Public Release Summary

Australian Pesticides & Veterinary Medicines Authority

Coopers Colleague Broad Spectrum Sheep and Lamb Drench

APVMA Product Number 59304

June 2007

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The APVMA invites comments on this PRS until 3 July 2007. Submissions should be sent to:

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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 Aging (Office of Chemical Safety), Department of Environment and Water Resources (Risk Assessment and Policy Section), and State Departments of Agriculture and Primary Industries

The APVMA has a policy of encouraging openness and transparency in its activities and of seeking community involvement in decision making. Part of that process is the publication of public release summaries for 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 *The Manual of Requirements and Guidelines for Veterinary Chemicals (Vet MORAG).*

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

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

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LIST OF ABBREVIATIONS AND ACRONYMS

ac	active constituent
ADI	Acceptable Daily Intake (for humans)
AchE	acetylcholinesterase
ALAT	alanine aminotransferase
AM	arithmetic mean
AP	alkaline phosphatase
ARfD	Acute Reference Dose
BCF	bioconcentration factor
bw	bodyweight
BZ	benzimadazole
CAS	Chemical Abstracts Service
ChE	cholinesterase
cm	centimetre
CXL	Codex maximum residue limits
d	day
DAT	days after treatment
DEW	Department Environment and Water Resources
DMW	dilute mineral water
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
ESI	export slaughter interval
EUP	end use product
F_o	original parent generation
F_1	first generation
g	gram
GAP	Good Agricultural Practice
GCP	Good Clinical Practice
GC-MS	gas chromatography –mass spectometry
GLP	Good Laboratory Practice
GM	geometric mean
GMP	Good Manufacturing Practice
GVP	Good Veterinary Practice
h	hour
ha	hectare
Hb	haemoglobin
HDPE	high density polyethylene

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
IUPAC	International Union of Pure and Applied Chemistry
kg	kilogram
K_{ow}	octanol water 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
LEV	levamisole
LOD	Limit of Detection – level at which residues can be detected
LOQ	Limit of Quantification – level at which residues can be quantified
meq	millequivalent
mg	milligram
mL	millilitre
ML	lega litre / macrocyclic lactone
MRL	Maximum Residue Limit
MSDS	Material Safety Data Sheet
NDPSC	National Drugs and Poisons Schedule Committee
NESTI	National Estimated Short Term Intake
ng	nanogram
NHMRC	National Health and Medical Research Council
NOEC/NOEL	no observable effect concentration level
OECD	Organisation for Economic Co-operation and Development
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
USEPA	United States Environmental Protection Agency
USFDA	United States Food and Drug Administration
µg	microgram
vmd	volume median diameter
WHP	withholding period

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1. INTRODUCTION

This publication provides a summary of the data reviewed and an outline of regulatory considerations for the proposed registration of *COOPERS® **COLLEAGUE™** Broad Spectrum Sheep and Lamb Drench*, hereinafter referred to as *Colleague*, containing the new active constituent, pyraclofos and the approved active constituent, albendazole. The product is for use as a broad spectrum oral drench for use in sheep and lambs for the control of sensitive gastrointestinal roundworms, lungworms and tapeworms. The product also claims to aid in the control of adult liver fluke, and reduce the output of viable worm and fluke eggs.

Colleague was previously registered by the NRA and released onto the Australian market by SmithKline Beecham in late December 1993. After the release, adverse events were reported which included the death of sheep. SmithKline Beecham chose to recall *Colleague* and withdrew the product from the market in 1994.

Gastrointestinal parasites in sheep throughout Australia have demonstrated resistance to many of the currently registered anthelmintics. The applicant contends the sheep industry needs access to another organophosphate formulation in order to provide parasite control without exerting further pressure on other drug classes as the justification for re-registering *Colleague.*

The information provided herein presents only the conclusions reached by various reviewers after consideration of the scientific database.

The Australian Pesticides and Veterinary Medicines Authority (APVMA) has completed an assessment of the data submitted by the applicant, Schering-Plough Animal Health in support of the registration of *Colleague* containing the active constituents pyraclofos and albendazole and now invites public comment before deciding whether to approve this product for use in Australia. The information contained in this document is provided for public comment.

Written comments should be received by the APVMA by 4 July 2007. Comments should be sent to:

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1.1. Applicant

Schering-Plough Animal Health
Locked Bag 5011
Baulkham Hills
NSW 2153

1.2. Formulated Product

Formulator	Bayer CropScience Pty Ltd	Autopak-Vetlab Group Pty Ltd	Argenta Manufacturing Ltd
Formulation plant	16 Lucca Road WYONG NSW 2259	39 Harris Street ST MARYS NSW 2760	2 Sterling Avenue Manurewa, Auckland NEW ZEALAND
Formulation Type	Liquid Suspension		

This formulation is not registered overseas.

2. CHEMISTRY AND MANUFACTURE

2.1. Active Constituent

The chemical active constituent pyraclofos is manufactured by Sumitomo Chemical Tadeka Agro Company Ltd, Nihonhashi Sunrise Building, 13-10 Nihonbashi 2-Chome Chuo-Ku,Tokyo, JAPAN; and has the following properties:

Common name: Pyraclofos

Chemical name IUPAC: (*RS*)-[*O*-1-(4-chlorophenyl)pyrazol-4-yl *O*-ethyl *S*-propyl phosphorothioate]

CAS Registry Number: 89784-60-1

Empirical formula: $C_{14}H_{18}ClN_2O_3PS$

Molecular weight: 360.8

Appearance: Pale yellow liquid

Boiling Point: 164^{o}C

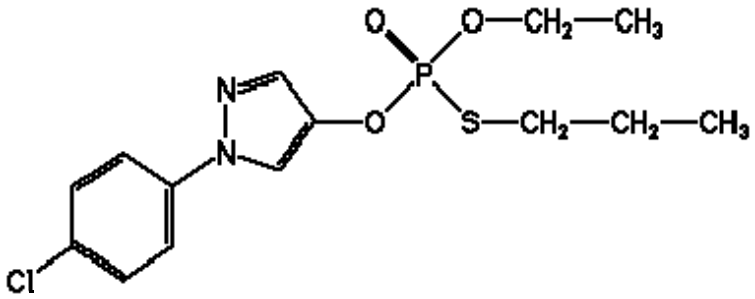
Relative Density: 1.271 g/cm^3 at 28^{o}C

Vapour Pressure: 1.2 x 10^{-8} mm.Hg at 20^{o}C

Water Solubility: 3.3 x 10^{-2} g/L at 20^{o}C (33 ppm)

n-Octanol/Water Partition Coefficient: log K_{ow} = 3.77 at 20^{o}C

Structural formula:



2.2. Formulated Product

Colleague is an oil suspension for oral administration containing 150 g/L pyraclofos and 19 g/L albendazole as the active ingredients. The product is an orange-brown liquid with a banana odour, it is non-volatile and immiscible with water. The Material Safety Data Sheets (MSDS) for each of the excipients was provided. All formulators of the product are Good Manufacturing Practice (GMP) licensed/accredited.

Colleague will be packed in either 5 L high-density polyethylene (HDPE) backpacks or 12 L HDPE drums. The product should remain within specifications for at least two years when stored below 30 ºC. The label should also contain an instruction to store out of direct sunlight.

The Chemistry and Residues Program (CRP) has evaluated the chemistry and manufacturing aspects of *Colleague* (composition and form of constituents, manufacturing process, quality control, stability, and specifications for containers for the product) in data submitted by Schering-Plough Pty Ltd to support their application for product registration. The CRP is satisfied that the chemistry requirements of Section 14(5) *Agricultural and Veterinary Chemicals Codes* have been met.

3. TOXICOLOGICAL ASSESSMENT

3.1. Evaluation of toxicity

3.1.1. Introduction

The toxicological assessment was conducted at the time of initial registration in 1993. No new assessment was conducted for this application and the findings from the original assessment are presented below.

The toxicological database for pyraclofos, which consists primarily of toxicity tests conducted using animals, is adequate to assess the likely public health implications of the proposed uses of *Colleague*. In interpreting these data, it should be noted that animal toxicity tests generally use doses or exposures which are high compared to likely human exposures. The use of high doses increases the likelihood that potentially significant toxic effects will be identified. Toxicity tests should also indicate dose levels at which the specific toxic effects are unlikely to occur. Such dose levels as the No-Observable Effect level (NOEL) are used to develop appropriate standards for exposures which can be tolerated by humans.

Pyraclofos is rapidly absorbed and is minimally retained in the tissues. It is almost completely metabolised and the metabolites are excreted in the urine and faeces. The acute toxicity of technical grade pyraclofos is moderate by oral and inhalation routes and low when applied to the skin. It is not an irritant to skin or eyes and is not a skin sensitiser.

In common with other organophosphate insecticides, pyraclofos is a powerful inhibitor of the family of enzymes which break down choline esters. Since the effects are only slowly reversible, the effects of chronic exposures tend to be cumulative. The main toxic effects are due to inhibition of acetylcholinesterase (AchE), the consequences of which are excessive and prolonged stimulation of nerves using acetylcholine as a neurotransmitter, resulting in salivation, sweating, slow pulse and muscle twitching as the more obvious effects.

The relative susceptibility of cholinesterases are plasma>red cell>brain. The inhibition of plasma cholinesterase was used to establish the NOEL, the lowest NOEL for this effect was 0.1 mg/kg body wt/day in a 2-year mouse feeding study. Cholinesterase inhibition is by far the most sensitive indicator of toxicity. In long-term oral dosing studies in mice, rats and dogs, there were relatively few toxic effects (mild anaemia, thyroid follicle enlargement in rats; mild liver toxicity in rats and dogs; none in mice) at dose rates 50-100 times higher than the threshold for inhibition of cholinesterases.

Pyraclofos was negative in tests designed to show the potential for this class of compounds to cause progressive nerve degeneration. Pyraclofos does not induce malignant cancers, have toxic effects on genetic material (DNA), or cause birth defects or reproductive toxicity in appropriate tests.

3.1.2. Toxicokinetics and Metabolism

Following oral administration of ^{14}C-pyraclofos (7-10 mg/kg) to rats, radioactivity in the blood rose to maximum levels within 4 hours and had practically disappeared within 24 hours. Excretion of radiolabel was rapid and extensive, and was mainly via the urine (85-90%) and the faeces (2-5%) in the first 24 hours. The excretion pattern was similar after a dose of 110 mg/kg po. Residual radiation in the tissues was generally very low. Following daily administration of pyraclofos (5mg/kg/day for 7 days) excretion was similar to that seen after a single dose.

The major urinary metabolites of pyraclofos following oral administration in rats were identified, and the metabolites resulted from several mechanisms, including cleavage of the P-O bond, cleavage of the P-S bond, O- deethylation and sulphate conjugation of some of the metabolites. Sex-based differences in the metabolism were noted at both 5 and 110 mg/kg dose levels, but the reason for the variations is unknown.

Kinetic studies on albendazole were not designed to measure the extent of absorption, but based on urinary recovery, at least 20-30% was absorbed in mice and rats, about 1% was absorbed in humans and 47% was absorbed in cattle. The degradation of albendazole followed similar pathways in rats, mice, cattle, sheep and humans. Oral doses resulted in very low plasma levels of unchanged drug indicating rapid first pass metabolism, probably by liver microsomes. The primary metabolic reactions were, oxidation of the sulphide to the sulphoxide and sulphone, followed by cleavage of the carbamate chain to form the 2-amino sulphone. The latter was found to be the main residue in the livers of sheep and cattle.

3.1.3. Acute Studies

The acute toxicity of *Colleague* was low and did not suggest any synergism between albendazole and pyraclofos. The product was a slight skin and eye irritant and a slight skin sensitizer, these actions are most likely due to an excipient used in the formulation. The acute oral toxicity was low in rats with an LD50 in males of 1818mg/kg, and in females, 1756 mg/kg. The rabbit acute dermal LD50 was low (>2000 mg/kg). There was evidence of slight ocular irritation rabbits, slight irritation to rabbit skin, and slight sensitisation in the guinea pig.

