen in rats at doses up to 5000 mg/kg bw. There were no effects on hexobarbital-induced sleeping time, convulsions or body temperature. No significant effects were seen on the cardiovascular system, autonomic nervous system, gastrointestinal system or skeletal muscle. Very slight increases in clotting time were seen at 5000 mg/kg bw in rats, however the significance of these effects was not clear.

DISCUSSION OF THE TOXICITY OF ACIBENZOLAR-S-METHYL

There is an extensive toxicological database for acibenzolar-S-methyl, allowing consideration of all aspects of the toxicological profile. Acibenzolar-S-methyl is rapidly absorbed in the rat, and relatively completely excreted in the urine following metabolism, involving cleaving the S-methyl group. The carboxylic acid produced is readily excreted in the urine, however conjugation with glycine also occurs, yielding a metabolite that is also readily excreted. The methanethiol group is rapidly oxidised, and either excreted as carbon dioxide via expired air and sulfur or sulfates via the urine or may be incorporated in serine, methionine, choline or creatine in the liver. The metabolic pathway in animals is sufficiently similar to that seen in plants, where cleavage producing the carboxylic acid is followed by conjugation with glycine, that long term studies using acibenzolar-S-methyl in animals will adequately reflect the effects of consumption of residues in food commodities.

In a range of studies, the primary effect observed was haemolytic anaemia, as evidenced by reduced erythrocyte count, haemoglobin and haematocrit and increased reticulocyte counts. This was seen in all species tested, with the dog being most sensitive in chronic studies. Associated with the anaemia were hypercellularity in the bone marrow along with a decreased myeloid:erythroid ratio was seen in dogs, the only species where this was investigated. Similarly, there was a tendency for increased severity of haemosiderosis in the spleen and also haemosiderosis in the liver. In rats at high doses in a chronic study, bilirubinuria was also seen, which may indicate a more severe effect in these animals. It is recognised that rats may be generally less sensitive than dogs to haemolytic effects, particularly at low doses. Humans are, in general, of slightly lower sensitivity than dogs, however there are a number of sensitive subpopulations which have particular susceptibility to the effects of oxidative stress on erythrocytes. These can include individuals with disruptions of the normal protective enzymatic functions, and individuals with abnormalities of erythrocyte morphology. There are also individuals in the population who, for reasons of cardiac or respiratory dysfunction, may experience difficulties following any reduction of the oxygen carrying capacity of the blood, and these individuals may be at more risk following exposure to acibenzolar-S-methyl.

A mechanistic study was done to attempt to determine how these effects were produced. In this study, both acibenzolar-S-methyl and methanethiol (a degradation product) resulted in decreased total glutathione content of erythrocytes. Reduced glutathione was reversibly bound to proteins in these incubations. Other effects seen with these compounds included the production of methaemoglobin and sulfhaemoglobin and also generalised signs of oxidative stress on the erythrocytes. The reduction of glutathione levels is a significant factor in the development of oxidative stress and ultimately haemolysis. Glutathione may be depleted following binding to toxic chemicals. In this case it appeared that there was additional binding to proteins which would increase the oxidative stress in the erythrocytes, in comparison to that seen in other metabolic cells such as hepatocytes. The reversible conversion of haemoglobin to methaemoglobin also contributes to oxidative stress, as it decreases oxygen availability. The effects of the irreversible conversion to sulfhaemoglobin are less clear, however it may also be associated with oxidative stress. In this study, the development of Heinz bodies was not reported. These are frequently seen in oxidative stress and as a precursor to haemolysis. It is not clear from the study designs whether erythrocytes were examined for the presence of Heinz bodies, although it was stated that erythrocyte morphology was examined in some studies. Haemosiderosis in organs associated with the metabolism of damaged erythrocytes, primarily liver and spleen, is a natural sequelae of this level of oxidative stress. The bilirubinuria seen in one rat study is a sign that the normal internal mechanisms of the body to deal with the breakdown products of haemolysis have been exceeded.

In the *in vitro* mechanistic study, the metabolite CGA 210007 did not produce any significant effects on erythrocytes. However, in a 28 day oral dosing study conducted only in female rats, CGA 210007 produced significant toxic effects, including decreased erythrocyte counts, and decreased haemoglobin and haematocrit levels. Hypocellularity of the bone marrow was seen, and there was no evidence of haemosiderosis in either liver or spleen. These effects are very similar to effects seen in a 28 day oral dosing study in rats with acibenzolar-S-methyl, and this may indicated that there are additional mechanisms of toxicity occurring other than oxidative stress.

There was no evidence of carcinogenicity with acibenzolar-S-methyl, and genotoxicity studies were negative. In genotoxicity studies using the metabolite CGA 210007, the Ames test using *S. typhimurium* strain TA98 was positive when tested with material of 95.1% purity, but was negative when retested with material of 99% purity. It therefore appears that the positive result may either have been incidental, or may have been associated with an impurity present in the material. It is unlikely that human exposure to any such impurity would occur following the agricultural use of products containing acibenzolar-S-methyl. CGA 210007 was negative in all other genotoxicity tests.

In a reproduction test in rats, there were no treatment-related effects on any reproductive parameters or on pup survival. In a developmental study in rats, a haemorrhagic discharge was seen in all treated groups. At doses up to 50 mg/kg bw/day, this was not associated with any embryo- or foeto-toxicity. At 200 mg/kg bw/day, skeletal effects were seen in the foetus, associated with delayed development, while at 400 mg/kg bw/day an increase in early resorptions was seen indicating embryotoxicity. Further range-finding studies, involving dosing for short periods of time (2 days) at various stages of gestation, showed that the haemorrhagic effect could be produced by dosing on days 6 and 7 or days 8 and 9 at 400 mg/kg bw/day. An increase in early resorptions was also seen in these groups. Dosing at 300 mg/kg bw/day also produced a haemorrhagic discharge, with embryotoxic effects seen after dosing on days 8 and 9 or days 12 and 13. In rabbits, a haemorrhagic discharge was only seen in dams prior to death at 300 and 600 mg/kg bw/day, with all other dams unaffected. The aetiology of the haemorrhagic discharge is not clear, as it was not consistently associated with resorptions, and was not related to foetal death. It therefore appears to be a maternotoxic effect with an unknown mechanism. No developmental effects were seen at doses which were not maternotoxic.

ADI and ARfD considerations

Haematological effects were seen in the dog at the lowest dose (5 mg/kg bw/day) in a 12 month study. No evidence of histopathological change associated with anaemia were seen at this dose. Clear NOELs were established in the mouse (1.14 mg/kg bw/day based on haematological changes at 10.8 mg/kg bw/day) and rat (7.77 mg/kg bw/day based on a range of effects including increased water consumption, bilirubinuria and haemosiderosis in the spleen at 96.9 mg/kg bw/day). Dogs are potentially more sensitive to effects on erythrocytes than are humans, however, there is potential for subpopulations of humans to be particularly sensitive. It is therefore appropriate to use the LOEL from the dog study of 5 mg/kg bw/day, and apply an additional 10 fold safety factor. Using a safety factor of 1000, the ADI is 0.005 mg/kg bw/day.

Acibenzolar-S-methyl is of low acute oral toxicity, and shows no neurotoxic potential. However, in developmental studies in rats, a haemorrhagic discharge was evident at all doses. In other studies, it was shown that the discharge was produced after only 2 days dosing during gestation. Given the short duration of treatment required for this effect, the ARfD may be based on this endpoint. The LOEL for haemorrhagic discharge in the rat developmental study was 10 mg/kg bw/day. Using a 1000-fold safety factor, the ARfD is 0.01 mg/kg bw/day.

PUBLIC HEALTH STANDARDS

Poisons Scheduling

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

On the basis of its toxicity, including maternotoxic effects, the NDPSC has included acibenzolar-S-methyl in Schedule 7 of the Standard for the Uniform Scheduling of Drugs and Poisons (SUSDP). There are provisions for appropriate warning statements and first-aid directions on the product label.

NOEL/ADI

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

The ADI for acibenzolar-s-methyl was established at 0.005 mg/kg bw/day based on a LOEL of 5 mg/kg bw/day in a 12-month dog dietary study and using a 1000-fold safety factor in recognition of the extensive toxicological database available for acibenzolar-s-methyl and the need to use a LOEL rather than a NOEL in the assessment of the ADI.

