Australian Pesticides & Veterinary Medicines Authority

EVALUATION OF THE NEW ACTIVE

PROHEXADIONE-CALCIUM

IN THE PRODUCT

REGALIS PLANT GROWTH REGULATOR

Public Release Summary

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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 Family Services (Chemicals and Non-prescription Drug Branch), Environment Australia (Risk Assessment and Policy Section), the National Occupational Health and Safety Commission (Worksafe Australia) and State departments of agriculture 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 publications *Ag Manual: The Requirements Manual for Agricultural Chemicals* and *Ag Requirements Series.*

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 First Floor, 22 Brisbane Avenue, Barton, ACT.

The APVMA welcomes comment on the usefulness of this publication and suggestions for further improvement. Comments should be submitted to:

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LIST OF ABBREVIATIONS AND ACRONYMS

[This list should be modified to include all the acronyms and abbreviations that actually appear in the publication.]

ac	active constituent
ADI	Acceptable Daily Intake (for humans)
AHMAC	Australian Health Ministers Advisory Council
ai	active ingredient
BBA	Biologische Bundesanalstalt fur Land – und forstwirschaft
bw	bodyweight
d	day
DAT	Days After Treatment
DT_{50}	Time taken for 50% 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
EEC	Estimated Environmental Concentration
E_rC_{50}	concentration at which the rate of growth of 50% of the test population is impacted
EUP	End Use Product
Fo	original parent generation
g	gram
GAP	Good Agricultural Practice
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
GVP	Good Veterinary Practice
h	hour
ha	hectare
Hct	Heamatocrit
Hg	Haemoglobin
HPLC	High Pressure Liquid Chromatography *or* High Performance Liquid Chromatography
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
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
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
NDPSC	National Drugs and Poisons Schedule Committee
ng	nanogram
NHMRC	National Health and Medical Research Council

NOEC/NOEL	No Observable Effect Concentration Level
OC	Organic Carbon
OM	Organic Matter
po	oral
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
SUSDP	Standard for the Uniform Scheduling of Drugs and Poisons
TGA	Therapeutic Goods Administration
TGAC	Technical grade active constituent
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
WHP	Withholding Period

INTRODUCTION

This publication provides a summary of data reviewed and an outline of the regulatory considerations for the proposed registration of REGALIS PLANT GROWTH REGULATOR as a foliar spray to apples to suppress shoot growth and lower the requirement for summer and winter pruning. The active constituent of the product is prohexadione-calcium, which has been approved by the APVMA. The APVMA also seeks public comment prior to the chemical product being registered for use in Australia.

Responses to public consultation will be considered prior to registration of the product detailed in this document. 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 the full technical reports on public health, occupational health and safety, environmental impact and residues in food are available upon request.

Written comments should be received by the APVMA by **8 September 2006** and should be addressed to:

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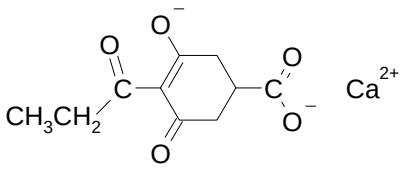
CHEMISTRY AND MANUFACTURE

ACTIVE CONSTITUENT

The active constituent prohexadione-calcium is manufactured in Japan by Ihara Chemical Industry Co., Ltd, 1800 Nakanoko, Fujikawa-cho, Ihara-gun, Shizuoka (Approval Number: 59700).

Chemical Characteristics of the Active Constituent

Common name:	Prohexadione-calcium
Synonyms and Code Number:	BX-112, KUH-883, KUM-883, LAB 285 342, BAS 9054 W, BAS 122 W, BAS 125 W
Chemical name (IUPAC):	Calcium 3-oxido-5-oxo-4-propionylcyclohex-3-enecarboxylate
(CA):	Calcium 3,5-dioxo-4-(1-oxopropyl)cyclohexanecarboxylate
Chemical Abstracts Service (CAS) Registry Number:	127277-53-6
Molecular formula:	$C_{10}H_{10}CaO_5$
Molecular weight:	250.26
Chemical structure:	



Physical and Chemical Properties of the Pure Active Constituent

Physical state:	Powder
Colour:	White to yellow brown
Odour:	None to sweet smelling
Optical rotation:	not optically active
Melting point (for solids):	>360 °C
Boiling point (for liquids):	Not applicable
Relative density:	1.44 at 22 °C
Solubility in water:	At 20°C, (96.6% purity) pH 5 (buffer): 1602 mg/L pH 7 (buffer): 786 mg/L pH 9 (buffer): 665 mg/L pH 6.5 (water): 174 mg/L
Solubility in organic solvents at 20 °C:	Acetone: 0.038 mg/L Toluene: 0.004 mg/L Dichloromethane: 0.004 mg/L Hexane: <0.003 mg/mL Methanol: 1.11 mg/mL Ethyl acetate: <0.010 mg/L

	Propan-2-ol: 0.105 mg/L
Vapour pressure:	1.335 x10^{-5} Pa @ 20^{o}C 1.737 x10^{-5} Pa @ 25^{o}C
Dissociation constant (pKa):	5.15
Surface Tension:	72.6 (0.02% in water), 72.3 mN/m (0.33% in water)
Photostability (DT_{50} in pure water):	~ 4 days
Octanol/Water partition coefficient:	log P_{ow} = -2.90 at 20 °C pH 7
pH:	Not applicable
Hydrolysis stability ($t_{½}$):	pH 5: 5 d at 20°C, <1 d at 50°C, <1 d at 70°C pH 7: 25 d at 20°C, 5 d at 50°C, 3 d at 70°C pH 9: 83 d at 20°C, 48 d at 50°C, 12 d at 70°C
Storage stability:	Prohexadione-calcium is chemically stable at temperatures of 54 °C for 2 weeks and is expected to be stable for at least 2 years when stored away from direct sunlight.
Corrosion characteristics:	Non-corrosive
Oxidizing properties:	Not oxidizing to cellulose
Flammability:	Not flammable
Auto-flammability:	Self-ignition at 335°C
Explosive properties:	Not explosive
Pesticide type:	Plant growth regulator

PHYSICAL AND CHEMICAL PROPERTIES OF THE PRODUCT

Distinguishing name:	Regalis Plant Growth Regulator
Formulation type:	Water dispersible granule
Active constituent concentration:	Prohexadione-calcium (100 g/kg)
Mode of Action:	Plant growth regulator and retardant. Foliar applied and absorbed via green tissue; translocated basipetally, as well as acropetally, within plants.
Physical state:	Solid
Colour:	Grey
Odour:	Moderate spicy
Relative density:	1.665
Bulk density:	755 g/L (loose), 784 g/L (tapped)
Acidity, alkalinity or pH value:	Not applicable
Viscosity:	Not applicable
Surface tension (at 20°C):	46.5 mN/m at 0.1% , 43.1 mN/m at 1.0%
Flash point:	Not applicable
Flammability:	Not highly flammable
Autoflammability:	Self-ignition at 371°C
Explosive properties:	Not explosive
Corrosion characteristics:	Not determined
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.

CONCLUSIONS OF THE ASSESSMENT OF CHEMISTRY AND MANUFACTURING

Based on a review of the chemistry and manufacturing details provided by the applicant, registration of REGALIS PLANT GROWTH REGULATOR is supported.

TOXICOLOGICAL ASSESSMENT

REGALIS PLANT GROWTH REGULATOR is intended for the bio-regulation of apple shoot growth. The recommended mixing rate of the product is 50-75 g per 100 L of water. The application rate is 800-3000 L/ha. The product will be applied in two or three spray programs, commencing when terminal shoots are 3 to 5 cm in length, with repeat applications at 3 to 5 weekly intervals. The higher rate is recommended on large, vigorous trees. The active constituent is leaf absorbed and best suited to dilute application using medium to coarse droplets. Users shall allow 6 h from application for the product to become rainfast.

Label restraints include warning against aerial application of the product, application of the product within 3 days of sprays containing calcium, ethylene or gibberellic acid, and application of the product more than 3 times per season.

METABOLISM AND TOXICOKINETICS ASSESSMENT

In a rat ADME study (Hallifax, 1992), it was established that prohexadione-calcium is rapidly absorbed following oral dosing. Excretion was extensive and rapid, mainly via urine at low doses. At higher doses there was a substantial increase of excretion via the faeces, with a concomitant decreased urinary excretion. Only a very small proportion of the administered dose was excreted via bile (0.2% and 0.3% in males and females respectively). The total bioavailability was approximately 80% in the low dose groups (single and repeated administration) which decreased to 37% at higher dose levels. In a tissue distribution study the highest levels of radioactivity were detected in the GI tract at both low and high dose levels, followed by skin, liver and kidney. The study also showed that prohexadione-calcium and its metabolites did not accumulate in the body.

A further ADME study investigated the metabolic pathways of prohexadione-calcium (Hallifax, 1993). The test substance was mainly excreted and detected in organs in the free acid form (approximately 40% of the administered dose). The main component in urine, feces, liver and kidney was identified as the free acid of prohexadione-calcium. There was one major unidentified metabolite in urine and several minor metabolites in both urine and faeces. One of these minor metabolites corresponded to the despropionyl free acid of prohexadione-calcium, accounting for about 1-2% of the administered dose. In the liver and kidneys, there was one major component which corresponded to the free acid of prohexadione-calcium. The metabolite profile in urine and faeces was independent of the dose or dosing regimen. The principle metabolic reactions included conjugation with glucuronic acid (a number of glucuronide-conjugated metabolites were identified), decarbamylation, and acyl migration.

ACUTE STUDIES

Prohexadione-calcium

Prohexadione-calcium was of low acute oral (LD_{50} >5000 mg/kg bw; mice and rats), dermal (LD_{50} >2000 mg/kg bw; rats), and inhalation toxicity (LC_{50} >4210 mg/m^3; rats). The compound was neither a skin irritant in rabbits, nor a skin sensitiser in guinea pigs, but caused slight eye irritation in rabbits.

Regalis Plant Growth Regulator

The product, REGALIS PLANT GROWTH REGULATOR containing 100 g/kg prohexadione-calcium, was of low acute oral (LD_{50} >2000 mg/kg bw), dermal (LD_{50} >2000 mg/kg bw) and inhalational toxicity (LC_{50} >5200 mg/m^3) in rats. The product was not a skin sensitiser in guinea pigs but caused slight eye and skin irritation in rabbits.