Observations following oral dosing were coarse tremors, dyspnoea, ocular discharge, oral discharge, urinary and faecal staining, soft stools, hypoactivity, partial alopecia and decreased food consumption. No gross pathology changes were observed. No mortalities or toxic signs were observed in rabbit acute dermal studies.

In an eye irritation study 3/6 rabbits developed conjunctival irritation consisting of slight redness. After 30 minutes of a single 4-hour dermal exposure, 6/6 rabbits had slight erythema. All animals were free of dermal irritation by day 7. When guinea pigs were challenged with 100% formulation, 3/20 animals showed equivocal signs of sensitisation consisting of slight confluent erythema, and 10/20 animals exhibited very slight non-confluent erythema. On rechallenge with formulation 4/20 animals showed confluent light erythema, and 9/20 animals were slight non- confluent erythema. It was concluded that the albendazole-Pyraclofos formulation showed a potential to produce dermal sensitisation.

Pyraclofos technical had moderate acute oral toxicity in the rat and mouse (237 and 420 mg/kg respectively), low dermal toxicity in the rat (<2000 mg/kg), moderate acute inhalation toxicity in the rat (1500 mg/m3), and did not cause skin or eye irritation in the rabbit.

At 50% EC pyraclofos formulation was moderately toxic to rats and mice via the oral route of administration, and had low acute dermal and inhalation toxicity in rats. A 35% W.P. formulation did not cause skin sensitisation in guinea pigs.

Acute oral tests in rats and mice indicated that pyraclofos metabolites and impurities were generally of low acute oral toxicity. Only one impurity, with an LD 50 of 82 mg/kg, was more toxic than pyraclofos.

The toxicity of single oral doses of albendazole was low. LD50 values were 1320 mg/kg in rats, over 3000 mg/kg in mice, between 500 and 1250 mg/kg in rabbits, 900 mg/kg in guinea pigs and over 10000 mg/kg in hamsters. Skin and eye irritation were not induced in rabbits.

3.1.4. Short-Term Repeat-Dose Studies

No short-term, repeat-does studies on Pyraclofos were performed.

Rats and dogs were administered albendazole in 4-week gavage studies. At a dose of 48 mg/kg and above, rats showed anaemia, leucopenia, and hypoplasia of testes and lymphoid organs, in animals that survived, these findings were generally reversible. The NOEL was 15 mg/kg. In dogs, leucopenia and decreased testicular weight were produced at 48 mg/kg and above while weight gain was reduced from 16 mg/kg. Some deaths occurred at 48 mg/kg and above. The NOEL was 4 mg/kg.

3.1.5. Subchronic Studies

In a 13-week study in mice, pyraclofos was administered at dietary levels of up to 200 ppm. While body weight and food consumption reductions were noted at the high dose level, the main treatment-related effect was the inhibition of plasma and RBC cholinesterase (ChE) activity. Plasma ChE activity was inhibited by greater than 35% at the low dose (1 ppm) level in males, and RBC ChE inhibition was about 50-60% at 10 and 20 ppm (40-50% inhibition. A NOEL was not demonstrated.

In a 3-month study in rats, animals were treated with pyraclofos at dietary levels of up to 1000 ppm. Reduced food consumption and body weights, decreased haematocrit and haemoglobin and decreases in alkaline phosphatase, total protein and globulin levels were noted at 100 and 1000 ppm. The main effect of pyraclofos administration was the inhibition of plasma and RBC cholinesterase activity at doses of 10 ppm and above, with inhibition of plasma enzyme activity up to 92% at 1000 ppm. Inhibition of brain cholinesterase activity was seen only at 1000 ppm, and female rats appeared to be more sensitive to the test compound than male animals. The NOEL for this study, based on inhibition of plasma and RBC cholinesterase activity, was 3 ppm (approx. 0.3 mg/kg/day).

A 3 month dietary study with albendazole in rats failed to reveal toxicity even at the highest dose of 30 mg/kg. In a 6 month dietary study in rats, the highest dose, 45 mg/kg, was highly toxic, causing emaciation, anaemia, neutropenia, small testes, liver necrosis, atrophy of lymphoid organs and finally death. Many organs showed bacterial colonization and death was probably related to the severe depression of the immune system. Slight haematological and hepatic effects were seen at 30 mg/kg, the NOEL was 5 mg/kg. Two 3 month dietary studies were conducted in mice. Death and reduced weight gain were induced at 1600 mg/kg, anaemia and leucopenia were seen from 800 mg/kg. Liver weights were increased at doses of 40 mg/kg and above. Histopathology was not carried out, but the NOEL for other effects was 20 mg/kg.

No toxicity was evident in a 3-month capsule study in dogs at doses up to 30 mg/kg. After dosing for 6 months in capsules, neutropenia was seen at 30 mg/kg and above. At 60 mg/kg there was also anaemia, decreased body, testes and uterine weights and bone marrow hypocellularity. The NOEL was 5 mg/kg.

3.1.6. Chronic/Carcinogenicity Studies

In a two-year mouse study, the administration of pyraclofos at dietary levels of up to 70 ppm did not result in any treatment-related changes to mortality, bodyweight, haematology, necropsy or histopathological findings or most biochemical parameters. The major treatment-related effect was inhibition of cholinesterase activity. Plasma enzyme activity was inhibited by greater than 80% at doses of 7 and 70 ppm at all sampling intervals, and RBC enzyme activity was inhibited at 70 ppm throughout the study. At 7 ppm, RBC cholinesterase activity was reduced by up to 40% during the first 12-18 months, but levels returned to greater than 80% of control levels by the termination of the study. No toxicologically significant inhibition of brain cholinesterase was noted at any dose level. The NOEL for this study was 0.7 ppm (equivalent to 0.1 mg/kg/day).

In a two-year rat study, pyraclofos was administered at doses of up to 300 ppm in the food. Treatment-related decreases in body weight gain and food and water consumption were seen at 300 ppm. Macrocytic anaemia, increased incidences of hepatocellular fatty change and increased large-sized thyroid follicles were seen at 100 and 300 ppm. No consistent treatment-related changes in tumour incidence or organ weight changes were noted. The major effect of pyraclofos administration was the inhibition of cholinesterase activity, with plasma and RBC activity inhibited at all sample intervals at doses of 10 ppm or greater. Brain ChE inhibition of 20-30% was noted in females in the high dose group. The NOEL for this study was 3 ppm (approx 0.15 mg/kg/day).

Pyraclofos was administered orally via capsules to beagle dogs at dose levels of up to 15 mg/kg/day for 1 year. No treatment-related changes were noted with respect to mortality, clinical observations, body weight gains or food consumption. At 15 mg/kg, increases in alanine aminotransferase (ALAT) and alkaline phosphatase (AP) were seen in males and females, and a decrease in albumin was noted in females at 3 and 15 mg/kg. Significant inhibition of plasma cholinesterase activity was seen in animals of both sexes at all dose levels and all sample intervals, with enzyme inhibition of about 40-60%, but no dose-response relationship was demonstrated. RBC cholinesterase activity was inhibited at 3 and 15 mg/kg by 60 and 80%, respectively, and both plasma and RBC enzyme activity tended to plateau from weeks 13-52. Brain cholinesterase

activity was inhibited by about 30% in females at 15 mg/kg. Deposition of haemosiderin in the spleens of males was seen at 3 and 15 mg/kg, and increased vacuolisation of the adrenal cortex was seen in females at 15 mg/kg. A NOEL was not demonstrated for this study, with inhibition of plasma cholinesterase activity at all dose levels.

In a 28-month dietary rat study with albendazole, the highest dose of 20 mg/kg caused mortality, neutropenia, hypercholesterolemia, testicular degeneration and hepatic fatty metamorphosis. Endometrial/cervical tumours and skin histiocytic sarcomas showed an apparent increase in certain treated groups. However, these findings were not statistically significantly different from the controls and were within the historical control range. The NOEL was 7 mg/kg.

Mice were treated with albendazole in the diet for 25 months. Anaemia, leucopenia and testicular degeneration were noted at 400 mg/kg. Hepatic vacuolation was produced at 100 and 400 mg/kg. Endometrial stromal polyps were somewhat increased over concurrent controls but statistical significance was not achieved and all incidences were within the historical control range. The NOEL was 25 mg/kg.

3.1.7. Reproduction Studies

In a 2-generation rat study, pyraclofos was administered to Charles River CD rats at dietary levels up to 1000 ppm. Consistently reduced body weights were seen in animals from the F0, F1 and F2b generations at 1000 ppm, and at 100 ppm reduced bodyweights were noted in males and females throughout the F1 generation, and in the F2b generation in its later stages. Reduced food consumption was also noted from 100 ppm in F1 and F2b generations. No treatment-related effects on reproductive parameters were noted at any dose. Pup survival indices and pup body weights were reduced at 1000 ppm, but no adverse treatment-related effects were noted at macroscopic and microscopic examination. The NOEL for pyraclofos from this study was 10 ppm (approx 0.5 mg/kg/day).

A 3-generation dietary study with albendazole was conducted in rats. There were no effects on fertility or reproduction indices, the only findings were reductions in post natal survival and growth at 11.6 mg/kg. The NOEL was 5.8 mg/kg.

Male rats were treated by gavage in a fertility study. Testicular hypoplasia was noted at 10 and 30 mg/kg, nevertheless impregnation ability was unaffected. Litter size and weight were reduced at 30 mg/kg, the former was probably due to a lower implantation rate. The NOEL was 1 mg/kg.

A gavage peri and post natal study in rats showed decreased survival and growth in utero and during lactation in the 40 mg/kg group. There was also some evidence for retarded organ development in the offspring of this same group. The NOEL was 20 mg/kg.

3.1.8. Developmental Studies

A range-finding developmental study with pyraclofos in the rabbit, using daily doses of up to 100 mg/kg by gavage from days 6-18 after mating, found no signs of toxicity in treated animals. Bodyweight reduction was noted at 100 mg/kg, with reduced body

weight gain at 40 mg/kg, and food consumption was markedly reduced at 100 mg/kg. Plasma cholinesterase activity was inhibited by 86% (15mg/kg) to 97% (100 mg/kg) on day 19 post-coitum. No treatment-related signs were noted at any dose level tested, and it was decided to use identical dose levels for the main study.

In a developmental study with Chinchilla rabbits, pyraclofos was administered by gavage at doses of up to 100 mg/kg/day on days 6-18 post-coitum. At 100 mg/kg one animal had diarrhoea, and animals in all dose groups, including controls, suffered weight losses at the onset of treatment. At 0 and 15 and 40 mg/kg, animals regained their pre-treatment weight after a few days, and at 100 mg/kg pre-treatment weight levels were not regained until after the dosing period. Marked reductions in plasma and RBC cholinesterase activity were noted in all treated groups. No treatment – or dose-related differences in uterine parameters were seen at any dose level, and no consistent, dose-related foetal anomalies or malformations were noted. Slight reductions in foetal bodyweights were probably a result of maternotoxicity. A maternal NOEL was not demonstrated, but no developmental toxicity was noted in this study.

In a rat developmental study, female rats received doses of pyraclofos up to 100 mg/kg/day on days 6-15 of gestation. Treatment-related maternotoxicity was noted at 100 mg/kg with tremors, decreased activity, lacrimation, emaciation and markedly reduced body weights observed during the treatment period. At 50 mg/kg, slight maternotoxicity was evident. No consistent treatment – or dose-related effects on uterine parameters or on foetal external, visceral or skeletal malformations or anomalies were noted. The NOEL for maternotoxicity was 10 mg/kg/day, and no developmental toxicity was noted at doses up to 100 mg/kg/day.

A gavage teratology study with albendazole in mice failed to reveal any adverse effects at doses up to 30 mg/kg. In rabbits, the maternotoxic dose of 30 mg/kg was associated with embryotoxicity and ectrodactyly. Foetal retardation was observed from 10 mg/kg. The NOEL was 5 mg/kg.

