



Australian Government
**Australian Pesticides and
Veterinary Medicines Authority**

Fipronil Review

Phase 2 Environmental Assessment Report:

Fipronil

Environmental Effects

prepared by

**Department of the Environment, Water, Heritage and the Arts
Environmental Branch**

**Canberra
June 2010**

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1. Introductory comments

Fipronil has been assessed for a number of end-use products since its original registration in the mid-1990s. At the time of the initial assessment for agricultural products, a data package addressing the environmental toxicity of fipronil was received and relied on in the assessment.

With subsequent applications for registration, some additional data were received. However, these could not be applied to earlier assessments already concluded.

In addition, there are now a number of studies, including those relied on in international assessments of fipronil, which have not been provided to the APVMA. The primary source document in this case is the draft assessment report (DAR) from the European Food Safety Authority (EFSA) review of fipronil¹. These data have the potential to alter previous conclusions relating to environmental risk of fipronil through changes in our understanding of the toxicity of fipronil and its metabolites to organisms in the environment.

This Appendix is designed to provide, in a step-wise format, the data that were available and relied on for different assessments of fipronil at the time, data that came available with later assessments, but could not be applied to earlier assessments, and data the APVMA/DEWHA is now aware of that may lead to a revision of the environmental risk of fipronil.

Due to the volume of available toxicity data, results are provided mainly in tabular form. Tables consist of results DEWHA has considered for different assessments. For these tables, it is important to observe the 'Notes' column for further detail as to when these data were provided or made available, as many results were not available at the time of initial crop protection assessments. The 'Notes' column provides a number, and the corresponding details are provided as follows:

Note 1: Study assessed in original data package (1996)

Note 2: Study assessed with turf submission (1997)

Note 3: Study assessed in extension of uses submission (cotton, sugarcane, sorghum etc.) 1998

Note 4 Study assessed in extension of seed dressing formulation to canola (2001)

Note 5 Study assessed in termiticide assessment (2002).

¹ Draft Assessment Report (DAR) – public version. Initial Risk Assessment Provided by the Rapporteur Member State France for the Existing Active Substance FIPRONIL of the second stage of the review programme referred to in Article 8(2) of Council Directive 91/414/EEC. Volume 3, Annex B, B.9. January 2005.

Additional ecotoxicity data became available to DEWHA before the 2002 assessment for fipronil use as a termiticide. However, these results were only available from a secondary literature source and actual tests were not available. Consequently, these results were not used in subsequent assessments. The secondary source and reported results are not provided in this Appendix. Rather, the individual papers and results that comprised this review were obtained to the extent possible, and these results are reported as new data not previously considered or available to DEWHA. These and later data were rated using DEWHA's data rating system as follows:

- | | |
|---------------------------------------|--|
| 1 Fully reliable: | GLP-compliant and fully compliant with the test guideline specified. |
| 2: Reliable with restrictions: | GLP-compliant but not fully compliant with the test guideline specified, but nevertheless judged to provide a reliable basis for regulatory decision-making. An asterisk is to be added to identify studies that are not standard that are judged to be reliable for the purpose conducted (such as mechanistic studies) |
| 3 Not reliable: | Not GLP-compliant and/or not compliant with the test guideline specified, and judged to not provide a reliable basis for regulatory decision-making. |
| 4 Not assignable: | Insufficient information provided to allow the reliability of the test or study report to be assessed (such as published literature). |

It should be noted, these ratings are derived from the OECD. Australia does not have mandatory GLP and consequently some allowances need to be made in addressing the validity of a study. For example, non-GLP studies cannot be considered unreliable on these grounds alone. Therefore, a degree of expert judgement was used in applying the validity rankings associated with studies assessed.

Data provided in the EFSA review were automatically rated as '4'. Where these studies are required (due to their results leading to a change in the risk assessment), the results only are included here and used within the risk assessment. Where they are not requested, further context from the studies is reported from the summaries in the EFSA review. This is proposed to be the final text for these and the studies summarised from the literature.

In terms of new data, particularly study results reported in the literature, it needs to be realised that not all new data are reported below. Many literature reports were considered by DEWHA. However, not all are useable. Generally, where good quality, regulatory studies that follow standard guidelines are available, these will take precedence for use in the risk assessment.

Where new data are likely to result in a change to the risk assessment, these will be identified and should be provided if not already available, for independent review. In the meantime, their results will be taken as given for use in the scoping risk assessment.

For chemical structures of fipronil and its metabolites, see Attachment 1, Volume 1.

2. Avian

2.1. Acute

2.1.1. Active constituent

The following acute avian toxicity studies were reviewed previously by DEWHA.