SHORT-TERM STUDIES

A 4-week rat study with dietary doses of 300, 1000, 3000 or 10000 ppm (equal to 30, 101, 301 or 1031 mg/kg bw/d in males; 30, 102, 310, or 1024 mg/kg bw/d in females) revealed treatment-related changes in haematological parameters (Inoue, 1988). These included decreased Hct and Hb levels, and RBC counts in male rats at 1031 mg/kg bw/d. In females no effects of toxicological significance were observed up to the top dose tested. The NOELs were 301 mg/kg bw/d (males) and 1024 mg/kg bw/d (females).

In a 4-week oral (gelatin capsules) study in dogs (Masseym et al., 1989) with dose levels of 0, 200, 600, or 2000 mg/kg bw/d, the effects on haematological parameters that were seen in rats, were not evident. Rather, the study revealed about 20% reduction in body weight gain at 600 and 2000 mg/kg bw/d (statistically significant at 2000 mg/kg bw/d). Although the decreased body weigh gain in animals at 600 mg/kg bw/d was not statistically significant, it was considered to be a biologically significant effect related to treatment. The lack of statistical significance is likely to be due to limited number of animals used. Therefore, the NOEL was set at 200 mg/kg bw/d.

SUBCHRONIC STUDIES

In a 13-week mice study with dietary doses of 0, 400, 2000, 10000, or 50000 ppm (equal to 0, 80, 389, 1986, or 10224 mg/kg bw/d in males; 0, 93, 454, 2256, or 11916 mg/kg bw/d in females) the kidney was found to be one of the main target organs of prohexadione-calcium toxicity (Inoue, 1991a). Incidence of fatty change of the renal tubular epithelium was significantly increased in a dose-responsive manner in females at 10000 and 50000 ppm. These effects were associated with increased relative kidney weight at 50000 ppm in females. Given that depressed WBC counts occurred in males treated at and above the lowest dose of 400 ppm (80 mg/kg bw/d), this study did not demonstrate a NOEL.

In a 13-week dietary rat study, administration of the prohexadione-calcium (0, 1000, 10000, 30000, or 50000 ppm, equal to 0, 73, 734, 2270 or 3924 mg/kg bw/d in males; 0, 80, 815, 2478 or 4221 mg/kg bw/d in females) resulted in decreased Hb level, MCH, MCHC values in males at all dose levels. Increased neutrophil and decreased lymphocyte counts, and decreased WBC counts occurred in males at 10000 ppm and above (Inoue, 1991b). The study also revealed that the stomach and kidneys were likely to be target organs in rats. The effects on the stomach included squamous cell hyperplasia at or near the limiting ridge of the forestomach in both males and females in a dose-related pattern from 10000 ppm, which occurred in parallel with increased food consumption in both sexes. The effects on kidneys included increased relative kidney weights in males, and slightly increased urine volume and decreased urine specific gravity in females, and increased blood sodium and chloride levels in all dosed male groups. Therefore, a NOEL was not established for male rats, based on the abnormalities in the haematology profile at ≥1000 ppm. The NOEL for female rats was 1000

ppm (equivalent to 80 mg/kg bw/d), based on the histological observations in forestomach at 10000 ppm and higher.

The effects on kidneys were also observed in a 3-month oral (gelatin capsules) study in dogs in which dose levels of 0, 80, 400, or 2000 mg/kg bw/d were administered (Horner, 1990). Cortical tubular basophilia and fibrosis and cortical areas of dilated basophilic tubules in kidneys were seen in both sexes at 2000 mg/kg bw/d, and in males at 400 mg/kg bw/d. These effects were seen with increased urine volume, decreased urinary specific gravity, and depressed blood potassium levels in both sexes at 400 and 2000 mg/kg bw/d. Moreover, clinical chemistry analysis revealed increased GPT activities in both sexes at same dose level. The NOEL in this study was 80 mg/kg bw/d, based on depressed serum potassium and increased GPT acitivites, and abnormal renal histology at 400 mg/kg bw/d and above.

CHRONIC AND CARCINOGENICITY STUDIES

In a 24-month mouse study with dose levels of 0, 400, 2000, 20000, or 40000 ppm (equal to 0, 55, 279, 2847 or 5911 mg/kg bw/d in males; 0, 68, 351, 3489 or 7334 mg/kg bw/d in females), impaired food conversion efficiency and depression in body weight occurred at and above 2000 ppm (Inoue, 1992). The haematological system and stomach were the main target tissues/organs. A variety of changes in haematological parameters were noted at weeks 52, 78, and 104. The most significant of these was a treatment-related reduction in WBC count at and above the lowest dose of 400 ppm in males. However, most of the other changes were not dose related or consistent over time. Histopathological examinations revealed ectopic proliferation of the mucosal and glandular epithelium in the submucosal layer of the glandular stomach in male and female mice in the highest dose tested. These changes were assessed to represent heteroplastic, ectopic proliferative changes accompanied by lumen dilatation and cytological degeneration. A higher incidence of hyperkeratosis of the forestomach was observed in both sexes at 20000 and/or 40000 ppm and hyperplasia of the squamous epithelium of the forestomach of female male mice was observed at the highest dose. In addition, a higher incidence of splenomegaly in treated males and vacuoler changes in the exocrine pancreas in females at the high-dose were observed. The study did not provide any convincing evidence that prohexadione-calcium has carcinogenic potential. Given that in males the WBC counts were significantly depressed at 400 ppm (55 mg/kg bw/d), the lowest dose tested, this study did not demonstrate a NOEL in male mice. This finding was a biologically significant effect which was consistently observed in the 13-week mouse study and the 2-year rat study. The NOEL in female mice was considered to be 400 ppm (68 mg/kg bw/d), based on decreased body weight at ≥2000 ppm (351 mg/kg bw/d).

In a 24-month dietary study in rats with dose levels of 0, 400, 2000, 10000 or 20000 ppm (equal to 0, 18.5, 93.9, 469.0, or 968.0 mg/kg bw/d in males; 0, 22.3, 114.0, 572.0, or 1180.0 mg/kg bw/d in females), increased food consumption, decreased food conversion efficiency and depressed body weight occurred at ≥2000 ppm, together with decreased MCH and MCHC values, and WBC and reticulocyte counts. Blood GOT and GPT activities were elevated, and blood sodium and chloride levels were increased in males at these doses. Urinary specific gravity was decreased and urinary volume was increased at 10000 and 20000 ppm. However, although there was some evidence of renal nephropathy and tubular dilation in decedent animals at 2000 and 10000 ppm, this was not found in rats that survived until termination. At 2000 ppm the stomach was a target organ of prohexadione-calcium, displaying injury seen as ectopic tissue, squamous cell hyperplasia and basal cell hyperplasia. In females, there were increased incidences of thyroidal C-cell hyperplasia at ≥10000 ppm

and follicular dilation and C-cell adenoma at ≥2000 ppm. Adrenal pheochromocytoma occurred with increased incidence at ≥10000 ppm in females. The NOEL was 400 ppm (18.5 mg/kg bw/d) based on depressed weight gain and food conversion efficiency, haematological and blood biochemical abnormalities and non-neoplastic and neoplastic lesions in the thyroid at ≥2000 ppm.

In a one-year dog study with oral (gelatin capsules) doses of 0, 20, 200 or 1000 mg/kg bw/d (Wrench, 1992), the most notable effects were in clino-chemical and/or hematological parameters and the kidney at the high dose, which were also recorded to a lesser extent at 200 mg/kg bw/d. The findings in clino-chemical and/or hematological parameters included decreased PCV, Hb, and RBC counts, decreased serum albumin and total protein and potassium, and increased phosphorus levels. Histopathological findings in the kidneys included increased incidence of dilated basophilic cortical tubules with or without fibrosis, which were associated with increased urine volume and decreased urine specific gravity. While water consumption was measured in this study, no statistically significant differences were observed because all animals were seen to have spilt or splashed their water, although increased water consumption could have occurred in the mid and high dose animals. The NOEL in this study was 20 mg/kg bw/d, based on the changes in haematological and clinical chemistry parameters, abnormal urinalysis findings, and histopathological findings in kidney at ≥200 mg/kg bw/d. The kidney is considered to be the primary target organ.

REPRODUCTION AND DEVELOPMENTAL STUDIES

A one-generation range-finding reproduction toxicity study was carried out in rats with dietary dose levels of 0, 400, 2000, 10000 and 50000 ppm (approximately 0, 40, 200, 1000 and 5000 mg/kg bw/d). Treatment-related effects were observed at 50000 ppm, as evidenced by decreased body weights, increased food consumption, and reduced pup growth. One death at this dose level and thickening of the stomach mucosa in three animals may have resulted from treatment. Based on the results, doses of 500, 10000 and 50000 ppm were selected for a two generation study in rats. Given that this was a range-finding study, a NOEL was not set (York and Schardein, 1990).

A 2-generation rat reproduction study was conducted with dietary dose levels of 0, 500, 5000 or 50000 ppm (approximately 0, 41, 377 or 3970 mg/kg bw/d). There were no adverse effects on reproduction parameters seen at any dose levels tested. Stomach lesions were observed at ≥500 ppm (thickening of the limiting ridge in the stomach in the F0 and F1 animals for both sexes; papillary acanthosis, diffuse acanthosis, and hyperkeratosis in the nonglandular stomach; hypertrophic hyperstaining gastric cells, glandular metaplasia, glandular dysplasia and glandular atrophy in the glandular stomach). Given the presence of histological lesions consistent with irritation and inflammation of the stomach at and above the lowest dose of 500 ppm (41 mg/kg bw/d), the study is considered not to have demonstrated a NOEL in the adult rats (NOEL <41 mg/kg bw/d). Mortality, and reduced bodyweight gain of females during gestation were noted in adults at 50000 ppm, together with transient inhibition in pup bodyweight gain. The NOEL for pup toxicity was 5000 ppm (377 mg/kg bw/d for females) based on decreased pup body weights (York, 1992).