Several rat teratology studies were conducted by gavage and dietary exposure. Embryotoxicity, foetotoxicity and external malformations were produced at doses down to 8.8 mg/kg. The most sensitive indicator of developmental toxicity was skeletal malformations, in particular limb defects, seen at doses down to 6.62 mg/kg. The NOEL was 5 mg/kg. Qualitatively similar findings were obtained with albendazole sulphoxide, 8 other metabolites were ineffective.

Other rat studies were conducted by exposing test animals to food containing liver from dosed cattle. Estimated doses of up to 0.42 mg/kg albendazole equivalents did not induce adverse effects in foetuses.

A sheep teratology study was conducted after a single oral dose of albendazole. Premature delivery, foetoxicity and post natal death were produced at 20 mg/kg and malformations were increased at 15 and 20 mg/kg. The NOEL was 10 mg/kg.

3.1.9. Genotoxicity Studies

Pyraclofos was negative for genotoxicity when tested in:

i) a DNA repair test (Rec-assay) with B.subtilis at doses of 50-20000 µg/disk, with and without S-9 metabolic activation, and

ii) in reverse mutation test with *E coli* and *S. typhimurium* at dose levels of 50-5000 µg/plate, with and without S-9 metabolic activation.

In an in vitro cytogenetics test, pyraclofos did not induce chromosomal aberrations in Chinese hamster lung cells, with or without metabolic activation, at concentrations of 1190 – 11900 µg/ml.

Albendazole was inactive in tests for bacterial mutation, chromosome aberrations and morphological transformation in cultured mammalian cells. The 2-amino sulphone metabolite was inactive in a bacterial mutation assay. Benzimidazoles, as a class, bind with tubulin and inhibit its polymerisation to microtubules.

3.1.10. Other Studies

No evidence of delayed neurotoxicity was noted in chickens that received pyraclofos by gavage at single dose levels of between 150-185 mg/kg. Treatment-related toxicity including death, ataxia and decreased limb tone were noted, but signs of toxicity disappeared within six days in surviving animals.

3.2. Public Health Standards

Pyraclofos is an organophosphate compound and is listed in Schedule 6 of the Standard for the Uniform Scheduling of Drugs and Poisons (SUSDP). It has a NOEL of 0.1 mg/kg/day and an Acceptable Daily Intake (ADI) of 0.001 mg/kg/day.

Albendazole is a member of the benzimidazole class of veterinary anthelmintics. It is listed in schedule 6 of the SUSDP with cut off to Schedule 5 for products containing 12.5% or less. It has a NOEL of 7 mg/kg/day and an ADI of 0.07 mg/kg/day. There are no reasons to alter the poisons schedule classifications, NOELs or ADIs. There are provisions for appropriate warning statements, first aid and safety directions on the product labels.

FIRST AID INSTRUCTIONS

If swallowed, splashed on skin or in eyes, or inhaled, contact a Poisons Information Centre Phone eg Australia 131 126; New Zealand 0800 764 766 or a doctor at once. Remove any contaminated clothing and wash skin thoroughly. If swallowed, activated charcoal may be advised. Give atropine if instructed.
CODES: m

There are no objections from a toxicological viewpoint to the registration of *Colleague*.

4. RESIDUES ASSESSMENT

4.1. Introduction

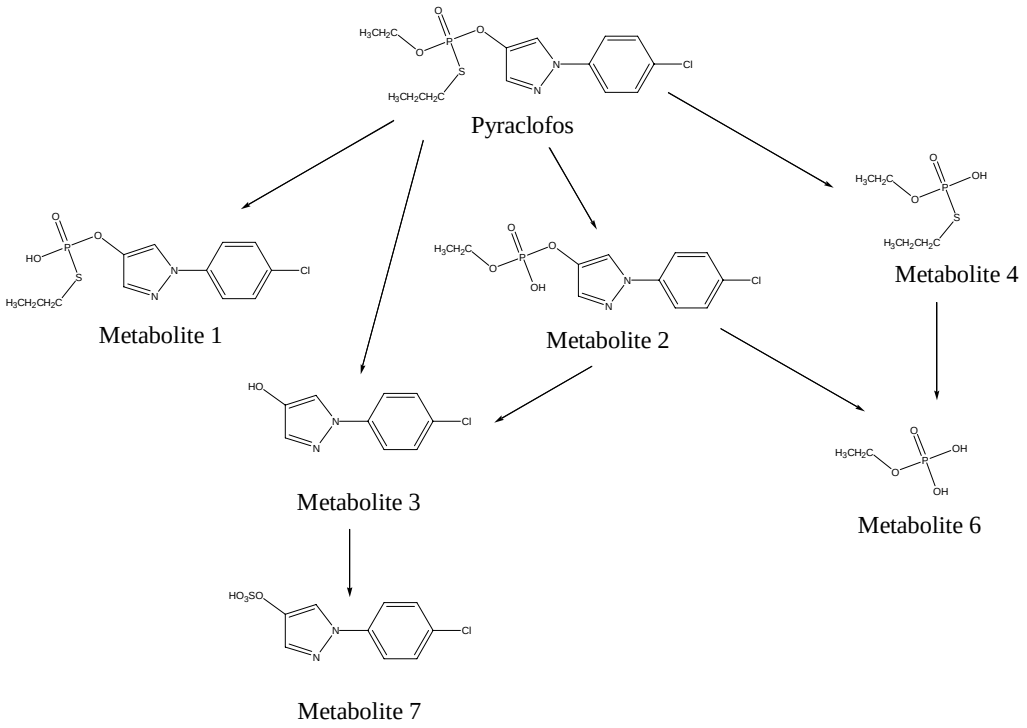
Colleague contains the active constituents, pyraclofos and albendazole, and is an oral drench that is to be used in sheep and lambs for the control of sensitive gastrointestinal roundworms, large lungworms and tapeworms. The product also claims to aid in the control of adult liver fluke, and reduce the output of viable worm and fluke eggs. As part of the residues assessment for **pyraclofos**, the Applicant submitted metabolism and residues data, along with details of the analytical methodology used to determine pyraclofos residues in edible sheep tissues. Details of these studies are provided below. Residues and trade aspects were also assessed for **albendazole**, which is co-formulated in the product. However, details of the albendazole assessment are not included in this Public Release Summary, as its use in the new product does not require any changes to the residue definition or the existing MRLs.

4.2. Metabolism of Pyraclofos

The available data for the metabolism of pyraclofos in sheep demonstrate that, after intraruminal dosing, the chemical is rapidly absorbed from the gut, with peak plasma levels occurring within 16 hours of dosing. Pyraclofos is then rapidly eliminated from the body, with ~80 % of the administered dose being eliminated via urine within 96 hours of dosing, and ~5 % of the administered dose being eliminated via the faeces in the same time frame.

The metabolism of pyraclofos involves a series of hydrolysis reactions centred around the phosphorothioate moiety (see the following figure): the reactions include cleavage of the entire moiety followed by conjugation with sulfate; de-ethylation of the phosphorothioate moiety; and cleavage of the P-S bond.

Proposed Metabolic Pathway for Pyraclofos



The three main metabolites comprising pyraclofos residues in edible sheep commodities are: (i) pyraclofos minus the phosphorothioate moiety (Metabolite 3); (ii) the sulfate conjugate of the de-phosphorothioate metabolite (Metabolite 7); and (iii) the P-S bond cleaved form of pyraclofos (Metabolite 2). The toxicity of pyraclofos and its metabolites appears to be associated with the phosphorothioate moiety. Thus, the toxicity of the main metabolites found in edible tissues (ie Metabolites 7, 2 and 3) is significantly lower than that of the parent molecule. It is concluded that the residue definition for pyraclofos should be the parent compound *per se*, as this is the toxicologically significant component of any residues.

The rank order of pyraclofos (parent) residues in edible tissues from treated sheep at 0 days post-treatment is: liver > fat > muscle > kidney. However, at 14 days post-treatment, highest residues of pyraclofos are observed in fat. Therefore, fat is considered to be the target tissue.

4.3. Analytical Methods

Details were provided for the validated analytical method used to determine the concentrations of pyraclofos residues in edible sheep tissues. Briefly, the method involves extraction of homogenised tissues with either diethyl ether/acetone[♣] (liver, kidney, muscle) or acetonitrile-saturated n-hexane (fat), followed by clean up using solid phase extraction cartridges. The pyraclofos content of the purified extracts is determined using a reverse phase HPLC method with UV detection. Residue concentrations are determined by comparison of the peak area ratio for pyraclofos to internal standard (naphthalophos) against an external standard calibration curve. The method LOQ was reported to be 0.01 mg/kg for all sheep tissues. The method LODs were 0.001 mg/kg for sheep liver, muscle and fat, and 0.003 mg/kg for sheep kidney.

4.3.1. Residue Definition

On the basis of the available metabolism and toxicology data for pyraclofos, it is concluded that the residue definition for pyraclofos should be the parent compound *per se*.

4.4. Residue Trials

The Applicant provided details of two (2) tissue residues trials conducted in Australia with *Colleague* In the first trial, Merino wethers (n=24) were administered a single oral dose of Colleague at either 1× the nominal dose rate (30 mg pyraclofos/kg bw and 3.8 mg albendazole/kg bw; equivalent to 0.73× the maximum dose rate) or 1× the maximum dose rate (40.9 mg pyraclofos/kg bw and 5.18 mg albendazole/kg bw). Animals were sacrificed at 10 and 14 days after treatment, and samples of liver, muscle, kidney, perirenal fat and subcutaneous fat were collected and analysed for their concentration of pyraclofos residues.

In the second trial, Merino wether hoggets (n=48) were administered a single oral dose of *Colleague* at the maximum 1× proposed label rate (equivalent to 40.9 mg pyraclofos/kg bw and 5.18 mg albendazole/kg bw). Groups of sheep (n=8) were sacrificed on days 10, 14, 21, 28, 35 and 42 after treatment, and samples of kidney,

[♣] It is noted that methanol was used as the extraction solvent in metabolism studies. The Applicant provided data to demonstrate that the extractability of incurred pyraclofos residues from liver, kidney and muscle using diethyl ether/acetone was comparable to that achieved using methanol. Similarly, the extractability of incurred pyraclofos residues from fat using acetonitrile-saturated n-hexane was comparable with that obtained when methanol was used as the extraction solvent. It is concluded that the use of diethyl ether/acetone (1:1) and n-hexane to extract incurred pyraclofos residues from sheep tissues will not compromise the accuracy of the residues results.

liver, muscle, peri-renal fat and subcutaneous fat were collected and analysed for their concentration of pyraclofos residues.

A summary of the results from these trials is tabulated below.

Pyraclofos residues in edible tissues from sheep treated with a single oral dose of 40.9 mg pyraclofos/kg bw (and 5.18 mg albendazole/kg bw; 1× the maximum proposed label rate).

Trial No.	Tissue	Sampling time (DAT)	n	Pyraclofos residues (mg/kg)	
				range	Mean ± SD
Trial No. 1	Liver	10	6	<0.01-<0.09	--
	Kidney	10	6	<0.01	--
	Muscle	10	6	<0.01	--
	Fat (subcutaneous)	10	6	<0.09-1.111	0.44 ± 0.38
		14	6	<0.01-0.175	--
	Fat (peri-renal)	10	6	0.093-0.360	0.20 ± 0.10
		14	6	<0.01-<0.09	--
Trial No. 2	Liver	10	8	<0.001	--
		14	8	<0.001	--
	Kidney	10	8	<0.003	--
		14	8	<0.003	--
	Muscle	10	8	<0.001	--
		14	8	<0.001	--
	Fat (subcutaneous)	10	8	<0.001-0.67	0.24 ± 0.24
		14	8	0.02-0.12	0.05 ± 0.04
		21	8	<0.001-0.25	0.08 ± 0.09
		28	8	<0.001-0.03	--
		35	6	<0.001	--
		42	6	<0.001	--
	Fat (peri-renal)	10	8	<0.001-0.47	0.15 ± 0.16
		14	8	<0.001-0.07	0.03 ± 0.03
		21	8	<0.001-0.04	--
		28	8	<0.001	--
		35	6	<0.001	--

DAT: Days After Treatment

Meat WHP*:* The Applicant proposed that a 14 day WHP be assigned to the use of *Colleague*.

