Public Release Summary

on

Evaluation of the new active

SPINOSAD

in the products

Laser Naturalyte Insect Control
Tracer Naturalyte Insect Control

National Registration Authority
for Agricultural and Veterinary Chemicals

October 1998

Canberra
Australia
FOREWORD

The National Registration Authority for Agricultural and Veterinary Chemicals (NRA) 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 NRA works in close cooperation with advisory agencies, including the Department of Health and Family Services (Chemicals and Non-prescription Drug Branch), Environment Australia (Risk Assessment and Policy Section), the National Occupational Health and Safety Commission (Worksafe Australia) and State departments of agriculture and environment.

The NRA 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 NRA 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 NRA’s publications Ag Manual: The Requirements Manual for Agricultural Chemicals and Ag Requirements Series.

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

More detailed technical assessment reports on all aspects of the evaluation of this chemical can be obtained by completing the order form in the back of this publication and submitting with payment to the NRA. Alternatively, the reports can be viewed at the NRA Library, Third floor, 10 National Circuit, Barton, ACT.

The NRA welcomes comment on the usefulness of this publication and suggestions for further improvement. Comments should be submitted to the Executive Manager—Registration, National Registration Authority for Agricultural and Veterinary Chemicals, PO Box E240, Kingston ACT 2604.
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# List of Abbreviations and Acronyms

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>µg</td>
<td>microgram</td>
</tr>
<tr>
<td>14C</td>
<td>carbon 14 isotope</td>
</tr>
<tr>
<td>ADI</td>
<td>acceptable daily intake (for humans)</td>
</tr>
<tr>
<td>ai</td>
<td>active ingredient</td>
</tr>
<tr>
<td>d</td>
<td>day</td>
</tr>
<tr>
<td>DAT</td>
<td>days after treatment</td>
</tr>
<tr>
<td>EC</td>
<td>emulsifiable concentrate</td>
</tr>
<tr>
<td>EC₅₀</td>
<td>concentration at which 50% of the test population are immobilised</td>
</tr>
<tr>
<td>g</td>
<td>gram</td>
</tr>
<tr>
<td>h</td>
<td>hour</td>
</tr>
<tr>
<td>Ha</td>
<td>hectare</td>
</tr>
<tr>
<td>HPLC</td>
<td>high pressure liquid chromatography or high performance liquid chromatography</td>
</tr>
<tr>
<td>kg</td>
<td>kilogram</td>
</tr>
<tr>
<td>L</td>
<td>litre</td>
</tr>
<tr>
<td>LC₅₀</td>
<td>concentration that kills 50% of the test population of organisms</td>
</tr>
<tr>
<td>LD₅₀</td>
<td>dosage of chemical that kills 50% of the test population of organisms</td>
</tr>
<tr>
<td>mg</td>
<td>milligram</td>
</tr>
<tr>
<td>mL</td>
<td>millilitre</td>
</tr>
<tr>
<td>MRL</td>
<td>maximum residue limit</td>
</tr>
<tr>
<td>MSDS</td>
<td>material safety data sheet</td>
</tr>
<tr>
<td>NDPSC</td>
<td>National Drugs And Poisons Schedule Committee</td>
</tr>
<tr>
<td>NER</td>
<td>non-extractable residue</td>
</tr>
<tr>
<td>ng</td>
<td>nanogram</td>
</tr>
<tr>
<td>NOEC/NOEL</td>
<td>no observable effect concentration/level</td>
</tr>
<tr>
<td>PHED</td>
<td>Pesticide Handlers Exposure Database</td>
</tr>
<tr>
<td>POEM</td>
<td>Predictive Operator Exposure Model</td>
</tr>
<tr>
<td>ppb</td>
<td>parts per billion</td>
</tr>
<tr>
<td>PPE</td>
<td>personal protective equipment</td>
</tr>
<tr>
<td>ppm</td>
<td>parts per million</td>
</tr>
<tr>
<td>s</td>
<td>second</td>
</tr>
<tr>
<td>SUSDP</td>
<td>Standard for the Uniform Scheduling of Drugs and Poisons</td>
</tr>
<tr>
<td>TGAC</td>
<td>technical grade active constituent</td>
</tr>
<tr>
<td>ULV</td>
<td>ultra low volume</td>
</tr>
<tr>
<td>WHP</td>
<td>withholding period</td>
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SUMMARY

The National Registration Authority for Agricultural and Veterinary Chemicals (NRA) has considered an application to register the new chemical spinosad for the control of cotton bollworm and native budworm in cotton, in New South Wales, Queensland and Western Australia, as specified in the directions-for-use table on the products’ labels.

This publication outlines the regulatory considerations and summarises the data reviewed by the NRA for the proposed registration of spinosad. Before deciding whether to approve this product for use in Australia, the NRA invites public comment. Comments should be submitted by 3 November 1998 to the NRA at the address indicated on page 1.

The NRA has assessed the data submitted by the applicant in support of this use of spinosad and provides the following information for public comment.

Public Health Aspects

Spinosad is comprised of approximately 10 related chemical factors of which two closely related factors, spinosyn A and spinosyn D, represent about 88% of the product's activity. The products to be registered, Laser Naturalyte Insect Control and Tracer Naturalyte Insect Control which contain spinosad at 125 g/L and 480 g/L, respectively will be used for the control of insects in the cotton industry.

Spinosad is of low acute oral, dermal and inhalational toxicity and is a slight eye irritant, but is not a skin irritant or sensitiser. Laser Naturalyte Insect Control is of low acute oral and dermal toxicity. It is a moderate skin irritant, a skin sensitiser, and is expected to be a moderate to severe eye irritant. Although Laser Naturalyte Insect Control is a skin sensitiser, two preparation similar to the diluted product, were not skin sensitisers. Tracer Naturalyte Insect Control is of low acute oral, dermal and inhalational toxicity, it is a slight skin and eye irritant, but is not a skin sensitiser.

In studies in mice, rats and dogs there was no evidence to suggest that spinosad is carcinogenic and this was further supported by negative findings in studies conducted to determine whether spinosad damages genetic material. A mechanism of action for spinosad has not been proposed, however, in mammals, the range of lesions induced and tissues affected suggest a non specific mechanism of cellular injury. Effects reported in reproductive and developmental etudes were of a kind that were consistent with parental/maternal toxicity, rather than a specific toxic effect on reproductive performance or behaviour or on the developing embryo or foetus. Although some damage to individual nerve fibres was reported in a 12-month neurotoxicity study in rats, there was no evidence to suggest that spinosad has potential to damage the nervous system in other specific studies.

Based on an assessment of the toxicology and the potential dietary intake of residues, it was considered that there should be no adverse effects on human health from the proposed use of the product.
**Residues in food and trade aspects**

Residue data were provided for cotton seed and the processed cotton seed oil from trials conducted in Australia and the USA.

**Cotton seed and processed fractions**

In the USA trials, the treatment regimes of 5 applications included 0.75x, 1x, 2x and 6x the maximum label rate (100 g ai/ha) at intervals of 7-17 days. Ginned cottonseed was collected 28 days after the last treatment. No residue was detected, except in the 6x study where the raw cottonseed commodity from ginned combine harvested cotton bolls contained residues at 0.073 mg/kg (limit of determination 0.003 mg/kg, limit of quantitation 0.010 mg/kg). No residues were found in the meal or soapstock samples. In the hulls, crude oil and refined oil, residues ranged from 0.012 to 0.018 mg/kg. Residues of Spinosad do not concentrate in the processed products hulls, meal, crude and refined oils or soapstock.

The Australian trials used 1-3 applications at 0.75x, 1x and 2x the label rate with intervals of 5-8 days. At 28-89 days after the last application no residues were found in cottonseed.

The residue data indicate that in accordance with the recommended use pattern the following withholding period is appropriate:

Cotton: DO NOT harvest for 28 days after application

**Trade**

As spinosad is a new pesticide and has only gained approval in the USA (for cottonseed) it is suggested that recognition for Australian MRL standards be sought from importing countries.

With regard to export animal commodities, residues are not likely to be a problem for animals fed a diet comprising up to 60% cottonseed by-products for which the level of residues is expected to be below the limit of quantitation, 0.01 mg/kg. Under these circumstances a pre-slaughter interval may not be required.

There is a potential problem of residues in cattle fed contaminated feed or pasture as a result of spray drift. It is difficult to assess the likely levels of spinosad on pasture/crops from spray drift. The worst case would be to assume the highest residue levels found on cotton leaves will also be found on crops/pasture affected by spray drift and that animals graze immediately after spraying. Using information from the residue depletion study conducted in dairy cows an interval free from contaminated feed of 4-5 weeks is necessary to guarantee the residue levels will fall to below the limit of determination.
**Occupational health and safety aspects**

Worksafe Australia has conducted a risk assessment on Laser Naturalyte Insect Control (Laser) and Tracer Naturalyte Insect Control (Tracer) for use in cotton. Laser is an aqueous suspension emulsion in oil (low volume) containing spinosad at 125 g/L and Tracer is an aqueous suspension concentrate containing spinosad at 480 g/L. Both products can be safely used by workers when handled in accordance with the control measures indicated in this assessment.

Spinosad and Tracer are not considered hazardous according to NOHSC criteria. However, Laser is determined to be a hazardous substance according to NOHSC criteria. Hazardous substances are subject to the workplace controls outlined in NOHSC (1994).

The active ingredient and the products will be formulated overseas and imported only as the finished, commercially packaged products.

Laser and Tracer will be applied to cotton by aerial spray or ground rig (boom spray).

Workers may be exposed to spinosad during mixing and loading, ground spray application, as well as during clean-up operations. The main hazards associated with Laser are moderate to severe eye irritation, skin sensitisation, slight skin irritation as well as the possibility of chronic effects. The main hazards associated with Tracer are slight eye and skin irritation. The risk assessment indicates that adequate worker controls can be instituted to enable safe use of the product.

Workers mixing and loading Laser should wear cotton overalls buttoned to the neck and wrist and a washable hat, elbow-length PVC gloves and face shield. During the ground application of Laser, workers should wear cotton overalls buttoned to the neck and wrist and a washable hat and elbow-length PVC gloves. During mixing/loading operations and ground applications with Tracer, workers should wear cotton overalls buttoned to the neck and wrist and a washable hat and elbow-length PVC gloves.

Re-entry workers do not require protective equipment.

**Environment aspects**

Spinosad, comprising spinosyn A and spinosyn D in a ratio of approximately 85:15, is the active ingredient in the formulations TRACER and LASER. TRACER is an suspension concentrate formulation with 480 g ai.L$^{-1}$, while LASER is an aqueous suspension emulsion in oil with 125 g ai.L$^{-1}$. It is to be used to control cotton bollworm (*Helicoverpa armigera*) and native budworm (*H. punctigera*) on cotton. LASER and TRACER are to be registered for use in NSW, Qld and WA only. The proposed maximum annual rate is 300 g ai.ha$^{-1}$ (no more than three applications of 100 g.ha$^{-1}$). LASER and TRACER (water based) can be applied by ground or by aerial spraying. LASER can also be aerially applied as an ULV formulation.
Environmental Fate

In laboratory studies, degradation of Spinosad appears restricted to aqueous and soil photolysis, and aerobic metabolism. There is some potential for Spinosad to be persistent in anaerobic sediment where degradation due to photolysis would be limited. Its movement off-site is probably limited to movement with substrate (eg soil). Degradation, irrespective of the mechanism, gives rise to intermediates in which the macrolide ring system is hydroxylated and/or the amino sugar is de-methylated (eg to form spinosyn B). Field dissipation studies, however, in which spinosyn A was applied to bare soil indicated no movement of Spinosad from the top layer of soil and with rapid degradation, (half-lives <1 d). The mineralisation half-life (assuming loss of label was due to production of CO₂), however, was 7 months. Spinosyn A did not bioaccumulate in fish. An aquatic microcosm test indicated that Spinosad residues could have a half-life of 3-5 d in relatively clear water. In aquatic sediment, however, it persisted and all samples had spinosyn levels of 15-20% of the applied dose, ie 24 h to 35 d after application, in agreement with the laboratory study result.

Environmental Effects

The ecotoxicity profile of Spinosad suggests that it is practically non-toxic in mammals and birds, and moderately toxic to fish, in acute studies. It is very slightly to slightly toxic to fish in chronic studies. No treatment-related mortality of oysters occurred, although a clear dose-response effect on new shell growth was evident. The acute toxicity of Spinosad to oyster is rated as very high.

The acute toxicity of Spinosad to water flea is rated as moderately toxic in a static test, while there is a marked difference in the static-renewal test which indicates only very slight to slight toxicity. The difference may be due to the formation of a more toxic metabolite or degradation product over the longer period of the static test.

In chronic studies using the mysid shrimp, the toxicity of Spinosad is rated as moderate. The chronic toxicity to water fleas is rated as high based on sub-lethal end-points, although the pattern of toxicity in the water flea tests indicated that mortality may be greater if the exposure period was extended. In the water flea, the chronic toxicity appears markedly different to that indicated from the acute toxicity tests, with acute to chronic ratios of the order of 10⁻³ X to 10⁻⁵ X.

Aquatic plant toxicity test results indicated that Spinosad is practically non-toxic to green algae, slightly toxic to the duckweed (based on growth and growth inhibition of both the plant and fronds), moderately toxic to blue-green algae, and highly toxic to freshwater and marine diatoms.

Spinosad appears to be only slightly harmful to beneficial arthropods, and potentially “soft” when compared with reference compounds now in use in cotton. Some effects of Spinosad on tachinid flies, ladybird beetles and particularly hymenoptera species (including bees and micro-hymenoptera parasitoids) were observed, although the occurrence of refugia may help in limiting the effects of Spinosad on these species. Spinosad is very slightly toxic to earthworms.
Environmental hazard

Environment Australia recognises, from discussions with the cotton industry, that there is an urgent need for replacement chemicals which have new chemistry and no cross resistance or bioaccumulation potential (in cattle), to control Helicoverpa. Spinosad apparently meets these criteria, and has certain advantages over present chemistries because it is potentially “softer” to some beneficial organisms.

When applied at the various rates, Spinosad will pose some hazard to aquatic organisms from acute exposure if directly sprayed, or if allowed to significantly drift (ie at 10% of application rate), onto water bodies. In the case of drift to more distant water bodies, either resulting in acute or chronic exposure, mitigation of effects may be possible through a number of factors (eg adsorption to sediment), which was demonstrated by modelling provided by the company, and Environment Australia’s own assessment. Therefore, acute and chronic risk to water column species would appear to be acceptable at all proposed rates for both the ULV and SC formulations. Hazard to benthic organisms also seems low.

Environment Australia concludes that Spinosad when directly sprayed on soil will not be a hazard to earthworms. Drift of Spinosad to adjacent properties or fields will also therefore not be a hazard. As persistence of Spinosad is not likely to occur in soil (eg due to photolysis or metabolism), earthworms are also not likely to be at risk from chronic exposure if chronic toxicity is similarly low as for acute toxicity.

Environment Australia also concludes that Spinosad when directly sprayed on avian food items will not be a hazard to birds. Drift of Spinosad to adjacent fields or habitats will also therefore not be a hazard. As persistence of Spinosad is not likely to occur on avian food items (eg short half-life on plants), avian species are also not likely to be at risk from chronic exposure.