In a range-finding developmental toxicity study in rats (0, 100, 250, 500 and 1000 mg/kg bw/d; gavage), no evidence of maternal toxicity was observed at any dose level. One and two out of 6 females at 250 and 500 mg/kg bw/d respectively were nongravid. The mean pre-implantation loss (16.5-25.2%) was higher at 100 to 500 mg/kg bw/d when compared to the

control group (10.8%) or the 1000 mg/kg bw/d group (12.9%). No treatment-related developmental toxicity and malformations or variations were apparent on examination of the foetuses. Based on the results, dose levels of 100, 300 and 1000 mg/kg bw/d were selected for a definitive developmental toxicity study in rats (Schardein, 1989).

In a developmental toxicity study in rats (0, 100, 300, or 1000 mg/kg bw/d; gavage), maternal toxicity was not evident at the highest dose of 1000 mg/kg bw/d. Caesarean section parameters were comparable to those of control animals. However, increased incidence of 14th rudimentary rib was observed at 300 mg/kg bw/d and above. The NOEL for maternal toxicity in rats was 1000 mg/kg bw/d, based on these findings. The NOEL for foetotoxicity was 100 mg/kg bw/d, based on increased incidence of rudimentary ribs at and above 300 mg/kg bw/d (Schardein and Fellow, 1990).

Three developmental toxicity studies in rabbits were performed at International Research and Developmental Corporation, USA (IRDC) in the USA. A range-finding developmental study in rabbits was conducted with dose levels of 0, 100, 250, 500 and 1000 mg/kg bw/d by gavage. Excessive maternal toxicity occurred at 500 and 1000 mg/kg bw/d and was manifested as death, sacrifice in extremis and abortion. Caesarean section examinations revealed an increase in postimplantation loss at 1000 mg/kg bw/d. This increase resulted in a decrease in the number of viable foetuses per doe. Treatment-related maternal bodyweight loss or depression in bodyweight gain occurred at all dose levels. Based on the results, dose levels of 0, 40, 200, and 750 mg/kg bw/d were selected for a developmental toxicity study in rabbits. No NOEL value was derived from this study because it was a range-finding study and only a limited number of animals was used (Schardein and Fellow, 1988).

In a developmental gavage study in rabbits (0, 40, 200, and 750 mg/kg bw/d), there was maternal toxicity at the mid- and high-dose level. These effects included increased mortalities, decreased defecation, and decreased body weight gains at ≥200 mg/kg bw/d; increased incidence of abortions at 200 mg/kg bw/d; stomach erosions, lung congestion, and slightly increased mean post-implantation loss at the high dose. There was a single maternal death from an incidental cause at 40 mg/kg bw/d. There was no evidence of a teratogenic effect at the high dose level (750 mg/kg bw/d), but data were limited to only 2 animals at the high dose (York, 1990a). Twenty female rabbits per group were used in this study. Given that maternal toxicity and abortions occurred at 200 mg/kg bw/d, the NOEL for maternal and foetal toxicity was set at 40 mg/kg bw/d.

Because only two dose levels (40 and 200 mg/kg bw/d) could be properly evaluated in the above study, it was repeated with lower doses. In the subsequent gavage study with dose levels of 0, 30, 75 and 150 mg/kg bw/d, maternal deaths occurred at dose levels ≥30 mg/kg bw/d, and bodyweight gains were reduced at 30 and 75 mg/kg bw/d but only reached statistical significance at gestation day 13-20 in the mid-dose group. In the absence of a significant effect at the high dose, the finding on maternal bodyweight at 30 and 75 mg/kg bw/d are probably not treatment-related. No developmental toxicity was seen at any dose level (York, 1990b). The single maternal death at 30 mg/kg bw/d is probably not treatment-related, given that the only maternal death at 40 mg/kg bw/d in the previous study was accidental. Therefore, the maternal NOEL was 30 mg/kg bw/d. The NOEL for developmental toxicity was 150 mg/kg bw/d, based on the absence of toxicity at any dose tested.

Given that somewhat conflicting results with respect to maternal toxicity in rabbits had been reported from the same laboratory (IRDC) at comparable dose levels using the same study protocol, a third teratology study on the same strain of rabbits under comparable experimental

conditions was performed in a Japanese laboratory (0, 30, 100, and 350 mg/kg bw/d) (Toxicology Research Center –The Imamichi Institute For Animal Reproduction, Japan; Kawanishi, 1992). There were no deaths in dams of treated groups up to the highest dose tested. The effects in dams were restricted to depressed body weight gain and premature delivery in 2 out of 18 dams in the high dose group. The premature deliveries may have resulted from maternal toxicity, but a direct foetotoxic effect can not be excluded. However, there were no treatment-related effects on any other indices of foetal development or growth. Therefore, NOEL for maternal and foetotoxicity was 100 mg/kg bw/d.

GENOTOXICITY STUDIES

Prohexadione-calcium was tested for mutagenic or genotoxic properties in five *in vitro* (Bacterial point mutation assay, Mammalian point mutation test, Cytogenetics Chromosome Damage test, Rat liver UDS DNA damage assay, Rec DNA damage assay) and two *in vivo* studies (Cytogenetics chromosome damage test and Micronucleus test), covering all standard end points for genetic toxicity. In a chromosome damage assay, some evidence of weak polyploidy-inducing activity was shown at short exposure times (6 h) both in the presence and absence of metabolic activation. However no evidence of polyploidy-inducing activity was seen at 24 or 48 h treatment times without metabolic activation and there was considerable variation in the amount of polyploidy within the different control cultures in this study. Therefore, the findings in this study were considered to be of relatively low toxicological significance. Negative results were obtained in all the other studies including the two *in vivo* tests.

NEUROTOXICITY STUDIES

No neurotoxic potential of the active substance is expected based on its chemical structure. Furthermore no signs of neurotoxicity were observed in acute toxicity studies or investigations after repeated administration. Therefore, investigations specifically designed to investigate the neurotoxic effects were not performed.

HAZARD CHARACTERISATION

Absorption, distribution, metabolism and excretion: When administered orally, prohexadione-calcium is rapidly absorbed from the GI tract of rats. The extent of absorption was high at moderate doses, but declined with increasing dose. It is mainly distributed in the GI tract, followed by skin, liver and kidney. Excretion was extensive and relatively rapid, mainly via urine at low dose levels. At high dose there was a substantial increase of excretion via the faeces, with concomitantly decreased urinary excretion. The potential for accumulation was considered to be very low, based on its relatively short time to maximum concentration in plasma (t_{max} =0.5 h) and low tissue residues. Nine metabolites were identified and the principlal metabolic reactions included conjugation with glucuronic acid, decarbamylation, and acyl migration. The free acid form of prohexadione-calcium was the principal component in excreta.

Acute toxicity of active prohexadione-calcium: Prohexadione-calcium was of low acute oral (LD_{50} >5000 mg/kg bw; in rats and mice), dermal (LD_{50} >2000 mg/kg bw; rats), and inhalation toxicity (LC_{50} >4210 mg/m^3; rats). The compound was neither a skin irritant in rabbits, nor a skin sensitiser in guinea pigs but exhibited slight eye irritation in rabbits. Prohexadione-calcium shares a similar acute toxicity profile with its structurally similar

compound trinexapac-ethyl (low acute oral, dermal, and inhalation toxicity, with slight to moderate eye irritation, and no skin irritation and sensitization).

Acute toxicity of product Regalis Plant Regulator: The product, REGALIS PLANT GROWTH REGULATOR containing 100 g/kg prohexadione-calcium, was of low acute oral (LD_{50} >2000 mg/kg bw), dermal (LD_{50} >2000 mg/kg bw) and inhalation toxicity (LC_{50} >5200 mg/m^3) in rats. The product was a slight skin and eye irritant in rabbits, but not a skin sensitiser in guinea pigs.

Repeat dose effects and other toxicological endpoints: At moderate to high doses the primary target organs were the stomach and kidneys. In addition, treatment-related changes in haematological and/or clinical-chemistry parameters were also noted in all species tested.

Treatment-related effects on the stomach were identified in mice and rats but not in dogs. Effects consisted of an increased incidence of ectopic proliferation of mucosal and glandular epithelium in the submucosa of the glandular portions of the stomach, an increased incidence of hyperkeratosis of the forestomach, and hyperplasia of the squamous epithelial cells of the forestomach. These effects were associated with administration by dietary admixture and were probably mediated directly on the stomach by the test chemical, occurring at and above 41 mg/kg bw/d in the 2-generation rat reproduction study, 2850 mg/kg bw/d in the chronic mouse study and 970 mg/kg bw/d in the chronic rat study. The marked disparity between the threshold doses at which the stomach lesions were found in the multi-generation and chronic rat studies is unexplained, but the abnormality were also seen at and above 80 mg/kg bw/d in the 13-week rat study. It is also noteworthy that stomach erosions/irritation occurred at ≥500 mg/kg bw/d in female rabbits in developmental toxicity studies. Given that prohexadione is an acid, oral administration of this compound may lead to decreased pH in the stomach and this could subsequently contribute to the stomach erosions/irritation and epithelium hyperplasia observed in rodents. Evidence suggests that repeated or persistent damage to cells of the forestomach epithelium and associated proliferative responses may be a common factor in rodent forestomach tumorigenesis (Harrison, 1992). In a 2-year rat study, administration of a structural analogue of prohexadione-calcium, trinexapac-ethyl resulted in treatment-related increased incidences of squamous cell carcinoma and basal epithelial cell hyperplasia of the nonglandular stomach, suggesting stomach could be one of common target organs of cyclo-hexane chemicals.

Treatment-related effects on the kidney were identified in mice, rats, and dogs. Effects on the kidneys consisted of increased relative kidney weights (mice and rats), cortical tubular basophilic change and fibrosis, cortical areas of dilated basophilic tubules (rats and dogs), increased incidence of fatty change of the renal tubular epithelium (mice), and increased incidence of chronic nephropathy and protein casts (rats). In rats and dogs these effects were usually associated with altered urinalysis findings (increased urine volume and decreased specific gravity), and altered blood electrolytes (increased sodium and chloride levels, and/or reduced potassium level). These kidney findings occurred in mice, rats, and dogs following treatment duration of 3 months or longer.