Tissue MRLs*:* Pyraclofos residues in liver, kidney and muscle from treated sheep are expected to be below the LOQ of the analytical method, when the proposed 14 day WHP is observed. Therefore, pyraclofos MRLs of *0.01 mg/kg are recommended for sheep liver, sheep kidney and sheep meat.

Pyraclofos residues in subcutaneous fat ranged from <0.001 to 1.111 mg/kg at 10 days after treatment. Residues declined to <0.01 to 0.175 mg/kg at 14 days after treatment, and <0.001 to 0.25 mg/kg at 21 days after treatment. Similarly, pyraclofos residues in perirenal fat ranged from <0.001 to 0.47 mg/kg at 10 days after treatment; <0.001 to <0.09 mg/kg at 14 days after treatment; and <0.001 to 0.04 mg/kg at 21 days after treatment. These data support the establishment of a pyraclofos MRL of 0.5 mg/kg for sheep fat, when the proposed 14 day WHP is observed.

Milk WHP*:* In the absence of any data for residues in sheep milk, the following milk WHP is recommended: "DO NOT use in ewes which are producing or may in the future produce milk or milk products for human consumption".

Proposed ESI*:* Fat was identified as the "target tissue" for pyraclofos residues, and is the tissue that governs the length of the export slaughter interval (ESI) assigned to the new product. It is recommended that an ESI of 35 days be assigned to *Colleague*, as pyraclofos residues in all edible tissues from treated sheep are below the LOD of the analytical method (<0.001 mg/kg) at 35 days after treatment.

4.5. Risk Assessment Conclusions

4.5.1. Dietary Risk Assessments

The chronic dietary exposure to veterinary chemicals is estimated by the National Estimated Daily Intake (NEDI) calculation encompassing all registered/temporary uses of the chemical and the mean daily dietary consumption data derived from the 1995 National Nutrition Survey of Australia. The NEDI calculation is made in accordance with WHO Guidelines[1] and is a conservative estimate of dietary exposure to chemical residues in food. The NEDI for pyraclofos is equivalent to 0.36 % of the ADI.

The acute dietary exposure is estimated by the National Estimated Short Term Intake (NESTI) calculation. The NESTI calculations are made in accordance with the deterministic method used by the JMPR[5] with 97.5th percentile food consumption data derived from the 1995 National Nutrition Survey of Australia. NESTI calculations are conservative estimates of acute exposure (24 hour period) to chemical residues in food. The Office of Chemical Safety (OCS) has not established Acute Reference Doses (ARfDs) for pyraclofos. Therefore, no NESTI calculations have been performed.

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

4.6. Recommended Amendments to the MRL Standard

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

Table 1

Compound	Food		MRL (mg/kg)
Pyraclofos			
DELETE:			
	MF 0822	Sheep fat	T*0.1
	MO 1288	Sheep kidney	T*0.1
	MO 1289	Sheep liver	T*0.1
	MM 0822	Sheep meat	T*0.1
ADD:			0.5
	MF 0822	Sheep fat	*0.01
	MO 1288	Sheep kidney	*0.01
	MO 1289	Sheep liver	*0.01
	MM 0822	Sheep meat	

5. ASSESSMENT OF OVERSEAS TRADE ASPECTS OF RESIDUES IN FOOD

5.1. Commodities Exported

Australian exports of sheep meat and offal, and live sheep could be affected by the use of *Colleague.*

5.1.1. Destination and Value of Exports

Mutton/lamb exports

Australia exported ~258 ktonne of mutton and lamb during 2004, which was valued at $AUS 1.07 billion. Details of the top export markets for Australian lamb/mutton are provided below.

Mutton and lamb exports in 2004 (Source ABARE 2005)

Rank (by $ value)	Importing country	Quantity (ktonne)	Value ($AUS million)
1	United States	46.5	308.5
2	European Union†	23.3	136.6
3	Japan	16.7	100.6
4	Saudi Arabia	19.5	53.0
5	Chinese Taipei	13.1	41.9
6	United Arab Emirates	5.8	27.0
7	Malaysia	6.6	22.6
8	Singapore	6.4	22.0
9	South Africa	14.9	17.6
Total		**257.6**	**1065.2**

†Regarded as 25 countries.

Live sheep exports

Australia exported approximately 3.4 million head of live sheep during 2004, which were valued at $AUS 225 million. Details of the top export markets for Australian live sheep are provided below.

Live sheep exports in 2004 (Source ABARE 2005)

Rank (by $ value)	Importing country	Quantity ('000 of animals)	Value ($AUS million)
1	Kuwait	1260	84
2	Jordan	930	58
3	Bahrain	490	33
4	Oman	289	20
5	United Arab Emirates	196	13
6	Qatar	137	10
Total		**3397**	**225**

5.1.2. Proposed Australian Use-Pattern

The maximum proposed Australian use-pattern for *Colleague* involves administering a single oral dose of 40.9 mg pyraclofos/kg bw and 5.18 mg albendazole/kg bw to sheep, with a 14 day WHP. The product is not intended for use in lactating ewes where milk or milk products are to be made available for human consumption.

5.2. Codex Alimentarius Commission (Codex) and Overseas MRLs

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

Codex MRLs have not been established for pyraclofos residues in any animal commodities. The Applicant indicated that pyraclofos is not registered in any overseas countries for use in sheep, and consequently no overseas tolerances/MRLs have been set for pyraclofos residues in edible sheep commodities.

5.3. Potential Risk to the Australian Export Trade in Edible Sheep Commodities

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

In the absence of any Codex MRLs or overseas tolerances for pyraclofos, the presence of any pyraclofos residues above the method LOQ in edible sheep commodities would constitute a risk to Australia's export sheep market.

The Applicant has proposed that an Export Slaughter Interval (ESI) of 35 days be assigned to *Colleague*, as pyraclofos residues in all edible tissues from treated sheep are below the LOD of the analytical method (<0.001 mg/kg) at this time.

Consequently, it is concluded that the risk to Australia's export trade in sheep meat and live sheep will be low when the proposed 35 day ESI is observed.

6. OCCUPATIONAL HEALTH AND SAFETY ASSESSMENT

Both the actives are manufactured overseas. The product will be formulated in Australia.

Hazardous/Non hazardous :

TGAC: Hazardous (pyraclofos, moderate acute oral toxicity) and (albendazole, low acute oral toxicity)

EUP: Hazardous, slight skin and eye irritant.

6.1. Category of Workers/Nature of the Work Done

Workers may be exposed to pyraclofos when formulating the end use product. The main risk at this stage is the possibility of dermal and inhalational toxicity.

Laboratory Staff – will carry out the sampling and chemical testing of the TGAC and EUP.

Storepersons – will be involved in the transport and storage of the TGAC and EUP on-site.

Formulators – will be engaged in the formulation of the EUP.

Packers – will be engaged in the packing of the EUP as sales units.

End-users – will be engaged in administering the EUP by drench gun to sheep and lambs.

To minimise worker exposure during formulation, the manufacturing process should be contained and well-ventilated. Approved personal protective equipment and clothing should be used where processes are contained. Personal hygiene procedures should always be followed to minimise exposure to pyraclofos and other substances used in the manufacture of *Colleague*. All personnel should be monitored for blood cholinesterase.

Fumed silica is one of the ingredients in the product. There is an exposure standard for fumed silica of 2 mg/m^3. Monitoring for fumed silica should be carried out routinely in the formulation plant to ensure that the exposure standard is not exceeded. A monitoring program for fumed silica is not considered necessary for end use, given the product formulation and use pattern.

The product will be used to control gastrointestinal roundworms, large lungworms, tapeworm and to reduce the output of viable worm and fluke eggs in sheep and lambs. The end users will administer the undiluted product by drench gun to sheep and lambs.

There is a possibility of eye and skin irritation if splashing or leakage occurs while administering the product. Therefore care should be taken to avoid contact with eyes and skin. The dose in the animals will be 1 mL/5 kg liveweight to give 30 mg pyraclofos/kg.

The company states that laboratory staff will be required to wear gloves, laboratory coat, dust mask and eye protection while sampling. Storemen will be required to wear overalls and safety boots.

All operators engaged in the formulation process and packaging will be required to wear overalls, safety boots, PVC or nitrile rubber gloves, eye protection and dust mask. The MSDS on the ingredients and the product should be made available to all workers.

For end-users the label safety directions are adequate to minimise exposure in normal use and additional information on the product is contained in the material safety data sheet.

Potential exposure to the end-users is low, as the product will be administered by automatic drench gun connected to a backpack. There is no data available on dermal absorption of either the active pyraclofos or the EUP, but dermal toxicity for both is low (>2000 mg/kg). In the event of a EUP splash on the skin, the potential for toxic effects of pyraclofos will be minimal as it constitutes only 15% of the product. However due to the dermatitis and hypersensitivity reported in humans exposed to albendazole, workers are required to wear PVC gloves.

SAFETY DIRECTIONS

The product will be used undiluted and administered using a drench gun. The main form of exposure is expected to be dermal, with the possibility of splashing into the eyes. Based on the use patterns, if recommended safety directions are followed, exposure to the product will be minimal.

Recommended Safety Directions are as follows:

HARMFUL [IF] SWALLOWED [OR] WILL IRRITATE THE EYES [AND] SKIN AVOID CONTACT WITH EYES AND SKIN [AND] REPEATED MINOR EXPOSURE MAY HAVE A CUMULATIVE POISONING EFFECT WHEN USING THE PRODUCT [AND] WEAR ELBOW-LENGTH PVC GLOVES [AND] WASH HANDS AFTER USE AFTER EACH DAY'S USE, WASH GLOVES [AND]
CODES: 129 133 161 162 164 210 211 190 279 283 290 294 351 360 361

7. ENVIRONMENTAL ASSESSMENT

7.1. Introduction

Schering-Plough Animal Health has applied for a re-registration of the product *Colleague* containing the active constituents (ac), the currently approved albendazole (19 g/L) and the previously withdrawn pyraclofos (150 g/L). The combination product is proposed for use in the control of sensitive gastrointestinal roundworms (including strains that have single or multiple resistance to benzimidazole, levamisole, closantel and macrocyclic lactone drenches), large lungworms and tapeworm. It also aids in the control of adult liver fluke and reduces the output of viable worm and fluke eggs.

The product was previously registered by the APVMA and marketed by SmithKline Beecham in 1993. After the release of the product, adverse events of death of sheep, mainly in WA, were reported. Complete investigations into the mortalities were not able to be established. Consequently, SmithKline withdrew the product in 1994. The applicant believes there were extenuating circumstances contributing to the adverse events and these could be addressed for any subsequent re-introduction.

Four classes of anthelmintics are currently marketed for the control of internal parasites in sheep. These are benzimidazoles, levamisole, macrocyclic lactones and organophosphates. Sheep parasites throughout Australia have demonstrated resistance to all drug classes except the organophosphates. The applicant contends the sheep industry needs access to another organophosphate formulation with high efficacy in order to provide parasite control without exerting further pressure on other drug classes. Pyraclofos, used overseas as a horticultural insecticide, is included in the formulation at a concentration shown to be highly efficacious against gastrointestinal parasites.

As per the agreed minutes of the meeting between Schering-Plough and the APVMA on the 23 June 2004, the original data package for the proposed product is not included as it is already held by the APVMA. Consequently, this submission contains only the additional data required to meet the current APVMA requirements. These additional data consist of toxicity studies of *Daphnia magna*, *Ceriodaphnia dubia*, algae and the soil micro-organisms nitrogen test. In addition, arguments are provided to address phytotoxicity and toxicity to earthworms.