Environment Australia concludes that while Spinosad may pose some hazard to hymenopteran species after acute exposure in laboratory tests, it appears to provide a softer option to other potential chemicals. Semi-field or field studies also confirmed the limited nature of effects of Spinosad on predators and parasitoids.

Efficacy and crop safety aspects

Data presented by Dow AgroSciences Australia Limited supported claims that LASER NATURALYTE INSECT CONTROL and TRACER NATURALYTE INSECT CONTROL adequately control Helicoverpa spp. in cotton. The data were gathered from a range of small plot replicated trials and commercial large-plot unreplicated treatments. The trial involved water based ground rig and aerial applications.

The data were adequate to satisfactorily assess efficacy when used according to the label directions (See pp. 36 - 51 ).

Safety to cotton has been demonstrated with no apparent adverse effects on a range of cotton cultivars, when used in accordance with directions.
INTRODUCTION

This publication provides a summary of the data reviewed and an outline of the regulatory considerations for the proposed application of the chemical spinosad as an insecticide for the control of cotton bollworm and native budworm in cotton. It also seeks public comment prior to the chemical product being approved for use in Australia.

Responses to public consultation will be considered prior to registration of the products detailed in this document. They will be taken into account by the NRA in deciding whether the product should be registered and in determining appropriate conditions of registration and product labelling.

Copies of full technical reports on occupational health and safety aspects, environmental impact, and residues in food are available from the NRA on request. They can also be viewed at the NRA Library located at the NRA’s offices on Level 1, Computer Associates House, 10 National Circuit, Barton, ACT 2604.

Written comments should be received by the NRA by 9 June 1997. They should be sent to:

Mr Graeme Barden
Senior Product Evaluator
Agricultural Chemicals Registration
National Registration Authority
PO Box E240
KINGSTON ACT 2604
FAX: (06) 272 3218

Applicant

Dow AgroSciences Australia Limited has applied for registration of two insecticide products containing a new active constituent, spinosad, a fermentation product of *Saccharopolyspora spinosa*.

Product details

Spinosad will be marketed under the trade names Laser Naturalyte Insect Control, a low volume product containing 125g/L spinosad, and Tracer Naturalyte Insect Control, a suspension concentrate product containing 480g/L spinosad.

Laser Naturalyte Insect Control and Tracer Naturalyte Insect Control will be imported fully formulated and packed outside Australia.

Dow AgroSciences Australia Limited intends to market Laser Naturalyte Insect Control and Tracer Naturalyte Insect Control in all States and Territories for the control of cotton bollworm and native budworm in cotton.
CHEMISTRY AND MANUFACTURE

Active constituent

The chemical active constituent spinosad has the following properties:

Common name (ISO): spinosad (spinosyn A and Spinosyn D, approximately 85:15)

Chemical name:
- spinosyn A
  \[2-[(6\text{-deoxy-2,3,4-tri-O-methyl\text{-(-L-mannopyranosyl)}}\text{oxy}]\text{-13-[[5\text{-}5\text{-dimethylamino\text{-}tetrahydro\text{-}6\text{-methyl\text{-}2H-pyran\text{-2-yl)}}\text{oxy}]\text{-9-ethyl\text{-}2,3,3a,5a,5b,6,9,10,11,12,13,14,16a,16b-tetradecahydro\text{-}14\text{-methyl\text{-}1H-as-indaceno}(3,2-d)oxacyclododecin}-7,15\text{-dione}}\]
- spinosyn D
  \[2-[(6\text{-deoxy-2,3,4-tri-O-methyl\text{-(-L-mannopyranosyl)}}\text{oxy}]\text{-13-[[5\text{-}5\text{-dimethylamino\text{-}tetrahydro\text{-}6\text{-methyl\text{-}2H-pyran\text{-2-yl)}}\text{oxy}]\text{-9-ethyl\text{-}2,3,3a,5a,5b,6,9,10,11,12,13,14,16a,16b-tetradecahydro\text{-}4,14\text{-dimethyl\text{-}1H-as-indaceno}(3,2-d)oxacyclododecin}-7,15\text{-dione}}\]

Product names:
- Laser Naturalyte Insect Control
- Tracer Naturalyte Insect Control

CAS Registry Number: 131929-60-7

Empirical formula:
- spinosyn A: \(C_{41}H_{65}NO_{10}\)
- spinosyn D: \(C_{42}H_{67}NO_{10}\)

Molecular weight:
- spinosyn A: 73.98
- spinosyn D: 746.00

Physical form: crystalline solid

Colour: light grey to white

Odour: slightly stale water

Melting point (°C):
- spinosyn A: 84-99.5
- spinosyn D: 161.5-170

Density (\(D^{20}_4\)):
0.152
Octanol/water partition coefficient ($K_{ow}$):

<table>
<thead>
<tr>
<th>pH</th>
<th>spinosyn A log Pow</th>
<th>spinosyn D log Pow</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>2.8</td>
<td>3.2</td>
</tr>
<tr>
<td>7</td>
<td>4.0</td>
<td>4.5</td>
</tr>
<tr>
<td>9</td>
<td>5.2</td>
<td>5.2</td>
</tr>
<tr>
<td>distilled water</td>
<td>3.9</td>
<td>4.4</td>
</tr>
</tbody>
</table>

**Vapour pressure at 25°C:**
- spinosyn A: $2.4 \times 10^{-10}$ mm Hg
- spinosyn D: $1.6 \times 10^{-10}$ mm Hg

**Structural formula:**

Spinosyn A ($R=H$) and Spinosyn D ($R=CH_3$)
TOXICOLOGICAL ASSESSMENT

The toxicological database for spinosad conducted using animals, is quite extensive. In interpreting the data, it should be noted that toxicity tests generally use doses 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 acceptable limits for dietary or other intakes at which no adverse health effects in humans would be expected.

Toxicokinetics and Metabolism

Over 50% of spinosad is absorbed in rats following oral dosing, most being eliminated via the faeces, urine, and bile in the first 24 hours. Spinosad is widely distributed to the tissues following absorption, however only low levels were detected in the rat 7 days following exposure. Spinosad is extensively metabolised in animals.

Acute Studies

Spinosad was of low acute oral toxicity (LD50 values: rats; between 2000 and 5000 mg/kg, mice; > 5000 mg/kg), low dermal (LD50; >2000 mg/kg in rabbits) and inhalational toxicity (LC50 (m+f); > 5180 mg/m$^3$ in rats). It was non irritating to the skin of rabbits, induced slight eye irritation in rabbits in one study, but was found non irritating in a second study, and was not a skin sensitisier in guinea pigs.

Tracer Naturalyte Insect Control, which contains spinosad at 480 g/L, was of low acute oral and inhalational toxicity in rats (LD50 value: > 5000 mg/kg, LC50 value: > 5000 mg/m$^3$), low acute dermal toxicity in rabbits (LD50 value: > 2000 mg/kg). It was a slight skin and eye irritant in rabbits, and was not a skin sensitiser in Guinea pigs.

Laser Naturalyte Insect Control, which contains spinosad at 125 g/L, was of low acute oral and dermal toxicity in rats (LD50 values of > 2000 mg/kg and > 4000 mg/kg, respectively). It was a moderate skin irritant in rabbits, and a skin sensitiser in guinea pigs. Although an eye irritation study was not conducted, since it is a moderate skin irritant the formulation is expected to be a moderate to severe eye irritant.

Although Laser Naturalyte Insect Control was found to be a skin sensitiser, two preparations which would be similar to the diluted product once it had been prepared for use, were not skin sensitisers in guinea pigs.
Repeat Dose Studies

Repeated dietary exposure to high levels of spinosad in mice, rats, and dogs resulted in marked reductions in bodyweight and/or death, and clinical signs of toxicity. Pathological examinations revealed that spinosad induced similar lesions in a wide range of tissues, and that these lesions were reported in all species studied. The major lesions were cellular vacuolation, inflammatory changes, necrosis, regenerative/degenerative changes, increased blood formation, and skeletal myopathy. Changes in haematological and clinical chemistry parameters were generally consistent with the type and extent of cellular injury or organ dysfunction reported. In chronic dietary studies in mice, rats and dogs there was no evidence to suggest that spinosad is carcinogenic.

Reproduction and Developmental Studies

In a 2-generation reproduction study in rats, reproductive effects reported at high dietary doses were attributed to non specific parental toxicity, rather than a specific toxic effect on the reproductive system. In rats, although spinosad induced an increase in delayed ossification of sternebrae in the absence of maternotoxicity from 50 mg/kg/day, this finding is usually attributable to delayed development and in this instance is most likely secondary to subclinical maternotoxicity occurring at that level of exposure. There was no evidence in rats or rabbits to suggest that spinosad has teratogenic potential.

Genotoxicity Studies

In a number of genotoxicity studies conducted with spinosad there was no evidence to suggest that spinosad damages genetic material.

Other Studies

Although in a 12-month rat neurotoxicity study there was some damage to individual nerve fibres in both sexes following dietary exposure to spinosad at 49 mg/kg/day, in other specific neurotoxicity studies conducted in rats there was no evidence to suggest that spinosad has potential to induce neurotoxicity.

PUBLIC HEALTH STANDARDS

Poisons Scheduling

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

The NDPSC recommended that spinosad be listed in poisons schedule 5 of the Standard for the Uniform Scheduling of Drugs and Poisons (SUSDP). There are provisions for appropriate warning statements and first aid directions on the product labels (see pp. 36 - 51 ).
The most sensitive species tested was the rat with a NOEL of 2.4 mg/kg/day. In order to calculate the acceptable daily intake (ADI) for humans, a safety factor is applied to the NOEL in the most sensitive species. The magnitude of the safety factor is selected to account for uncertainties in extrapolation of animal data to humans; variation within the human population; the quality of the experimental data, and the nature of the potential hazards. Using a safety factor of 100, an ADI of 0.02 mg/kg/day was established.
RESIDUES ASSESSMENT

DowAgroSciences Australia Limited has applied for registration of Laser Naturalyte Insect Control and Tracer Naturalyte Insect Control for use in the control of cotton bollworm (*Helicoverpa armigera*) and native budworm (*Helicoverpa punctigera*) on cotton crops. Registration of Spinosad in the USA has been gained and a temporary MRL set for cottonseed (26 February 1997), to expire on 15 November 1999.

Appropriate residue and metabolism studies were provided in accordance with the *Requirements for Clearance of Agricultural and Veterinary Products*, to support the use of Spinosad on cotton in Australia.

**Residues in Food Commodities**

Residue data were provided for cotton seed and the processed cotton seed oil from trials conducted in Australia and the USA.

**Cotton seed and processed fractions**

In the USA trials, the treatment regimes of 5 applications included 0.75x, 1x, 2x and 6x the maximum label rate (100 g ai/ha) at intervals of 7-17 days. Ginned cottonseed was collected 28 days after the last treatment. No residue was detected, except in the 6x study where the raw cottonseed commodity from ginned combine harvested cotton bolls contained residues at 0.073 mg/kg (limit of determination 0.003 mg/kg, limit of quantitation 0.010 mg/kg). No residues were found in the meal or soapstock samples. In the hulls, crude oil and refined oil, residues ranged from 0.012 to 0.018 mg/kg. Residues of Spinosad do not concentrate in the processed products hulls, meal, crude and refined oils or soapstock.

The Australian trials used 1-3 applications at 0.75x, 1 x and 2x the label rate with intervals of 5-8 days. At 28-89 days after the last application no residues were found in cottonseed.

The residue data indicate that in accordance with the recommended use pattern the following withholding period is appropriate:

Cotton: DO NOT harvest for 28 days after application

**Metabolism and Animal Transfer Studies**

Studies on Spinosad metabolism under field trial conditions were conducted in the USA using radioactivity labelled spinosyn A and spinosyn D. Five applications at rates 5x the Australian label rate and at 6-8 day intervals were made. Samples of the seed boll were taken 48-49 days after the last application. Spinosyn A and D metabolise similarly. Radioactivity was primarily located in fatty acids of the cottonseed oil,
(linoleic, oleic, palmitic acid) as well as in glucose subunits of cellulose in the lint fibres. Natural products derived from the metabolised spinosyns accounted for 60% of the radioactivity. Residues of spinosyn A and D were detected on the leaves up to defoliation. The leaf surface is important in degradation, allowing the spinosyns to be partially decomposed by sunlight so they can be metabolised by the plant. Ultimately, small fragments enter the plant carbon pool and are transformed into the fatty acids, glucose, etc. that are detected in cotton seed.

Metabolism studies for animal were conducted in rats, hens and lactating goats. For each animal type common metabolic pathways were identified. At all doses most of the spinosyns were excreted via the faeces and urine, >86%, >70% and > 40-70% for rats, hens and lactating goats respectively. The highest levels of spinosyns A and D were detected in the fat followed by the liver, kidney, milk and muscle. In each tissue the major residue was the parent spinosyns. In the case of the liver and kidney significant metabolism of the spinosyns occurred via N-demethylation, O-demethylation, hydroxylation etc.

A detailed transfer study in dairy cows was presented. Four groups of cows were fed at various dose levels of spinosad in the feed for 28 days. The levels of residues were greatest in the fat followed by the liver kidney and muscle. A further group of cows were withdrawn from oral feeding at an exaggerated dose rate and used to study the residue decay over a 56 day period. Although the residue partitions differently amongst the tissue types, the rate of residue decay was similar for all tissues studied. Low levels of residues were still detected in the fat after 56 days.

When the residue decline study is scaled to likely feed levels for livestock fed cottonseed commodities, such as cottonseed meal or hulls, there is no residue implication. However, in the case of spray drift onto feed crops or pasture it is possible livestock could be subjected to significant residues if grazing occurs within several days of the application. In this case a pre-slaughter interval of a minimum of 4-5 weeks should enable any residues in animal commodities to fall below detectable limits. This is an important factor when considering export commodities and trade implications.

Analytical methods for the determination of the residues, defined as the sum of spinosyn A and spinosyn D, were provided. After appropriate extraction and solid-phase clean-up, the parent spinosyns A and D were detected directly using gas chromatography. The limit of determination was 0.003 mg/kg.
**MRL Standard**

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

### Table 1

<table>
<thead>
<tr>
<th>Compound</th>
<th>Food</th>
<th>MRL (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>ADD:</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Spinosad</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ML 0106</td>
<td>milks</td>
<td>0.02</td>
</tr>
<tr>
<td>MM 0095</td>
<td>meats (mammalian)[in the fat]</td>
<td>0.2</td>
</tr>
<tr>
<td>MO 0105</td>
<td>edible offal (mammalian)</td>
<td>0.05</td>
</tr>
<tr>
<td>SO 0691</td>
<td>cottonseed</td>
<td>*0.01</td>
</tr>
<tr>
<td>PM 0110</td>
<td>poultry meat</td>
<td>*0.01</td>
</tr>
<tr>
<td>PO 0111</td>
<td>poultry, edible offal</td>
<td>*0.01</td>
</tr>
<tr>
<td>PE 0112</td>
<td>eggs</td>
<td>*0.01</td>
</tr>
</tbody>
</table>

### Table 3

<table>
<thead>
<tr>
<th>Compound</th>
<th>Residue</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>ADD:</strong></td>
</tr>
<tr>
<td><strong>Spinosad</strong></td>
<td>Sum of spinosyn A and spinosyn D</td>
</tr>
</tbody>
</table>
Assessment of Overseas Trade Aspects of Residues in Food

Trade Implications

As spinosad is a new pesticide and has only gained approval in the USA (for cottonseed) it is suggested that recognition for Australian MRL standards be sought from importing countries.