Prohexadione-calcium consistently caused haematological abnormalities in mice and rats following repeat-dose administration. These abnormalities consisted of depressions in Hb, MCH and MCHC levels and reductions in WBC counts. These effects were strongest in male mice and rats, and were seen at doses of ≥55 and ≥73 mg/kg bw/d, respectively. In dogs, the haematological effects were confined to depressed RBC counts, and occurred at 200 mg/kg bw/d and above. The toxicity studies did not identify the mechanism by which prohexadione-

calcium caused the haematological abnormalities, nor why male rodents were more sensitive than females. NOELs of 20 mg/kg bw/d for haematological effects were demonstrated in rats and dogs, but the effects occurred at the lowest dose of 55 mg/kg bw/d to which mice were exposed in the mouse chronic/carcinogenicity study.

Evidence of carcinogenic potential was confined to small non dose-related increases in the incidence of adrenal pheochromocytoma at ≥572 mg/kg bw/d, and thyroid C-cell adenoma at ≥114 mg/kg bw/d in female rats. The thyroidal adenomas were associated with hyperplastic abnormalities within the organ. From a regulatory perspective, concern is reduced because of the relatively high doses at which these phenomenon occurred, doses of which would never be achieved by humans under normal conditions of use. Furthermore, the weight of evidence from genotoxicity studies was that prohexadione-calcium is not genotoxic.

In a two-generation reproduction study in rats, toxicity was recorded in parental animals [mortality (8%) in F0 males, reduced bodyweight gains in F0 adult and F1 offspring, increased water consumption in F0 and F1 females, decreased food consumption during the first few weeks after weaning for the F1 adults and test article-related thickening of the limiting ridge in the stomach in the F0 and F1 animals for both sexes] at the high dose level (3970 mg/kg bw/d). Similar effects were observed at the mid dose (377 mg/kg bw/d) but with lessened severity. Microscopically, treatment-related lesions were observed in the nonglandular stomach (papillary acanthosis, diffuse acanthosis, and hyperkeratosis) and in the glandular stomach (hypertrophic hyperstaining gastric cells, glandular metaplasia, glandular dysplasia and glandular atrophy). These were present in adult male rats at the lowest dose of 41 mg/kg bw/d.

Even at the high dose, there were no adverse effects on most reproductive parameters. Effects on developing pups were restricted to the high dose level and consisted of reduced bodyweight and lactation weight gain. The NOEL for effects on pups was 377 mg/kg bw/d. The weight of evidence from this study is that prohexadione-calcium has no selective effects on the reproductive system, or on behaviour of adults or on their offspring.

Six studies [2 in rats (including one range-finding study) and 4 in rabbits (including one range-finding study)] were performed to examine the developmental toxicity of prohexadione-calcium. In a 1990 teratology study in Sprague-Dawley rats, no maternal toxicity was evident at the highest dose tested, 1000 mg/kg bw/d. However increased incidence of 14th rudimentary rib was observed in foetuses at ≥300 mg/kg bw/d and above, suggesting that delayed foetal development occurred. However the effect seen in rat foetuses was not seen in rabbits, at the highest dose tested (1000 mg/kg bw/d).

Interpretation of the 3 rabbit studies performed at IRDC in the USA was difficult because of large numbers of unexplained maternal deaths and wide between-group variation in maternal weight gain within and between experiments. However, the following conclusions can be drawn from the IRDC studies:

Pregnant rabbits are significantly more sensitive to prohexadione-calcium than pregnant rats.

Doses of ≥200 mg/kg bw/d were clearly maternally toxic, causing death or requiring does to be euthanised in moribund condition.

Doses at 200 mg/kg bw/d caused abortions. This may have been caused by maternotoxicity, but direct toxicity to the foetuses cannot be excluded.

- The highest dose at which abortion did not occur was 150 mg/kg bw/d. Thus, this dose may be taken as being the NOEL for foetal toxicity, given that there was no evidence of adverse effects on foetal development.

- There was a single unexplained maternal death at 30 mg/kg bw/d. However, although one doe treated at 40 mg/kg bw/d had to be sacrificed moribund, its clinical signs were inconsistent with those seen in does that died or became unwell at higher doses. The illness was attributed to physical injury, rather than the test chemical. No other mortality or evidence of maternal toxicity was recorded at 40 mg/kg bw/d. Therefore the maternal death seen at 30 mg/kg bw/d is unlikely to have been treatment-related.

- However, some maternal deaths at doses of 75 and 150 mg/kg bw/d were unexplained and could have arisen from treatment. Hence, the NOEL for maternal toxicity in the three IRDC studies was considered to be 40 mg/kg bw/d.

The fourth rabbit developmental study performed at a Japanese laboratory (to follow up the IRDC studies) did not result in maternal deaths, although maternal toxicity (depressed bodyweight gain) and premature foetal delivery did occur at the highest dose of 350 mg/kg bw/d. The NOEL for materno- and foetotoxicity in this study was 100 mg/kg bw/d (Kawanishi, 1992).

Comparison between the IRDC studies (York 1990 a & b) and Kawanishi (1992) suggest that rabbits in the Japanese study were less sensitive to the test chemical than those used at IRDC. The reason for this is unknown. Environmental differences between the two laboratories could have occurred. However, in the absence of any explanation, the results of the IRDC studies can not be discounted, and the overall NOELs for materno- and foeto-toxicity should be set at 40 and 150 mg/kg bw/d, respectively.

Prohexadione-calcium exhibited negative results in a bacterial point mutation assay (Ames test, in a HPRT locus mammalian cell mutation assay in V79 cells, in a rat liver UDS DNA damage assay in primary rat hepatocytes, and in a Rec DNA damage assay in *B. subtilis* with or without metabolic activation. The test compound exhibited weak positive results in a cytogenetics chromosome damage assay at 6 h but showed negative results in the presence of metabolic activation system at 24 and 48 h incubation. Given that no evidence of polyploidy-inducing activity was seen at 24 or 48 h treatment times without metabolic activation (24 and 48 h), the OCS considers that the finding is of relatively low toxicological significance. In an *in vivo* rat cytogenetics chromosome damage assay and in mice micronucleus test, prohexadione-calcium gave negative results. It is, therefore, concluded that prohexadione-calcium is not genotoxic *in vivo*.

No signs of neurotoxicity were observed in the acute toxicity studies or investigations after repeated administration, indicating that prohexadione-calcium is unlikely to be selectively toxic to the nervous system.

ADI AND ARFD CONSIDERATIONS

The Acceptable Daily Intake (ADI) for man is the estimate of the amount of a substance in the diet in food or drinking water, expressed on a milligrams per kilogram of bodyweight basis, that can be taken daily over a lifetime without risk.

The studies most relevant to setting the ADI are sub-chronic, chronic, multi-generation reproduction and developmental toxicity studies via the oral route. In the toxicological database on prohexadione-calcium, the lowest NOELs in chronic studies were 18.5 mg/kg bw/d in rats (based on abnormal haematology, clinical chemistry and thyroid histopathology and decreased bodyweight gain and food conversion efficiency at ≥94 mg/kg bw/d) and 20 mg/kg bw/d in dogs (based on abnormal haematology and clinical chemistry and renal histopathology at ≥200 mg/kg bw/d). When the outcomes of these two chronic exposure studies are considered together, it is reasonable to set the pivotal NOEL at 20 mg/kg bw/d, to which a safety factor of 100 should be applied, comprising a 10-fold uncertainty factor for extrapolating from experimental animals to humans, and a 10-fold uncertainty factor to allow for variation in the human population. Therefore, the ADI for prohexadione-calcium is 0.2 mg/kg bw/d.

The acute reference dose (ARfD) is the estimate of the amount of a substance in food or drinking water, expressed on a milligram per kilogram body weight basis, that can be ingested over a short period of time, usually one meal or during one day, without appreciable health risk to the consumer on the basis of all known facts at the time of evaluation. The TGA considers that where there are data and toxicological endpoints, an ARfD should be set for all compounds that can potentially be present as residues in food or drinking water. An exception would be where there is so little toxicity that is difficult to establish endpoints relevant to single-dose exposure.

The studies most relevant to setting an ARfD are acute toxicity or neurotoxicity studies, and developmental toxicity studies (given that foetal malformation or death could occur in response to a single day's exposure during a critical period of development). Effects seen at the outset of repeat-dose toxicity studies may also be relevant if they could have arisen from a single day's exposure.

Prohexadione-calcium is of demonstrably low acute oral toxicity, having an LD_{50} of >5000 mg/kg bw in mice and rats. At this dose, no clinical signs or effects on internal organs were observed. However, although chemicals with such low acute toxicity would not normally require an ARfD to be set, prohexadione-calcium caused foetal abortions in rabbits at ≥200 mg/kg bw/d, an end-point which could potentially arise from a single exposure. The NOEL for this effect was 150 mg/kg bw/d. Therefore, an ARfD of 1.5 mg/kg bw/d should be set for the protection of women of childbearing age, applying a 100-fold safety factor (incorporating 10-fold factor for interspecies extrapolation and variation within the human population) to the NOEL of 150 mg/kg bw/d for developmental toxicity in rabbits. Therefore, the ARfD for prohexadione-calcium is 1.5 mg/kg bw/d.

POISONS SCHEDULE

In summary, prohexadione-calcium is of low acute toxicity, is a slight eye irritant, would pose a relatively low hazard from repeated use and is unlikely to produce irreversible toxicity. At its 46th meeting, on 21-23 February 2006, the NDPSC agreed that, based on the low acute toxicity and slight eye irritancy, prohexadione-calcium be included in Schedule 5 of the SUSDP.

CONCLUSIONS OF THE TOXICOLOGICAL ASSESSMENT

There are no objections on public health grounds to the registration of REGALIS PLANT GROWTH REGULATOR containing 100 g/L prohexadione-calcium. REGALIS PLANT GROWTH REGULATOR can be used safely if handled in accordance with the instructions and safety directions on the product label and is not an undue hazard to the safety of people exposed to it during its handling and is not be likely to have an effect that is harmful to human beings

RESIDUES ASSESSMENT

REGALIS PLANT GROWTH REGULATOR contains the new active constituent prohexadione-calcium and is used to reduce stem length in apple trees. As part of the residues assessment for prohexadione-calcium, plant and animal metabolism studies, supervised residue trials, processing studies, trade aspects, environmental fate and chemistry were considered, and details are provided below.