This report is an update of the original report conducted in 1993, and only the additional material has been assessed and incorporated.

According to the submission there are no overseas registration of the proposed product.

7.2. Environmental Exposure

7.2.1. Environmental Release

Volume

It is expected that 9100 L of *Colleague* will be sold in each of the first two years of sale. These quantities would require import of 1.365 tonnes of pyraclofos per year. Product maturity is expected to occur in year 5 which will be 71,000 L (10,650 kg ac/year). It is expected that 30% of these sales will come from users who currently do not use organophosphates. The remaining 70% will be current organophosphate users who change to *Colleague.*

Application and use pattern

Application is by oral drench to sheep for the control of a range of internal parasites. The dose rate is 1 mL of the product (0.15 g pyraclofos) per 5 kg body weight (bw) of the sheep with 2 to 4 dosings per year. The product is applied directly without dilution. The main environmental exposure to pyraclofos will occur by excretion of unchanged chemical and metabolites in the urine and faeces. Spillage of the chemical during the act of drenching sheep is usually only the occasional single sheep dose (1.5 g pyraclofos for a 50 kg sheep), misapplied when the animal to be treated moves suddenly. Spillage when decanting from the 20 litre container into the applicator backpack is not common as a tap can be inserted into the drum for this purpose.

Formulation, handling and disposal

The formulation of the imported active ingredients will take place either at St Marys or Wyong NSW and at Auckland New Zealand. These plants usually have closed formulating facilities with bunding and waste water treatment facilities. Rinsate from drum and equipment washing is recycled to the next formulation batch.

7.3. Metabolism / Excretion by Animals

Pyraclofos was administered as an oral drench to sheep, at a dose rate of 30 mg/kg body weight in combination with the already cleared compound albendazole. A total of 81 % of the administered dose was recovered in the excreta. Urine and faeces contained 90.3% and 9.7% of the recovered radioactivity respectively. Analysis showed that pyraclofos is extensively metabolised in sheep to more polar products resulting from hydrolysis of the phosphate ester bonds. In urine the major metabolites are #2 (unidentified) and #7 [sulphate conjugate of 1-(4-chlorophenyl)-4-hydroxypyrazole] while in the faeces metabolite #3 (1-(4-chlorophenyl)-4-hydroxypyrazole) and unchanged pyraclofos are the major components detected. Neither pyraclofos nor metabolites were found in the 7 day post treatment sampling.

Studies indicate approximately 5% of the administered dose for rats absorbed from the gastrointestinal tract is retained in the body, the remainder (95%) being excreted in the urine and faeces (urine > 87%), mainly in the form of metabolites.

7.4. Environmental Fate

Studies submitted in this area may be summarised as follows:

7.4.1. Hydrolysis

The rate of hydrolysis was variable depending on the pH of the buffered test solutions (see table below). Maximum duration was 49 days and analysis was by HPLC. Breakdown products formed were by de-alkylation of the phosphate group (I, II and III in Attachment A).

In the unbuffered natural water sample the rate of hydrolysis was initially as fast as the similar pH buffered laboratory sample, but decreased in rate as hydrolysis by-products lowered the pH of the sample. This would not be likely to occur in the field as dilution would be greater.

Table 1: Hydrolysis of pyraclofos

SOLUTION	**T_{50}**	**days**
pH	**25 ^{o}C**	**37 ^{o}C**
1.2	37	14
5	112	28
7	29	8
9	0.5	0.13
7.5 (natural)	41	

7.4.2. Photolysis

Aqueous and dry glass photolysis, after an exposure of 32 days to natural light on the rooftop of the laboratory in Osaka Japan, 35^{o}N latitude, was calculated to give the results tabulated below. Care should be taken with these figures as they represent a sampling period of less than one half life. However, timing of the experiment was in the northern winter.

In natural water of pH 7.4 degradation by photolysis was similar to the sterile buffered pH 7 sample.

Table 2: Photolysis in water at various pH and on dry glass

pH	**T_{50} days**
7	37
5	36
7.5 (natural)	26
Dry glass	40

7.4.3. Degradation in Soil and Water

Five soils as described below were utilised for the soil test work reported.

Table 3: Test soils physical and chemical properties.

Soil	Texture	Clay %	Carbon %	pH	CEC meq/100 g
Aichi	Clay loam		1.13	4.7	
Ibaraki	loam	10	6.2	6.3	22.8
Kagawa	loam	13.1	1.2	6.0	7.5
Kouchi	Clay loam		2.29	6.25	10.7
Niigata	Sandy loam	12.3	.95	5.5	8.0

The studies of five soils treated with ^{14}C labelled pyraclofos under both aerobic and anaerobic conditions all reported a half life of between 40 and 50 days over the 120 day sampling period. Sampling took place every 10 days and the extract was analysed by HPLC. After 120 days >50% of the radioactivity was bound to soil with unchanged pyraclofos (25%) the major extractable constituent. Approximately 0.1% of ^{14}C volatiles and 7-8% of $^{14}CO_2$ were collected after 120 days. Proposed degradation pathways are in Attachment B.

7.4.4. Mobility in Soils

Adsorption/Desorption

The test utilised 5 g soil samples and 100 mL of test solutions at concentrations of 3, 6 and 12 µg/mL of ^{14}C labelled pyraclofos. Samples were shaken for 0.5, 1, 2 and 3 hours, separated by centrifuge and the supernatant analysed by liquid scintillation to determine equilibrium times.

Values of soil sorption coefficient, (K_{OC}) for the five soils were calculated. K_{OC} values were obtained with a range from 585 to 2984 in the Ibaraki and Aichi soils respectively, indicating moderate to strong adsorption. Equilibrium time was determined to be 3 hours for all soils tested.

Leaching potential

Soil column leaching studies were performed using 300 mm soil filled columns which were eluted with 200 mL of distilled water (said to be the equivalent of 786 mm of rainfall) over a 6.7 hour period. The column was then fractionated into 20 mm lengths with the soils and eluate analysed by sample oxidiser and liquid scintillation system.

The results showed that 80 to 90% of the applied ^{14}C labelled pyraclofos was bound in the top 4 cm of the column. Radioactivity concentrations equivalent to less than 1% of those applied from the soils tested was found in the eluate. Again this supports moderate to strong adsorption.

7.4.5. Field Dissipation Studies

No field dissipation studies were reported in this submission. This is acceptable for the proposed use considering the low amount of environmental exposure to the unchanged active.

7.4.6. Accumulation in Soils

The substance is unlikely to accumulate in soils because of the moderate to low persistence of pyraclofos. The infrequent drenching (maximum of four per year), low amount of unchanged substance excreted and the usual farm practice of rotational grazing resulting in treated sheep excreting on different parts of the farm after each of these drenchings, also ensures there should be no accumulation.

7.4.7. Bioaccumulation

No data were available with the original submission as the low quantities reaching the aquatic compartment at infrequent intervals would preclude the need for bioaccumulation testing for the proposed use. However, a bioaccumulation study has now been completed for pyraclofos in common carp (*Cyprinus carprio*) as presented below:

Report: Handley et al (1992)

Guidelines: OECD Guideline No. 305 C and the requirements of the Japanese Ministry of International Trade an Industry's chemical substance Control Law Clause No. 117, 1973

GLP: Yes

Test System
The study involved 24 common carp per test concentration being placed into test media containing 0 (control), 1 and 5 µg/L pyraclofos for a test period of 56 days. Three fish were selected at random for analysis on days 14, 28, 42, 49 and 56. All remaining fish were sacrificed on day 56. The temperature, pH and dissolved oxygen levels were within acceptable limits throughout the duration of the test.

The mean measured test concentration for the 1 and 5 µg/L test groups were 86.4 and 84.4% of the nominal concentration, respectively. There were no mortalities or adverse reactions to exposure observed in any of the fish in the control and test vessels throughout the duration of the study.

Results from the analysis of fish tissues showed that pyraclofos accumulated in the body tissues of the fish. The 56 day bioconcentration factors (BCFs) were 117 and 208 at test concentrations of 1 and 5 µg/L, respectively. The results of the study up to day 49 indicate that the BCF attained an approximate constant level. However, the results from the 5 µg/L test group indicate that there is an increase in the Day 56 BCF over that obtained from the previous sampling occasion. As all the fish were sacrificed on Day

56, no further investigation for the increase in the Day 56 BCF at concentration of 5 µg/L was performed.

The active constituent is not considered to be bioaccumulative given that the BCF values at the concentrations investigated were determined to be <500.

7.4.8. Summary of Environmental Fate

Degradation rates and routes

The main degradation pathway for pyraclofos is within the treated animal. This material is then released to soil via the excreta with less than 10% of the administered dose expected to arrive in this compartment as unmodified pyraclofos. Hydrolysis, photolysis and soil breakdown mechanisms under both aerobic and anaerobic conditions give a half life of between 40 and 50 days, except for hydrolysis under alkaline conditions which is considerably faster.

Metabolites

The formation and excretion of metabolites by the treated animal lead to a set of non active polar metabolites that are broken down further in the soil and water compartments to carbon dioxide and phosphoric acid. Metabolites resulting from breakdown of any unchanged pyraclofos that reaches the environment follow a similar path.

Mobility

Soil binding by pyraclofos is moderate to strong and the chemical would be classed as immobile.

Bioaccumulation

A bioaccumulation study in fish indicates that pyraclofos is unlikely to bioaccumulate.

7.5. Environmental Effects

Two reports were provided to address the deficiencies identified in the original assessment. These reports include toxicity studies on daphnia, cladoceran, algae and soil microorganisms nitrogen test, in addition to the previous ecotoxicological data from the original submission. Arguments were also provided to address phytotoxicity and toxicity to earthworms.

7.5.1. Avian Toxicity

Single dose testing of avian species was carried out in accordance with US EPA FIFRA guideline 71-1. The birds in all the tests were dosed by gavage with the substance dispersed in corn oil and their health and mortality was monitored for 14 days.

Table 4: Acute Toxicity of pyraclofos to bird species

Species	LD_{50} (mg/kg)
Hen	182
Mallard	>120
Bobwhite Quail	31

As might be expected a number of manifestations of toxicity were observed in surviving birds. There are no results for dietary studies. The substance is rated as highly toxic to birds. However, the opportunity for exposure to birds is limited by the method of administration of the end use product as an internally applied sheep drench.

7.5.2. Aquatic Toxicity

Acute toxicity of pyraclofos to three species of fish and the water flea *Daphnia pulex* was tested using well described test methods in close conformity to the currently established guidelines. All concentrations quoted were nominal. Hydrolysis should not have unduly influenced the integrity of the results due the length of the test and pH of the test water (6-7), though some adsorption to vessel walls or test organisms/excreta may have occurred.

Table 5: Acute toxicity to aquatic organisms

Species	EC_{50} mg/L	Exposure hr	Test Method
Rainbow Trout	0.059	96	Flow through
Carp	0.044	48	Still Water
Killifish	1.91	72	Still Water
Daphnia pulex	0.0035	6	Still Water

The test results exhibit a range of toxicities ranging from very highly toxic for *Daphnia pulex* (note 6 hour results only), carp and rainbow trout to moderately toxic for killifish.

Exposure of aquatic species to pyraclofos will not be by a direct route but at the end of a sequence that would involve substantial degradation of the chemical before it reached water.

The applicant has provided additional studies on the acute toxicity of pyraclofos to *Daphnia magna*, *Ceriodaphnia dubia* and freshwater green alga (*Selenastrum capricornutum*) to update the original submission of the proposed product.