With regard to export animal commodities, residues are not likely to be a problem for animals fed a diet comprising up to 60% cottonseed by-products for which the level of residues is expected to be below the limit of quantitation, 0.01 mg/kg. Under these circumstances a pre-slaughter interval may not be required.

There is a potential problem of residues in cattle fed contaminated feed or pasture as a result of spray drift. It is difficult to assess the likely levels of spinosad on pasture/crops from spray drift. The worst case would be to assume the highest residue levels found on cotton leaves will also be found on crops/pasture affected by spray drift and that animals graze immediately after spraying. Using information from the residue depletion study conducted in dairy cows an interval free from contaminated feed of 4-5 weeks is necessary to guarantee the residue levels will fall to below the limit of determination.
OCCUPATIONAL HEALTH AND SAFETY ASSESSMENT

The active ingredient spinosad is not determined to be a hazardous substance by Dow AgroSciences Australia Ltd according to National Occupational Health and Safety Commission (NOHSC) Approved Criteria for Classifying Hazardous Substances.

Spinosad is light grey to white crystalline solid with low vapour pressure. It is not classed as a dangerous good under the Australian Code for the Transport of Dangerous Goods by Road and Rail (ADG Code).

Laser is an aqueous suspension emulsion in oil containing 125 g/L spinosad and 457 g/L hydrocarbon liquid. Dow AgroSciences Australia Ltd has classified Laser as a hazardous substance in accordance with NOHSC Approved Criteria based on its irritant effects.

Tracer is an aqueous suspension concentrate containing 480 g/L spinosad. Dow AgroSciences Australia Ltd have classified Tracer as not being hazardous according to NOHSC Approved Criteria.

The active ingredient and the products will be formulated overseas and imported only as the finished, commercially packaged products -- Laser in 20L wide neck containers and 200L drums with 2” BSP bung. Tracer will be available in 5L, 10L and 20L wide neck containers. Neither product is classified as a dangerous good under the ADG Code.

Transport, storage and retailing

Sea transport employees, dock workers, road transport workers and store persons will handle packaged product only. Exposure of these workers will only occur if packaging is breached.

Advice on safe handling and storage of Laser and Tracer is provided on the respective labels and Material Safety Data Sheets (MSDS).

End use

Laser and Tracer will be applied to cotton by aerial spray or ground rig (boom spray).

Laser will be mixed in water and applied by aerial spray or ground rig, or mixed with DC-Tron NR oil and applied by aircraft ULV. A product rate of 600-800mL will be used for all methods of application. The aqueous spray mixture will be applied at 30L/ha by aerial spray resulting in a maximum product concentration of 2.67% (0.33% ai) or at 50L/ha by ground rig resulting in a maximum product concentration of 1.6% (0.2% ai). The mixture in oil will be applied at 5L/ha, being equivalent to a maximum final product concentration of 16% (2% ai).

Tracer will be mixed with water and applied at a rate of 150-200mL product/ha. The
aerial spray volume will be 30L/ha resulting in a maximum product concentration of 0.67% (0.32% ai). For ground application a spray volume of 50L/ha will be used which is equivalent to a 0.4% product maximum (0.19% ai).

Workers may be exposed to spinosad during mixing and loading, spray application, as well as during clean-up operations. Transfer to aircraft (and in some cases ground rig) tanks will be conducted using a pump.

Exposure to end users will be predominantly through the dermal route. Inhalation and eye exposure to spray mist may occur during spray application. Inhalational exposure during mixing and loading should be negligible based on the low vapour pressure and use pattern of the products.

Aerial application is not expected to result in any significant exposure to pilots.

The main hazards associated with Laser are moderate to severe eye irritation, skin sensitisation and slight skin irritation while the main hazards associated with Tracer are slight eye and skin irritation. There is little concern of prolonged or repeat exposure to spinosad by the dermal route. However, due to the high proportion of hydrocarbon liquid in Laser, chronic exposure to this product should be considered a potential concern to workers.

The dermal absorption of spinosad is not known. A conservative estimate of 10% absorption was used in the risk assessment.

No relevant exposure studies were available. Estimates of exposure and risk for workers involved in aerial and ground boom application were provided by the applicant in a study based on exposure modelling using the Pesticide Handlers Exposure Database (PHED ). Estimates were also calculated by Worksafe Australia using the UK Predictive Operator Exposure Model (UK POEM) and a surrogate mixer/loader study. The risk assessment indicated that both products pose no undue risk during use on cotton at the proposed rates, provided gloves are worn during all mixing/loading operations as well as during ground application. The risk assessment suggested that additional protective equipment will be required to minimise risk during mixing/loading and ground application of both products.

**Entry into treated areas or handling treated crops**

No re-entry exposure information is available for spinosad. Cotton chippers, crop checkers, growers, advisers and workers operating harvesting equipment have potential for exposure to spinosad in treated areas.

Cotton harvesting will be mechanical and is not expected to result in any significant worker exposure. Crop checkers and chippers, however, may handle treated crops or become contaminated with leaf residues for significant periods of time. Based on exposure estimates from surrogate studies, workers should be able to safely handle
treated foliage as early as the treatment day.

**Recommendations for safe use - all workers**

**End users**

End users should follow the instructions and Safety Directions on the product labels.

Workers mixing and loading Laser should wear cotton overalls buttoned to the neck and wrist and a washable hat, elbow-length PVC gloves and face shield.

During the ground application of Laser, workers should wear cotton overalls buttoned to the neck and wrist and a washable hat and elbow-length PVC gloves.

During mixing/loading operations and ground applications with Tracer, workers should wear cotton overalls buttoned to the neck and wrist and a washable hat and elbow-length PVC gloves.

**Personal protective equipment**

The personal protective equipment worn by all workers should meet the relevant Standards Australia standard specified below:

- **Gloves** - AS 2161-1978, Industrial Safety Gloves and Mittens (Excluding Electrical and Medical Gloves)
- **Goggles** - AS 1337-1992, Eye Protection for Industrial Applications
- **Overalls and aprons** - AS 3765-1990, Clothing for protection against hazardous chemicals

**MSDS**

The MSDS for Laser and Tracer were provided by Dow AgroSciences Australia Ltd as part of the submission for registration.

Manufacturers and importers should produce an MSDS for the hazardous product, Laser. The MSDS should contain information relevant to Australian workers, as outlined in NOHSC National Code of Practice for the Preparation of Material Safety Data Sheets. Employers should obtain the MSDS from the supplier and ensure that their employees have ready access to it.
Conclusions

Worksafe Australia supports registration of spinosad in Laser Naturalyte Insect Control at 125 g/L as an aqueous suspension emulsion in oil and Tracer Naturalyte Insect Control at 480 g/L as a suspension concentrate, for use on cotton.

Future products containing spinosad at different concentrations, with different formulations or altered packaging, use patterns or application methods, will require a separate occupational health and safety risk assessment.

Products containing different non-active constituents or different concentrations of existing non-active constituents, will require an assessment in order to assign safety directions.
ENVIRONMENTAL ASSESSMENT

Spinosad comprising spinosyn A and spinosyn D in a ratio of approximately 85:15 is the active ingredient in the formulations TRACER and LASER. TRACER is an suspension concentrate formulation with 480 g ai.L⁻¹, while LASER is an aqueous suspension emulsion in oil with 125 g ai.L⁻¹. It is to be used to control cotton bollworm (*Helicoverpa armigera*) and native budworm (*H. punctigera*) on cotton. LASER and TRACER is to be registered for use in NSW, Qld and WA only. The proposed maximum annual rate is 300 g ai.ha⁻¹ (no more than three applications of 100 g.ha⁻¹). LASER and TRACER (water based) can be applied by ground or by aerial spraying. LASER can also be aerially applied as an ULV formulation (its main market position).

Summary of Environmental Fate Studies

**Hydrolysis**

Spinosyn A and spinosyn D are hydrolytically stable in buffered solutions with a pH of 5 and 7. Some degradation of either spinosyn (about 10% over 30 to 70 d) occurs at pH 9, in which the amino sugar is cleaved and a double bond formed in the aglycone ring system, most likely at the 16,17-position.

**Photolysis**

Preliminary photolysis studies

A study was performed in which Spinosad or various spinosyns (ie spinosyn A, spinosyn B, and spinosyn C with the latter two spinosyns thought, from an earlier study, to be photodegradation products of spinosyn A) were exposed to UV light after placement on glass slides. Results could not be definitively interpreted because of the lack of suitable controls, and merely act as indicative and preliminary to aquatic and soil photolysis experiments. The degradation pathway seemed to indicate that while spinosyn A would degrade to, in turn, spinosyn B then spinosyn C, each spinosyn would also degrade to other UV-inactive products. The results also indicated that the stability of Spinosad was seemingly affected by the type of light source and type of formulation or formulation component. Most noticeably, photodegradation in summer sunlight was around 2-14 X faster than in spring sunlight for Spinosad (depending on formulation), while the suspension concentrate had an half-life up to 33 X greater than the emulsion (in artificial light and spring sunlight - only 13 X greater in summer sunlight but all <10 h). In terms of environmental significance, it is clear that half-life will be greatly influenced by light intensity.

The company, in its response to *Environment Australia*'s request to comment on how relevant the formulation used in field studies were for the Australian situation, expected that the impact of formulation would be most significant within the first few hours. Relative differences in the formulations, however, were shown to be less under summer daylight exposure in the above study. *Environment Australia* agrees that it is likely the impact of formulation is most significant within the first few hours when considering photolysis.
Aqueous photolysis studies

In a 48 h aquatic photolysis study, the half-lives of spinosyn A and spinosyn D were determined in a buffer of pH 7 to be 1.92 and 1.69 d, respectively, assuming a 12:12 photo:scotophase. Exposure of spinosyn A to sunlight produced three degradation products, while exposure of spinosyn D to sunlight produced 4 degradation products.

One photoproduct of spinosyn A (a hydroxylated pseudoaglycone) was 16% and 24% of initial levels after 48 h in the absence and presence of a solvent, respectively. The remainder of the photoproducts were 12% and 14% of the initial level (but with no one individual photoproduct greater than 10%), with 40% and 25% of the activity not characterised in the absence and presence of a solvent, respectively. One photoproduct of spinosyn D (equivalent to the major spinosyn A photoproduct) was 15 and 19% of initial levels after 30 d in the absence and presence of a solvent, respectively. The remainder of the photoproducts were 10% and 15% of the initial level (but with no one individual photoproduct greater than 10%) with 55% and 45% of the activity not characterised, in the absence and presence of a solvent, respectively.

In contrast to the predictions of the indicative study above, no spinosyn B or spinosyn C were formed, while UV-inactive products were apparently formed. No volatiles were formed. Some solvent effects were observed with degradation slightly enhanced in treatments in which solvent was not used (by about 20% for either spinosyn). This did not result in higher concentrations of the degradation products.

Soil photolysis studies

Two soil photolysis studies were performed: study 1 using separately spinosyn A and spinosyn D on dry soil, study 2 using spinosyn A on wet soil. Spinosyn A and spinosyn D on dry soil (study 1) had calculated half-lives of 82 d and 44 d, respectively under autumn sunlight, but did not follow first order kinetics. This is reflected in half-lives, when calculated by the authors of the study for the last two data points, of 164 d and 127 d for spinosyn A and spinosyn D, respectively. If the half-lives are recalculated assuming that a percentage of the spinosyn is “unavailable” for photodegradation (ie it is assumed the spinosyns were in two fractions, that of “available” and “and unavailable” for photodegradation, with the amount of spinosyn left at the end of the study assumed to be the latter), then the half-lives are 8.7 d and 9.4 d for spinosyn A (assuming 67% unavailable) and spinosyn D (assuming 49% unavailable), respectively. Similarly in study 2, spinosyn A on wet soil had a calculated half-life of 14.9 d, but did not follow first order kinetics reflected in a half-life (calculated for the last two data points) of 64 d. Re-calculation of the half-life assuming 12% is “unavailable” gives a half-life of 9.7 d, almost identical for the recalculated half-life of spinosyn A on dry soil.

At the end of the 30 d Study 1 period, 69% and 53% of the initial radioactivity remained as parent material (spinosyn A and spinosyn D, respectively). Spinosyn B or spinosyn B(D) was ≈3% in the Study 1 when corrected for levels in dark controls. At the end of the 30 d Study 2 period, 21% and 12% of the initial radioactivity was respectively left as parent and spinosyn B in the exposed samples compared to 80% and 8% in the dark controls.

In study 1 and study 2, low levels of CO\textsubscript{2} were detected with mean levels of 1.7% and 5.2% produced by the end of each study, respectively, for spinosyn A (1.8% was
produced from spinosyn D on dry soil). Compared to the predictions of the indicative study above, no spinosyn C was formed. Three and four photoproducts were found and characterised when spinosyn A was exposed on dry and wet soil, respectively. One of the photoproducts was probably spinosyn B (formed through mono-N-de-methylation of the amino sugar of spinosyn A), and was found at time 0 at 1-3% of initial level, increasing to a mean level of 6.6% and 12.1% in dry and wet soils, respectively. The levels of the other photoproducts were generally low (1-2% and 2-3% in study 1 and study 2, respectively). Spinosyn B was found in the dark controls in study 2, however, reaching a mean level of 8.3%, potentially indicating a similar level of production from light exposure as in study 1 (ie around 6%); the soils were not sterile and the authors of the study concluded that metabolism was the likely cause for the production of spinosyn B in the control soil (and to a similar extent in the test soil). Only two photoproducts were found when spinosyn D was exposed, one the equivalent of spinosyn B (both were <10% of initial levels). The level of radioactive material not identified in Study 1 is about 5-10% for spinosyn A and 20-25% for spinosyn D (5% and 6% in the dark controls, respectively). The level of radioactive material not identified in Study 2, however, is 40% and 15% in the exposed and dark samples, respectively.

To explain the field results (see below), the company’s current hypothesis is that photolysis is “that photolysis is responsible for the initial cleavage or other alteration (such as oxidation) of the macrolide system at one or more positions and that this altered entity is then more readily susceptible to further extensive metabolism by soil microbes.” Also, they argue that “this photolytic process can occur either when the Spinosad molecule is present in the solution phase or after it has been initially bound on the outer surface of soil particles” with binding to soil possibly even catalysing ring cleavage.

Metabolism

Aerobic Metabolism

In one study, the half-lives of $^{14}$C-spinosyn A and $^{14}$C-spinosyn D were relatively short on a silt loam soil (half-lives of 17 d for spinosyn A and 15 d for spinosyn D), and a sandy loam soil (9.4 d for spinosyn A) at 75% moisture holding capacity at 0.33 bar. The major breakdown products, spinosyn B or spinosyn B(D) (from spinosyn A and spinosyn D, respectively), appear to be relatively long lived (half-lives range from 100 to >356 d), with concentrations in soil reaching 50-70% of the applied radioactivity. First order kinetics were observed for the periods selected to determine half-lives. The level of non-extractable residues (NER) increased steadily over the study period, reaching a maximum of 40-50% by the end of the study, irrespective of soil/spinosyn treatment. Metabolism of spinosyn A on either soil led to production of 15-20% of the applied activity as volatiles by the end of the study, while for spinosyn D, there was little production of volatiles (maximum of 3% over 6 months). There was significant production of other metabolites and other degradation products in spinosyn A treatments (20-25% of applied activity).