Metabolism of prohexadione-calcium

Plants

When applied to barley and rice, prohexadione-calcium is rapidly absorbed and translocated mainly to the leaf and stem. A relatively small proportion of the applied dose (~15% total radioactive residues (TRR)) is translocated to the root and developing head. Prohexadione-calcium undergoes conversion to its free acid form followed by rapid depletion within the first week. However, after this initial decline residues plateau and no substantial radioactive decrease in stem, leaf, straw, grain or root was observed after 8 days. This may indicate binding of the free acid moiety to insoluble plant fractions. Prohexadione free acid was the major metabolite in barley and rice at all time points assayed (2, 8 and 66 days after treatment (DAT)). All other metabolites were present at levels below 10 % TRR.

The metabolism of prohexadione-calcium in apple is extensive and results in the formation of numerous components at relatively low levels. Two primary metabolites; 4,5-dicarboxy-2-pentone (BX112-M10, 9.21% TRR, 0.028 mg/kg equivalents) and calcium 4-acetyl-3-oxido-5-oxo-3-cyclohexene (BX112-I5, a desmethyl metabolite, 11.78% TRR, 0.036 mg/kg equivalents) were identified in organosoluble fractions. The remaining identifiable metabolites (n=7) accounted for 27% TRR (individually not more than 8%). A large number of unidentified metabolites (n = 20; individually no more than <5% TRR) were also accounted for. Overall 97% of the radioactivity was accounted for following extraction. The free acid prohexadione constitutes only a minor portion of the extractable radioactivity at 1.8% of TRR.

Animals

In lactating goats, ingested prohexadione-calcium is rapidly absorbed from the gastro-intestinal tract. Maximum concentrations of radioactivity in blood and plasma occurred by about eight hours after dosing.

The major route of excretion was via the urine (73-83%) with a minor proportion excreted in the faeces (11-12%). Elimination was rapid with 95% eliminated within eight hours and 98 % eliminated within 23 hours following a single dose exposure. Negligible enterohepatic recycling was observed (<1% TRR, as free acid in bile). Elimination via milk was negligible (0.1% TRR cumulative, equivalent to a mean daily excretion of 0.328 mg/L equivalents).

Kidney contained the highest residues found in edible tissues and this is consistent with the major route of elimination. Total radioactive residues (TRR) for all tissues based on a cumulative dose was <1%, as such no bioaccumulation is expected to occur from continual dosing.

Total radioactivity in kidney at 8 hours after the final dose was 25.9 mg/kg equivalents. This residue consisted of 5.7 mg/kg equivalenta prohexadione free acid (BX-112) and 4.1488 mg/kg equivalents desisopropionyl metabolite (KI 1902).

Liver accounted for 3.87 mg /kg equivalents and muscle 0.68 mg/kg equivalents primarily as the free acid.

The residue components in the remaining tissues and milk were not elucidated due to low analyte levels and interference from protein and fats in the remaining tissues.

Given the nature of the study, the persistence of residues cannot be determined. However, there is a strong indication that absorption and elimination are rapid with no bioaccumulation. Enterohepatic recirculation was negligible.

Rat metabolism studies demonstrated a very similar metabolic profile in that the free acid comprises a majority of the residue in rat tissues.

Analytical methods

Details were provided for two validated analytical methods used to determine the concentrations of the prohexadione free acid and its desisopropionyl metabolite in plant and animal commodities. Briefly, the methodology involves extraction with acidified acetonitrile followed by solid phase separation (SPE) purification and either assay using HPLC/UV (parent free acid in plant commodities only) or methylation to enable analysis using GC/MS (parent free acid and desisopropionyl metabolite in both plant and animal commodities). An inter-laboratory study was also submitted for the GC/MS method. Limits of quantitation (LOQs) for the GC/MS method in mammalian tissues, milks and apple were 0.05, 0.01 and 0.05 mg/kg, respectively. The limit of Quantitation for the HPLC/UV method in apple was 0.05 mg/kg.

Residue definition

Methods used to detect residues in apple were limited to quantification of the free acid (<2% TRR). The results indicate that even if the method were to include the two remaining primary metabolites (~22% TRR), it is unlikely that total residues would be present at levels above the limit of detection (LOD, 0.02 mg/kg) in apple. In apple the free acid is confirmed as the target residue.

In rice and barley the major metabolite detected was the free acid in free or conjugated form and this is confirmed as the target residue.

In mammals (rats and lactating goats) the major metabolite detected was the free acid in free or conjugated form[1]. While the desisopropionyl metabolite was observed at 16% in kidney it was not observed in rat tissues and the rapid excretion of the active indicates that this metabolite may be transient in nature. Any long-term residues (post 24 hours) of prohexadione will consist primarily of the conjugated and unconjugated free acid moieties of prohexadione. The free acid is confirmed as the target residue in livestock tissues.

The following residue definition is recommended for prohexadione-calcium:

[1] The Analytical method reduces bound and unbound free acid residues to the free acid moiety for quantification

Compound	Residue definition
prohexadione-calcium	Sum of the free and conjugated forms of prohexadione expressed as prohexadione

Residues trials

The Applicant has proposed that REGALIS PLANT GROWTH REGULATOR be applied to apples as a 2-3 spray program at intervals of up to 5 weeks, commencing when shoots are 3-5 cm long. As a result of this use pattern application may occur within a month of harvest for early season varieties. However, the Applicant has proposed a withholding period (WHP) of 8 weeks (56 days) to restrict late season use of the product in apples.

In Australian trials (n=2) REGALIS PLANT GROWTH REGULATOR was applied as per the proposed use pattern. Treated fruit were sampled from 63 - 121 days after treatment (DAT). Residues occurring at these time points were below the LOD (<0.02).

The Australian trials submitted by the Applicant support the proposed MRLs of <0.02 mg/kg. However, as the earliest sampling point in these trials occurs 63 DAT, these trials cannot be used in support the proposed WHP.

In New Zealand trials (n=3) REGALIS PLANT GROWTH REGULATOR was applied with a similar use pattern but at rates equivalent to or up to 2.3x (g ai/100L) the proposed Australian rate. Treated fruit were sampled from 0 - 70 DAT. Residues occurring 56 DAT were below the LOD (<0.02 mg/kg) at all application rates.

The New Zealand trials submitted by the Applicant support the proposed maximum residue limit (MRL) of *0.02 mg/kg at the proposed WHP of 56 days when the product is applied as per the Australian use pattern.

In European trials (France, Germany, Italy, n=18) REGALIS PLANT GROWTH REGULATOR was applied twice at rates equivalent to 2x (g ai/100L) the Australian use pattern. All trials were sampled immediately after application and at intervals up to 114 DAT. In all trials, samples taken at 42 – 44 DAT demonstrated no prohexadione residues above the LOQ (<0.05 mg/kg). In one trial (Champagne-Ardennes, France), residues were detected in apples sampled at 40 DAT at a level of 0.052 mg/kg.

The European trials submitted by the Applicant support an MRL of *0.05 mg/kg (LOQ for European trials) at the proposed WHP of 56 days when the product is applied at rates up to twice the Australian use rate. However, REGALIS PLANT GROWTH REGULATOR was only reapplied twice in these trials (the Australian use pattern is for application up to 3 times). This disparity in the use pattern is not expected to have a significant bearing on the residues levels observed. It can also be inferred from the New Zealand trials that residues would most likely not occur above the analytical method LOD (0.02 mg/kg) which equates to the proposed Australian MRL.

In one processing study prohexadione-calcium was applied to apple at a rate 24x that proposed under Australian conditions. Following harvest 45 DAT significant residues were found in all processed fractions (washed fruit, wet pomace and juice). Washing removed a significant proportion of the residues (18.0 – 23.3%). The residues remaining in the fruit (initial wt. 19.73 kg) constituted 16.30 – 22.6% in the wet pomace (2.44 kg, 39.6% dry

matter) and 39.0 – 44.4% in the juice (15.46 kg). This equates to concentration factors[2] of 0.79, 0.90 and 0.58 in washed apple, wet pomace and juice. As no concentration occurs in the processed commodities separate MRLs for wet pomace and juice are not required. However, dry pomace (39.6% dry matter) is calculated to contain residues up to 2.25x that of the whole fruit.

The American processing study submitted by the Applicant can be used to support MRLs in processed commodities as the study allows processing factors to be determined for the separate processed commodities. Application of these processing factors to residues observed in other trials demonstrates that residues in all processed fractions except dry apple pomace are estimated to be below LOQ at 40 DAT. The data indicate that prohexadione-calcium residues in dry pomace may exceed LOQ at day 40 and a conservative MRL of 0.1 mg/kg is recommended to account for this possibility at the proposed WHP of 56 DAT.

Based on the data submitted from Australian, New Zealand and European residues trials, a withholding period of 56 days is supported with the establishment of MRLs as follows

Table 1

Compound	Food		MRL (mg/kg)
prohexadione-calcium	FP 0226	Apple	*0.02

Table 4

Compound	Animal feed commodity	MRL (mg/kg)
ADD: prohexadione-calcium	Apple pomace (dry)	0.1

Animal commodity MRLs

Exposure estimates

A processing study examining prohexadione-calcium residues in apple commodities was submitted for evaluation. The application rate used in this study was 24 times higher than the proposed Australian use pattern.

An estimate of the exposure of cattle and poultry to prohexadione-calcium residues (through consumption of contaminated pomace) is provided in the following two tables.

Cattle- 500 kg bw, feed intake 20 kg DM/day

Feed group	**Commodity**	**% in diet**	**Feed intake (kg/day)**	**Highest Residue, mg/kg**	**% DM**	**Livestock dietary exposure**	
						mg/animal	**mg/kg bw**
Fruit by-product	Apple Pomace (dry)	20	4	0.1	100	0.4	0.0008

[2] Processing factor = Ave residue in processed fraction/Ave. residue in raw agricultural commodity

Poultry- 2 kg bw, feed intake 0.15 kg DM/day

Feed group	Commodity	% in diet	Feed intake (kg/day)	Highest Residue, mg/kg	% DM	Livestock dietary exposure	
						mg/animal	mg/kg bw
Fruit by-product	Apple pomace (dry)	5	0.1	0.1	100	0.01	0.005

The data indicate cattle and poultry may be exposed to residue levels of 0.0008 and 0.005 mg/kg bw, respectively following exposure to pomace treated at the proposed Australian rate. Review of the metabolism trials shows that goats exposed for 10 days to residues equivalent to 0.45 ppm in the feed demonstrate TRRs below the analytical method LOQ for prohexadione-calcium. Based on these data, it is recommended that prohexadione-calcium MRLs be set at or about the LOQ of the analytical method for milks (*0.01 mg/kg), mammalian meat (*0.05 mg/kg) and edible mammalian offal (*0.05 mg/kg).