Acute Reference Dose (ARfD)

The acute reference dose 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 single or short term dose which causes no effect in the most sensitive species of experimental animal tested, together with a safety factor which reflects the quality of the toxicological database and takes into account the variability in responses between species and individuals.
The highest acute dose of a compound at which no evidence of toxicity was detected is the starting point for the estimation of the ARfD. In this case there was no acute dose of acibenzolar-s-methyl free of evidence of toxicity. In lieu of this absence, a LOEL of 10 mg/kg bw in a rat teratogenicity study was used as the basis for setting an ARfD. The ARfD was established at 0.01 mg/kg bw on the basis of this LOEL using a 1000-fold safety factor in recognition of the extensive toxicological database available for acibenzolar-s-methyl and the need to use a LOEL rather than a NOEL in the assessment of the ARfD.

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RESIDUES ASSESSMENT

Introduction

Bion® Plant Activator Seed Treatment contains the active constituent acibenzolar-S-methyl and is to be used as a seed treatment in cotton for the suppression of Fusarium Wilt (*Fusarium oxysporum* f. sp. *vasinfectum*) and Black Root Rot (*Thielaviopsis basicola*). As part of the residues assessment for acibenzolar-S-methyl, plant and animal metabolism studies, supervised residue trials, trade aspects, environmental fate and chemistry were considered. Details are provided below.

Metabolism

Wheat was treated with [Phenyl-(U)-^{14}C]-acibenzolar-S-methyl by foliar application at a rate of 50 g ai/ha. Approximately 50% of the applied dose was lost within 14 days by evaporation and transpiration from the plants. The majority of the TRR after 14 days was found in non-extractable plant material (62.7%), indicating extensive metabolism. The metabolite pattern of the extracted residue showed 6 major fractions, two of which were identified as the acid metabolite (i) and the 5-hydroxylated acid metabolite (ii). Hydrolysis of the other four fractions formed the common acid metabolite. Characterisation of the non-extractable material in grains showed that acibenzolar-S-methyl was extensively metabolised into sugar esters of the acid (15.8%), starches (5.8%) and protein (8.7%). The majority of the residue found in grains (39.3%), husks (40.9%) and straw (45.3%) consisted of, or could be converted to the acid metabolite (i).

Tomato plants growing under greenhouse conditions were treated with [Phenyl-(U)-^{14}C]-acibenzolar-S-methyl at 637 g ai/ha, with application repeated 14 and 28 days after the first application. Samples of tomatoes and foliage were collected at regular intervals to measure the uptake and distribution of the radioactivity. The results indicate that acibenzolar-S-methyl is extensively metabolised, predominantly to the acid metabolite (i) and subsequent conjugated forms (sugar conjugate and glycoside ester). Also found in significant amounts were the 5-hydroxylated metabolite (ii) and the 4-hydroxylated metabolite (iii).

Tobacco plants growing under greenhouse conditions were treated by foliar application with [Phenyl-(U)-^{14}C]-acibenzolar-S-methyl at a total rate of 170 g ai/ha, sprayed on 3 occasions. Radioactivity was measured in lower and upper leaves and the stem. The results indicate acibenzolar-S-methyl is metabolised predominantly to the acid metabolite (i), which includes sugar and ester conjugates. Also found in significant amounts were the 4-hydroxylated and 5-hydroxylated acid metabolites (ii and iii). The non-extracted activity amounted to less than 10% of the total activity found.

The metabolic pathway of acibenzolar-S-methyl in wheat, tomato and tobacco proceeded via the hydrolysis of parent molecule to the acid metabolite (i) followed by ester conjugation with sugars. Subsequent oxidation of the phenyl ring led to the 5-hydroxylated acid metabolite (ii) and to the 4-hydroxylated acid metabolite (iii) (tomato and tobacco) followed by sugar conjugation as O-glycoside. The metabolic pathway in **plants** is qualitatively similar in all crops tested (Figure 1).

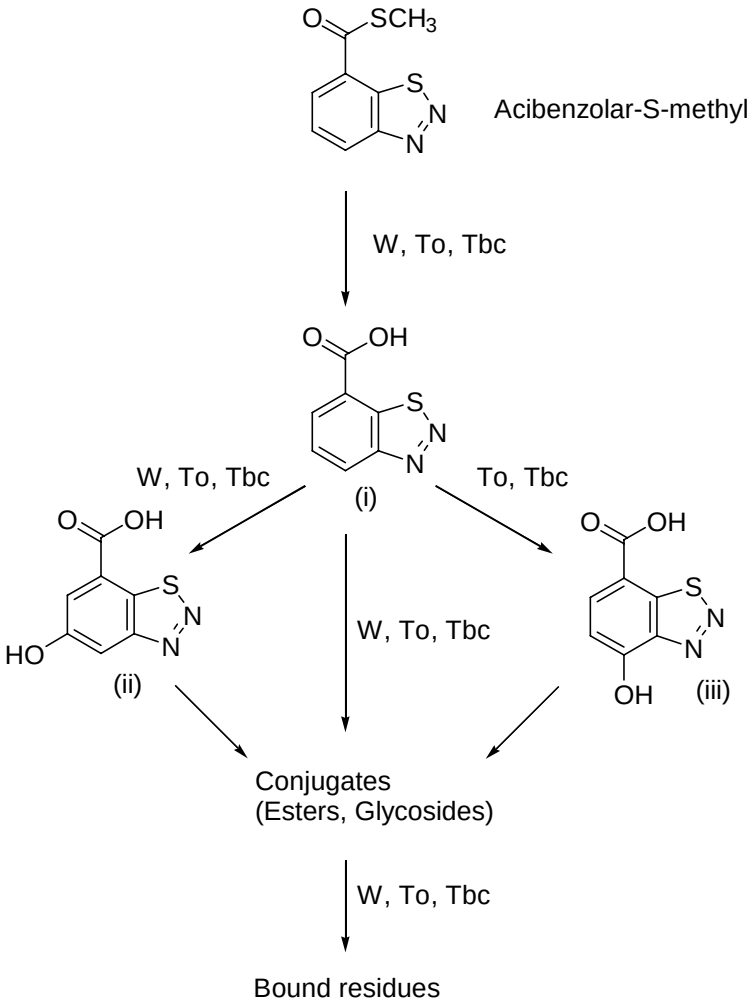

Figure 1: Proposed metabolism of acibenzolar-S-methyl in wheat (W), tomato (To) and tobacco (Tbc)

Lactating goats were dosed with [Phenyl-(U)-^{14}C]-acibenzolar-S-methyl for 4 consecutive days at the equivalent of 11.9 ppm in the feed. Excretion of acibenzolar-S-methyl was primarily via the urine (63.5% of the total dose) and faeces (11.6%). Residues found in tissues were less than 0.2% of the total dose. These were predominantly in the blood, kidney and liver. Tissue residues found were 3 μg/kg acibenzolar-S-methyl equivalents in leg muscle and tenderloin, 2 μg/kg in omental fat, 3 μg/kg in perirenal fat, 41 μg/kg in liver and 283 μg/kg in kidney. Residues in milk were also low (12 μg/kg at 0 – 78h) with less than 0.2% of the dose eliminated via the milk.

The major component of the residue was the acid metabolite (i) in the faeces (68.9%), milk (70.3%), tissues (65-90%) and urine (96.8%). Also identified was the glycine conjugate of the acid metabolite (iv) which was found in significant amounts in the milk (22.1%) and minor amounts in tissues (2.3-4.4%). The 5-hydroxylated acid metabolite (ii) was found as a minor metabolite eliminated in the faeces (1.6%). Acibenzolar-S-methyl was only found in the faeces (20.6%).

Laying hens were dosed with [Phenyl-(U)-^{14}C]-acibenzolar-S-methyl for 4 days at the equivalent of 19.1 ppm in the diet. The majority of the dose was recovered in the excreta (87% of the dose), with traces recovered in the tissues (<0.4%) and eggs (<0.003%). Tissue residues 6 hours after the final dose were 13 μg/kg acibenzolar-S-methyl equivalents in lean meat, 13 μg/kg in peritoneal fat, 45 μg/kg in skin and attached fat. Higher levels were found in the liver (332 μg/kg equivalents) and the kidneys (903 μg/kg equivalents). Egg white and egg yolk contained 1 and 2 μg/kg, respectively (interval 0-78 h).