Table V3.1: Summary of avian acute toxicity data previously reviewed by DEWHA

Species	Result	Reference	Notes*
Mallard duck	LD50 > 2150 mg/kg	Pedersen, 1990a	1
Pigeon	LD50 > 2150 mg/kg	Hakin and Rodgers, 1991	1
House sparrow	LD50 = 1120 mg/kg	Pedersen and Helston, 1991	1
Bobwhite quail	LD50 = 11.3 mg/kg	Pedersen, 1990b	1
Bobwhite quail	LD50 = 17.3 mg/kg	Pedersen and DuCharme, 1993	2
Pheasant	LD50 = 31 mg/kg	Hakin and Rodgers, 1992a	1
Red-legged partridge	LD50 = 34 mg/kg	Hakin and Rodgers, 1992b	1

* - see introductory comments

No signs of toxicity were apparent during 21 days of observation following administration in gelatin capsules to mallards.

Toxicity to pigeons was evident as regurgitation occurred between about 1 hour and 2.5 hours after oral administration in corn oil at the two highest doses (1000 and 2000 mg/kg) and food consumption was reduced in birds treated at 250 mg/kg or higher. Body weight gain after 7 days was retarded at all treatment levels, the lowest being 125 mg/kg, but effects after 14 days were only apparent at the highest dose. No mortality was recorded.

Sparrows were subdued after administration in gelatin caps, and mortality occurred at all doses tested (464–2150 mg/kg) within 24 hours, but with complete remission in survivors by 24 hours. Clinical signs of toxicity included piloerection, wing-beat convulsions, asthenia and dyspnoea. Food consumption was normal and no effects on body weight were apparent, but a number of abnormalities (friable liver, enlarged or mucus filled gizzard, abdominal haemorrhage) were evident in some dead birds on pathological examination.

Clinical signs of toxicity in bobwhites dosed by gelatin capsule (Pedersen 1990b) included anorexia, loss of balance, piloerection, wing-beat convulsions, laboured breathing and listlessness. Deaths occurred 3–18 days after dosing at 10, 21.5 and 46.4 mg/kg, and dose correlated reductions in food consumption were evident for 3 days in all test groups (lowest dose tested was 1 mg/kg). Abnormalities were evident in most of the dead birds (18 from 23) on pathology.

The second result for bobwhite quail (Pederson & DuCharme 1993) differs from the remaining acute oral results in the use of formulated material (1.6% granules) rather than technical active. High toxicity to this species is confirmed.

Pheasants were subdued after oral intubation of fipronil in corn oil at 10 mg/kg and above, with the onset of reduced food consumption in male birds observed at 20 mg/kg and in females at 40 mg/kg. Lower bodyweights were apparent by day 7 as a result. Mortalities occurred sporadically through 3 weeks of observation, the first on day 3. No abnormalities were apparent at macroscopic post mortem examination.

A similar response was observed in partridges, with subdued behaviour and unsteadiness observed soon after administration at 24 mg/kg and above, and reduced food consumption in all treated birds. Mortalities occurred sporadically through 2 weeks of observation. Dead birds were noticeably thin.

The following new information is available from the literature:

Table V3.2: Summary of avian acute toxicity literature data not previously reviewed by DEWHA

Species	Result	Reference	Rating
Zebra finch (<i>Taeniopygia guttata</i>)	LD50 = 310.2 mg/kg	Kitulagodage et al. 2008	4

This study considered toxicity of technical fipronil compared with a commercial formulation, Adonis 3UL Insecticide used in Australia for plague locust control. The formulation results are reported below. The test was performed following OECD TG425 for acute oral toxicity up-and-down method. This test procedure is of value in minimising the number of animals required to estimate the acute oral toxicity of a chemical. While the guideline discusses rodent testing, theoretically it should not preclude avian testing for acute oral toxicity. However, the establishment of the fipronil LD50 is not described adequately in the paper as it uses a previously derived LD50 from unpublished data by the same authors.

2.1.2. Formulations

The following new information is available from the literature:

Table V3.3: Summary of avian acute formulation toxicity literature data not previously reviewed by DEWHA

Species	Result	Reference	Rating
Zebra finch (<i>Taeniopygia guttata</i>)	LD50 = 15.14 mL product/kg LD50 = 45.4 mg fipronil/kg	Kitulagodage et al. 2008	4

This study considered toxicity of technical fipronil compared with a commercial formulation, Adonis 3UL Insecticide used in Australia for plague locust control following OECD TG425. The results suggest the formulation provides much

higher toxicity of fipronil to this species than technical fipronil alone. However, this assumes toxicity is imparted from the fipronil component of the formulation. Adonis 3UL also contains 12.5% diacetone alcohol, and this substance was tested individually for its toxicity to Zebra finch. It was shown to have an LD50 of 15.14 mL/kg, the same as the test formulation, indicating it is more responsible for bird toxicity than fipronil in the formulation tested here.