Spray drift

REGALIS PLANT GROWTH REGULATOR is to be applied by ground application. The Applicant did not provide any residues data to address the issue of spray drift. Therefore, in the absence of actual data, an estimate of likely drift is considered.

If 10 % of the prohexadione-calcium applied to one hectare of orchard were assumed to drift uniformly onto an adjacent pasture of area of one hectare, then the amount of prohexadione-calcium deposited would be 10 g prohexadione-calcium/ha. If pasture contains 1500 kg dry matter per hectare, the resultant residue level in the pasture would be 6.6 mg/kg on a dry weight basis i.e. 6.6 ppm in the feed.

Degradation in animals
Data from the animal transfer study conducted in lactating goat show that, when fed the equivalent of 380 ppm in the feed, the levels of prohexadione-calcium residues in milk and edible tissues were above the method LOQ. Extrapolation of these residues to a 6.6 ppm feed equivalent demonstrate residues above the method LOQ may occur in animal commodities. However, metabolism data demonstrate that prohexadione-calcium undergoes rapid absorption and excretion as the free acid metabolite (bound or unbound to glucuronides).

It may be concluded that consumption of spray drift-contaminated feed by livestock may result in detectable prohexadione-calcium residues in animal commodities. However, given the rapid absorption and excretion of the active, these residues would be transient in nature and it is concluded that residues above the LOQ would not present after a day or two.

The label statements and standard industry practice in relation to spray applications are adequate to address possible spray drift issues.

Risk Assessment Conclusions

Dietary risk assessments

The chronic dietary exposure to prohexadione-calcium 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[3] and is a conservative estimate of dietary exposure to chemical residues in food. The NEDI for prohexadione-calcium is equivalent to 0.1% of the acceptable daily intake (ADI).

It is concluded that the chronic dietary exposure of prohexadione-calcium is acceptable and residues in food will not pose an undue hazard to the safety of people.

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[3] 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 NESTIs for all relevant commodities are summarised in the following table. The highest acute dietary intake was estimated at 1.8% of the ARfD. It is concluded that the acute dietary exposure is acceptable.

Commodity	**Acute exposure (% of the acute reference dose)**	
	Children (2-6 years of age)	**Whole population (2 years and above)**
Apple	1.82	1.29
Meat (mammalian)	0.27	0.16
Edible offal (mammalian)	0.02	0.06
Milks	0.76	0.30

Standards

The following changes to the *MRL Standard* are recommended:

Table 1

Compound	Food		MRL (mg/kg)
ADD: prohexadione-calcium	FP 0226	Apple	*0.02
	MM 0095	Meat (mammalian)	*0.05
	MO 0105	Edible offal (mammalian)	*0.05
	ML 0106	Milks	*0.01

Table 3

Compound	Residue definition
ADD: prohexadione-calcium	Sum of the free and conjugated forms of prohexadione expressed as prohexadione

Table 4

Compound	Animal feed commodity		MRL (mg/kg)
ADD: prohexadione-calcium		Apple pomace (dry)	0.1

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

ASSESSMENT OF OVERSEAS TRADE ASPECTS OF RESIDUES IN FOOD

Use of the product in accordance with the label instructions is unlikely to risk Australian trade as chemical residues are expected to remain below the limit of quantitation for the commodities of concern (apple, mammalian meat, milks and offal).

OCCUPATIONAL HEALTH AND SAFETY ASSESSMENT

BASF Australia Ltd has submitted a data package seeking registration of a new product, REGALIS PLANT GROWTH REGULATOR, which is intended for the bio-regulation of apple shoot growth. The product will be mixed at 50 - 75 g/100 L and applied as an air-assisted spray using 800 - 3000 L/ha spray volume (0.04 – 0.225 kg ai/ha) in apple orchards with maximum of 3 sprays per season with 3-5 week intervals between applications. The higher application rate is recommended on large, vigorous trees.

Label restraints include warning against aerial application of the product, application of the product within 3 days of sprays containing calcium, ethylene or gibberellic acid, and application of the product more than 3 times per season.

A withholding period of 8 weeks is recommended for apple harvesting. The applicant has stated that no post application activities will be necessary in apple orchards until the spray has dried.

Contract workers may apply the product in various apple orchards. Considering the time length from shoot growth to fruit set (which is ~3 months), a NOEL established in a sub-chronic study is considered more appropriate for the OHS risk assessment of prohexadione-calcium.

Based on the findings of toxicological studies evaluated, prohexadione-calcium has low acute oral, dermal and inhalation toxicity. It is not a skin irritant or a skin sensitiser, but a slight eye irritant. REGALIS PLANT GROWTH REGULATOR has low acute oral, dermal and inhalational toxicity. It is not a skin sensitiser but a slight eye and skin irritant.

Based on the product use pattern information and toxicology data provided, the proposed use of the product would not cause any undue health hazard to workers. Personal protective equipment has been recommended based on acute and repeat dose risk assessments. Based on estimated post application dermal exposure for workers undertaking crop management activities, a re-entry statement was recommended.

There are no objections on OHS grounds to the registration of the product, REGALIS PLANT GROWTH REGULATOR, containing 100 g/L of prohexadione-calcium. The following first aid instructions and safety directions should appear on the product label:

FIRST AID INSTRUCTIONS

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

SAFETY DIRECTIONS

The hazard-based safety directions, together with any PPE requirements established during the OHS assessment, form the safety directions which will be included in the FAISD Handbook, and should be included on the product label:

Will irritate the eyes and skin

Avoid contact with eyes and skin

When opening the container and preparing spray, wear elbow-length PVC gloves

Wash hands after use

THE FOLLOWING RE-ENTRY STATEMENT IS RECOMMENDED ON THE PRODUCT LABEL.

Do not allow entry into treated areas until the spray has dried, unless wearing cotton overalls (or equivalent clothing) and chemical resistant gloves. Clothing must be laundered after each day's use.

CONCLUSIONS OF THE OH&S ASSESSMENT

The data provided and considered in this assessment justify the Safety Directions established in the present evaluation. The proposed use of Regalis Plant Growth Regulator will not be an undue hazard to the safety of people exposed to it during its handling and it is not be likely to have an effect that is harmful to human beings.

ENVIRONMENTAL ASSESSMENT

INTRODUCTION

BASF Australia Ltd has applied for the registration of a new product, REGALIS PLANT GROWTH REGULATOR, containing the new active constituent prohexadione-calcium at 100 g ai/kg. REGALIS PLANT GROWTH REGULATOR is to be used to inhibit vegetative growth in apples and for the optimisation of fruit yield. The active ingredient is mainly absorbed and translocated in the plant where it inhibits the biosynthesis of gibberellin. It will be applied at either 50 g/100 L spray, which at 2000 L/ha corresponds to 1.0 kg/ha of Regalis (100 g ac/ha), or 75 g/100 L spray for larger trees, which at 3000 L/ha corresponds to 2.25 kg/ha of Regalis (225 g ac/ha).

ENVIRONMENTAL FATE

Hydrolysis

In a study conducted to meet German BBA Guidelines the hydrolysis of prohexadione-calcium was pH-dependent and followed first order kinetics. The half lives were 5, 25 and 83 days at 20°C and pH 5, pH 7 and pH 9 respectively. The proposed degradation pathway involved protonation followed by cleavage of the propionyl side chain to give KI-5376 (3,5-dioxocyclohexanoic acid). Prohexadione-calcium is rated as fairly hydrolysable at pH 5, moderately hydrolysable at pH 7 and slightly hydrolysable at pH 9.

Photolysis

The aqueous irradiation of prohexadione-calcium, prohexadione and KI-5376 with natural sunlight was studied at pH 6.1 for 9 weeks. The pathway proposed for photolysis of prohexadione-calcium was the same as for hydrolysis with formation of KI-5367, which then degrades under light to give tricarballylic acid and glutaric acid together with some minor degradates. This was confirmed in the studies where prohexadione and KI-5367 were exposed to natural sunlight. The half-lives under irradiated conditions were estimated as 4.2 days but the half-life for the dark control was not given. Prohexadione-calcium will hydrolyse/photolyse with a half-life of <1 week.

The half-life for reaction of prohexadione-calcium with hydroxyl radicals in the atmosphere was calculated as 31 hours but it is not expected to be volatile.

Metabolism
Aerobic soil

The metabolism of prohexadione-calcium was studied in 4 soils, classified as loamy sand, loam, silt loam and loam sand, under aerobic conditions at 20°C according to BBA Guidelines. Metabolism was fast, with all the DT50s calculated as <2 days, with an average of about 1 day. The only significant metabolite was carbon dioxide which was approximately 60% of the applied radioactivity for all soils after 16 days. Soil bound radioactivity reached maxima of between 19 and 27% of applied for all soils. At 10°C, degradation of prohexadione-calcium was slower, as expected, with the half-lives estimated as up to 8 days and evolution of carbon dioxide reached between 54 to 60% of applied after 32 days. The Department of Environment and heritage (DEH) concludes that in microbially active soils prohexadione-calcium degrades rapidly and is rated as readily degradable.

Aerobic aquatic metabolism

The aerobic aquatic metabolism of prohexadione-calcium was conducted according to BBA Guidelines using two sediment-water systems from Scotland. No metabolites were detected in the overlying water, only parent compound. Evolution of carbon dioxide reached 76% and 84% of the applied radioactivity for both systems. There was a maximum of 12.5 % of the applied radioactivity found in the sediments. The DT50s were calculated as 32 and 12 hours respectively using a second order kinetic analysis.

Anaerobic soil metabolism

The anaerobic aquatic metabolism of prohexadione-calcium was conducted according to BBA Guidelines using 4 soils (same soils as used for the aerobic study). The soil was incubated under nitrogen before being dosed with prohexadione-calcium and incubated at 25°C under nitrogen for 32 days. The major degradation product was carbon dioxide with some soil bound residues. The average half life was determined using first order analysis as 2 days and the longest as 4.2 days.

Ready biodegradation

The biodegradability of prohexadione-calcium was determined in a manometric respirometry test according to OECD Guidelines. It is not readily biodegradable according to the test requirements.