Characterisation of the residual activity in edible tissues and eggs found the acid metabolite (i) was the most abundant: 73% in meat, 77% in liver, 77% in skin/fat and 68% in egg white. The following conjugates of the acid metabolite were also found: glycine conjugate (iv), δ conjugate of ornithine (v), bis – acid (α, ε) conjugate of lysine (vi), bis – acid (α, δ) conjugate of orthinine (vii) and acid δ conjugate of α-benzoyl-ornithine (viii).

The metabolic pathway of acibenzolar-S-methyl in **animals** (figure 2) proceeds via:

1. Hydrolysis to the acid metabolite (i) as the major metabolite in excreta, milk and tissues.
2. Conjugation of the acid metabolite with glycine (iv), which was found in significant amounts in milk and in minor amounts in tissues.
3. Conjugation in the liver and muscle, which quantitatively released the acid metabolite upon hydrolysis.
4. Hydroxylation of the acid metabolite to 5-hydroxylated acid metabolite (ii), which was eliminated via faeces.

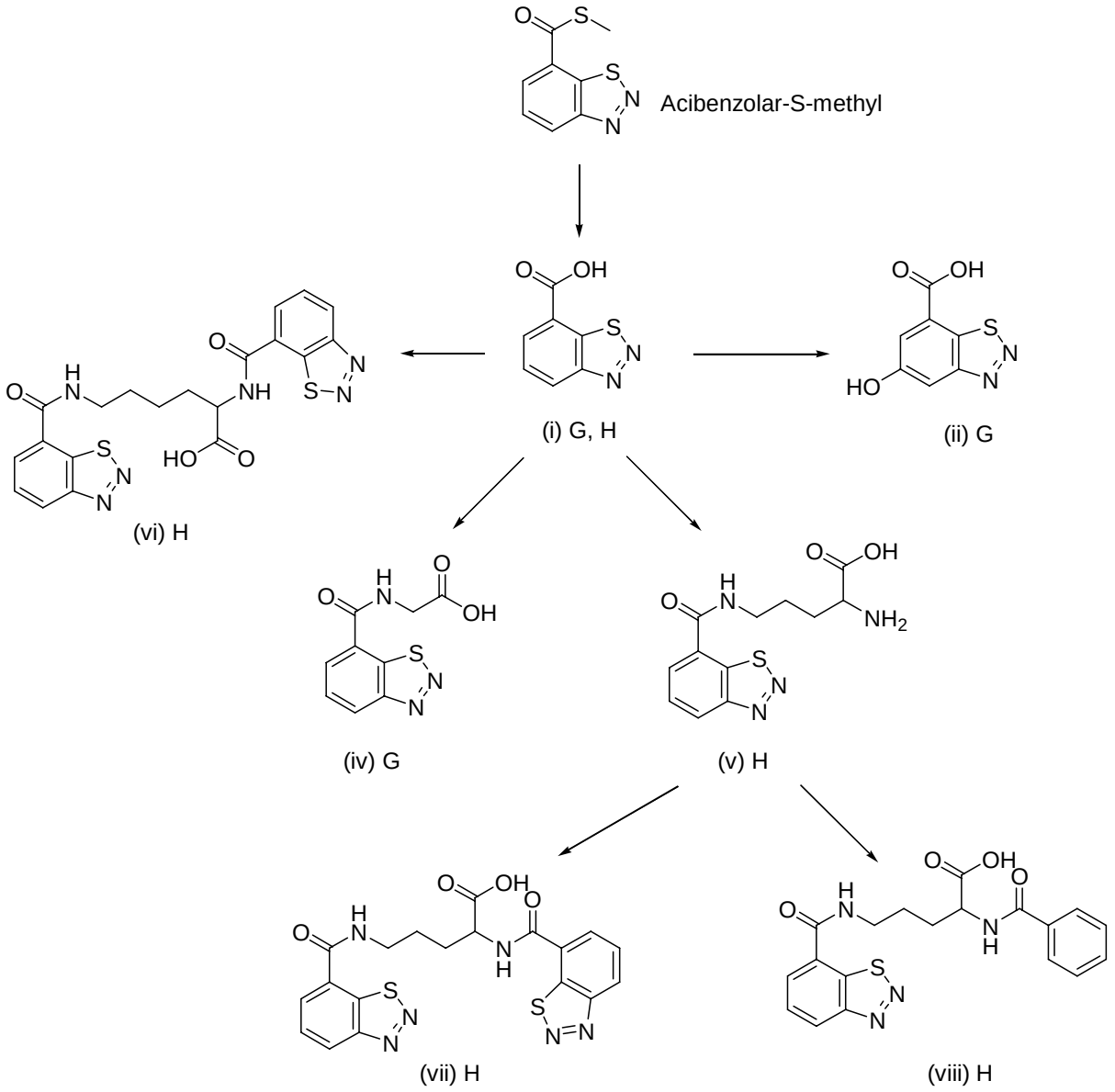

Figure 2 : Proposed metabolism of acibenzolar-S-methyl in goats (G) and hens (H)

Analytical methods

Cotton seed and trash

Residues of acibenzolar and metabolites containing the benzo[1,2,3]thiadiazole-7-carboxy moiety were hydrolysed to benzo[1,2,3]thiadiazole-7-carboxylic acid by heating the homogenised samples of cotton seed or trash in the presence of sodium hydroxide. The extract was purified by partition between aqueous acid and hexane/tert-butyl-methyl ether (TBME), followed by a second partition between aqueous alkali and hexane/TBME. Quantitation of the residue was by liquid chromatography with detection by triple quadrapole mass spectrometry. Residues present were expressed as acibenzolar-S-methyl equivalents. The LOQ for the method was 0.02 mg/kg for both acibenzolar-S-methyl and its acid metabolite in cotton seed and trash, when expressed as acibenzolar-S-methyl.

Other substrates

Validated methods were also provided for the determination of residues of acibenzolar and its metabolites in meat, eggs, fat, liver, kidney and milk. Again the methods involved hydrolysis to benzo[1,2,3]thiadiazole-7-carboxylic acid. After extraction and clean up, quantitation was performed using a 2 column HPLC switching system with UV detection. The LOQs for the method for the various sample matrices are summarized below:

Sample matrix	Technique	LOQ, mg/kg
Meat	HPLC-UV	0.02
Eggs		0.02
Fat		0.02
Liver		0.02
Kidney		0.02
Milk		0.005

Storage stability

The storage stability of residues of acibenzolar-S-methyl and its acid metabolite in samples of wheat grain was shown to be satisfactory over 2 years at –18 °C. In the residue trials submitted, all samples were maintained under freezer conditions, (i.e.<–15 °C) prior to analysis and tested within 17 months of collection. This is acceptable for the purposes of the current application.

Residue definition

The residue definition for acibenzolar-S-methyl is the "*sum of acibenzolar-S-methyl and benzo[1,2,3]thiadiazole-7-carboxylic acid metabolite, expressed as acibenzolar-S-methyl*".

Residue trials

Six trials were conducted in Australia in which a product identical to Bion® Plant Activator Seed Treatment (containing 500 g/L acibenzolar-S-methyl) was applied to cottonseed at rates of 1.2 and 2.4 mL/100 kg seed. (up to 2 × proposed rate). Treated seed was sown using commercial sowing/planting equipment. Samples of seed and trash were collected at a time equivalent to commercial harvest. Samples of seed were ginned using small scale ginning equipment to remove the lint from the seed. Samples were analysed for acibenzolar-S-methyl and its acid metabolite.

No quantifiable residues of acibenzolar-S-methyl or its acid metabolite were found in any of the samples of cotton seed or trash at harvest after application of product at rates up to 2.4 mL/100 kg seed (1.2 g ai/100 kg, 2× proposed rate). An MRL of *0.02 mg/kg is appropriate for cotton seed. Feeding of cotton trash to grazing animals is not currently considered Good Agricultural Practice in Australia. An MRL for cotton trash will therefore not be established.