Report: Sewell *et al* (1992)

Guidelines: OECD Guideline No. 202 and Method C2 of Commission Directive 84/449/EEC

GLP: Yes

Test System
Acute toxicity of pyraclofos was tested on *Daphnia magna* in a 48 h static test. Twenty daphnids (2 replicates of ten each) were used with nine test concentrations (ranging from 0.10 to 10 µg ac/L - nominal) plus control and solvent control (DMF).
Test conditions included use of dechlorinated and aged laboratory tap water. The total hardness is approximately 50 mg/L as $CaCO_3$. The stability of the test substance was also determined in the light and dark conditions over a 48 h test period. Stability studies were conducted three times to determine whether the test compound is stable when dissolved in aged dechlorinated tap water over a 48 h period. The EC50 was calculated using the method of Thompson.

Findings:
Water quality parameters such as temperature, pH and dissolved oxygen were unaffected by the addition of test substance and remained within acceptable ranges for *Daphnia* survival during the course of the study. The test samples from the stability studies were shown to be unstable in aqueous solution in both light and dark under laboratory conditions. It was considered that the loss is not due to hydrolysis or photolysis. However, the main study test samples were shown to be stable and within 20% of the nominal concentration and thus nominal concentrations were used to report results.

Following 48 h, immobilisation of 50, 100 and 100% was observed in the 3.2, 5.6 and 10 µg ac/L groups, respectively, with no immobilisation observed in any of the lower treatment groups or controls. No observations were made in the report with respect to sub-lethal effects.

Conclusions:
Based on the results of the study, the 48 h EC50 was calculated to be 3.2 µg ac/L (95% CI 2.8-3.6 µg ac/L). The NOEC was determined to be 1.8 µg ac/L.

Report: Smith and Atkinson (2004)

Guidelines: OECD Guideline No. 202 *Ceriodaphnia dubia* assay

GLP: No

Test System
Acute toxicity of pyraclofos was tested on the freshwater cladoceran *(Ceriodaphnia dubia)* in a 48 h static test based on the method described by the USEPA (1993) and adapted for use with the locally collected *C. dubia* (Sydney Clone). Based on the range finding test nominal concentrations of 10, 3.0, 1.0, 0.3, 0.1, 0.03, 0.01 and 0.0 (control) µg/L of pyraclofos were tested. Dilute Mineral Water (DMW) was used as the diluent for the toxicity tests and as the culture medium for the test organisms. Four replicates were used for each test concentration and control. Five *C. dubia* neonates were used for each test vial. The test vials were incubated in a chamber at 25ºC. A 16:8 h light/dark cycle was provided. The condition of the test animals were assessed at 24 h and 48 h and the number of immobilised animals was recorded. The pH, temperature dissolved oxygen were measured. The test concentrations were determined by (GC-MS). Note that the stability of the test compound in solution after 72 h of exposure was not performed. The EC50 estimates (with 95% confidence limits) were determined using the trimmed Spearman-Karber method.

Findings:
Following 48 h, no survival were observed in the 1.0, 3.0 and 10.0 µg ac/L groups. 100% survival was observed in any of the lower treatment groups or controls. No observations were made in the report with respect to sub-lethal effects.

Conclusions:
Based on the results of the study, the 48 h EC50 was calculated to be 0.55 (95% CI: 0.3-1.0) µg ac/L. The NOEC was determined to be 0.3 µg ac/L.

Report: Smith and Atkinson (2004)

Guidelines: OECD Guideline No. 201 and USEPA OPPTS 840.5400 and USEPA Method 1003.0

GLP: No

Test System
The inhibitory effect of the test compound on the growth of green alga was tested over a period of 72 h. The test consisted of 3 replicates in glass scintillation vials per test concentration. The test organisms were exposed to nominal concentrations of 0 (control), 10, 30, 100, 300, 1,000, 3,000 and 10,000 µg/L. Each vial was inoculated with 1-2 X 10^4 cells/mL of a pre-washed *Selenastrum* suspension. Vials were incubated at 24ºC under continuous light. Cell density in each treatment was determined after 72 h by counting cells using a haemocytometer. The pH was determined at 0 and 72 h of exposure. Three concentrations of the copper reference toxicant were included in the bioassays to validate the test. The pyraclofos concentrations were determined by HRC GC-MS. Note that the stability of the test compound in solution after 72 h of exposure was not performed. The 72 h IC50 was calculated using Linear Interpolation. After testing the data for normality and homogeneity of variance, Dunnett's Multiple Comparison Test was used to determine NOEC.

Findings:
The data indicate that there are significantly lower cell yields at nominal concentrations of 1,000, 3,000 and 10,000 µg/L compared with solvent control treatment results. The reference toxicant is within the acceptable cusum chart limits of 0.250-0.461 thus confirming the validity of the test.

Conclusions:
Based on the results of the study, the 72 h IC50 was calculated to be 1724 µg/L (95% CI 990-2207 µg/L) for alga. The NOEC was determined to be 300 µg/L.

7.5.3. Non-target Invertebrates

Honey bee

Toxicity to European honey bee was tested by feeding a spiked sucrose solution. Observations were continued for 48 hours after removal of the test solution and the toxicity figures established.

Table 6: Toxicity of pyraclofos to honey bee

Species	LC_{50}	LD_{50}
Honey Bee	79.6 ppm	1.3 µg/bee

Results show pyraclofos to be highly toxic to honey bee according to the comparative scale developed by (Atkins et al 1976).

Earthworms

The toxicity of pyraclofos formulated as a 35% wettable powder was tested on one of the standard species of earthworm *Eisenia foetida*, by two methods. The first was a soil mix in which the earthworms were introduced and monitored for seven days after application. The second method involved surface application of the substance to the soil in pots then introduction and monitoring of the earthworms for seven days. Benomyl, known to be of high toxicity, was used as a standard.

Table 7: Effect of pyraclofos on earthworm

Chemical	Soil mix	Surface spray
Benomyl	>67;<670 mg/kg	> 2 kg/ha
Pyraclofos	> 610 mg/kg	> 2 kg/ha

The results show that pyraclofos is of low toxicity to earthworms under field conditions as no mortalities were recorded at the highest rate tested.

Arthropods

Safety to other arthropods is summarised in a table provided as an extract from the leaflet (Japanese Pesticide Information Service Information No 53 1988). In this summary the activity of pyraclofos expressed as an LD50 or LC50 in ppm for several orders of insect pest (part of table is reproduced below). There is a wide range of susceptibility between orders and amongst species in the orders. In the order diptera the mosquito larvae *Culex* is the most susceptible (<0.1 ppm). In the order coleoptera the adult insects are the most tolerant of pyraclofos.

Table 8: Insecticidal activity of pyraclofos against various species of pest

Pest (order and species)	Stage	Treatment	LD_{50} ppm
Lepidoptera			
Spodoptera litura	L (3rd)	Foliar spray	12.1
Plutella xylostella	L (3rd)	Foliar spray	11.7
Peris rapae	L (3rd)	Foliar spray	<50
Coleoptera			
Epilachna varivestis	L	Foliar spray	3.3
Aulacophona femoralis	A	Foliar spray	250
Diptera			
Culex pipiens molestus	L	In water	<0.10
Musca domestica	A	Topical appn.	10
Ortphoptera			
Blattella germanica	A	Dry film	50

Soil micro-organisms

Report: Smith and Atkinson (2004)

Guidelines: OECD Guideline No. 216

GLP: Not stated

Test System

The nitrogen transformation test was used to determine the toxic effect of pyraclofos on soil microbial activity. Concentrations of 56 and 560 mg pyraclofos/kg soil were used in the test on the basis that 1.05 g of pyraclofos is likely to be excreted in the first 24 h in 18.75 kg of soil mixed with 187.5 g of sand (for an area of 50 X 50 cm at a depth of 5 cm). A control soil sample was also analysed. The soil was characterised as follows:

Table 9: Characteristics of the soil

Sand (%)	Silt (%)	pH	Organic Carbon Content (%)	Kjeldahl Nitrogen as N (ppm)	Microbial Biomass Nitrogen (µg/N/g)	Microbial Biomass Carbon (µg/C/g)	Initial Nitrate Concentration (mg/kg)	CEC meq/100 g
80	20	6.5	1.8	490	25	260	96	4.2

The pyraclofos was applied to the soil by spiking a concentrated solution into sand, then allowing the solvent to evaporate before mixing the spiked sand with the test soil. The test soils were incubated at 20ºC. Test containers were covered with aluminium foil that had small holes to allow some air circulation. The soil moisture was measured with each nitrate determination and the moisture was adjusted with distilled water if necessary. A bulk soil sample was prepared for each of the test concentrations. After thorough mixing, two sub-samples were taken and analysed on days 0, 7, 14 and 21 from each of the two test samples and the control sample. The sub-samples were extracted for nitrate by using 0.1 M potassium chloride. The solutions were filtered and analysed by HPLC. A recovery experiment spiked with nitrate was performed to determine the % recovery.

Findings:
The applicant claimed that recoveries were found to be within an acceptable range but no recovery results were provided. The soil samples showed no lasting adverse effect on microbial nitrification or respiration process 21 days post treatment at 56 and 560 mg/kg of pyraclofos with a <10% deviation in soil nitrate concentration compared to the control.

Conclusions:
On the basis of the data provided, pyraclofos has no adverse effect on microbial nitrification at pyraclofos concentrations of 56 and 560 mg/kg.

7.5.4. Mammals

Colleague (19 g/L albendazole and 150 g/L pyraclofos) is listed in Schedule 6 of the SUSDP. LD_{50} for the rat was found to be 473 mg/kg on a combined sex basis.

7.5.5. Phytotoxicity

The data relating to metabolic fate in cabbage plants provides information on the possible persistence and breakdown of pyraclofos under field conditions when excreted from the sheep in the urine. When applied to the leaf surface at the rate of 2 µg/cm, pyraclofos disappeared with a half life of approximately one week. Very little translocation occurred. Similarly there was very little uptake in cabbage plants grown in soil treated with pyraclofos after 30 days after treatment. No symptoms of phytotoxicity appear to have been observed.

In use as an insecticide in other countries, pyraclofos is registered on field crops, vegetables and orchards trees for repeated applications at rates of exposure higher than any likely from use as a sheep drench (Royal Society of Chemistry 1993). The only mention of phytotoxic effects is to orchard trees at certain stages of growth, this, however, is typical of most organophosphate compounds.

7.5.6. Summary of Environmental Toxicity

Pyraclofos is highly to very highly toxic to birds, aquatic organisms and bees but of low toxicity to the earthworm. The data supplied for effect on other invertebrates is that the chemical is used in other parts of the world as an insecticide/acaricide with high to moderate toxicity to various orders of arthropod. Phytotoxicity is likely to be limited due to use as a commercial insecticide in many crops at far higher rates than the environmental exposure likely from use as a sheep drench.

7.6. Environmental Risk Assessment

7.6.1. Risk arising from use

From the available data the highest toxicity to the environment is expressed in the aquatic compartment where all species tested showed a relatively high degree of susceptibility to pyraclofos. The most sensitive end point in the aquatic environment is the 48 h EC_{50} of 0.55 µg/L for freshwater cladoceran which will be used in the aquatic risk assessment. This compartment, however, is not likely to be highly exposed to pyraclofos due to the mode of application as an internally applied drench to sheep. The substance is also highly toxic to birds and bees but again exposure is likely to be very low. The soil compartment is the one of highest concern.

Soil Compartment

The company provided has a model based on "worst case" figures generated from physico-chemical data and the laboratory work reported above. The amount of pyraclofos being excreted over a hectare of land at a stocking rate of 10 sheep/ha from four dosings per year was calculated to be 57 g/ha. This calculation assumes no metabolism and provides a theoretical end point concentration for terrestrial ecosystem of 25 ppb if spread evenly in the top 15 cm of soil.

While mixing is likely to be limited to the top 5 cm (resulting in a worst case calculation of 75 ppb), this simple model exaggerates the amount reaching soils as no allowance is made for metabolic breakdown during the excretion time of the product (1 week). If only 10% is assumed to be excreted unchanged a worst case concentration of 7.5 ppb is calculated if mixed in the top 5 cm of soil. In commercial use, however, the time between dosing would be at a minimum of 21 days and dissipation and breakdown would have occurred in this period, thus reducing the end point concentrations substantially.