In sterile soils, there was also production of spinosyn B: spinosyn B reached levels of 12.6% and 8.5% on Commerce and Hanford soils at Day 240 and Day 93, respectively, while spinosyn B(D) increased over the 6 month study period to reach 33.4% of the
applied activity by the end of the test. Non-extractable residues in the spinosyn A treatments reached 18% and 35% in the Commerce and Hanford sterile soils, respectively, while they only reached 4% in the spinosyn D treatment in the Commerce sterile soil. Various degradation products (ie those formed by abiotic, or chemical, processes) were formed at a level of 30-35% of applied activity.

The proposed pathway consists of either 1) de-methylation of the amino sugar (forming spinosyn B), hydroxylation of the aglycone ring system, then removal of water (to form a double bond in the aglycone ring system), or 2) hydroxylation of the aglycone ring system. None of these steps involves ring cleavage, although the extent of conformational change of the structure and effect on activity is unknown.

Another (second) study on four soils which were treated with labelled spinosyn A or spinosyn D at a rate of 1 mg ai.ha\(^{-1}\). The half-lives of spinosyn A, spinosyn D and their de-methylated products, spinosyn B or spinosyn B(D), were calculated to be 24-76 d, 15-65 d, 72-271 d, 54-736 d, respectively. The data clearly indicate that in the dark the total spinosyn levels in the soil do not decrease markedly over time, as the major degradation product is through de-methylation to spinosyn B or spinosyn B(D): spinosyn B and spinosyn B(D) reached levels of between 40% to 70% and 30% to 50% of the applied radioactivity, respectively. There was no correlation of half-life of spinosyn A, spinosyn B, spinosyn D or spinosyn B(D) with biomass. Non-extractable residues increased over time to reach levels at the end of the study generally between 25% to 35% of the applied radioactivity. The production of volatiles (CO\(_2\)) was more variable with degradation of spinosyn A giving maximum levels at the end of the study between 5.8% and 26%, while spinosyn D degradation gave levels of between 4.8% and 25%. Numerous other metabolites (<10% of applied activity) were formed and as they were similar in identity to the first study, the same pathway is proposed.

**Anaerobic aquatic metabolism**

In an anaerobic water-sediment (clay) system, labelled spinosyn A or spinosyn D was added to give a final concentration of 0.85 mg.L\(^{-1}\) in the water phase. The half-lives of spinosyn A and spinosyn D were 161-250 d and 250-495 d - the lower figure was calculated for the more rapid, initial degradation. The half-lives are essentially those for the spinosyns in sediment, as less than 10% of the radioactivity (either treatment) was found in the water phase 7 days after treatment (DAT) and had decreased to around 2% from 84 to 365 DAT. Around 80% of the radioactivity was extracted from sediment from 3 DAT through the rest of the study period. The level of activity associated with non-extractable residues was variable through the study period, but reached maxima of 17.4% and 15.5% 365 DAT in the spinosyn A and spinosyn D treatments, respectively. Less than 1% of the activity was found as volatiles. Three degradation products in the spinosyn A treatment and one degradation product in the spinosyn D treatment were found at (maximum) levels between 10-15% of applied activity in the sediment phase, at various times through the study. In contrast to the above, the maximum levels of the de-methylated products (ie spinosyn B or spinosyn B(D) at 170 DAT in sediment) were 5.8% and 6.5% in the spinosyn A and spinosyn D treatments, respectively. A pathway was not proposed but appeared to involve the de-methylation of either of the sugar groups and/or the formation of the reverse-pseudoaglycone (ie the spinosyn with the rhamnose sugar cleaved) and its keto derivative.
**Conclusion for degradation studies**

The above aerobic metabolism studies conducted in the dark indicate that while spinosyn A and spinosyn D have similar half-lives and may break down relatively quickly (ie half-life of 10-20 d but up to 65-70 d, possibly as a result of drier soils and/or due to the 5 °C difference in temperature), the de-methylated products, spinosyn B and spinosyn B(D), can persist and reach high concentrations in the soil (half-lives of 50-736 d, reaching concentrations of 30-70% of the applied activity). Further, a significant level of the radioactivity was not extracted after one year (generally 25-50% of applied activity), although some volatiles might be produced (up to 26%). The pathway is likely to be either 1) de-methylation of the amino sugar (forming spinosyn B), hydroxylation of the aglycone ring system, then removal of water (to form a double bond in the aglycone ring system), or 2) hydroxylation of the aglycone ring system.

Anaerobic metabolism of the spinosyns is slow with half-lives >160 d, with most of the radioactivity found in sediment (less than 10% was found in the water phase 7 DAT and had decreased to around 2% from 84 to 365 DAT). A pathway was not proposed but appeared to involve the de-methylation of either of the two sugar groups and/or the formation of the reverse-pseudoaglycone and its keto derivative.

**Mobility**

In a soil flask adsorption/desorption studies with spinosyn A and spinosyn B, both processes were described by Freundlich isotherms. The adsorption and desorption coefficients for 5 soils, $K_{ads}$ and $K_{des}$, were calculated to be 8.3 to 323 for spinosyn A and 4.3 to 179 for spinosyn B. For adsorption based on organic matter, $K_{om}$, the coefficients for the 5 soils were calculated to be 491-80750 for spinosyn A and 391 to 44750 for spinosyn B. These high coefficients suggest that both spinosyn A and spinosyn B are likely to be strongly bound in soils. The adsorption of the spinosyns were not correlated to organic matter content but was weakly correlated to the cation exchange capacity of the soil. A stronger correlation, however, exists for $K_{oc}$ when plotted against CEC X pH, possibly indicating that the charged form and high CEC is necessary for extensive binding at lower pH.

Based on laboratory studies, spinosyn A would be classed as a “transition leacher”, based on a worst case aerobic metabolism half-life of 70 d and $K_{oc}$ of 500, while spinosyn B would be classed as a “probable leacher”, based on a worst case aerobic metabolism half-life of 356 d (see above) and $K_{oc}$ of 400. This is in marked contrast when using the field dissipation half-life (see below) of <1 d for spinosyn A which would clearly cause it to be classed as an “improbable” leacher.

The maximum Henry’s Law Constant for spinosyn A and spinosyn D was respectively $1.4 \times 10^{-5}$ and $2.8 \times 10^{-3}$ Pa.m$^3$.mole$^{-1}$ at pH 9. As chemicals with $H < 3 \times 10^{-2}$ Pa.m$^3$.mol$^{-1}$ are regarded as having low volatility, *Environment Australia* concludes that both spinosyn A and spinosyn D are unlikely to be volatile under field conditions, particularly when the pH is less than the p$K_a$ leading to a predominance of the N$^+$ species.

**Field dissipation**
A study, conforming to USEPA guidelines, was conducted on the dissipation of Spinosad under “field” conditions at two sites: Wayside (Mississippi) and Fresno (California). The soil at these sites (silt loam and sandy loam, respectively) had relatively high CEC values and were alkaline (pH 8.1-8.2 and 7.6-8.7, respectively), with both these parameters interacting and weakly correlating with higher adsorption in the laboratory mobility studies. The soil moisture content at 0.33 bar (ie field capacity) was 18.9% in the top 15 cm of soil. The company has no data for soil moisture at the time of application in the field soil dissipation studies but that “it is probably safe to assume that the soils at the surface of the plots were significantly drier than any of the soils in the laboratory studies” with the potential to affect the rate of microbial degradation.

Radiolabelled spinosyn A was applied to bare soil in a single application was made at a rate of 500 g.ha$^{-1}$ was made, reflecting the potential field use of 4 to 5 applications at 10 d to 14 d intervals (ie maximum single rate of 100 g.ha$^{-1}$). Spinosyn A was formulated as an emulsifiable concentrate (not equivalent to the LASER or TRACER formulation) and applied with a back-pack sprayer.

Half-lives of spinosyn A determined from these studies were <1 d for either soil with little label observed deeper that the top layer (0-15 cm). When label was observed, it accounted for less than 5% and 10% of the total applied radioactivity below 15 cm at any given sampling time for the Wayside and Fresno test sites, respectively. The label appeared much later in the study, and according to the company, probably reflects movement of degraded material as the Kd was high for spinosyn A, with some degradates more polar than the parent material likely representing “either highly modified degradates of spinosyn A or small polar fragments of the molecule”.

The level of extractable residues decreased markedly over the study period with a corresponding increase in non-extractable residues, and also total residues, with up to 75% and 73% of the applied label was removed from the Wayside and Fresno sites, respectively.

**Metabolite profile**

Characterisation of soil extracts (either site) indicated a large number of minor products that were a mixture of spinosyn A and spinosyn B isomers which contain one, two, or more hydroxyl groups on the macrolide portion of the degradates; all degradates were <10% of initial levels (spinosyn B was only 4.4% of initial levels 1 DAT). The maximum uncharacterised activity was reached 4 DAT and 14 DAT for Wayside and Fresno soils, respectively, with 36% and 28% of initial applied levels.

**Attempt to measure CO$_2$ evolution**

A laboratory study was performed on samples taken from the field sites at various times to determine the half-life for mineralisation of spinosyn A on aged soils. The moisture content of the soils was adjusted to 75% 0.33 bar prior to use and inoculated with fresh soil from the appropriate site. The samples were incubated for a maximum of 85 d at 25 °C under laboratory conditions in the dark. The amounts of radioactivity lost was based on the initial radioactivity present at the start of the laboratory incubation period. The amount of lost radioactivity ranged from 4% after 85 days in the 0 DAT Wayside sample to 22% after 71 days in a 14 DAT Wayside sample with mineralisation half-lives of 1442 d to 240 d, respectively, based on the initial amount of radioactivity present in
the soil and total radioactivity from the soil to the trap.

Assuming the decline in the field radioactivity is assumed to be due to mineralisation, however, then mineralisation half-lives (ie 50% CO$_2$ formation) based on the field radioactivity decline in situ were 205 d and 218 d for label applied to Wayside and Fresno soils, respectively. The percentage degradation to determine in situ field mineralisation was calculated at any given sampling time by “dividing the sum of the total radioactivity accounted for in each soil fraction by the appropriate 0 Time recovery value. The correlation co-efficients for the first order regression lines were 0.6969 and 0.8294 for Wayside and Fresno soils, respectively, although the decay curves also exhibit 2nd order kinetics (ie slowing of degradation over the latter part of the study). This indicates a fairly complex pathway between initial rapid loss of parent activity and complete breakdown to CO$_2$.

Discussion on field dissipation pathway
The authors acknowledge that the apparent rapid mineralisation was a surprise as “no single laboratory environmental fate study completed at the time the field study was initiated suggested” suggested that this would occur. They argue that the mineralisation of spinosyn A under field conditions is probably due to photodegradation on the surface, followed by movement of the degradates into the soil profile where microbial action can occur. Environment Australia acknowledged that the half-life for spinosyn A appears very rapid, and surprising but had difficulty in accepting this conclusion and asked the company to comment. In response, the company agreed that “just as the long term losses of radioactivity observed in the field dissipation studies were not due solely to photolysis but rather to some interaction of metabolism and photolysis, it is also possible that the initial rapid loss of spinosyn A that was observed in these studies was due to some interaction of metabolism and photolysis”. The company maintains, however, that “photolysis by itself is a key factor in the initial phase of the dissipation process for Spinosad”, even if some interaction with another process might occur they argue that “photolysis must still play a dominant role”. Further, the company considers the field moisture levels as insufficient for microbial degradation for it to be the main degradation pathway, as well as the very short half-lives at both sites (on dry soils) precluding a major role for microbial degradation as the initiator of breakdown.

There is limited literature evidence that soil photolysis is a possible degradation route in the field, and that this can occur in the adsorbed state, but with Environment Australia accepting that this may be occurring and be the main degradation route in this case. The major evidence is the apparent different metabolic profile formed in laboratory photolysis and field studies, in contrast to the lack of extensive formation of the significantly more persistent spinosyn B in these studies, and spinosyn B being the major degradation product (50-70%) in laboratory soil metabolism studies performed in the dark. However, a number of anomalies and uncertainties remain including 25% of spinosyn B was still being formed in the glass plate photolysis studies; 67% of parent remaining after 30 d in the dry soil laboratory study; and the calculations used to determine the half-life for the “available” fraction appearing to be a gross oversimplification and possibly scientifically invalid. However, taken in its totality despite the highly favourable test conditions (ie bare soils
with summer sunlight), it appears that Spinosad is not likely to be persistent in the field, particularly as extensive formation of the persistent spinosyn B fraction and other spinosyn isomers seems to be ruled out.

**Accumulation/Metabolism**

**Plant Studies - residues on leaves**

When Spinosad was sprayed on cotton at 5 nominal weekly rates to give 2000 and 1000 g.ha\(^{-1}\) for spinosyn A and spinosyn D, 10-15% of the total leaf residue (recovery was 95.3% and 97.7% for spinosyn A and spinosyn D) was found as spinosyn A, although the presence of spinosyn D could not be confirmed. Results indicated that no spinosyn A or spinosyn D, or closely related metabolites, were found in any of the major seed fractions, although some radioactivity (55-60%) was incorporated into natural products (fatty acids). As the period between the last application and the pre-harvest sampling was 34 d, the field half-life for spinosyn A on plants is approximately 10-13 d. This does not, however, account for the nature of the residue (ie some residues may remain bound on, or incorporated in, the leaf (see below).

In another study in which spinosyn A was applied to cotton at 4 nominal weekly rates to give 2000 g.ha\(^{-1}\), more information was obtained on the half-life of spinosyn A. In this study, 25-30 ppm of spinosyn A (or equivalents) would be applied to the leaf surface, with a loss of 80-90% of radioactivity prior to the next application, giving a half-life of 2-3 d. At the end of the study, there was about 6% residues left on, or incorporated into, the leaf.

**Environment Australia** notes that while the disappearance of label may be rapid, the mechanism by which this occurs (ie photodegradation and/or metabolism) is unclear, ie it does not rule out incorporation prior to metabolism. Also, the lack of influence of rainfall events was not clearly demonstrated.

From other unverified data taken from Dow “in house” publications, the following gives some idea of rainfastness and degradation of Spinosad on plant surfaces: Mortality of 2nd instar, even after 10 mm of rainfall applied 2 h after insecticide application, was significant, implying that Spinosad had some degree of rainfastness. Other data indicated that Spinosad may first bind to the leaf surface and then penetrate the cuticle which protects it from further breakdown. In another review article, however, the extent of degradation was stated to be dependent on the degree of shading of the fruit or leaves (after looking at cotton, turnip and cabbage leaves, and apple fruit), with degradation half-lives ranging from 1.6 to 16 d (no rainfall).

**Fish bioaccumulation**

Uptake, depuration, bioconcentration and metabolism of \(^{14}\)C spinosyn A in rainbow trout (*Oncorhynchus mykiss*) under flow-through test conditions was studied following USEPA guidelines. Two concentrations of 5 ± 0.6 \(\mu\)g.L\(^{-1}\) and 19 ± 5.6 \(\mu\)g.L\(^{-1}\) were used. An equilibration period (chemical only in test apparatus), uptake phase and depuration phase were part of the test design with metabolites both quantified and identified where possible in the high dose treatment.