A harvest withholding period is not required when the product is used as directed.

Processing studies

Since residues in cotton seed were less than the LOQ, processing studies were not conducted and are not required.

Animal commodity MRLs

Potential animal feed commodities derived from cotton include cotton seed, meal and hulls. Detectable residues are not expected to occur in these commodities at harvest. The livestock dietary exposure from consumption of cotton seed will be negligible. In addition, metabolism studies suggest that there is no potential for bio-accumulation. Residues are therefore unlikely to occur in animals fed on produce grown from treated seed. Animal commodity MRLs will be established at the limit of quantitation of *0.02 mg/kg for the determination of acibenzolar-S-methyl in the relevant substrates (*0.005 for milk).

Estimated dietary intakes

The chronic dietary exposure to acibenzolar-S-methyl is estimated by the National Estimated Daily Intake (NEDI) calculation encompassing all registered/temporary uses of the chemical and the mean daily dietary consumption data derived from the 1995 National Nutrition Survey of Australia. The NEDI calculation is made in accordance with WHO Guidelines[1] and is a conservative estimate of dietary exposure to chemical residues in food. The NEDI for acibenzolar-S-methyl is equivalent to 1.9% of the ADI.

It is concluded that the chronic dietary exposure of acibenzolar-S-methyl is acceptable.

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 with 97.5th percentile food consumption data derived from the 1995 National Nutrition Survey of Australia. NESTI calculations are conservative estimates of acute exposure (24 hour period) to chemical residues in food.

The acute exposure to acibenzolar-S-methyl residues in cottonseed is considered acceptable, ranging from 0.2 to 1.6% of the ARfD for the general population and from 0.2 to 3.8% for infants (2-6 years).

Bioaccumulation potential

Acibenzolar-S-methyl has a K_{ow} log P = 3.1 (25 °C), suggesting fat solubility. After oral administration to animals, acibenzolar-S-methyl is rapidly absorbed and also rapidly and

1.Guidelines for predicting dietary intake of pesticide residues, WHO, 1997.

almost completely eliminated with urine and faeces. Residues in tissues were generally low and there was no evidence of accumulation or retention of acibenzolar-S-methyl or its metabolites. In the goat feeding study most of the radioactivity was found in the liver and kidneys (41 and 283 µg/kg respectively). This is as expected when the majority of the dose is eliminated, as these are the organs of transformation and clearance. Residues in fat were low and were at similar concentrations to those in muscle at 2-3 µg/kg.

In soil, the compound dissipates via hydrolysis to the acid; DT_{50} 0.3 d (pH 9). The product further degrades, DT_{50} 20 d; metabolites become completely degraded and mineralised (pH 9). Potential for bio-accumulation is therefore low.

Spray drift

Spray drift is not an issue as the proposed use is for seed treatment only.

Recommendations

The following amendments will be made to the MRL standard:

Table 1

Compound	Food		MRL (mg/kg)
DELETE:			
Acibenzolar-S-methyl	SO 0691	Cotton seed	T*0.02
ADD:			
Acibenzolar-S-methyl	SO 0691	Cotton seed	*0.02
	MO 0105	Edible offal (mammalian)	*0.02
	PE 0112	Eggs	*0.02
	MM 0095	Meat [mammalian]	*0.02
	ML 0106	Milks	*0.005
	PO 0111	Poultry, edible offal of	*0.02
	PM 0110	Poultry meat	*0.02

The following withholding periods are required in relation to the above MRLs:

Withholding periods:
Harvest: Not required when used as directed.

Export Slaughter Interval
An export slaughter interval is not required when used as directed.

ASSESSMENT OF OVERSEAS TRADE ASPECTS OF RESIDUES IN FOOD

Relevant Export Commodities, Overseas Registration Status and MRLs, and Potential for Undue Risk to Australian Trade

Commodities exported
Cottonseed, meal, oil and animal commodities derived from livestock fed on cotton seed, meal and hulls are the commodities exported.

Destination and Value of Exports
In 2004-2005 Australia produced 912 kt of cottonseed, of which 214 kt was exported. [2] Exports of cottonseed oil and cottonseed meal in 2004-2005 amounted to 2.19 kt and 6.42 kt respectively.[3] The actual values of Australia's exports of cottonseed, meal and oil were not reported. The major exports for cottonseed were Japan (144.7 kt), Korea (56 kt) and Saudi Arabia (10.6 kt).

Overseas registrations
The applicant indicated that there are no overseas registrations for the use of acibenzolar-S-methyl on cotton, nor as a seed treatment.

Comparison of Australian MRLs with Codex and overseas MRLs.
The Codex Alimentarius Commission (Codex) is responsible for establishing Codex Maximum Residue Limits (CXLs) for pesticides. Codex CXLs are primarily intended to facilitate international trade, and accommodate differences in Good Agricultural Practice (GAP) employed by various countries. Some countries may accept Codex CXLs when importing foods.

Acibenzolar-S-methyl has not been considered by Codex.

Potential risk to trade
Export of treated produce containing finite (measurable) residues of acibenzolar-S-methyl may pose a risk to Australian trade in situations where (i) no residue tolerance (import tolerance) is established in the importing country or (ii) where residues in Australian produce are likely to exceed a residue tolerance (import tolerance) established in the importing country.

Six trials were conducted in Australia in which a product identical to Bion® Plant Activator Seed Treatment (containing 500 g/L acibenzolar-S-methyl) was applied to cottonseed prior to sowing. No quantifiable residues of acibenzolar-S-methyl or its acid metabolite were found in any samples of cotton seed or trash at harvest after application of product at rates up to 2.4 mL/100 kg seed (1.2 g ai/100 kg, 2× proposed rate). An MRL of *0.02 mg/kg is appropriate for cotton seed. Feeding of cotton trash is not currently considered good agricultural practice in Australia. Therefore an MRL will not be set for this commodity. As no quantifiable residues were found in cotton seed, residues are unlikely in processed commodities that may be used as animal feeds. Separate MRLs are therefore not required for cotton seed meal and hulls.

Animal commodities
Detectable residues are not expected to occur in harvested cotton seed, cotton seed meal and hulls. Therefore the livestock dietary exposure from consumption of produce derived from crops grown from treated plant seed will be negligible. Residues are unlikely to occur in

[2] Source: Australian Commodity Statistics, 2002, abareconomics.

[3] Cottonseed meal figure includes sunflower seed meal.

animals and animal commodity MRLs will be established at the limit of quantitation for acibenzolar-S-methyl in the relevant substrates. A protection statement advising not to feed treated seed directly to animals is included on the draft label.

Use of the product in accordance with the label instructions is unlikely to risk Australian trade as chemical residues in cottonseed and commodities derived from livestock fed on produce grown from treated seed are expected to be not detectable.

Residues above quantifiable levels are not expected to occur in cotton seed at harvest or in animal commodities derived from livestock fed on harvested cotton seed or its processed commodities. Detection of residues by importing countries is therefore unlikely to occur and the proposed use is not considered to present an undue risk to Australia's export trade.

The APVMA welcomes comment with regard to whether the proposed use of acibenzolar-S-methyl on cotton seed, processed commodities, or livestock fed on commodities produced from crops grown from seed treated with acibenzolar-S-methyl poses an undue prejudice to Australia's trade in these commodities.

Assessment of Occupational Health & Safety

Acibenzolar-S-methyl (CAS: 135158-54-2) is listed on the ASCC Hazardous Substances Information System (HSIS) Database (NOHSC, 2005) with the following risk phrases:
R36/37/38 Irritating to eyes, respiratory system and skin
R43 May cause sensitisation by skin contact
Based on the product toxicology information and concentrations of acibenzolar-S-methyl and other ingredients in the product (50%), the OCS classified Bion® Plant Activator Seed Treatment as a hazardous substance in accordance with ASCC Approved Criteria for Classifying Hazardous Substances (NOHSC, 2004) with the following risk phrase:
R36 Irritating to eyes

Formulation, Packaging, Transport, Storage and Retailing

Bion® Plant Activator Seed Treatment will be formulated overseas and imported into Australia, fully packaged and ready for sale. Transport and warehouse workers and storepersons will handle the packaged product and will be exposed to the product only if packaging is breached.