A more realistic figure might be derived as follows:

Amount excreted per hectare following a single dose (assumes 10 sheep per hectare and about 10% excreted unchanged) = 1.5 g

Concentration in top 5 cm of soil = 0.625 ppb

Therefore there appears to be a significant safety factor in soils. Concentrations that affect soil and plant inhabiting arthropods are not likely to be exceeded in the course of normal application and excretion.

The applicant has provided arguments to substantiate the low toxicity of pyraclofos to earthworms. This is based on the criterion used by the US EPA and US FDA in environmental risk assessment ie if the estimated environmental concentration (EEC) of a substance does not exceed 1% of the LC_{50}, it is generally concluded that there is little or no environmental risk from this substance. The EEC in the terrestrial compartment was estimated to be 25 ppb based on 100% of the excreted drug corresponding to the parent compound. On the basis of the metabolism data provided only 10% of the excreted residues are due to unchanged drug and the metabolites are inactive. Furthermore, seasonal usage in sheep coupled with hydrolysis, photolysis, adsorption to soil particles and microbial transformation will impact on the final EEC. Consequently, exposure of the soil dwelling invertebrates will be transient and localized and accumulation of inhibitory concentrations over time is highly unlikely. Additionally, the binding of pyraclofos to soils may reduce its availability if ingested by soil dwelling organisms. Therefore, the ECC is likely to be <2.5 ppb and <1% of the lowest LC_{50} observed in the species studied.

Based on the calculations, the risk quotient Q for earthworm is likely to be <<0.1 in view of the low toxicity of pyraclofos to earthworm (>610 mg/kg) and the low PEC of <2.5 µg/kg soil. As for other soil organisms, the nitrogen transformation test at 560 mg/kg and 56 mg/kg and the lowest LC_{50} of 3.3 mg/L for Coleoptera (*Epilachna varivestis*, see Table 8) indicate that there is unlikely to be an environmental risk at the PEC of 2.5 µg/kg soil.

Aquatic compartment

Similarly the company has (using the above assumptions) derived a “worst case” concentration in water by assuming an average of 100 cm of rain per year and calculating the likely level in (interstitial) water by use of the partitioning co-efficient between water and soil. In this way a value for the aquatic ecosystem of 1.2 ppb is derived.

The calculation presented below is based on a run-off scenario. This assumes limited use in sheep feedlots and that sheep are not yarded after treatment.

Assuming that the run-off from a stocking rate of 10 sheep per hectare runs into pond 1 ha in size and 15 cm deep and that pyraclofos run-offs in the run-off water at 5% of applied, then

Weight of pyraclofos in pond water = 1.5 g X 5%
= 0.075 g

Volume of water = 10000 m^2 X 0.15 m X 1000 L/m^3
= 1.5 ML

Concentration in pond = 0.075 g/1.5 ML
= 0.05 µg/L

The above calculation can readily be further refined. A US review reported run-off of an organophosphate after rainfall was only 0.3-0.6% of applied (ATSDR, 1997). Using a figure of 0.5%, the EEC in water from run-off is 0.005 µg/L and for freshwater cladoceran Q = 0.005/0.55 <0.1 indicating an acceptable risk to aquatic invertebrates.

Vegetation

Envrionmental risk would appear to be low. The chemical is applied to many field pasture and orchard crops in other parts of the world at rates well in excess of the expected environmental exposure to pasture plants as a component of sheep excreta.

The applicant has provided argument that it is generally considered that organophosphates are not particularly toxic to plant species. It has been reported that among 131,596 chemicals tested on five species of plant seeds (corn, wild oats, cotton, soybean and radish) less than 1% of the chemicals tested killed 100% of any species at a concentration <1 mg/kg and nearly none (0.006%) killed <0.1 mg/kg (Kenaga, 1981). Therefore, at the worst case EEC of 25 µg/kg (see above), pyraclofos will not likely to pose an environmental risk to terrestrial plant species. However, a more realistic figure can derived as follows:

Amount excreted per hectare following a single dose (assuming 10 sheep per hectare and about 10% excreted unchanged) = 1.5 g

Concentration in top 5 cm of soil = 0.625 µg/kg

It may be concluded that there appears to be a significant safety factor in soils although concentrations may be higher if use in sheep feedlots is proposed.

7.6.2. Risk arising from formulation, handling and disposal

Formulation/Packaging

Formulation will take place in well established facilities that have experience, equipment and emergency control facilities to handle this class of pesticide.

Formulation is a suspension liquid packed in Ready-to-Use 5 L HDPE backpacks that would only require connection to the drench gun for application to the sheep and 12 L HDPE drums. The formulation in the 12 L drums would be transferred to the backpacks as required.

Use

The proposed product is in a ready to use form. The contamination dangers usually associated with chemical mixing on the farm are minimised.

Disposal

The disposal statement supplied in the label is considered acceptable.

7.7. Conclusions And Recommendations

The re-introduction of pyraclofos as component of a sheep anthelmintic is unlikely to pose an unacceptable environmental risk when used and disposed of according to label directions.

DEH recommends that APVMA can be satisfied that the proposed use of pyraclofos would not be likely to have an unintended effect that is harmful to animals, plants, or things or to the environment.

In the future if a wider use for pyraclofos as a foliar applied insecticide/acaricide is envisaged the environmental risk should be reassessed. Depending on the proposed use additional fate and ecotoxicity data may be required.

8. EFFICACY AND SAFETY ASSESSMENT

8.1. Efficacy:

The APVMA approved the following claims for *Colleague* (45463) when initial registration was approved in 1993: "For the control of sensitive gastrointestinal roundworms, large lungworms and tapeworms. Aids in the control of adult liver fluke and reduces the output of viable worm and fluke eggs."

Following discussions with Schering-Plough Animal Health the APVMA agreed that re-assessment of these claims was not required for the second registration. However, a new claim against resistant species was proposed which required assessment. The claim was "including strains that have single or multiple resistance to benzimidazole, levamisole, closantel and macrocyclic lactone drenches."

Schering-Plough supported this claim with data from an artificial infection and pen trial (04/0208) and a confirmatory field trial (04/0183) conducted at two sites in NSW and one site in WA. These three sites were chosen as they were believed to have pre-existing resistance.

The pen trial (04/0208) compared the efficacy of *Colleague* with Rametin (35800) combined with a benzimidazole (BZ), Triton (55285) and Q-Drench (56573). 18-month old Merino wethers were artificially infected with *Haemonchus contortus*, *Teladorsagia circumcincta* and *Trichostrongylus colubriformis*. The Reviewer reported *Colleague* was effective (>99.9% GM) against all three multiple resistant species when compared to untreated controls.

In the field trial (04/0183), efficacy of *Colleague* was compared with ML, BZ, levamisole (LEV), BZ+LEV drenches, Rametin alone and Rametin+BZ. At one of the NSW sites *Trichostrongylus* and *Oesophagostomum* were the dominant species with minor population of *Teladorsagia*. *Colleague* was fully effective (100%). However, due to the low egg count and proportion of *Teladorsagia*, the results were not considered reliable.

The helminth population at the other site in NSW was predominantly *Haemonchus* with minor representations of *Trichostrongylus* and *Teladorsagia*. The Reviewer disagreed that true resistance, by definition, to BZ existed on this site, but the product was 100% effective. *Trichostrongylus* and *Teladorsagia* were the dominant population at the WA site. Resistance to BZ and LEV existed, but none or marginal resistance to ML and BZ+LEV combination. *Colleague* demonstrated efficacy (95% AM) against the species present.

The Reviewer reported that *Colleague* was highly effective at each site, but the level of resistance was lower than expected.

When the field studies are considered in conjunction with the evidence from the pen trial, the claim of effectiveness against GIT roundworms with resistance to benzimidazole, levamisole, closantel and macrocyclic lactone drenches is supported.

8.2. Target Animal Safety:

Colleague (45463) was originally registered by Smith, Kline and Beecham on 6 December 1993 and was recalled following a series of adverse events, which included the deaths of sheep; on 6 January 1994. Prior to the original registration and the launch of *Colleague* safety testing in sheep was performed over an eight-year period. This testing included pen trials (margin of safety studies at 3 and 5 times the recommended dose) with no reported adverse reactions. Some deaths did occur in the field trials at 2 and 3 times the recommended dose. The original trial work indicated that caution was needed for those sheep with lupinosis, in poor condition or that had been held off feed. The approved label (dated 3 December 1993) therefore reflected these concerns with precautions that included:

- Sheep with severe liver disease eg. lupinosis should not be treated.
- Care should be taken when drenching sheep recently introduced to a high grain diet or when drenching severely debilitated or stressed sheep.
- During the first month of pregnancy all medication should be administered with care. It is particularly important not to exceed the recommended dosage at this time.
- The dosing schedule suggested that only lambs over 10kg in weight should be treated.

Following the launch of the product on 6 December 1993 an overall mortality rate of 1% (1676) was reported from a total of 166,443 sheep treated on 81 properties. Mortality rates in individual mobs were higher, rising to as much as 40%. The product was voluntarily recalled and withdrawn on 6 January 1994. Many of the deaths were attributed to organophosphate (OP) poisoning, but were not confirmed. Environmental conditions (drought), stress, pre-existing liver diseases, on-farm practices such as overnight yarding, withholding of feed and water, introduction to high grain diet, and user unfamiliarity with OP drenches were offered as reasons for the occurrence of the adverse events although some of these adverse events were due to practices contrary to the precautions on the approved label.

A comparative trial was provided in the submission to re-register *Colleague*. The level of safety of *Colleague* (pyraclofos) was compared with an 800 g/kg naphthalopos (NAP) containing drench (OP), and with non-OP containing drenches. The study was conducted on commercial sheep properties and the cooperating graziers were trained in the use of *Colleague* using a training manual developed by Schering-Plough. This included ensuring that sheep which had been grazing Paterson's Curse, heliotrope, St

John's Wort or supplemented with lupin grain were not included in the study. Sheep that belonged to one of the following categories below were therefore not included:

- Severe liver disease (lupinosis)
- Recently introduced onto a high grain diet
- Severely debilitated or stressed
- Lambs < 10 kg liveweight
- Yarded overnight and held off feed and water

Treatment groups consisted of mixed sex and age sheep. Where a wide variation in weight existed within groups, sheep were drafted by weight into sub-groups to prevent overdosing. Each group was monitored on the afternoon of treatment, then 24 and 48 hours post-treatment for deaths and signs of OP poisoning. A post-mortem was conducted on any dead sheep that were found within 48 hours post treatment, and the possible cause of death was determined. Sheep that died after 48 hours were not investigated, and Schering-Plough did not provide the rationale for failing to investigate these or for not including sheep dying with clinical signs of OP poisoning. Blood cholinesterase levels of some members of treated flocks were determined in some instances to indicate whether the cause of death was due to OP poisoning. A drench history of the mobs was also recorded.

A total of 72,659 sheep were treated with currently registered products with no deaths recorded. Of these sheep, 29,321 were treated with an organophosphate drench containing naphthalaphos to, and a further 43,338 sheep were treated with Non-OP drenches such as those containing levamisole, benzimidazoles, and macrocyclic lactones and combinations of the above. No deaths in the above control groups were recorded.

Of the 57,581 sheep that were drenched with *Colleague*, a total of 59 deaths (0.102 %) were recorded.

Further data was submitted and the total number treated with *Colleague* was revised up to 75,874 - with a total number of 61 deaths or an overall mortality of 0.08%. The total number in the control groups was also revised – up to 74,283 . In the control groups, again there was zero mortality in a total of 74,283 sheep treated with either the other OP product containing naphthalaphos (30,173) or the non-OP products (44,110).

An observed mortality rate of 1 % was considered a sufficient reason by the manufacturer to stop sales and to recall the product in December 1993.