Mobility
Adsorption/desorption

The adsorption/desorption of prohexadione-calcium was studied by the batch equilibrium method using 4 sterilized agricultural soils. Prohexadione-calcium moderately adsorbed onto all soils with K_{oc}s ranging from 82 to 307 and was classified as having medium to high mobility (McCall classification). Desorption showed that between 44 to 87% desorbed from the soils.

Leaching potential

A column leaching study was conducted using two soils in accordance with BBA Guidelines. The soils used were a loam and a loamy sand, the same that had been used previously. The majority of the applied radioactive, >72%, was recovered in the gas traps or eluents and identified as carbon dioxide. There was significant radioactivity recovered as soil bound residues (maximum as 33% of applied).

A second leaching column study was conducted using three standard German soils, Speyer 2.1, 2.2 and 2.3. Prohexadione–calcium underwent rapid mineralisation to give mainly carbon dioxide for Speyer 2.1 and 2.2 soils, with some radioactivity being retained in the soil. For the Speyer 2.3 soil, the leaching conditions affected the result with those that were free draining giving mainly CO_2 and some soil bound radioactivity while for those that were not free draining there was significantly less radioactivity recovered as CO_2. DEH concluded that the study shows very rapid degradation of prohexadione-calcium no leaching occurs under aerobic conditions and but in waterlogged/anaerobic conditions, prohexadione-calcium (or prohexadione) can leach through the soil profile.

Two aged leaching column studies were conducted using the two agricultural soils that where used in the first study and the Speyer 2.1 soil in accordance with BBA Guidelines. The soils were aged for 13 hours before being applied to the top of the corresponding soil column. During the aging there was significant mineralisation, and further mineralisation occurred during the elution phase. Total CO_2 recovered was up to 61% of applied radioactivity. Extraction of the soil sections showed that almost the entire radioactivity in the soil sections was as soil bound residues.

Field Dissipation

No studies were presented. Given the rapid degradation and mineralisation in the laboratory studies, the absence of field studies is acceptable.

Bioaccumulation

No bioaccumulation studies were presented. This is acceptable given that the water solubility and rapid degradation indicates that bioaccumulation is highly unlikely.

ENVIRONMENTAL TOXICITY

Avian

Prohexadione-calcium was found to be practically non-toxic to bobwhite quail in a single oral dose acute study conducted to meet US EPA requirements, with an LD50 estimated to be greater than 2000 mg/kg, the highest dose tested. Two 5-day dietary studies in bobwhite quails and in mallard ducks, conducted to US EPA requirements, similarly showed that prohexadione-calcium was practically non-toxic to the test species, with LC50 values estimated to be greater than 5200 ppm, the highest concentration tested, in both cases. A six week study testing subchronic toxicity and reproductive effects on Japanese quail fed prohexadione-calcium in the diet, found no clear dose-response effects of prohexadione-calcium. The NOEC to be greater than the highest concentration tested of 1000 mg/kg in the diet.

Aquatic

Acute toxicity studies on bluegill sunfish (*Lepomis macrochirus*) and rainbow trout (*Oncorhynchus mykiss*) determined that prohexadione-calcium is practically nontoxic to fish, with 96 LC50s estimated at greater than 100 mg/L in a limit test conducted to US EPA and OECD requirements.

An acute toxicity study on rainbow trout conducted to OECD requirements determined that the formulated product is practically non-toxic to fish, with a 96 LC50 of 117 mg/L (actual concentration). Persistent turbidity was observed at concentrations of 25 mg/L and above. Sublethal effects such as restlessness were noted at concentrations of 63 mg/L BAS 125 10 W and greater. Therefore, the NOEC of the formulated product is 63 mg/L.

A chronic study on survival and growth of juvenile rainbow trout demonstrated little toxic effect of prohexadione-calcium. There were no significant effects of exposure to prohexadione-calcium over 28 days on survival, growth rates or weight, when compared to controls. Therefore, the 28 d NOEC was set at 100 mg/L, the maximum nominal concentration tested.

For aquatic invertebrates the acute 48 h daphnia toxicity test conducted according to US EPA and OECD Guidelines using technical prohexadione-calcium gave an EC50 of > 100 mg ac/L and prohexadione-calcium was rated as non-toxic. Using the formulated product BAS 125 10 W, again tested according to US EPA and OECD Guidelines, the acute 48 h daphnia toxicity test gave an EC50 of >100 mg/L and NOEC of 100 mg/L, equivalent to 9.72 mg ac/L, and was rated as non-toxic. In the chronic 21 day study, conducted according to EEC guideline, the NOEC and LOEC for daphnia was > 100 mg ac/L with effects on survival, mobility and reproduction being not effected. In the mysid 96-h study conducted according to USA EPA guideline, the EC50 of >125 mg/L and NOEC of 125 mg/L and was rated as non-toxic.

Technical prohexadione-calcium was rated non toxic to marine diatom algae (*Skeletonema costatum*), freshwater algae (*Selenastrum capricornutum*), freshwater diatom (*Naviculla pelliculoa*) and blue-green algae (*Anabaena flos-aquae*), with measured 120-h E_bC50 of > 1.2 mg ac/L and NOEC of 1.2 mg ac/L in limit tests conducted according to USA EPA guidelines. Technical prohexadione-calcium was rated as practically non toxic to freshwater algae (*Selenastrum capricornutum*) with measure 120-h EbC_{50} of > 100 mg ac/L and a NOEC of 100 mg ac/L in a limit test conducted according to OECD and EPA Guidelines.

The formulated product BAS 125 10 W (9.72 mg ac/L) was rated as moderately toxic to freshwater algae (*Pseudokirchirchneriella subcapitata*) with a 72-h E_bC50 of 50 mg/L and a NOEC of 3.13 mg/L in a static test conducted according to EEC Directive 92/69/EEC and OECD 201 guidelines.

Technical prohexadione-calcium was rated at worst as moderately toxic to duckweed (*Lemma gibba G3*) with measured 14 days E_bC50 of >1.2 mg ac/L and a NOEC of 1.2 mg ac/L in a static-renewal limit test conducted according to USA EPA guidelines.

Non-Target Terrestrial Invertebrates

The NOEC for BAS 125 10 W (9.72 mg ac/L) to bees was > 65.28 and > 100 μg/bee for the oral and contact exposure routes respectively, tested according to OECD 213 and 214 guidelines. The tested substance can be rated as harmless to bees.

The toxicity of the formulated product BAS 125 10 W, containing prohexadione-calcium at a concentration of 9.74%, was also tested on several beneficial insect species. At rates of 5 kg BAS 125 10 W/ha, the effects of BAS 125 10 W overall were found to be minimal and was classified as harmless to lacewings, spiders, rove beetles, ground beetles and parasitise aphids but slightly harmful to predatory mites, as summarised below. The proposed rate for the Australian product is 2.25 kg/ha.

Survival of larval lacewing *Chrysoperla carnea* was unaffected by exposure to residues of BAS 125 10 W at double the Australian field application rates. Although not statistically analysed, there was also no effect on reproduction. Similarly, exposure to the formulated product BAS 125 10 W had little effect on mortality and food consumption in the wolf spider *Paradosa spp*. (Araneae, Lycosidae) when tested over two weeks.

Survival of parasitic wasps *Aphidius rhopalosiphi* was significantly reduced by exposure to BAS 125 10 W at 5 kg/ha in one test, but not in a second test. Nevertheless, behavioural abnormalities were observed in wasps exposed to the two highest concentrations of 2.5 kg/ha and 5 kg/ha in the second test.

Residues of BAS 125 10 W, when applied to glass plates, were found to be slightly harmful to predatory mites, *Typhlodromus pyri*, by causing a 38.7% reduction in beneficial capacity over two weeks. The reduction was predominantly driven by a reduction in reproductive output after exposure to BAS 125 10 W.

The formulated product BAS 125 10 W had little effect on mortality and food consumption in the ground beetle *Poecilus cupreus* when tested over two weeks. By contrast, BAS 125 10 W significantly reduced reproduction in the rove beetle, *Aleochara bilineata*, which parasitizes aphids, when tested over ten weeks. However, BAS 125 10 W had little effect on survival.

Earthworms

In tests on the effect of prohexadione-calcium technical on earthworms (*Eisenia fetida*) conducted according to OECD guidelines using artificial soil with five concentrations ranging from 198-1000 mg/kg artificial soil, there was no mortality and the LC50 was determined as > 1000 mg ac/kg and the NOEC = 1000 mg ac/kg.

The formulated product was also found to be non-toxic to earthworms (*E. fetida*) over 14 days, when maintained in artificial soil. Because no significant mortality or other treatment related effects occurred at concentrations ranging from 198 to 1000 mg BAS 125 10 W/kg soil, an LC50 could not be calculated. The NOEC was set at 1000 mg/kg, the highest nominal concentration tested.

Soil micro-organisms

Prohexadione-calcium did not show any adverse effects on soil micro-organisms respiration and nitrogen fixing in standard soil microbial tests at 500 g ac/ha. It and did not affect sewage sludge micro-organisms at concentrations up to 1000 mg/L, the soil bacteria (*Pseudomonas putida*) up to concentrations of 10 000 mg/L. The formulated product did not affect soil microbial process (respiration and nitrogen fixing) at approximately 10 times the proposed Australian rate.

Non-target vegetation

The effect of BAS 125 10 W on growth and biomass on non-target plants was evaluated at rates up to 250 and 750 g ac/ha according to OECD Guideline 208: Terrestrial plant growth. The plant species tested were the same in each; cabbage (*Brassica oleracea*), pea (*Pisum sativum*), carrot (*Daucus carota*), corn (*Zea mays*), oats (*Anena satina*) and onion (*Allium cepa*). After treatment with BAS 125 10 W damage (growth reductions) could be observed for cabbage, carrot, corn and oats (mean damage 14-50%) in the 7.5 kg/ha treatment group. The lower treatment rate (2.5 kg/ha) also caused slight to moderate injuries in cabbage, carrot, corn and oats (15-44%). In peas and onion neither concentration caused symptoms of phytotoxicity. For all plant species a normal development of control plants was observed. No statistically significant differences between the control and the test substance were observed for carrot, pea, corn and onion. For cabbage treated at 7.5 kg/ha compared to control a reduction of 27% was detected. And for oats both rates tested caused a significant reduction in biomass (22.1% lower rate and 22.2 higher rates).