Both doses reached equilibrium in the fish body after 4 days of exposure in whole fish,
muscle and “remainder” tissue. Measured concentrations of label in the test water showed a decrease to below detection limits by Day 2 in the depuration period. Mean residue levels (as 14C ng per g fish tissue) in each of the tissues were, for the low dose: 365, 79 and 547, respectively, and for the high dose: 1647, 412 and 2159, respectively. BCFs were therefore generally low (a range of 80-185 in the “remainder” tissue of the high dose treatment, with a mean of 2621 when corrected for lipid content), and were similar to those predicted by computer modelling. In the depuration phase, half the label removed from the tissues in 4 to 5 d, and 90% of the label removed in 12 to 16 d. The level of label attributable to spinosyn A was >65% on Day 1 of the exposure phase, but from Day 4 to 28, 70-80% of the label consisted of numerous metabolites. The major metabolite was characterised as spinosyn J and exceeded 50 ng.g\(^{-1}\). To account for the metabolites, a four-compartment model (ie allowing for elimination through excretion of metabolites) was used, and gave steady-state BCFs for spinosyn A in whole fish, muscle, and “remainder” tissue of 19, 6 and 19, respectively, indicating that significant bioaccumulation of spinosyn should not occur.

**Accumulation potential in an aquatic microcosm**

An outdoor aquatic microcosm study was performed to help determine the fate of Spinosad in water. The microcosms were stainless steel-lined tanks buried in the ground to a depth of 58 cm and with a surface area of 2.2 m\(^2\). Water had a pH of 7.6 and total suspended solids of 24 mg.L\(^{-1}\). The sediment was a clay loam with a pH of 7.6 and CEC of 15.9 mEq.100 g\(^{-1}\). An experimental suspension concentrate formulation using Spinosad at 480 g.L\(^{-1}\) at a rate of 100 g.ha\(^{-1}\) (200 mL of solution applied in total) was applied using a pressurised pack (nitrogen) and a single flat fan nozzle on a 90 cm spray boom. HPLC was used to analyse water and sediment for spinosyn A, spinosyn B, spinosyn D and spinosyn B(D). Immunoassay was used to determine spinosyn-like compounds.

The expected concentration in water at the start of the test was 20 µg.L\(^{-1}\). The dissipation curves showed distinct second order kinetics, with the initial rapid removal from water probably due to dissipation of chemical through the water body as spraying was banded rather than uniform (assuming none went to sediment with subsequent rapid degradation). The half-lives that more probably reflect actual dissipation or degradation are those determined from 8 hours to the end of the study period, giving 4.7 d and 2.7 d for spinosyn residues (immunoassay) and total measured spinosyns (HPLC), respectively. Spinosyn A was the major species with mean levels at 8 h of 11.8 µg.L\(^{-1}\), and not detectable 15 d after application.

In sediment, residues (measured by immunoassay) were detected 24 h after application to the end of the study (sediment samples were collected for 35 d after application) but were relatively low, with values ranging from 32.1 to 56.0 ng.g\(^{-1}\) (limit of quantitation was 50 ng.g\(^{-1}\), both species detected from 24 h after application through to the end of the study). Levels of Spinosyn A and spinosyn B (HPLC) ranged from 10.7 to 14.9 ng.g\(^{-1}\) and 3.6 to 11.1 ng.g\(^{-1}\) respectively, with a combined mean at the end of the study of 21.9 ng.g\(^{-1}\) \(\pm\) 6.4 ng.g\(^{-1}\), or about 14-15% of the applied Spinosad (immunoassay gave 15-20% total spinosyn residues in sediment).

The study authors conclude that, because only “trace” levels (ie 15-20% of applied dose)
of Spinosad were found in sediment, adsorption to sediment can be “eliminated as a major dissipative route” for Spinosad. They therefore state “it appears most likely that rapid dissipation from the water column is caused by photolysis”. Environment Australia agreed that for this particular study, aqueous photolysis might be a reasonable explanation, and 3-5 d a reasonable estimate of half-life for spinosyn residues (cf 1.5-2 d in the aqueous photolysis study) - it did not, however, address the potential for degradation or movement to sediment in extremely turbid waters, typical of Australian cotton growing areas. Environment Australia also noted that the study did indicate that Spinosad will be stable in sediment, as indicated in anaerobic aquatic studies (in which studies were conducted in the dark and where 80% of applied had moved to sediment by 3 days after application), at so-called “trace” levels. The company has agreed that the dissipation half-life of Spinosad under the high turbidity levels in water predicted for the Australian cotton growing area would be longer than that observed in the aquatic microcosm study, and also a greater portion of any Spinosad would move into the sediment phase.

**Summary of Environmental Effects Studies**

**Avian toxicity studies**

**Avian acute toxicity studies**

Northern Bobwhite quail and Mallard ducks were used in oral dose acute toxicity studies. The LD$_{50}$s determined for these species were both greater than 2000 mg ai.kg body weight$^{-1}$, indicating that Spinosad is practically non-toxic (USEPA classification) to birds when orally dosed.

Ten day old Northern Bobwhite quails and Mallard ducks were used in acute dietary toxicity studies. The LC$_{50}$s determined for these species were >5156 and 5253 ppm in the feed, respectively. These results indicate that Spinosad is practically non-toxic (USEPA classification) to birds when ingested with food.

**Avian reproduction toxicity studies**

Results of reproductive studies on the Mallard duck and Northern Bobwhite quail indicate a NOEC of 550 ppm for both species, based on clinical observations, gross necropsy results or reproductive effects, although clear concentration-effects curves were observed in the mallard test for egg-laying, hatchlings and 14 d old survivors, indicating the potential for effects at concentrations below the NOEC.

**Fish toxicity tests on Spinosad**

Acute LC$_{50}$s for carp (*Cyprinus carpio*), sheepshead minnow (*Cyprinodon variegatus* - marine species), bluegill sunfish (*Lepomis macrochirus*) and trout (*Oncorhynchus mykiss*) were 4.99, 7.87, 5.94 and 30 mg ai.L$^{-1}$, respectively. The NOECs, based on mortality, were 0.7 mg ai.L$^{-1}$ for the carp, 1.80 mg ai.L$^{-1}$ for the minnow, 2.10 mg ai.L$^{-1}$ for the sunfish and 5.2 mg ai.L$^{-1}$ for the trout. Therefore, Spinosad has moderate acute toxicity.

In a 62 d trout (*Oncorhynchus mykiss*) ELS toxicity test, no significant statistical
differences were observed for percent of embryos hatched, percent normal larvae at hatch or percent survival to thinning (day-16). The NOEC and LOEC, based on all end-points tested, were 0.498 and 0.962 mg ai.L\(^{-1}\), respectively. Environment Australia estimated an 62 d LC50 of 3.9 mg.L\(^{-1}\). In the sheepshead (Cyprinodon variegatus) 35 d minnow ELS toxicity test, sublethal effects were observed at 9.63 mg.L\(^{-1}\) and one fish exhibited loss of equilibrium and lethargy at 4.84 mg.L\(^{-1}\). Sub-lethal affects were followed by mortality, with 66% mortality in the 4.84 mg.L\(^{-1}\) treatment at end of the study). The NOEC and LOEC, based on growth end-points, were 1.15 and 2.38 mg ai.L\(^{-1}\), respectively. Environment Australia estimated a 32 d LC50 of 4.6 mg.L\(^{-1}\).

In a trout (Oncorhynchus mykiss) sub-chronic toxicity (21 d flow-through) test, the LC50 decreased from ~8 mg.L\(^{-1}\) to ~4.8 mg.L\(^{-1}\) from day-8 to day-21. The NOEC based on growth endpoints was 3.7 mg.L\(^{-1}\), whilst based on lethal and sublethal effects was 1.2 mg.L\(^{-1}\). The difference between the acute toxicity test LC50 (30 mg ai.L\(^{-1}\)) when compared to sub-chronic test LC50 (4.8 mg ai.L\(^{-1}\)) was about 6.25X.

A feature of the chronic fish tests is the slow rate at which maximum mortality was reached, though it should be noted that concentrations were well above those that maybe encountered in the field and where extended exposure is highly unlikely.

**Invertebrate aquatic toxicity tests on Spinosad**

The water flea \textit{(Daphnia magna)} was used in a 48 h static test and 48 h static-renewal test (solutions renewed after 24 h), while the grass shrimp \textit{(Palaemonetes pugio)} and Eastern oyster \textit{(Crassostrea virginica)} were respectively used in a 96 h static-renewal and 96 h flow-through test. The results of the water flea 48 h static test and 48 h static-renewal test (renewed at 24 h) were 48 h EC\textsubscript{50}'s of 1.48 mg ai.L\(^{-1}\) and 14.0 mg.L\(^{-1}\), respectively. A LC\textsubscript{50} was also determined for the static-renewal test, resulting in a LC\textsubscript{50} of 93 mg.L\(^{-1}\). The NOEC and LOEC for the static test, based on mortality/hypoactivity, were 6.7 and 52 µg ai.L\(^{-1}\), while for the static-renewal test, they were 300 and 450 µg.L\(^{-1}\), respectively, based on sub-lethal effects. The acute toxicity of Spinosad to grass shrimp in a static-renewal (daily) test was determined, resulting in a 96 h EC\textsubscript{50}. NOEC and LOEC of >9.76 mg ai.L\(^{-1}\), 1.66 mg.L\(^{-1}\), and 2.71 mg ai.L\(^{-1}\), respectively, based on mortality. In a 96 h flow-through test, the oyster had a reduction in new shell growth ranging from 0% at 93 µg.L\(^{-1}\) to 63% at 530 µg.L\(^{-1}\). The EC\textsubscript{50}, NOEC and LOEC, based on new shell growth, were 295 µg ai.L\(^{-1}\), 110 and 220 µg ai.L\(^{-1}\), respectively.

The acute toxicity of Spinosad to water flea is rated as moderate in the static test, while there is a marked difference in the static-renewal test which indicates only slight toxicity (although there was an apparent difference between lethal and sub-lethal effects when the LC\textsubscript{50} and EC\textsubscript{50} for the static-renewal test are compared). Given that the half-life range for Spinosad was similar between the two tests, one conclusion could be that the difference is due to the formation of a more toxic metabolite or degradation product over the longer period of the static test (ie solutions were not renewed for 48 h cf renewal after 24 h).

In a 28 d mysid \textit{(Mysidopsis bahia)} chronic test, significant differences for the effect of Spinosad on mysids were observed on the mean number of live young per female reproductive day (YPFRD), survival and dry weight. The mean survival ranged from 1.7% at 1500 µg.L\(^{-1}\) to 97% at 84 µg.L\(^{-1}\), with all concentrations = 360 µg ai.L\(^{-1}\) significantly different to the control. Environment Australia estimated a 28 d LC\textsubscript{50} of
The lowest NOEC and LOEC, based on YPFRD, were 84 and 170 µg ai.L\(^{-1}\), respectively.

Two 21 d chronic tests were performed on water flea (Daphnia magna): one in which water flea were exposed to Spinosad in a flow-through system (standard chronic test design), the other in which water fleas were exposed to pulses of Spinosad at five-day intervals. Significant differences for the effect of Spinosad on water fleas in the flow-through test were observed on survival, length and total number of progeny. Survival ranged from 65% to 100% in treatments (5.8 and ≥0.62 µg ai.L\(^{-1}\), respectively, concentration response) compared to no mortality in controls. The LC\(_{50}\) was therefore > 5.8 µg L\(^{-1}\). The lowest NOEC and LOEC, based on length, was 0.62 and 1.2 µg ai.L\(^{-1}\), respectively, reflecting a nine percent reduction in length between the LOEC and controls.

Significant differences for the effect of Spinosad on water fleas in the pulse test were observed on length and total number of progeny. Survival was apparently affected by low oxygen levels (the test system was not aerated) and no treatment-related deaths occurred. The LC\(_{50}\) was therefore > 57 µg L\(^{-1}\). The lowest NOEC and LOEC, based on length, was 1.8 and 3.7 µg ai.L\(^{-1}\), respectively, reflecting a nine percent reduction in length between the LOEC and controls. Degradation of Spinosad in the test vessels was generally linear, giving half-lives of 22 to 40 h. Extrapolating using the half-lives derived for the test solution, Spinosad would only be detected at between 3.3% and 11% of time zero levels at 120 h after test solution renewal.

The chronic toxicity appears markedly different to that indicated from the acute toxicity tests. It is evident from the data that the acute to chronic ratio of the two standard tests (ie 48 h static-renewal EC\(_{50}\) cf 21 d flow-through MATC growth) is of the order of 10\(^5\)X difference. Even using the much more sensitive 48 h static EC\(_{50}\), the ratio is in the order of 10\(^3\)X difference. Also, in the acute static-renewal test, there was a distinct difference between the pattern of mortality and immobility. While only a very low level of mortality was observed in the test, a much higher level of immobilisation occurred at 48 h. This is also illustrated by the 100-fold difference in NOECs (0.30 mg.L\(^{-1}\) cf 33 mg.L\(^{-1}\), immobilisation versus mortality). This, together with the large acute to chronic ratios, caused Environment Australia to be concerned about these sub-lethal and sub-chronic effects and speculate whether there is a possibility that the parent compounds, degradates and/or impurities are causing these sublethal and subchronic effects.

Aquatic plant toxicity tests using Spinosad

Measured test concentrations indicated that Spinosad was not stable in most of the five plant tests' media, either degraded or precipitated from solution, or possibly adsorbed to the test organism. One exception was the cyanobacteria test in which degradation was not observed and evaporation not stated to have occurred.

The average specific growth rate (relative to the controls) of the green algae, Selenastrum capricornutum, was significantly reduced at test concentrations = 11 mg.L\(^{-1}\). There was a small but statistical increase in cell count at 110 mg L\(^{-1}\). The EC\(_{50}\), NOEC and LOEC are respectively therefore >110 mg.L\(^{-1}\), 4.3 mg.L\(^{-1}\) and 11 mg.L\(^{-1}\), but the results should be viewed with caution given the unreliability of the test conditions (evaporation and
precipitation of test compound, and possibly adsorption of Spinosad to the algal surface coat).

The inhibition of growth rate, relative to the controls, of the freshwater diatom (Navicula pelliculosa) ranged from 7.80% to 99.9%, with statistically significant reduction in the mean standing crop at 120 µg.L\(^{-1}\) and 344 µg.L\(^{-1}\). The 5-day EC\(_{50}\), NOEC and LOEC are respectively therefore 110 µg.L\(^{-1}\), 49 µg.L\(^{-1}\) and 120 µg.L\(^{-1}\) using the initial measured concentrations.

Complete inhibition of population growth of the marine diatom (Skeletonema costatum) occurred at the three highest test concentrations (= 744 µg.L\(^{-1}\)), while there was also statistically significant reduced growth at 342 µg.L\(^{-1}\). The 5-day EC\(_{50}\), NOEC and LOEC are respectively therefore 230 µg.L\(^{-1}\), 170 µg.L\(^{-1}\) and 340 µg.L\(^{-1}\), using the initial measured concentrations.