The overall mean mortality in the subsequent study undertaken in the summer and autumn of 2003/4 was reduced to around 0.1%, but there was a clustering effect and some mobs treated with *Colleague* had mortalities ranging up to 1.8 % (5 sheep died from 274 treated) and in one group of 50 sheep drafted away from a larger mob 2 sheep died (4% mortality).

The study detailed mortality only and did not detail the incidence of clinical cases of OP poisoning which later recovered nor how this morbidity might impact on production losses nor animal welfare implications.

The applicant has argued that the level of resistance to other classes of anthelmintics and associated losses in terms of economics and animals justifies the registration of *Colleague* despite the risk to target animal safety. An extensive education of the end users associated with the safety trial was conducted using the manual developed by Schering-Plough and followed the precautions previously outlined above. The study showed that there was an observed mortality rate associated and directly attributable to the use of this product that was not seen with the other registered anthelmintics used under similar conditions. However the treatment and control groups could not be directly compared because of the absence of clearly defined criteria for the inclusion/exclusion of adverse events.

The classification of mortalities *a posteriori* represents a potential for significant bias in statistical conclusions. The trial design and conduct therefore precluded a valid statistical analysis of the mortality data from the use of Colleague in sheep being undertaken

Considering the demonstrated toxicity of the product for the target animals and the need for a diagnostic and informed approach to limit that risk, it is proposed that the product be used by or under the direction of registered veterinary surgeons. The following directions are therefore proposed to be included on the label:

TO BE SUPPLIED ONLY ON THE WRITTEN ADVICE OF A REGISTERED VETERINARY SURGEON.
TO BE USED BY OR UNDER THE DIRECTION OF A REGISTERED VETERINARY SURGEON.

9. LABELLING REQUIREMENTS

POISON

KEEP OUT OF REACH OF CHILDREN
READ SAFETY DIRECTIONS BEFORE OPENING OR USING
FOR ANIMAL TREATMENT ONLY

COOPERS®
COLLEAGUE ®
Broad Spectrum Sheep and Lamb Drench.

ACTIVE CONSTITUENTS: 150 g/L PYRACLOFOS
(An Anticholinesterase Compound)
19 g/L ALBENDAZOLE

PICTURE

An oral drench for the control of sensitive gastrointestinal roundworms (including strains that have single or multiple resistance to benzimidazole, levamisole, closantel and macrocyclic lactone drenches), large lungworms and tapeworm. Aids in the control of adult liver fluke and reduces the output of viable worm and fluke eggs.

IMPORTANT:
READ PRECAUTIONS BEFORE USE.
MIX PRODUCT WELL BEFORE USE. INVERT CONTAINER AT LEAST 10 TIMES AND SHAKE VIGOROUSLY BEFORE DRENCHING.

5 Litres
12 Litres

COOPERS LOGO

COOPERS®
COLLEAGUE® *Broad Spectrum Sheep and Lamb Drench*
When used as an oral drench COLLEAGUE is effective against sensitive strains of the following internal parasites of sheep:

Roundworms: Mature and immature	
Barber's pole worm	*Haemonchus contortus*
Stomach hair worm	*Trichostrongylus axei*
Small brown stomach worm	*Ostertagia* spp
Black scour worm	*Trichostrongylus* spp
Small intestinal worm	*Cooperia* spp
Thin-necked intestinal worm	*Nematodirus* spp
Hookworm	*Bunostomum* spp
Nodule worm	*Oesophagostomum columbianum*
Large bowel worm	*Oesophogostomum venulosum*
Large mouthed bowel worm	*Chabertia ovina*
Lungworms	
Large lungworm	*Dictyocaulus filaria*
Tapeworms	
(Heads and Segments)	*Moniezia expansa*
Liver Fluke (adult)	*Fasciola hepatica*
Ovicidal	Reduces the output of viable worm and fluke eggs

Pyraclofos is a member of the organophosphate family of drenches. Albendazole is a member of the benzimidazole family of drenches.
Resistance may develop to any drench. Ask your local veterinarian or animal health advisor for recommended parasite management practices for your area to reduce resistance development. It is advisable that a drench resistance test should be conducted before any drench is used.

DIRECTIONS FOR USE
TO BE SUPPLIED ONLY ON THE WRITTEN ADVICE OF A REGISTERED VETERINARY SURGEON.
TO BE USED BY OR UNDER THE DIRECTION OF A REGISTERED VETERINARY SURGEON.
Restraints:
DO NOT use in ewes which are producing or may in the future produce milk or milk products for human consumption.

Precautions:
This product is an organophosphate containing product. Deaths may occur following the use of this product. Label directions should be strictly followed.
REVIEW NORMAL ORAL DRENCHING MANAGEMENT PRACTICES PRIOR TO THE USE OF COLLEAGUE. Management practices should ensure that all drenching procedures are correctly carried out. During the first month of pregnancy all medication should be administered with care. It is particularly important not to exceed the recommended dosage at this time.
COLLEAGUE must not be used to treat sheep with severe liver disease (eg. lupinosis).
COLLEAGUE must not be used to treat sheep recently introduced to a high grain diet.
COLLEAGUE must not be used to treat severely debilitated or stressed sheep.
COLLEAGUE must not be used to treat lambs < 10 kg liveweight.
COLLEAGUE must not be used to treat sheep that have been yarded overnight and held off feed and water.
Allow animals access to feed and water IMMEDIATELY following treatment.
Animals in poor condition, during severely cold weather, may be predisposed to OP toxicity.
Sheep should be weighed and dosed accurately.
Minimise stress to sheep by avoiding administering other treatments at the same time as COLLEAGUE.

SHAKE CONTAINER BEFORE USE.
Dose Rates: Sheep and Lambs. 1 mL per 5 kg body weight, for example:

[*5L and 12L Pack Treats to be included on appropriate label*]

Bodyweight (kg)	Dose Rate mL	5L Pack Treats	12L Pack Treats	Bodyweight (kg)	Dose Rate mL	5L Pack Treats	12L Pack Treats
10	2	2500	6000	41 - 45	9	555	1333
11 - 15	3	1666	4000	46 – 50	10	500	1200
16 - 20	4	1250	3000	51 – 55	11	454	1090
21 - 25	5	1000	2400	56 – 60	12	416	1000
26 - 30	6	833	2000	61 – 65	13	384	923
31 - 35	7	714	1714	66 – 70	14	357	857
36 - 40	8	625	1500	71 - 75	15	333	800

Sheep over 75 kg should be dosed at 1mL per 5 kg bodyweight. Sheep should be weighed and dosed accurately.

GENERAL INSTRUCTIONS
MIXING: BEFORE USE ENSURE PRODUCT IS WELL MIXED BY INVERTING THE CONTAINER AT LEAST 10 TIMES AND SHAKING VIGOROUSLY.

COLLEAGUE Aids in the Control of Adult Liver Fluke
COLLEAGUE aids in the control of adult liver fluke. COLLEAGUE reduces the output of viable fluke eggs. Contamination of pasture will be reduced by avoiding grazing recognised fluke areas for 24 hours after dosing.

WARNING: Users should be aware that there is potential toxicity to humans if they are accidentally exposed to product while dosing. Due care should be taken that sheep are not overdosed and that label safety directions for users are strictly followed.

For further information contact Coopers Customer Service on 1 800 226 511.

WITHHOLDING PERIODS
MEAT: DO NOT USE less than 14 days before slaughter for human consumption.
MILK: DO NOT USE in ewes which are producing or may in the future produce milk or milk products for human consumption.

TRADE ADVICE:
EXPORT SLAUGHTER INTERVAL (ESI) – DO NOT slaughter for export for 35 days after treatment.

SAFETY DIRECTIONS:

Harmful if absorbed by skin contact or swallowed. Will irritate the eyes and skin. Avoid contact with eyes and skin. Repeated minor exposure may have a cumulative poisoning effect. When using the product wear elbow-length PVC gloves. Wash hands after use. After each day's use, wash gloves.

FIRST AID:
If poisoning occurs, contact a doctor or Poisons Information Centre. (Phone 131126). If swallowed, give one atropine tablet every 5 minutes until dryness of the mouth occurs – if poisoned by skin absorption or through lungs, remove any contaminated clothing, wash skin thoroughly and give atropine tablets as above. Get to a doctor or hospital quickly.
Additional information is listed in the material safety data sheet.

COLLEAGUE 5L/12L – IMMEDIATE CONTAINER REAR PANEL (continued)

SPECIALIST ADVICE IN EMERGENCY ONLY
Schering-Plough Pty Ltd
1 800 226 511
ALL HOURS - AUSTRALIA-WIDE

DRUMMUSTER ELIGIBLE CONTAINER

WARRANTY
Schering-Plough Pty Ltd (SPPL) warrants that this product is of merchantable quality and fit for its intended purpose. SPPL's liability for any loss, including consequential losses or injury caused by any act or omission, including negligent acts or omissions, by SPPL or its agent, is limited to replacing or repairing the product at the option of SPPL. If possible, a sample of any product causing concern should be retained or delivered to SPPL within 30 days for a scientific examination.

DISPOSAL:
This container can be recycled if it is clean, dry, free of visible residues and has the ***drum****MUSTER* logo visible. Triple or pressure rinse container for disposal. Dispose of rinsate or any undiluted chemical according to State legislative requirements. Wash outside of the container and the cap. Store cleaned container in a sheltered place with cap removed. It will then be acceptable for recycling at any ***drum****MUSTER* collection or similar container management program site. The cap should not be replaced but may be taken separately.

COOPERS ANIMAL HEALTH
A Division of Schering-Plough Pty Ltd
11 Gibbon Road, Baulkham Hills NSW 2153

STORAGE
Store below 30°C (Room Temperature). Store in original container, tightly closed in a safe place.
DO NOT STORE DRUM OR EQUIPMENT CONTAINING PRODUCT IN DIRECT SUNLIGHT

APVMA Approval No. 59304/5/_____
APVMA Approval No. 59304/12/_____

SEE BELOW FOR BATCH AND EXPIRY

® Coopers is a Registered Trademark of Schering-Plough Animal Health Corporation.
® Colleague is a Registered Trademark of Schering-Plough Ltd (Switzerland)
® Yellow (PANTONE® 123) the predominant colour of Coopers packaging is a Registered Trademark of Schering-Plough Animal Health Corporation. PANTONE is a registered trademark of Pantone, Inc.
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5L
12L

Batch and Expiry to be screen printed on immediate container

Barcodes and printer codes to be assigned

COLLEAGUE SWINGTAG AND STICKER 5L & 20L

SWINGTAG

COOPERS®
COLLEAGUE ®
Broad Spectrum Sheep and Lamb Drench.

IMPORTANT:
READ PRECAUTIONS BEFORE USE.
MIX PRODUCT WELL BEFORE USE. INVERT CONTAINER AT LEAST 10 TIMES AND SHAKE VIGOROUSLY BEFORE ORAL DRENCHING.

APVMA Approval No. 59304/_____

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® Colleague is a Registered Trademark of Schering-Plough Ltd (Switzerland)

COOPERS LOGO

STICKER

IMPORTANT:
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APVMA Approval No. 59304/_____

10. 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.

11. References

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Australian Pesticides and Veterinary Medicines Authority *MRL Standard: Maximum Residue Limits in Food and Animal Feedstuffs,* APVMA, Canberra. (See footnote below)

Australian Pesticides and Veterinary Medicines Authority *Vet Labelling Code—Code of Practice for Labelling Veterinary Chemical Products,* APVMA, Canberra. (See footnote below)

Kenaga E E 1981. Comparative toxicity of 131,596 chemicals to plant seeds, Ecotoxicology and Environmental Safety, 5, 4496-4475.

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WHO 1997, *Guidelines for predicting dietary intake of pesticide residues* (revised), prepared by the Global Environment Monitoring System - Food Contamination Monitoring and Assessment Programme (GEMS/Food) in collaboration with Codex Committee on Pesticide Residues, WHO/FSF/FOS/97.7.

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