Use and Exposure

Bion® Plant Activator Seed Treatment is intended as a seed treatment in the control of two diseases in cotton crops, namely, Fusarium Wilt caused by *Fusarium oxysporum f.sp. vasinfectum* and Black Root Rot caused by *Thielaviopsis basicola*. Bion® Plant Activator Seed Treatment can be safely used by workers when handled in accordance with the control measures indicated in this assessment.

The rate of application is 1.2 mL of the product/100 kg seed. The product is to be diluted with water before being applied to seed in specialised seed-treatment equipment. Treated seeds are not to be used for human or animal consumption, or be allowed to contaminate grain or other seed intended for animal or human consumption.

The treatment solution is prepared by filling the solution tank with the required volume of water and mixing in the appropriate amount of the product. The mixture is then stirred using the stirring system of the tank. Total volumes of 500 mL - 1.2 L water/100 kg seed are recommended on the product label. The prepared solution must be used within a week.

Once the seeds are thoroughly coated, they move over a drying table where air-assisted drying of the seeds takes place. Dry treated seed emerges from the machine through a funnel into sacks, which are sealed mechanically and stacked automatically onto pallets. Treated seeds are normally packed in 20 kg bags. Apart from the initial connection of the peristaltic pump to the chemical drum, users have no contact with the chemical.

The operator comes in contact with the product only during calibration of the machine and replacement of the chemical drum. Bion® Plant Activator Seed Treatment forms a dry film coating over the surface of the treated seed. Because the treatment process is enclosed there is little risk of worker exposure. Approximately 200 tonnes of seed are treated per day at commercial seed treatment plants, using 2.4 L products (1.2 kg acibenzolar-S-methyl).

Commercial seed treaters conduct seed treatments for approximately five to six months of the year.

For planting purposes, bags of treated seeds are emptied into the hopper of a seed planter. Workers are only expected to be exposed to the treated seed while filling the planter box. They do not need to be in close proximity to the treated seed during planting.

In the absence of worker exposure data or an appropriate model to estimate worker exposure during seed treatment, OCS used the Pesticide Handlers Exposure Database (PHED) Surrogate Exposure Guide to estimate worker exposure to Bion® Plant Activator Seed Treatment during mixing and loading the product. Exposure during seed treatment is expected to be minimal by virtue of the fully enclosed treatment equipment.

Based on the risk assessment, cotton overalls buttoned to the neck and wrist (or equivalent clothing) should be worn when opening the container and preparing treatment solution.

Seed baggers and farmers will handle treated seed. The storage of treated seed for long periods and breakage of seed may produce some amounts of product dust and workers would therefore need to wear disposable dust masks when pouring seeds into the hopper of the seed planter.

Recommendations for Safe Use

Users should follow the instructions and Safety Directions on the product label. Safety Directions include the use of cotton overalls buttoned to the neck and wrist (or equivalent clothing) when opening the container and preparing the treatment solution.

Re handling treated seed

The label recommends that a disposable dust mask should be worn when handling treated seeds for sowing.

Conclusion

Bion® Plant Activator Seed Treatment is for use in commercial agricultural situations only. The primary issue for users as a result of the overall toxicological profile of the product is slight eye irritancy. The product is likely to be of low oral, dermal and inhalational toxicity and not a skin irritant or skin sensitiser. The product can be safely used by workers when handled in accordance with the instructions on the product label and any other control measures described above.

Additional information is available in the MSDS provided for Bion Plant Activator Seed Treatment.

ENVIRONMENTAL ASSESSMENT

Environmental Exposure

Acibenzolar is a benzothiadiazole ester plant host defence inducer that imitates the natural pathogen initiated plant immune defence mechanism ("systemic activated resistance" (SAR)) leading to increased plant resistance against disease. Registration is sought for use as a 500 g/L flowable seed treatment formulation for use in cotton with the product name, Bion® Plant Activator Seed Treatment. The prescribed application rate is 0.6 g active per 100 kg seed.

Environmental Chemistry and Fate

Hydrolysis

One hydrolysis study was provided. The outcome of this study showed acibenzolar-S-methyl was likely to hydrolyse within the environmental pH range. Extrapolated half-lives at 20°C for pH 5, 7 and 9 were 3.8 years, 23.1 weeks and 19.4 hours respectively. Over the pH range, only one hydrolysis product was found in appreciable amounts, namely, the acid metabolite, CGA 210007.

Photolysis

Aqueous photolysis

Two studies were provided. The first test irradiated ^{14}C-acibenzolar-S-methyl in a "Suntest" accelerated exposure table unit. Parent compound degraded rapidly with a half-life of around 1 hour. Volatiles were produced (around 20% after 30 days). The acid metabolite was the only metabolite identified, at levels <5%. This is a known hydrolysis product, and is likely photolysed at a rate similar to parent compound. The second study determined rate and quantum yield. The quantum yield of direct photolysis was 0.0356 and 0.0208 at 289 and 327 nm respectively. Half-lives estimated in shallow waters in summertime were <0.5 h (estimations for latitudes 40°N and 50°N).

Soil photolysis

Two studies were provided, both using ^{14}C-acibenzolar-S-methyl. The first study was performed on a silt loam, either as a dry or viable soil. During the study (720 hours of continuous irradiation), minimal mineralisation was found with <5% CO_2 in the irradiated viable soil. The overall half-life was <1 day. However, this was not due to photolysis as transformation was mainly to the acid metabolite, even in the dark control. The metabolite degraded in the irradiated soil with a half-life around 8 days, but did not degrade to any significant extent in the dark control. Similar results were found for the second study, conducted on a sandy loam over 30 days. This study, using a more intensive extraction process, showed high levels of "other" metabolites (~20%) at the end of the study, that were mainly polar in nature. There was an apparent photolytic effect as in both the viable and sterile soils, a different metabolite profile was found in the extracts. The half-life of acibenzolar-S-methyl was calculated to be around 1 day (DT90 39 days). However, this was not due to photolysis as similar results for the parent compound were found in the dark samples. The main acid metabolite did not degrade significantly in this study in the irradiated soil (maximum 59% after 7 days and found at 48% after 30 days).

Atmospheric photochemical oxidation

The half life for atmospheric degradation of acibenzolar-S-methyl through reactions with OH-radicals was calculated to be 39 hours, but low volatility will limit this route of degradation and it is unlikely to be significant.

Metabolism

Aerobic soil metabolism

Three studies were provided with aerobic soil degradation assessed on a total of 5 soils. Transformation of the parent compound was rapid in all soils, primarily corresponding to an increase in the acid metabolite, CGA 210007. Over time, this metabolite decreased in concentration as levels of bound residues and CO_2 increased. In one study (360 days), bound residues accounted for around 53% and CO_2 for 39% at the end of the study (1 soil). The half-life of CGA 210007 in this soil was 17.5 days. A second study (3 soils, 120 days) also resulted in very fast transformation of parent to the acid metabolite. Two of these soils showed a high level of both bound residues and CO_2 at the end of the study (35-50%). The sandy soil (low %OM and microbial activity) produced much lower volatiles (10% at the end of the study) and 31% bound residues. In this soil, degradation of the acid metabolite was much slower (106 days) than the other two soils (around 20 days). An unknown polar metabolite was found at levels >10% AR in two soils. The final aerobic soil study considered the influence of soil moisture, temperature and concentration on degradation (silty loam soil, 182 days). Regardless of incubation conditions, hydrolysis of the parent compound to the acid was quick (half-lives <1 day). Incubation conditions did appear to impact on the degradation of the acid. Fast degradation was found at 20^{o}C (DT50 10-20 days for initial concentration of 0.1 and 1 ppm respectively). However, at lower moisture content (30% FC) or lower temperature (10^{o}C), degradation was significantly reduced with half-lives of 92 and 72 days respectively.

Anaerobic soil metabolism

No anaerobic study was provided. However, as a component of one aerobic metabolism study, anaerobic conditions were introduced into a set of incubation vessels after 28 days aerobic conditions (1 soil, total 120 days incubation). After the 28 days aerobic incubation, levels of acid metabolite were around 40% with around 7% CO_2 and 44% bound residues. The onset of anaerobic conditions essentially halted further degradation of the acid metabolite with no real change in levels of this compound, volatiles or bound residues at the end of the study.