Cyanobacteria (Anabaena flos-aquae) exhibited marked inhibition of population growth and significantly different mean standing crop values (cells.mL\(^{-1}\)) at the three highest test concentrations, ie those = 7.94 mg.L\(^{-1}\). The EC\(_{50}\), NOEC and LOEC are respectively therefore 8.1 mg.L\(^{-1}\), 3.9 mg.L\(^{-1}\) and 7.9 mg.L\(^{-1}\) based on mean measured values.

The EC\(_{50}\) values for duckweed (Lemma gibba) plant growth (ie total number of plants or fronds) were 12.4 mg.L\(^{-1}\) for plants and 10.6 mg.L\(^{-1}\) for fronds. The EC\(_{50}\) values for inhibition of growth (number of plants or fronds on Day 14 as a percentage of Day 0 values) were 19.7 mg.L\(^{-1}\) for plants and 12.7 mg.L\(^{-1}\) for fronds. There was significant growth reduction in the fronds from 4.31 mg.L\(^{-1}\) (22.8%). Considerable growth inhibition was exhibited at the highest test concentration (14.3 mg.L\(^{-1}\)), 68.2% for plants and 74.5% for fronds. The NOEC for plants and fronds using growth as an endpoint, are respectively therefore 7.4 mg.L\(^{-1}\) and 1.9 mg.L\(^{-1}\), using the initial measured concentrations. Considerable degradation, however, was observed for Spinosad in this test, with as little as 25% of Spinosad left at the end of the test.

Aquatic plant toxicity test results therefore indicate that Spinosad is practically non-toxic to green algae, slightly to moderately toxic to the duckweed (based on growth and growth inhibition of both the plant and fronds), moderately toxic to blue-green algae, and highly toxic (bordering very highly toxic) to freshwater and marine diatoms. Environment Australia notes that these classifications would change if something other than the initial test concentrations were used in the calculation of results (ie based on mean measured values), Spinosad would clearly be very highly toxic to diatoms.

Predators and parasitoids studies

Predators and parasitoids laboratory studies

Laboratory toxicity tests on the honey bee (contact and contact with aged residues); Predatory mites (Phytoseiulus persimilis); Whitefly parasitoid (Encarsia formosa); Minute pirate bug (Orius insidiosus); and Ladybird beetles (Hippodamia convergens) were performed using International Organisation for Biological Control (IOBC) guidelines. Spinosad was applied to test vessels and/or prey as the SC formulation (480 g ai.L\(^{-1}\)) or as part of Spinosad fermentation broth (killed S. Spinosa with 3.75% Spinosad). Two reference compounds were used to indicate “margins of safety”: cypermethrin and carbaryl. The tests give an idea of acute toxicity only, as the possible sub-chronic
effect of Spinosad was not determined since the tests’ exposure period was mostly 24 h (predatory mites had an exposure period of 72 h).

The results of the laboratory studies indicate that Spinosad (either formulation) is potentially “soft” on beneficials when compared to cypermethrin, with a margin of safety of about 10 for the most sensitive beneficials (both hymenopteran species), *Apis mellifera* and *Encarsia formosa*. Results from the aged residue contact toxicity study with the honey bee also gave a similar impression for honey bees when exposed to material collected from the field at different times after application - at worst, the level of mortality is 20-25% up to 24 h after application at the highest rate of 400 g.ha⁻¹.

**Predators and parasitoids semi-field and field studies**

The following semi-field or field studies on the effect of Spinosad on predators and parasitoids were performed:

1) Spinosad was applied to cotton at rates of 75 and 125 g ai.ha⁻¹ at Fresno (California) and Wayside (Mississipi) sites with observations made on the following arthropods: assassin bug (*Reduviidae*), big-eyed bug (*Geocoris* spp), lacewing (*Chrysopa* spp), ladybird beetle (*Hippodamia* spp), minute pirate bug (*Orius tristicolor*), parasitic wasp (*Lysiphlebus testaceipes*), and western plant bug (*Lygus hesperus*);

2) Spinosad was applied to cotton at rates of 25, 50 and 100 g ai.ha⁻¹ at Fresno (California) and Burdett (Mississippi) sites with observations made on the following arthropods: assassin bug (*Zelus* spp), damsel bug (*Nabis* spp), big-eyed bug (*Geocoris* spp), lacewing (*Chrysopa* spp), ladybird beetle (*Hippodamia convergens* and other genera/species), pirate bug (*Orius* spp), and lygus bug (*Lygus* spp);

3) Studies in which the beneficials, the predacious ladybird beetle, *Hippodamia convergens*, or the ectoparasitic tachinid fly, *Lespesia archippivora*, were placed on cotton sprayed at 25, 50 and 100 g ai.ha⁻¹ in cages which were also treated with Spinosad or left untreated;

4) Spinosad was applied to cotton at rates of 50, 100 and 150 g ai.ha⁻¹ at a furrow irrigated site in the Namoi Valley, NSW, with observations made on the following arthropods: thrips (*Thrips* spp), jassids (*Austroasca* spp), apple dimpling bug (*Campylomma liebknechti*), aphids (*Aphis gossypii*), mites (*Tetranychus urticae*), spiders (*Araneidae & Clubionidae*), hoverfly larvae (*Syrphidae*) and other predators (ants, beetles); and

5) Spinosad was applied to cotton at rates of 50, 100 and 150 g ai.ha⁻¹ at a furrow irrigated site on the Darling Downs, NSW, with observations made on a range of arthropods.

In comparison to reference compounds (eg synthetic pyrethroids, profenofos, endosulfan), Spinosad was generally “soft” on beneficials, or at least no worse than the reference compounds. Most noticeable was its lack of control of some bug (true bug and aphid) pests. Some effect of Spinosad on parasitic wasps was noted after the first application in Study 1, but this could not be demonstrated after the second application because of low host (aphid) numbers. In study 5, Spinosad possibly affected
trichogrammatids and other microhymenoptera, although the level of egg parasitism was similar between the control and Spinosad treatments.

Study 3 clearly indicated that Spinosad could have an effect on ladybird beetles and tachinid flies. It appears, however, that when ladybird beetles were given a refugia (ie clean surface), they did not acquire a dose to manifest effects (perhaps because of avoidance of the treated surface). In contrast, when tachinids were given refugia, the manifested effects appeared dependent on the dose acquired (ie demonstration of lack of an avoidance behaviour of treated surfaces). The effects of Spinosad on tachinids, however, demonstrated Spinosad was no worse than cypermethrin.

In conclusion, Spinosad appears to be relatively safe to beneficial arthropods, although some effects might be noted on tachinid flies, ladybird beetles and particularly hymenoptera species. The occurrence of refugia may help in limiting the effects of Spinosad on these species.

Earthworm studies

Two acute toxicity tests were performed using *Eisenia foetida* with Spinosad as the fermentation broth or formulated product dispersed on an artificial medium. The 7 d LC$_{50}$, 14 d LC$_{50}$ and NOEC respectively were 93 g.kg$^{-1}$ (95% confidence limits of 76 and 142 g.kg$^{-1}$), 48 g.kg$^{-1}$ (95% confidence limits of 40 and 76 g.kg$^{-1}$), and 20 g.kg$^{-1}$ for Spinosad when tested as a broth. If the fermentation broth is assumed to have roughly 4% Spinosad, then the 7 d LC$_{50}$, 14 d LC$_{50}$ and NOEC respectively would be 4, 2 and 0.8 g Spinosad.kg$^{-1}$. No treatment-related effects were recorded when Spinosad was tested as the formulated product, so the LC$_{50}$ and NOEC respectively were >>970 mg.kg$^{-1}$ and 970 g.kg$^{-1}$. These test results would therefore classify Spinosad as very slightly to slightly toxic to earthworms.

No other data for soil dwelling biota, including soil micro-organisms, have been made available, although *Environment Australia* notes that results from a soil micro-organism test should be available at the end of 1998.

Plant toxicity

No terrestrial plant toxicity studies were provided, although aquatic plants (various algae, cyanobacteria and duckweed) were tested (see above) and indicated that Spinosad was highly (possibly very highly) toxic to diatoms. Efficacy studies, however, indicated no terrestrial plant toxicity and *Environment Australia* is satisfied that phytotoxicity does not need to be further addressed.

Hazard arising from use

*Environment Australia* recognises, from discussions with the cotton industry, that there is an urgent need for replacement chemicals which have new chemistry and no cross resistance or bioaccumulation potential (in cattle), to control *Helicoverpa*. Spinosad apparently meets these criteria, and has certain advantages over present chemistries because it is potentially “softer” to some beneficial organisms.

Contamination of non-target areas is likely to be through surface-water run-off (with
Spinosad in solution or, more likely, adsorbed to suspended sediment) and/or spray drift, with the highest application rate in cotton of 300 g.ha\(^{-1}\) (ie 3 X 100 g.ha\(^{-1}\)). This cumulative total is warranted as a worst case given the short periods between applications that are expected.

**Aquatic hazard**

Clearly, Spinosad when applied at the various rates, will pose some hazard to aquatic organisms from acute exposure if directly sprayed, or if allowed to significantly drift (ie at 10% of application rate), onto water bodies. In the case of drift to more distant water bodies, either resulting in acute or chronic exposure, mitigation of effects may be possible through a number of factors (eg adsorption to sediment). The following summarises more “realistic” scenarios (applicable to both chronic and acute exposure) using modelling provided by the company, and secondly the assessment performed by Environment Australia with attention to the Australian use pattern.

**Refined modelling using GENEEC, GLEAMS and EXAMS**

The model used was GENEEC in a USEPA Tier 1 assessment and, because the derived Q-values were of concern (worst case Q-value of 3.73 and a typical Q-value of 1.67 for water flea), a Tier 2 assessment was also performed. The Tier 2 modelling for run-off and/or spray drift used soil, pond and meteorological data relevant to cotton growing areas in the United States (from various databases) as input (the assumed half-life for photodegradation is 1 d). Aerial drift was also considered, with 5% of the applied Spinosad added to the pond for each application. Using the most sensitive MATC end-point (growth from the water flea 21 d flow-through test), Q-values ranged from 0.016 at 56 d to 0.072 at 21 d for the highest concentration of 125 g.ha\(^{-1}\) when run-off plus spray drift was considered. When the fate of 5% spraydrift of Spinosad into a 15 cm deep, 2023 m\(^2\) pond was modelled (assuming no outflow), the Q-value was 0.002 using the oyster EC\(_{50}\) for shell deposition - using other endpoints also indicated that Q-value would be <0.04.

**Assessment of acute hazard with an understanding of mitigating and other factors**

At the highest cumulative rate, with 1% spray drift (no degradation assumed), Spinosad as an ULV formulation presents a marginally unacceptable acute risk while the SC formulation (ie 30% that of the ULV hazard) presents an acceptable acute risk based on the *Daphnia* (sublethal) MATC of 18.6 µg.L\(^{-1}\). All other lower rates were acceptable for both ULV and SC formulations.

Several other factors would influence the ultimate effects of Spinosad, including possible binding of Spinosad to sediment (about 15% when applied to a moderately clear water but up to 80% as indicated in the anaerobic study). In the case of 80% of the applied Spinosad being bound, Q would range from <0.001 to 0.022 when considering 1% (ULV) spray drift of the various application rates possible. Therefore, risk to water column species would appear to be acceptable at all rates for both the ULV and SC formulations. Even assuming only 15% is bound to sediment, risk to water column species is still acceptable at all rates for the ULV and SC formulation. *Environment Australia* considers that this result is congruent with the likely Australian scenario of waters leaving cotton fields having high turbidity, limited photolysis, and with the high sediment load.
readily adsorbing any free Spinosad, but that it suggests that benthic organisms might be at risk.

**Assessment of chronic hazard with an understanding of mitigating and other factors**

Only the scenario of 3 sprays at the highest rate is the situation which is most likely to give rise to chronic exposure of Spinosad of 28 d (the same length of time as the study period from which the most sensitive MATC, *Daphnia* length, is derived).

For direct spraying (from each of three applications: application at a week’s then fortnight’s interval), a risk is considered unacceptable when the half-lives are \( \geq 1 \) d. It is arguably a more realistic situation (eg reasonable management practices are unlikely to lead to the same spot being sprayed directly on each of three successive applications) to consider 1% spray (ULV) drift which indicates an unacceptable chronic risk for half-lives \( \geq 2 \) d (for the SC formulation, this would be \( > 4 \) d), but does not allow for adsorption to sediment.

As indicated above, several other factors would influence the ultimate effects of Spinosad, including the Q further reduced by 80% (assuming adsorption to sediment) leaving only the half-lives \( = 4 \) d (even longer half-lives for the SC formulation) above levels of concern. Given that a MATC was used and compared against a lower Q-value limit of 0.1, as well as a shallow water depth of 15 cm, and that this Q-value limit is arguably very stringent for areas not considered pristine, *Environment Australia* considers that it is unlikely that Spinosad presents a chronic hazard to water column dwelling organisms in the cotton growing area.

**Hazard of Spinosad to benthic organisms from acute and chronic exposure**

It was estimated that eroded material from 1 Ha could be deposited to a depth of 0.2 cm in a large pond 60 m X 30 m. Given that 1 Ha would have been sprayed, and the amount of soil eroded in one season was about 5 tonne, the concentration of Spinosad would be 60 µg Spinosad.g sediment\(^{-1}\) (ie 300 g ai applied in one season giving \( 300 \times 10^6 \) µg Spinosad divided by \( 5 \times 10^5 \) g sediment). While it is difficult to draw any firm conclusions without sediment toxicity data, *Environment Australia* notes that the acute effects of sensitive water column organisms (ie in the ppb range) are similar to the expected range for Spinosad residues in sediment. This would lead to Q-values of \( > 0.1 \) and therefore of concern (eg the acute Q-value for the oyster would be 0.2 while using the water flea chronic MATC of 0.863 µg.L\(^{-1}\), the Q-value would be 3.2).

While the estimated concentration of Spinosad in sediment is relatively high given the above scenario, Environment Australia acknowledges that this assumes that all the Spinosad applied to the one hectare field is washed off with the sediment, and that this results in only a very shallow deposition layer. Any degree of crop interception or degradation in soil is not taken into account. Further, it is likely that this layer would undergo some degree of mixing (either in situ or with sediment washed off from fields not sprayed with Spinosad) that would further dilute the Spinosad residues. Spinosad and its residues is also likely to be strongly bound to the sediment resulting in very low interstitial pore water concentrations, again possibly further mitigating the risk (acute or chronic) of Spinosad residues to benthic organisms.

**Terrestrial (soil) hazard scenarios**
The soil estimated environmental concentration is 0.2 mg kg\(^{-1}\) using a soil depth and density of 10 cm and 1.5 g cm\(^{-3}\). Considering the effect concentration for \textit{Eisenia fetida}\(^{1}\) of 2 mg ai kg\(^{-1}\), the Q-value would be 1 X 10\(^{-3}\). As the Q-value is well below 0.1, \textit{Environment Australia} concludes that Spinosad when directly sprayed on soil will not be a hazard to earthworms. Drift of Spinosad to adjacent properties or fields will also therefore not be a hazard. As persistence of Spinosad is not likely to occur in soil (e.g., due to photolysis or metabolism), earthworms are also not likely to be at risk from chronic exposure if chronic toxicity is similarly low as for acute toxicity.