Mammals

The result showed that prohexadione has little acute toxicity to rats. The chronic toxicity is also relatively low.

RISK ASSESSMENT

Risk to Terrestrial Organisms
Birds

Based on the typical diet of northern bobwhite quail and the EEC of prohexadione-calcium in food items, the concentration in the diet was calculated as 23.6 mg ac/kg diet. With the dietary LD_{50} for quail and mallards of >5620 mg ac/kg bw and NOECs of 5600 mg ac/kg bw significantly above the dietary EEC, clearly there is no hazard to birds from feeding on food items directly oversprayed.

Earthworms

The 14-d NOEC for the earthworm was >1000 mg ac/kg soil and is at least 1000 times higher than the predicted soil concentration. Thus the proposed use is not expected to pose an acute hazard to earthworms. There were no studies on chronic toxicity to earthworms but given the large safety margin for acute effects and the very rapid aerobic soil degradation, chronic effects are unlikely.

Beneficial arthropods

The hazard to honey bees is also expected to be low as the application rate of 225 g ac/ha (equivalent to 2.25 μg ac/cm^2) is approximately 50 times lower than the contact NOEC of 100 μg ac/bee, assuming that a honeybee is approximately 1 cm^2 in surface area. Effects on species such as parasitic wasps, green lacewing, ground beetles and spider are not expected as applications at rates twice to that proposed had no or limited effect in laboratory studies. There were effects on rove beetles and predatory mites in laboratory tests but at 2500 g Regalis/ha, these effects were rated as harmless.

Soil micro-organisms

The information presented on the effect of prohexadione-calcium on soil micro-organisms showed these organisms were only minimally affected at 25 kg Regalis/ha in laboratory tests. Therefore a hazard to soil micro-organisms and soil microbial processes is unlikely at the proposed rate of 2.25 kg/ha.

Hazard to Aquatic Organisms
Direct overspray

The worst-case scenario of a direct overspray of a 15 cm deep body of water with an application rate of Regalis would result in a maximum predicted environmental concentration (PEC) of 166 μg ac/L. Using the most sensitive effect on fish with the acute LC50 of 119 mg/L and NOEC of 40 mg/L, calculations indicated a low risk. Also, the 28-day NOAEL for chronic toxicity was 100 mg ac/L which indicates no hazard for chronic exposure. Acute effects on water fleas and presumably other aquatic invertebrates are also unlikely, with a 48 hour EC_{50} of >100 mg ac/L and the chronic NOEC of 100 mg ac/L.

For algae, the EC_{50} (cell density) was >100 μg ac/L and NOEC = 100 mg ac/L in one test and three limit tests showed no effect on algae and diatoms at 1.2 mg/L. As the PEC is less than the lowest NOEC (0.177 mg/L verses 1.2 mg/L), effects on these organisms are unlikely. For duckweed the EC50 was determined as > 1.2 μg ac/L but NOEC = 0.12 mg ac/L and as the PEC is larger than the NOEC (0.177 mg/L verses 0.12 mg/L), effects on duckweed cannot be ruled out. However, at 3 metres away spraydrift is estimated as 15.7% of application rate using German data. The PEC at 3 metres away is then calculated as 28 μg/L, below the NOEC of 0.12 mg/L and therefore the risk is acceptable. In addition, the rapid degradation in water/sediment (ie natural water systems) would limit the impact of any spraydrift event.

Run-off

Run-off from apple orchards could occur but this is unlikely when the rapid degradation of prohexadione-calcium is considered. A simple model showed that the initial concentration in water in a pond reached by runoff is 8.3 μg/L, significantly below the most sensitive EC50 and the NOEC for duckweed and thus the risk is acceptable.

Leaching

Prohexadione-calcium is water soluble, with limited binding to soil but a very short half-life in soil (<2 days). As indicated by the applicant, the potential concentrations in groundwater are likely to be extremely low, calculated as <0.01 μg/L due to rapid degradation. Significant leaching under field conditions appears unlikely.

Multiple Applications

The label does give directions for a maximum of 3 applications per season 3 weeks apart. Given that prohexadione-calcium degrades rapidly in soil with half-life of < 2 days, the carryover in soil between applications is negligible, <1% assuming first order degradation. Degradation in water is also rapid and the peak concentration was estimated as 150 μg ac/L. Therefore the additional hazard from 3 possible applications per year is considered very low and acceptable.

Chronic hazard

Prohexadione-calcium is unlikely to be a chronic toxicant. With the rapid degradation and given the very low acute hazard, prohexadione-calcium is not expected to cause a chronic hazard.

Desirable vegetation

At the proposed maximum spray rate of 2.25 kg Regalis/ha, adverse effects on non-target plants are considered possible and direct overspray is a hazard. In tests using both dicotyledonous and monocotyledonous seedlings, there were phytotoxic effects and the most sensitive at 250 g ac/ha were cabbage, corn and oats, though only oats was statistically significant. There may be temporary effect on some species in the orchard sod areas. Assuming that non-target plants are outside the apple orchard, spray drift at 3 m from the BBA standard spraydrift is just 15.7% of applied and effect on non-target plants outside of the sprayed orchards is unlikely.

Mammals

There is unlikely to be any significant effect on non-target mammals. As there was no acute toxicity to rats even at 2000 mg/kg bw, the hazard to mammals is expected to be low. Also chronic toxicity is unlikely given the rapid degradation and that the lowest NOAEL is 1000 ppm for prohexadione-calcium (3-month feeding trial with rats).

CONCLUSIONS OF THE ASSESSMENT CONDUCTED BY THE DEPARTMENT OF ENVIRONMENT AND HERITAGE

It was concluded by the DEH that provided the product is applied according to the proposed label requirements then there is unlikely to be an unintended effect that is harmful to animals, plants or things or to the environment.

EFFICACY AND SAFETY ASSESSMENT

The product REGALIS PLANT GROWTH REGULATOR is used for the suppression of shoot growth in apples. The applicant has provided data which show the product reduces shoot growth and lowers the requirement for summer and winter pruning. Seventeen replicated experiments, undertaken in commercial orchards, were described. Trials were conducted in Tasmania, Queensland and Victoria and comprised 4 rates of REGALIS PLANT GROWTH REGULATOR (0, 50, 100 and 150 g/L) applied initially to young shoots around the end of blossoming.

REGALIS PLANT GROWTH REGULATOR consistently and effectively suppressed shoot growth in apples in all trails except one, at Toolamba REGALIS PLANT GROWTH REGULATOR was only partially effective. Two applications of REGALIS PLANT GROWTH REGULATOR at 50 g or 75 g per 100 L were sufficient to produce a 50 % reduction in shoot length during late spring and autumn. Directions present on the draft label are adequate to cater for vigorous trees and no detrimental effects were observed on crop health or fruit quality following application of REGALIS PLANT GROWTH REGULATOR at doses up to twice the recommended rate of application.

The label claims that the product is ‘for the bio-regulation of apple shoot growth’ are supported by overseas data and results from the Australian trials. The efficacy reviewer supported the application for registration

CROP SAFETY

In terms of leaf colour, tree health, and fruit quality, there were no detrimental effects of the use of REGALIS PLANT GROWTH REGULATOR on apple trees. The application method is high volume and is unlikely to cause problem with nearby non-target organisms.

LABELLING REQUIREMENTS

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

REGALIS®
Plant Growth Regulator

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ACTIVE CONSTITUENT: 100 g / kg PROHEXADIONE-CALCIUM

For the bio-regulation of apple shoot growth,
as specified in the DIRECTIONS FOR USE table.

Net contents 500 g, 1 kg, 3 kg, 10 kg

® = Registered trademark of BASF
DIRECTIONS FOR USE:

RESTRAINTS:
DO NOT apply by aerial application.
DO NOT apply REGALIS within 3 days of sprays containing calcium , ethylene or gibberellic acid.
DO NOT apply more than 3 applications per season.

CROP	PURPOSE	RATE	CRITICAL COMMENTS
Apples	Shoot growth reduction	50 to 75 g /100L	Apply in a two or three spray program commencing when terminal shoots are 3 to 5 cm in length. Repeat applications at 3 to 5 weekly intervals. Use the higher rate on large , vigorous trees. Apply a third application if required on vigorous trees. Regalis is leaf absorbed and best suited to dilute application using medium to coarse droplets. Thorough coverage of leaves under slow drying conditions will aid uptake. Apply in water with a pH of 7 or less, use LI 700 to acidify alkaline water. Allow 6 hours from application for the product to become rainfast.

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

WITHHOLDING PERIODS: DO NOT HARVEST FOR 8 WEEKS AFTER APPLICATION

GENERAL INSTRUCTIONS

MIXING

Slowly pour the REGALIS Plant Growth Regulator into the spray tank three-quarters filled with water, with the agitation system running. Fill tank and commence spraying.

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- **APPLICATION**

Apply by ground application equipment only. Use commercial orchard equipment set to deliver a high spray volume with a medium to coarse droplet range.
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COMPATIBILITY
Regalis is compatible with most commonly used pesticides. Do not apply within 3 days of sprays containing calcium , ethylene or gibberellic acid.

RE-ENTRY PERIOD
Do NOT allow entry into treated areas until the spray has dried, unless wearing cotton overalls (or equivalent clothing) and chemical resistant gloves. Clothing must be laundered after each day's use.

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 the closed, original container, in a cool, well-ventilated area, out of direct sunlight. 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 not available, bury the containers below 500 mm in a disposal pit specifically marked and set up for this purpose clear of waterways, vegetation and roots. Empty containers and product should NOT be burnt.

SAFETY DIRECTIONS
Will irritate the eyes and skin. Avoid contact with the eyes and skin. When opening the container and preparing spray, wear elbow-length PVC gloves. Wash hands after use.

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

MSDS
Additional information is listed in the Material Safety Data Sheet.

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

THIS PRODUCT IS NOT CONSIDERED TO BE A DANGEROUS GOOD UNDER THE AUSTRALIAN CODE FOR THE TRANSPORT OF DANGEROUS GOODS BY ROAD AND RAIL

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APVMA Approval No.: **59683**

® = Registered trademark of BASF
Label Version: V060606

Product number:
Batch Number:
Date of Manufacture:
Customer Service Hotline: 1800 635 550

BASF Australia Ltd
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Noble Park
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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.

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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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