Water/Sediment Metabolism

One study was provided considering degradation, distribution and metabolism of ^{14}C-acibenzolar-S-methyl in two water/sediment systems. The test substance was applied to the water column, and partitioned quickly to sediments with 32-36% parent found in sediments and 39-52% remaining in the water at day 0. In both systems, the rate of degradation of parent compound was fast (average DT50 and DT90 values around 0.7 and 2.5 days respectively). Apart from the acid metabolite, the formation of which corresponded to decline in the parent compound, no other metabolites were found at any level >0.5%. Production of volatiles was not significant with around 9.5% CO_2 found in both systems after 1 years incubation. The acid metabolite gradually declined from both the water and sediment phases in both systems. Half-lives were in the order of 347 and 495 days from the pond and river systems respectively.

Mobility

Soil adsorption/desorption (batch equilibrium) studies

Two batch equilibrium adsorption/desorption studies were provided with testing covering a total of 11 soil types. Due to the instability of acibenzolar-S-methyl, equilibration times were only a few hours duration. Based on all results, the range of Koc values was 492-3288. The mean Koc was 1666. Acibenzolar-S-methyl is expected to have low to slight mobility based on these findings.

A further study was provided where the mobility of the acid metabolite CGA 210007 was assessed in a batch equilibrium study with 6 soil types. This compound exhibited much higher mobility with Koc values ranging from 40 to 312 (mean 138). These values are indicative of high to very high mobility.

Soil Column Leaching

Three column leaching test reports were provided. The first was a comparative study where movement of acibenzolar and the CGA 210007 metabolite were compared in four soil types to movement of monuron, a substance known to be a moderate leacher. Movement of acibenzolar-S-methyl through the 30 cm soil columns was minimal in all soil types, and had a relative mobility factor of 0.11 compared to monuron, and classed as immobile. CGA210007 was found in leachates from one soil, and moved considerably through the soil profiles with leaching comparable to Monuron. Relative mobility factors compared to monuron in all soils ranged from 0.07-1.6 with a mean of 0.99. CGA 210007 was classed as a moderate leacher based on these results.

In the other two experiments, two soils were tested with acibenzolar-S-methyl aged (5 hours) and leached with either 200 or 508 mm of artificial rainfall. In both, no movement of acibenzolar-S-methyl was found below 2 cm confirming the immobility of this compound. When leached with 200 mm, CGA 210007 was found as low as 26 cm, but only limited radioactivity was found in leachates (<0.5%) and was not characterised. In the experiment using 508 mm artificial rain, CGA 210007 was found down to 24 cm in one soil and all through the soil profile and in leachates (~14%) in the other, again confirming the leaching potential of this metabolite.

Volatility

One study was provided addressing the volatilisation of acibenzolar-S-methyl from plant and soil surfaces following a post-emergent spray. Overall volatilisation from wheat plants (2-leaf stage) and soil when sprayed as a WG50 formulation under controlled climatic conditions was found to be <10% in 24 hours after application. Volatilisation primarily occurred from plants (38%) with no significant volatilisation from the soil surface.

The Henry's Law Constant indicates volatility from water surfaces is likely to be low. This and the low vapour pressure indicate acibenzolar-S-methyl is unlikely to partition to the atmospheric compartment. Modelling indicates that where the chemical is present in the atmosphere in its gaseous phase, it is unlikely to persist with an estimated half-life through reaction with hydroxyl radicals of around 39 hours.

Field dissipation

The results from several field trials were provided. These reports were invariably in summary form, and consequently lacked detailed experimental information. However, the results were relatively consistent thereby building confidence in their usability to predict behaviour of acibenzolar-S-methyl in the field. When applied to bare ground plots, results tended to mirror those found in laboratory soil metabolism studies with a rapid transformation of parent compound to CGA 210007. Where levels of the metabolite were measured in soil over 30 days showed this compound to dissipate with a half-lives of around 6-38 days. Movement below 10 cm was uncommon. Some studies only measured levels in soils and plants (grain and straw from wheat or barley crops) once only and up to three months after application. Not surprisingly, no residues were found in the soil samples this long after application. On one occasion, residue levels of 0.065 mg/kg was found in wheat straw. No residues were found at quantification limits in wheat grain, or barley grain or straw at 119 and 78 days after application respectively.

Bioaccumulation in aquatic organisms

A bioconcentration study was provided to address potential accumulation in aquatic biota, using bluegill sunfish as the test organism. The average of the actual BCFs determined for the two test concentrations at or near steady state were 118, 47 and 197 for the whole body, edible and non-edible tissues respectively, indicating the chemical be slightly to moderately concentrating. The study demonstrated an elimination half-life of less than 1 day following cessation of exposure.

Environmental Toxicology

Avian

Acibenzolar-S-methyl is not toxic to birds based on two acute, two short term dietary and two reproductive studies. The acute oral LD50s for mallard duck and bobwhite quail were determined to be >2000 mg/kg. Dietary toxicity over 5 days was >5000 ppm for both mallard duck and bobwhite quail with a NOEC of 2600 ppm for mallard duck. Reproductive toxicity testing demonstrated NOECs of 600 mg/kg diet and 1000 mg/kg diet (the highest rate tested) for bobwhite quail and mallard duck, respectively.

Aquatic

Fish

Two freshwater fish and one saltwater fish were tested for acute toxicity. The freshwater tests were performed under both static and flow-through conditions for both species with the salt-water fish tested under flow-through conditions. All results were in very close agreement with LC50s ranging from 0.4-2.8 mg/L. The lowest LC50 of 0.4 mg/L was found for rainbow trout (static exposure). This test also provided the lowest NOEC (<0.1 mg/L) due to a slight pigmentation effect on fish later in the exposure time at the lowest test concentration of 0.1 mg/L. Based on the toxicity to rainbow trout (a second test under flow-through conditions resulted in an LC50 of 0.88 mg/L), acibenzolar-S-methyl is considered highly toxic to fish under acute exposure. One chronic study was provided for rainbow trout (early life stage, 87 days duration). Growth was the most sensitive biological end-point and results showed a NOEC of 0.026 mg/L (MATC 0.037 mg/L), indicating the substance is moderately toxic to fish under chronic exposure.

Under static conditions, the metabolite CGA210007 was practically non-toxic to fish based on one study to rainbow trout.

Aquatic invertebrates

Acibenzolar-S-methyl is considered highly toxic to aquatic invertebrates. The parent compound was tested acutely on three species (*D. magna*, mysid shrimp and eastern oyster) with EC50s ranging from 0.61-2.9 mg/L. Chronic testing on *D. magna* further confirmed this toxicity with NOECs of 0.048 and 0.044 mg/L in two separate studies. In one of these studies growth was the most sensitive biological parameter although reproduction was statistically reduced at 0.18 mg/L. In the second study, reproduction was the most sensitive end-point for both time to first brood and cumulative number of young produced per female.

A single acute, static exposure test to *Daphnia magna* with the CGA 210007 metabolite showed this substance to be slightly toxic with an EC50 of 58 mg/L (NOEC 10 mg/L).

Algae and an aquatic macrophyte (duckweed)

Two algal species and one aquatic plant study were provided. For the algal species, EC50s ranged from 0.5 mg/L (biomass) to 3.31 mg/L (cell density). However, acibenzolar-S-methyl was more toxic to the aquatic plant, *Lemna gibba* with an EC50 of 0.31 mg/L. The substance is therefore considered highly toxic to aquatic plants and algae.

Based on one study with one algal species, the metabolite CGA 210007 is slightly toxic to algae with a 72 h EC50 of 90 mg/L (biomass).

Terrestrial Invertebrates

Honey bees were tested for acute toxicity by both oral and contact routes. Bees were exposed to 5 concentrations and no mortality or sub-lethal effects were observed at any time. The 48 h LD50 and NOEC for oral and contact toxicity were >128.3 and 100 µg/bee, respectively. Testing in earthworms showed no dose related impacts on mortality up to the highest treatment level of 1000 mg/kg dw soil. However, there did appear to be an effect on worm weights at the end of the study, and while not compared statistically, the mean weights at all treatment levels were at least 15% lower than control levels (16% reduction at the lowest test rate and 31% reduction at the highest test rate). This suggests a NOEC of <12.3 mg/kg soil based on weight reductions.