\textit{Terrestrial (avian) hazard scenarios}

To determine hazard to birds, the analyses to determine a Q-value used estimated residues in feed based on a modified Kenaga nomogram\(^{2}\). The lowest dietary acute effects for Mallard ducks and Bobwhite quail were LC\(_{50}\)s of >5156 mg ai kg\(^{-1}\) and >5253 mg ai kg\(^{-1}\), respectively. The estimated dietary environmental concentration for the Mallard and Bobwhite is 11.5 mg ai kg\(^{-1}\) and 31.1 mg ai kg\(^{-1}\), respectively. Using the above effect concentrations, but bounded by arbitrarily using the lower limit, the Q-value for the Mallard and Bobwhite would respectively be 2.19 X 10\(^{-3}\) and 5.92 X 10\(^{-3}\). As the Q-values are well below 0.1, \textit{Environment Australia} concludes that Spinosad when directly sprayed on avian food items will not be a hazard to birds. Drift of Spinosad to adjacent fields or habitats will also therefore not be a hazard. As persistence of Spinosad is not likely to occur on avian food items (e.g., short half-life on plants), avian species are also not likely to be at risk from chronic exposure.

\textit{Terrestrial (above ground invertebrates) hazard scenarios}

Laboratory toxicity tests in which Spinosad was compared to two reference compounds to indicate “margins of safety” demonstrated that Spinosad was potentially “soft” on beneficials after acute exposures, although some effects were noted for honey bees. \textit{Environment Australia} therefore concluded that while Spinosad may pose some hazard to hymenopteran species after acute exposure in laboratory tests, it provides a softer option to other potential chemicals.

Semi-field or field studies also confirmed the limited nature of effects of Spinosad on predators and parasitoids, although some effects were noted on tachinid flies, ladybird beetles and particularly hymenoptera species. The effects on ladybird beetles and tachinid flies were not noticed when refugia were available. Other studies clearly indicated that ladybird beetles were not affected.

\textit{Potential effects on eutherian and marsupial mammals}

Spinosad (and TRACER and LASER formulations) was demonstrated to be practically non-toxic to rats and mice. Converting the oral LD\(_{50}\) to an estimated dietary LC\(_{50}\), gives a LC\(_{50}\) of around 75000 mg kg\(^{-1}\) food\(^{-1}\). Comparing this to residues estimated by the Kenaga nomogram, the hazard posed by Spinosad by the ingestion of residues to small rodents would appear to be extremely low (Q-value of 3.9 X 10\(^{-4}\)). While some uncertainty remains with regards to marsupials, given their apparent difference in reproduction and development, and detoxification, even with a large margin of safety (e.g,
100), the risk to non-eutherian mammals would be very low (Q-value of $3.9 \times 10^{-2}$).

**Conclusion**

*Environment Australia* has assessed data in support of Spinosad, and in recognition of the favourable ecotoxicity profile and low rates of use, as well as use of best management practices, concludes that environmental hazard is acceptable for the proposed use in cotton.

It notes that soil photolysis appears to be responsible for the rapid degradation in the field, although the mechanism is not well characterised.
Efficacy and Safety Assessment

Justification for use

LASER NATURALYTE INSECT CONTROL is a low volume product containing 125g/L of the new insecticide, spinosad, a fermentation product of *Saccharopolyspora spinosa*. TRACER NATURALYTE INSECT CONTROL is a suspension concentrate product containing 480g/L spinosad.

Insecticide resistance in *Helicoverpa armigera* can cause significant damage to the cotton industry. The new products have been developed in an attempt to provide an extra tool in integrated pest management and insecticide resistance management.

Registration is supported by Australian agricultural authorities.

Proposed use pattern

Spinosad is proposed to be used to control *Helicoverpa* spp. in cotton. This use is proposed for New South Wales, Queensland and Western Australia, as specified in the directions for use table on the products’ labels (See pp. 36 - 51).

LASER NATURALYTE INSECT CONTROL will be available in 20L and 200L packsizes. TRACER NATURALYTE INSECT CONTROL will be available in 5L, 10L and 20L packsizes.

The use rate for TRACER NATURALYTE INSECT CONTROL is 150 – 200mL/ha.

A harvest withholding period of 4 weeks has been recommend for cotton treated with TRACER NATURALYTE INSECT CONTROL. Also, a recommendation that livestock be prevented from grazing cotton crop, stubble or gin trash following treatment by TRACER NATURALYTE INSECT CONTROL has been made. These appear on the products’ labels (See pp. 36 - 51).

Evaluation of efficacy

Data presented by Dow AgroSciences Australia Limited supported claims that LASER NATURALYTE INSECT CONTROL and TRACER NATURALYTE INSECT CONTROL adequately control *Helicoverpa* spp. in cotton. The data were gathered from a range of small plot replicated trials and commercial large-plot unreplicated treatments. The trial involved water based ground rig and aerial applications.

The data were adequate to satisfactorily assess efficacy when used according to the label directions (See pp. 36 - 51).
**Phytotoxicity**

Safety to cotton has been demonstrated with no apparent adverse effects on a range of cotton cultivars, when used in accordance with directions.

**Resistance management**

The labels recommend that LASER NATURALYTE INSECT CONTROL and TRACER NATURALYTE INSECT CONTROL be applied no more than three (3) times in any one cropping season. Additional information is given on the labels (See pp. 36 - 51).

The Insecticide Resistance Management Strategy for Cotton will include specific information on timing of applications through each season. This strategy is reviewed annually to optimise use of the products with a view to avoiding resistance development.
Laser Naturalyte Insect Control

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

Dow AgroSciences

Laser *
Naturalyte* Insect Control

ACTIVE CONSTITUENT: 125 g/L SPINOSAD
457 g/L HYDROCARBON LIQUID

For the control of cotton bollworm (*Helicoverpa armigera*) and native budworm (*Helicoverpa punctigera*) in cotton.

IMPORTANT: READ THE ATTACHED BOOKLET BEFORE USE.

Net Contents: 20 Litres
200 Litres

SHAKE WELL BEFORE USE

Dow AgroSciences Australia Limited
A.C.N. 003 771 659
26 Rodborough Road
FRENCHS FOREST NSW 2086

CUSTOMER SERVICE TOLL FREE 1-800 700 096

* Trademark of Dow AgroSciences

NRA Approval No.: GMID
STORAGE AND DISPOSAL

- Store in the closed, original container in a cool, well-ventilated area. Do not store for prolonged periods in direct sunlight.
- Triple rinse or preferably pressure rinse containers before disposal. Add rinsings to spray tank. Do not dispose of undiluted chemicals on site. If recycling, replace cap and return clean containers to recycler or designated collection point.
- If not recycling, break, crush or puncture and bury empty containers in a local authority landfill. If no landfill is available, bury the containers below 500 mm in a disposal pit specifically marked and set up for this purpose clear of waterways, desirable vegetation and tree roots. Empty containers and product should not be burnt.
- This is a recyclable container. Free phone Dow AgroSciences (1-800 700 096) for advice and information on the recycling service closest to you.

SMALL SPILL MANAGEMENT
Clean up small spill by applying absorbent material such as earth, sand, clay granules or cat litter to the spill. Sweep up material for disposal when absorption is completed and contain in a refuse vessel for disposal in the same manner as for containers (see Storage and Disposal Section). If necessary wash the spill area with an alkali detergent and water and absorb as above the wash liquid for disposal.

SAFETY DIRECTIONS

- Will irritate the eyes and skin.
- Avoid contact with eyes and skin.
- Repeat exposure may cause allergic disorders.
- When preparing spray, wear cotton overalls buttoned to the neck and wrist and a washable hat, and elbow-length PVC gloves.
- If product on skin, immediately wash area with soap and water.
- If product in eyes, wash it out immediately with water.
- Wash hands after use.

FIRST AID

- If poisoning occurs, contact a doctor or Poisons Information Centre. (Ph: 13 1126)

MATERIAL SAFETY DATA SHEET
Additional information is listed in the Material Safety Data Sheet for Laser Naturalyte Insect Control which is available from Dow AgroSciences on request. Call Customer Service Toll Free on 1-800-700 096.

NOTICE
Seller warrants that the product conforms to its chemical description and is reasonably fit for the purposes stated on the label when used in accordance with directions under normal conditions of use. No warranty of merchantability for a particular purpose, express or implied, extends to the use of the product contrary to label instructions, or under off-label permits not endorsed by Dow AgroSciences, or under abnormal conditions.

EMERGENCY RESPONSE
(All Hours)
RING FROM ANYWHERE IN AUSTRALIA
1-800 033 882
(LOCAL CALL FEE ONLY)
IN A TRANSPORT EMERGENCY ONLY
DIAL 000
FOR POLICE OR FIRE BRIGADE

NRA Approval No.
GMID
D.O.M./Batch No
Laser *
Naturalyte* Insect Control

ACTIVE CONSTITUENT: 125 g/L SPINOSAD
457 g/L HYDROCARBON LIQUID

For the control of cotton bollworm (*Helicoverpa armigera*) and native budworm (*Helicoverpa punctigera*) in cotton.

IMPORTANT : READ THIS BOOKLET BEFORE USE.

Dow AgroSciences Australia Limited
A.C.N. 003 771 659
26 Rodborough Road
FRENCHS FOREST  NSW  2086

CUSTOMER SERVICE TOLL FREE  1-800 700 096

* Trademark of Dow AgroSciences

NRA Approval No.: GMID
DIRECTIONS FOR USE

DO NOT apply more than 3 applications to any field in any one season (see the RESISTANCE statement).

<table>
<thead>
<tr>
<th>CROP</th>
<th>PEST</th>
<th>STATE</th>
<th>RATE</th>
<th>CRITICAL COMMENT</th>
</tr>
</thead>
</table>
| Cotton | Bollworm (*Helicoverpa armigera*) Native budworm (*Helicoverpa punctigera*) | NSW, Qld and WA only  | 600-800 mL/ha | Use the low rate against light infestations and higher rates when infestation is heavy (see guidelines below). Carefully monitor eggs and larvae of *Helicoverpa* species by regular field scouting. Target sprays against brown eggs and newly hatched very small larvae. **Guidelines**

*Light Infestations:* Use 600 mL/ha when infestation of *Helicoverpa* species is less than 10 eggs and 2 larvae/m of row.

*Heavy infestations:* Use 800 mL/ha when infestation of *Helicoverpa* species exceeds 10 eggs and/or 2 larvae per metre of row.

Larvae larger than 8 mm in length and larvae feeding within bolls and squares may not be controlled.

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

WITHHOLDING PERIOD: DO NOT HARVEST FOR 28 DAYS AFTER APPLICATION.

DO NOT ALLOW LIVESTOCK TO GRAZE COTTON CROP, STUBBLE OR GIN TRASH WHICH HAS BEEN TREATED WITH LASER Naturalyte INSECT CONTROL
GENERAL INSTRUCTION

- Laser Naturalyte Insect Control is formulated as an aqueous suspension emulsion in oil that is suitable for application by either Ultra Low Volume method or as a conventional application in water. The active constituent is derived from the fermentation of a naturally occurring micro-organism. Laser Naturalyte Insect Control may be used in integrated pest management (IPM) and conventional insect control programmes in cotton. It has a unique mode of action and controls *Helicoverpa* species that are resistant to conventional insecticides.
- Laser Naturalyte Insect Control works by both contact and ingestion. Exposed larvae stop feeding almost immediately but can take up to 3 days to die.

MIXING

- Agitate or shake the container immediately prior to use.
- Half fill the spray tank and add the appropriate amount of accurately measured Laser Naturalyte Insect Control, then complete filling the spray tank.
- Ensure thorough agitation by mechanical or hydraulic action at all times during mixing and application.
- Use only clean water within the range pH 5-9, or DC-Tron® NR oil to dilute Laser Naturalyte Insect Control.

STORAGE OF DILUTED SPRAY MIX

- Whenever possible use the spray mix immediately after it is prepared. However, when weather conditions or mechanical breakdown prevent immediate use, the spray mix may be stored for up to 72 hours without loss of activity.
- The spray mix should be agitated thoroughly by mechanical or hydraulic action at regular intervals during storage to prevent phase separation or sediments forming. Ensure that the stored spray mix is thoroughly agitated at least once every 8 hours.
- The spray mix must be stored out of direct sunlight.
APPLICATION
- **DO NOT** apply when conditions are unsuitable for water or oil based spray applications. Avoid high temperature, strong winds, inversion conditions, imminent rain or any other conditions that may reduce the quality of spray coverage or result in drift from the area to be treated.
- A strategy to minimise spray drift should be employed at all times when aerially applying sprays to, or near, sensitive areas. Such a strategy is illustrated by the cotton industry’s Best Management Practice Manual.

Spray Volumes
- Thorough cover of the crop is essential. For optimum results follow the application specifications listed below:

Application in Water from the Ground or Air
- Apply a minimum spray volume of 50 L/ha by ground rig or 30 L/ha by aircraft.
- Use nozzle configurations which produce droplets with a volume median diameter (VMD) of 150-250 microns. Under conditions of low humidity and high temperatures adjust spray volume and droplet size upward. Do not spray when the temperature exceeds 28°C or when the relative humidity is less than 40%. Best results are achieved when the temperature is below 25°C and the relative humidity is greater than 65%.

Aircraft Ultra Low Volume Application
- Apply a minimum spray volume of 5 L/ha using DC-Tron NR oil as the carrier.
- Use Micronair AU5000 nozzles (or similar) with blade angles set to produce a droplet size of 100 micron VMD. Do not spray when the temperature exceeds 32°C or when the relative humidity is less than 40%.

RAINFASTNESS
- Rain can wash Laser Naturalyte Insect Control from treated plant surfaces and result in reduced insect control. Avoid making spray applications when rain is expected within 6 hours of spraying.

COMPATIBILITY
- Laser Naturalyte Insect Control is compatible with Pix® Plant Growth Regulator when applied as a water based spray.

® Registered Trademark
RESISTANCE

- Laser Naturalyte Insect Control has a unique mode of action and controls *Helicoverpa* species that are resistant to conventional insecticides. To avoid the development of resistance to Laser Naturalyte Insect Control follow the steps listed below:
- **DO NOT** apply more than 3 applications of Laser Naturalyte Insect Control, or any product containing an active constituent from the same class or mode of action, to any field in any one season.
- Use Laser Naturalyte Insect Control strictly in accordance with the current Insecticide Resistance Management Strategy for cotton.
- Cultivate all cotton fields as soon as possible after picking to destroy overwintering pupae of *Helicoverpa armigera*.

PROTECTION OF LIVESTOCK

- Dangerous to bees. Avoid direct application or drift of the spray mix onto bee hives.
- Bees foraging in sprayed crops will not be affected once the spray deposit has dried.

PROTECTION OF WILDLIFE, FISH, CRUSTACEA AND THE ENVIRONMENT

- **DO NOT** allow the product or used containers the enter dams, ponds, waterways or drains.
- **DO NOT** allow irrigation water from treated paddocks to enter adjacent pastures, crops or water supplies.
- **DO NOT** apply in strong winds, inversion conditions or any other conditions that may result in drift onto adjacent pastures, crops, or water supplies.
- **IT IS ESSENTIAL** to retain the first flush of tailwater/stormwater in the tailwater dam after application.