Several laboratory and field tests were conducted on beneficial arthropods. For all tests, acibenzolar-S-methyl was applied as a WG50 formulation with exposure being through contact with dried residues or through the soil depending on the test organism. Based on the IOBC classification, the chemical was shown to be harmless to all tested organisms at the maximum rates tested (aphid predator *O. insidiosus* up to 30 g ac/ha; predatory mite *T. pyri* up to 200 g ac/ha; carabid beetles *P. curpeus* up to 30 g ac/ha; parasitic wasp *A. rhopalosiphi* up to 200 g ac/ha; and aphid parisitoid *A. matricariae* up to 30 g ac/ha). For a classification of "Harmless" in the IOBC framework, the total reduction in beneficial capacity (usually combined effect of adult mortality and reproduction) can not exceed 30% of control values.

Microorganisms

In a test considering impacts on respiration rates and N-mineralisation from soil microorganisms, incorporation of acibenzolar-S-methyl showed no effects on short-term respiration rates up to 300 g/ha in two soil types. N-mineralisation affected in a transient nature in a silt loam, but not in a loamy sand. Therefore, it can be concluded that acibenzolar-S-methyl showed tolerable, no long lasting effects on N-mineralisation of the tested agricultural soils.

Non-target vegetation

No test data were provided for non-target terrestrial plants.

Environmental Risk Assessment

The only use pattern considered was as a seed treatment to cotton. Consequently, exposure to the environment will low. As no spraying of this material is considered, risks to environmental organisms resulting through spray drift were not applicable. Risks to aquatic organisms potentially exposed through sub-surface run-off were considered for both the parent compound and the main metabolite, and it was concluded that the risk posed through this route was acceptable.

Risks to birds (consumption of treated seed), soil microorganisms and non-target terrestrial arthropods were considered based on the use pattern, by comparison with expected residues on food items or by deriving an expected soil concentration. In all cases, risks were shown to be acceptable.

No phytotoxicity test data were available to predict risk to non-target terrestrial vegetation. However, the use pattern is such that exposure will be negligible, so risk to non-target terrestrial plants is considered acceptable.

Conclusions

Studies have shown that under field situations acibenzolar-S-methyl and its main metabolite will be degraded rapidly. The parent compound will not move down the soil profile. Based on comparisons of predicted concentrations in the environment with ecotoxicity test results, when used in the proposed manner, the risk to birds, fish, aquatic invertebrates, algae/aquatic plants, terrestrial organisms and soil microorganisms has been deemed acceptable. While no data are available for non-target terrestrial plants, risk to these is low due to the use pattern resulting in insignificant exposure.

DEW has recommended that the APVMA be satisfied that the proposed use of acibenzolar in Bion® Plant Activator Seed Treatment in accordance with good agricultural practices would not be likely to have an unintended effect that is harmful to animals, plants or things, or to the environment.

EFFICACY AND SAFETY ASSESSMENT

Justification for use and Mode of Action

Bion® Plant Activator Seed Treatment is proposed as a seed treatment in cotton for the suppression of Fusarium Wilt (*Fusarium oxysporum* f.sp. *vasinfectum*) and Black Root Rot (*Thielaviopsis basicola*).

The area of cotton grown in Australia in recent years has been as high as 500,000 ha, depending on the available water for irrigation and the world cotton price. Cotton is a high value crop, generating over 1 billion dollars in export earnings for Australia each year. Over the last ten years there has been a growing incidence in the number of cotton crops infected either with Fusarium Wilt or Black Root Rot. Both these diseases have the potential to cause severe yield losses due to the lack of current control mechanisms. Fusarium Wilt survives in infected cotton trash and is easily transported between farms on machinery and other transport. The disease can cause up to 100% loss of plant stands through the destruction of the vascular tissue. Black Root Rot is more widespread, covering NSW and Southern QLD. Black Root Rot does not grow on plant residues, rather it survives as spores in the soil. Back-to-back cotton crops allow a build-up of spores and disease severity. The typical root-blackening and damage causes loss of vigour but, more importantly, allows the entry of secondary pathogens into the plant which can lead to major yield loss.

Currently no specific control options exist for Fusarium Wilt or Black Root Rot. The industry relies on a combination of crop rotation, strict farm hygiene procedures and the selection of less susceptible varieties. This control program, despite reducing disease levels still does not provide adequate protection. The registration of Bion® Plant Activator Seed Treatment will add another component to this combined approach to the control of these diseases in Australian cotton. It is believed that Bion® Plant Activator Seed Treatment in combination with existing control options will allow the continued production of cotton in areas that are badly infected with these diseases.

Bion® Plant Activator Seed Treatment is a novel seed treatment containing acibenzolar-S-methyl which is claimed to stimulate the plant's natural defence mechanisms by inducing Systemic Acquired Resistance (SAR) in the plant. Plant Activators are compounds that can activate the plant's natural defence mechanisms and result in Systemic Acquired Resistance (SAR) against pathogens. The phenomenon has been demonstrated in a range of crop species and widely published, a selection of the key reviews and papers (Kessmann et al., 1994, Ryals et al., 1994, Friedrich et al., 1996, Lawton et al., 1996 and Gorlach et al., 1996) have been included to support the application. The applicant has provided information on the registration of products containing acibenzolar-S-methyl in many overseas countries including Europe and the Americas however none pertain to cotton or seed treatment application. Overseas registrations include use in bananas, various tree and nut crops including citrus, pome fruit and hazelnuts, cereals such as barley and wheat , various vegetable and fruit crops and tobacco.

Bion® Plant Activator Seed Treatment has been shown to give good suppression of Fusarium Wilt (*Fusarium oxysporum* f.sp. *vasinfectum*) and Black Root Rot (*Thielaviopsis basicola*) in cotton when used as part of an integrated plant disease management strategy.

Registration is supported by Australian agricultural authorities.

Proposed use pattern

Bion® Plant Activator Seed Treatment will be applied to cotton seed to suppress infection by Fusarium Wilt caused by *Fusarium oxysporum* f.sp. *vasinfectum* and Black Root Rot caused by *Thielaviopsis basicola*.

Application is at the rate of 1.2 ml per 100kg of cotton seed. The product should be applied diluted with water in specialised seed treatment equipment. A good flow and metering system for the initial prepared solution is important. Depending on the type of seed treatment equipment, it may be necessary to increase the recommended amount of water slightly in order to ensure an optimal flow of the solution and an even treatment of seed. Total volumes of not less than 500mL, or not more than 1.2 L per 100 kg of seed, are recommended when Bion® Plant Activator Seed Treatment is used alone. If additional seed treatments are applied in conjunction with Bion® Plant Activator Seed Treatment a higher total application volume may be required to ensure even coverage of seed.

Use is proposed for all State and Territories.

Evaluation of efficacy

The data presented support the claims for good suppression of Fusarium Wilt (*Fusarium oxysporum* f.sp. *vasinfectum*) and Black Root Rot (*Thielaviopsis basicola*) in cotton when used as part of an integrated plant disease management strategy. Detailed efficacy data was presented including results from a range of Australian field trials.

The application provides data from 21 field trials conducted throughout northern New South Wales and southern Queensland from 2003 to 2005. Field trials were sown into fields naturally infected with *Fusarium oxysporum* f.sp. *vasinfectum* or *Thielaviopsis basicola*. Trial plots ranged in size from 1 row x 10 metres to 4 rows x 915 metres (length of field). Five trials consisted of 1 row x 10 metre plots in randomised blocks, these trials examined a large number of treatments including varying rates of active ingredient, length of active ingredient application time and varieties. The other field trials presented consisted of combinations of multiple rows and large replicated plots / strips and assessed a smaller number of treatments.
Twelve cotton varieties (Sicala 45, Sicala 60BR, Sicot 289BR, Sicot 189, Sicot 71BR, Sicot 14B, Sicot F-1, DPL570B, DPL556BR, CSX407, CSX407BR, CSX47RR) were sown in the trials. Sicot 189 was the predominant variety sown in the field trials.
Treatments included: untreated, Seed treatment @ 3, 6, 9 and 12 mg a.i. /kg seed and varying times of pre-seed treatment application. The predominant seed treatment rate assessed was 6 mg ai /kg seed. All field trials had at least four replications of each treatment.

Disease assessments for the Fusarium field trials were conducted at seedling and adult plant stages. They consisted of stem cutting and vascular tissue discoloration assessment on a 0-4 scale. Final yield was measured in six trials. The Black Root Rot field trials were assessed using establishment count, growth stage, root discoloration assessment on a 0-10 scale and above ground tissue dry weight. One of the trials also assessed final yield.

Twenty field trials evaluated Bion® Plant Activator Seed Treatment @ 6 mg a.i. / kg seed for control of *Fusarium oxysporum* f.sp. *vasinfectum*. Data from fifteen trials was relied upon in the review. Four of the field trials demonstrated statistically significant increases in total percent plant survival following seed treatment compared with the untreated control. Data

from a further six field trials indicated increased total percent survival, though not at statistically significant levels from the untreated control. Yield measurements from six field trials showed a slight increase in yield following seed treatment.

Bion® Plant Activator Seed Treatment @ 6 mg a.i. / kg seed was evaluated in six field trials for control of *Thielaviopsis basicola*. Statistically significant reductions occurred in root discoloration in four trials following seed treatment when compared with the untreated control. Plant establishment was significantly higher in two trials and greater, though not statistically significantly in a further trial compared with the untreated control. Yield in one trial was statistically significantly greater than the untreated control.

The trial data demonstrated that the use of Bion® Plant Activator Seed treatment applied at the rate of 1.2mL per 100kg of seed gives a commercially acceptable level of suppression of the two pathogens, *Fusarium oxysporum* f.sp. *vasinfectum* and *Thielaviopsis basicola* in cotton under field situations. The suppression was exhibited as increased percent plant survival and establishment, reduced black root rot severity and some yield benefits. The label claim that the product, as an activator of systemic acquired resistance, is for disease suppression and is to be used as a component of an integrated management strategy for the control of Fusarium wilt and Black Root Rot, is supported.

Crop Safety

Treatments were applied at up to 12 mg a.i. per kg seed (i.e. twice the proposed label rate) and no negative impacts on seedling survival or crop yield were noted.

Resistance management

Acibenzolar-S-methyl has not been allocated to a resistance management group.

Conclusion

Sufficient statistically analysed data from suitably designed and scientifically conducted trials has been presented to substantiate the claims for use shown on the draft labels. As long as the product is used according to label instruction and Good Agricultural Practice it should be suitable for the proposed purposes.

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LABELLING REQUIREMENTS

DANGEROUS POISON

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

BION®
Plant Activator

SEED TREATMENT

ACTIVE CONSTITUENT: 500 g/L ACIBENZOLAR-S-METHYL

For suppression of Fusarium Wilt and Black Root Rot of Cotton by activating the plant's natural resistance mechanisms

IMPORTANT: Read the attached booklet before use

1, 2 or 5 LITRES

Syngenta Crop Protection Pty Limited
Level 1, 2-4 Lyon Park Road, North Ryde NSW 2113

In a transport emergency dial 000, Police or Fire Brigade.
For specialist advice in an emergency only, call 1800 033 111 (24 hours).

APVMA Approval No.: 60556/
Item number

syngenta

STORAGE AND DISPOSAL

Store in closed original container in a cool, well ventilated area as cool as possible. DO NOT store for prolonged periods in direct sunlight.

Triple or preferably pressure rinse containers before disposal. Add rinsings to spray tank. DO NOT dispose of undiluted chemicals on site. If recycling, replace cap and return clean containers to recycler or designated collection point. If not recycling, break, crush or puncture and bury empty containers in a local authority landfill. If no landfill is available, bury the containers below 500 mm in a disposal pit specifically marked and set up for this purpose clear of waterways, desirable vegetation and tree roots. Empty containers and product should not be burnt.

SAFETY DIRECTIONS

Will irritate the eyes. Avoid contact with eyes. Wash hands after use.

When opening the container s and preparing the treatment solution, wear:

- **cotton overalls buttoned to the neck and wrists (or equivalent clothing)**

FIRST AID

If poisoning occurs contact a doctor or Poisons Information Centre. Phone 131 126.

MATERIAL SAFETY DATA SHEET

If additional hazard information is required refer to the Material Safety Data Sheet. For a copy phone 1800 067 108, or visit our website at www.syngenta.com.au

MANUFACTURER'S WARRANTY AND EXCLUSION OF LIABILITY

Syngenta has no control over storage, handling and manner of use of this product. Where this material is not stored, handled or used correctly and in accordance with directions, no express or implied representations or warranties concerning this product (other than non-excludable statutory warranties) will apply. Syngenta accepts no liability for any loss or damage arising from incorrect storage, handling or use.

Batch No.	
Date of Manufacture	

DANGEROUS POISON

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DIRECTIONS FOR USE

Crop	Disease	Rate	Critical Comments
Cotton	Suppression of: Fusarium Wilt caused by *Fusarium oxysporum* f.sp. *vasinfectum* Black Root Rot caused by *Thielaviopsis basicola*	1.2 mL /100 kg seed	Apply diluted with water to clean seed before sowing. This seed treatment should be used as part of an integrated management strategy to control Fusarium Wilt and Black Root Rot.

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

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

EXPORT SLAUGHTER INTERVAL
An export slaughter interval is not required when used as directed

GENERAL INSTRUCTIONS
BION is an inducer of host plant resistance. BION exhibits a unique mode of action that stimulates the natural systemic acquired resistance (SAR) response found in most plant species. BION has no direct activity against target pathogens.

Application
The product should be applied diluted with water in specialised seed-treatment equipment. As for all such seed treatments, a good flow and metering system for the initial prepared solution is important. Depending on the type of seed treatment equipment, it may be necessary to increase the recommended amount of water slightly in order to ensure an optimal flow of the solution and an even treatment of seed.
Prepare the solution as follows:
1. Fill the solution tank with the required volume of water and mix with the appropriate volume of BION. Total volumes of not less than 500 mL nor more than 1.2 L water/100 kg of seed are recommended when BION is applied alone. If additional seed treatments are applied in combination with BION a higher total application volume may be required to ensure even coverage of seed.
2. Switch on the stirring system and stir.

The prepared solution must be used within 1 week.

PRECAUTION
DO NOT use treated seed for animal or human consumption.
DO NOT allow treated seed to contaminate grain or other seed intended for animal or human consumption.
DO NOT feed treated seed, or otherwise expose, to wild or domestic birds.
When treated seed is stored it should be kept apart from other grain and the bags or other containers should be clearly marked to indicate the contents have been treated. Bags which have held treated seed should not be used for any other purpose.

Re-handling treated seed: Wear disposable masks when handling treated seeds for sowing.

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

STORAGE AND DISPOSAL

Store in closed original container in a cool, well ventilated area as cool as possible. Do not store for prolonged periods in direct sunlight.

Triple or preferably pressure rinse containers before disposal. Add rinsings to spray tank. Do not dispose of undiluted chemicals on-site. If recycling, replace cap and return clean containers to recycler or designated collection point.

If not recycling, break, crush or puncture and bury empty containers in a local authority landfill. If no landfill is available, bury the containers below 500 mm in a disposal pit specifically marked and set up for this purpose clear of waterways, desirable vegetation and tree roots. Empty containers and product should not be burnt.

SAFETY DIRECTIONS

Will irritate the eyes. Avoid contact with eyes. Wash hands after use.

When opening the container s and preparing the treatment solution, wear:

- **cotton overalls buttoned to the neck and wrists (or equivalent clothing)**

FIRST AID

If poisoning occurs contact a doctor or Poisons Information Centre. Phone 131 126.

Material Safety Data Sheet

If additional hazard information is required refer to the Material Safety Data Sheet. For a copy phone 1800 025 931 or visit our website at www.syngenta.com.au

Batch No.:	
Date of Manufacture:	

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GLOSSARY

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

References

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Footnote:
Updated versions of these documents are available on the APVMA website *http://www.apvma.gov.au*

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Australian Pesticides and Veterinary Medicines Authority
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