PROTECTION OF NON-TARGET INSECTS

- Laser Naturalyte Insect Control applications do not significantly reduce populations of natural predatory arthropods including; ladybird beetles (*Coccinella, Diomus* and *Harmonia* spp.), lacewings (*Chrysopa* sp.), big-eyed bug (*Geocoris* sp.), pirate bugs (*Orius* spp.), damsel bug (*Nabis* sp.), apple dimpling bug (*Campylomma* sp.), and spiders. When preserved, these beneficial arthropods can aid in the extended natural control of insect pests and reduce the likelihood of secondary pest outbreaks.
- If Laser Naturalyte Insect Control is mixed with any product that is not selective to predatory insects then the full benefit of Laser Naturalyte Insect Control to IPM programs may be lost.

CLEANING SPRAY EQUIPMENT

- After using Laser Naturalyte Insect Control empty the tank and completely drain the system. Rinse the tank, pumps, lines, hoses, filters and nozzles by circulating clean water through the system. Drain and repeat the rinsing procedure twice.
STORAGE AND DISPOSAL

• Store in the closed, original container in a cool, well-ventilated area. Do not store for prolonged periods in direct sunlight.
• Triple rinse or preferably pressure rinse containers before disposal. Add rinsings to spray tank. Do not dispose of undiluted chemicals on site. If recycling, replace cap and return clean containers to recycler or designated collection point.
• If not recycling, break, crush or puncture and bury empty containers in a local authority landfill. If no landfill is available, bury the containers below 500 mm in a disposal pit specifically marked and set up for this purpose clear of waterways, desirable vegetation and tree roots. Empty containers and product should not be burnt.
• This is a recyclable container. Free phone Dow AgroSciences (1800 700 096) for advice and information on the recycling service closest to you.

SMALL SPILL MANAGEMENT
Clean up small spill by applying absorbent material such as earth, sand, clay granules or cat litter to the spill. Sweep up material for disposal when absorption is completed and contain in a refuse vessel for disposal in the same manner as for containers (see Storage and Disposal Section). If necessary wash the spill area with an alkali detergent and water and absorb as above the wash liquid for disposal.

SAFETY DIRECTIONS

• Will irritate the eyes and skin.
• Avoid contact with eyes and skin.
• Repeat exposure may cause allergic disorders.
• When preparing spray, wear cotton overalls buttoned to the neck and wrist and a washable hat, elbow-length PVC gloves and a face shield.
• When using the prepared spray, wear cotton overalls buttoned to the neck and wrist and a washable hat, and elbow-length PVC gloves.
• If product on skin, immediately wash area with soap and water.
• If product in eyes, wash it out immediately with water.
• Wash hands after use.

FIRST AID

• If poisoning occurs, contact a doctor or Poisons Information Centre (Ph: 13 1126)

MATERIAL SAFETY DATA SHEET
Additional information is listed in the Material Safety Data Sheet for Laser Naturalyte Insect Control which is available from Dow AgroSciences on request. Call Customer Service Toll Free on 1-800-700 096.
Tracer Naturalyte Insect Control

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

Tracer*
Naturalyte* Insect Control

ACTIVE CONSTITUENT: 480 g/L SPINOSAD

For the control of cotton bollworm (*Helicoverpa armigera*) and native budworm (*Helicoverpa punctigera*) in cotton

IMPORTANT : READ THE ATTACHED BOOKLET BEFORE USE

Contents: 5 Litres
10 Litres
20 Litres

SHAKE WELL BEFORE USE

USE
Dow AgroSciences Australia Limited
A.C.N. 003 771 659
26 Rodborough Road
FRENCHS FOREST NSW 2086

CUSTOMER SERVICE TOLL FREE 1 800 700 096

* Trademark of Dow AgroSciences

NRA Approval No.: 49066/1298
GMID
STORAGE AND DISPOSAL

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

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

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

SMALL SPILL MANAGEMENT

Clean up small spill by applying absorbent material such as earth, sand, clay granules or cat litter to the spill. Sweep up material for disposal when absorption is completed and contain in a refuse vessel for disposal in the same manner as for containers (see Storage and Disposal Section). If necessary wash the spill area with an alkali detergent and water and absorb as above the wash liquid for disposal.

SAFETY DIRECTIONS

- May irritate the eyes and skin

- Avoid contact with eyes and skin

- When preparing spray and using the prepared spray, wear cotton overalls buttoned to the neck and wrist and a washable hat and elbow-length PVC gloves.

- After each day’s use, wash gloves and contaminated clothing

- Wash hands after use.

FIRST AID

If poisoning occurs, contact a doctor or Poisons Information Centre. (Ph: 13 1126)

MATERIAL SAFETY DATA SHEET

Additional information is listed in the Material Safety Data Sheet for Tracer Naturalyte Insect Control which is available from Dow AgroSciences on request. Call Customer Service Toll Free on 1-800-700 096.

NOTICE

Seller warrants that the product conforms to its chemical description and is reasonably fit for the purposes stated on the label when used in accordance with directions under normal conditions of use. No warranty of merchantability or fitness for a particular purpose, express or implied, extends to the use of the product contrary to label instructions, or under off-label permits not endorsed by Dow AgroSciences or under abnormal conditions.

EMERGENCY RESPONSE

(All Hours) RING FROM ANYWHERE IN AUSTRALIA 1-800 033 882 (LOCAL CALL FEE ONLY)

IN A TRANSPORT EMERGENCY ONLY DIAL 000 FOR POLICE OR FIRE BRIGADE

Bar for stock identification

NRA Approval No.: GMID
D.O.M./Batch No.:
CAUTION
KEEP OUT OF REACH OF CHILDREN
READ SAFETY DIRECTIONS BEFORE OPENING OR USING

Tracer*
Naturalyte* Insect Control

ACTIVE CONSTITUENT: 480 g/L SPINOSAD

For the control of cotton bollworm (*Helicoverpa armigera*) and native budworm (*Helicoverpa punctigera*) in cotton

IMPORTANT : READ THIS BOOKLET BEFORE USE

Dow AgroSciences Australia Limited
A.C.N. 003 771 659
26 Rodborough Road
FRENCHS FOREST NSW 2086

CUSTOMER SERVICE TOLL FREE 1 800 700 096

* Trademark of Dow AgroSciences

NRA Approval No.: GMID
DIRECTIONS FOR USE

DO NOT apply using Ultra Low Volume methods. DO NOT apply more than 3 applications to any field in any one season (see the RESISTANCE statement).

<table>
<thead>
<tr>
<th>CROP</th>
<th>PEST</th>
<th>STATE</th>
<th>RATE</th>
<th>CRITICAL COMMENT</th>
</tr>
</thead>
</table>
| Cotton | Bollworm \( \textit{Helicoverpa armigera} \)  
Native budworm \( \textit{Helicoverpa punctigera} \) | NSW, Qld and WA only | 150-200 mL/ha | Use the low rate against light infestations and higher rates when infestation is heavy (see guidelines below).  
Carefully monitor eggs and larvae of \textit{Helicoverpa} species by regular field scouting. Target sprays against brown eggs and newly hatched very small larvae.  
Guidelines:  
\textit{Light infestation:}  
Use 150 mL/ha when infestation of \textit{Helicoverpa} species is less than 10 eggs and 2 larvae/m of row.  
\textit{Heavy infestation:}  
Use 200 mL/ha when infestation of \textit{Helicoverpa} species exceeds 10 eggs and/or 2 larvae per metre of row.  
Larvae larger than 8 mm in length, and larvae feeding within bolls and squares may not be controlled. |

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

WITHHOLDING PERIOD: DO NOT HARVEST FOR 28 DAYS AFTER APPLICATION.

DO NOT ALLOW LIVESTOCK TO GRAZE COTTON CROP, STUBBLE OR GIN TRASH WHICH HAS BEEN TREATED WITH TRACER Naturalyte INSECT CONTROL
GENERAL INSTRUCTIONS

- Tracer Naturalyte Insect Control is formulated as a suspension concentrate that is suitable for application in water by aircraft or ground rig. The active constituent is derived from the fermentation of a naturally occurring micro-organism. Tracer Naturalyte Insect Control may be used in integrated pest management (IPM) and conventional insect control programmes in cotton. It has a unique mode of action and controls *Helicoverpa* species that are resistant to conventional insecticides.

- Tracer Naturalyte Insect Control works by both contact and ingestion. Exposed larvae stop feeding almost immediately but can take up to 3 days to die.

MIXING

- Agitate or shake the container immediately prior to use.
- Half fill the spray tank and add the appropriate amount of accurately measured Tracer Naturalyte Insect Control, then complete filling the tank.
- Ensure thorough agitation by mechanical or hydraulic action at all times during mixing and application.
- Use only clean water within the range pH 5-9 to dilute Tracer Naturalyte Insect Control.

STORAGE OF DILUTED SPRAY MIX

- Whenever possible use the spray mix immediately after it is prepared. However, when weather conditions or mechanical breakdown prevent immediate use, the spray mix may be stored for up to 72 hours without loss of activity.
- The spray mix should be agitated thoroughly by mechanical or hydraulic action at regular intervals during storage to prevent sediments forming. Ensure that the stored spray mix is thoroughly agitated at least once every 8 hours.
- The spray mix must be stored out of direct sunlight.
APPLICATION

- Thorough coverage of the crop is essential. For optimum results follow the application specifications listed below:
- Apply a minimum spray volume of 50 L/ha by ground rig or 30 L/ha by aircraft.
- Use nozzle configurations which produce droplets with a volume median diameter (VMD) of 150-250 microns. Under conditions of low humidity and high temperatures adjust spray volume and droplet size upward. Avoid spraying when the temperature exceeds 28°C or when the relative humidity is less than 40%. Best results are achieved when the temperature is below 24°C and the relative humidity is greater than 65%.
- **DO NOT** apply when conditions are unsuitable for water-based spray applications. Avoid high temperature, strong winds, inversion conditions, imminent rain or any other conditions that may reduce the quality of spray coverage or result in drift from the area to be treated.
- A strategy to minimise spray drift should be employed at all times when aerially applying sprays to, or near, sensitive areas. Such a strategy is illustrated by the cotton industry’s Best Management Practice Manual.
- **DO NOT** apply Tracer Naturalyte Insect Control using Ultra Low Volume methods.

RAINFASTNESS

- Rain can wash Tracer Naturalyte Insect Control from treated plant surfaces and result in reduced insect control. Avoid making spray applications when rain is expected within 6 hours of spraying.

COMPATIBILITY

- Tracer Naturalyte Insect Control is compatible with Pix® Plant Growth Regulator when applied as a water based spray.
- Tracer Naturalyte Insect Control is compatible with Uptake* Spraying Oil (1L/ha) applied as a water based spray.
- Tracer Naturalyte Insect Control is not compatible with ULV formulations.

CLEANING SPRAY EQUIPMENT

- After using Tracer Naturalyte Insect Control empty the tank and completely drain the system. Rinse the tank, pumps, lines, hoses, filters and nozzles by circulating clean water through the system. Drain and repeat the rinsing procedure twice.

* Trademark of Dow AgroSciences
Registered Trademark of AgrEvo
RESISTANCE

- Tracer Naturalyte Insect Control has a unique mode of action and controls *Helicoverpa* species that are resistant to conventional insecticides. To help prevent the development of resistance to Tracer Naturalyte Insect Control follow the steps listed below:
  - DO NOT apply more than 3 applications of Tracer Naturalyte Insect Control, or any product containing an active constituent from the same class or mode of action, to any field in any one season.
  - Use Tracer Naturalyte Insect Control strictly in accordance with the current Insecticide Resistance Management Strategy for cotton.
  - Cultivate all cotton fields as soon as possible after picking to destroy overwintering pupae of *Helicoverpa armigera*.

PROTECTION OF LIVESTOCK

- Dangerous to bees. Avoid direct application or drift of the spray mix onto bee hives.
- Bees foraging in sprayed crops will not be affected once the spray deposit has dried.

PROTECTION OF WILDLIFE, FISH, CRUSTACEANS AND ENVIRONMENT

- DO NOT allow the product or used containers to enter dams, ponds, waterways or drains.
- DO NOT allow irrigation water from treated paddocks to enter adjacent pastures, crops or water supplies.
- DO NOT apply in strong winds, inversion conditions or any other conditions that may result in drift onto adjacent pastures, crops or water supplies.
- IT IS ESSENTIAL to retain the first flush of tailwater/stormwater in the tailwater dam after application.

PROTECTION OF NON-TARGET INSECTS

- Tracer Naturalyte Insect Control applications do not significantly reduce populations of natural predatory arthropods including; ladybird beetles (*Coccinella, Diomus* and *Harmonia* spp.), lacewings (*Chrysopa* sp.), big-eyed bug (*Geocoris* sp.), pirate bugs (*Orius* spp.), damsel bug (*Nabis* sp.), apple dimpling bug (*Campylomma* sp.), and spiders. When preserved, these beneficial arthropods can aid in the extended natural control of insect pests and reduce the likelihood of secondary pest outbreaks.
- Tracer appears toxic to parasitic wasps and some effects may be anticipated.
- If Tracer Naturalyte Insect Control is mixed with any product that is not selective to predatory insects then the full benefit of Tracer Naturalyte Insect Control to IPM programs may be lost.
STORAGE AND DISPOSAL

• Store in the closed, original container in a cool, well-ventilated area. Do not store for prolonged periods in direct sunlight.
• Triple rinse or preferably pressure rinse containers before disposal. Add rinsings to spray tank. Do not dispose of undiluted chemicals on site. If recycling, replace cap and return clean containers to recycler or designated collection point.
• If not recycling, break, crush or puncture and bury empty containers in a local authority landfill. If no landfill is available, bury the containers below 500 mm in a disposal pit specifically marked and set up for this purpose clear of waterways, desirable vegetation and tree roots. Empty containers and product should not be burnt.

SMALL SPILL MANAGEMENT
Clean up small spill by applying absorbent material such as earth, sand, clay granules or cat litter to the spill. Sweep up material for disposal when absorption is completed and contain in a refuse vessel for disposal in the same manner as for containers (see Storage and Disposal Section). If necessary wash the spill area with an alkali detergent and water and absorb as above the wash liquid for disposal.

SAFETY DIRECTIONS

• May irritate the eyes and skin
• Avoid contact with eyes and skin
• When preparing spray and using the prepared spray wear cotton overalls buttoned to the neck and wrist and a washable hat and elbow-length PVC gloves.
• After each day’s use, wash gloves and contaminated clothing.
• Wash hands after use.

FIRST AID
If poisoning occurs, contact a doctor or Poisons Information Centre. (Ph: 13 1126)

MATERIAL SAFETY DATA SHEET
Additional information is listed in the Material Safety Data Sheet for Tracer Naturalyte Insect Control which is available from Dow AgroSciences on request. Call Customer Service Toll Free on 1-800-700 096.
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Suggested Further Reading


National Registration Authority for Agricultural and Veterinary Chemicals 1996, *MRL Standard: Maximum Residue Limits in Food and Animal Feedstuffs*, NRA, Canberra.

NRA PUBLICATIONS ORDER FORM

To receive a copy of the full technical report for the evaluation of [active constituent] in the product [product name], please fill in this form and send it, along with payment of $30 to:

[name]
[section]
National Registration Authority for Agricultural and Veterinary Chemicals
PO Box E240
Kingston ACT 2604

Alternatively, fax this form, along with your credit card details, to: [name and section] at (06) [number].

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