2.1.3. Metabolites

DEWHA has not previously received any acute avian toxicity data related to fipronil metabolites.

The following regulatory studies are considered new data and are reported in the ESFA review. The summaries from this review follow. Despite data for the desulfinyl metabolite, MB46513, appearing to be more toxic to mallard duck and bobwhite quail than the parent compound, DEWHA has not requested these studies. The metabolite was most toxic to bobwhite quail (about twice as toxic as the parent compound). However, information provided in Volume 2 indicates that while this is the main photolysis product, it is not expected to be produced at levels exceeding 10% parent:

Table V3.4: Summary of acute avian toxicity data (metabolites) – new information

Metabolite	Species	Result (mg/kg bw)	Reference	Rating
MB46513	Bobwhite quail	21 d LD50 = 5.41	Pedersen and Solatycki, 1993	4
MB46513	Mallard duck	14 d LD50 = 437	Helsten and Solatycki, 1994	4
MB46136	Bobwhite quail	14 d LD50 = 41	Gallagher et al. 2001a	4
MB46136	Mallard duck	14 d LD50 = >2000	Gallagher et al. 2001b	4

Pedersen and Solatycki (1993): MB46513 (98.6% pure) was administered in gelatine capsules to bobwhite quail, 20 weeks old; initial body weight range 159–258 g/bird; fasted around 21 hours prior to dosing. Test groups consisted of a control and five doses of 1.47, 3.16, 6.81, 14.7 or 31.6 mg/kg bw. Each test group consisted of five male and five females in a single pen. Daily observations were made over 21 days for mortalities or clinical signs of intoxication. Individual bodyweight was determined on days 1, 3, 7, 14 and 21. Group food consumption was recorded on test days 3, 7, 14 and 21. Gross pathological examinations were performed on all 21 birds that died during the investigation, four arbitrarily-selected birds from the controls and the test groups of 1.47, 3.16 and 6.81 mg/kg bw, and on all four survivors from the 14.7 mg/kg bw group. Data on mortality and body weight were statistically evaluated based on regression analysis and analysis of variance respectively.

A single mortality was recorded in the control group while mortality of 0%, 40%, 0%, 60% and 100% was recorded in the 1.47, 3.16, 6.81, 14.7 or 31.6 mg/kg bw groups respectively. Significant effects on the body weight were noted on day 7 in birds dosed at the two highest rates. Reduced food consumption was

noted during days 1–3 at 3.16 mg/kg bw and above. Food consumption remained low at the two top dose groups until day 7. Signs of toxicity included abnormal excreta, lethargy, wing-beat convulsions. Slight chalky excreta as observed at 1.47 mg/kg bw were considered not treatment-related, but to fasting prior to dosing. Gross pathology revealed abnormal findings (emaciation, empty crops, coloured gizzards) in 19 of the 21 birds that died during the investigation. Examination of the 20 arbitrarily-selected survivors revealed abnormal findings of miscellaneous nature only in four birds from the dose groups above 1.47 mg/kg bw. The LD50 was calculated to be 5.41 mg/kg bw (95% CI 2.44–12.0 mg/kg). The NOEL was determined at 1.47 mg/kg bw based on the absence of observations of lethal or sublethal effects at this level that could be attributed to the test material. Despite the greater toxicity than parent, the full study will not be requested as contribution of this metabolite to overall residues in the environment is not expected to exceed 10%.

Helsten and Solatycki (1994): MB46513 (99.7% pure) was administered in gelatine capsules to mallard ducks, 26 weeks old; initial body weight range 2801–2884 g/bird; fasted around 21.5 hours prior to dosing. Test groups consisted of a control and six doses of 147, 215, 464, 1000, 1470 and 2150 mg/kg bw. Each test group consisted of five male and five females in a single pen. Daily observations were made over 14 days for mortalities or clinical signs of intoxication. Individual bodyweight was determined on days 1, 3, 7 and 14. Group food consumption was recorded on test days 3, 7 and 14. Gross pathological examinations were performed on all 36 birds that died during the investigation, four arbitrarily-selected birds from the controls and the test groups of 147, 215 and 464 mg/kg bw, and on single surviving bird from the 1000 mg/kg bw group. Data on mortality and body weight were statistically evaluated based on analysis of variance.

Reduced feed consumption and significant reduction of body weight was noted in all test groups over days 1–3 and at 215, 464 and 1000 mg/kg over days 4–7. All birds were dead in the two highest test groups after 3 days. Signs of toxicity included lethargy, lack of coordination, convulsions and death. Remission of clinical signs was achieved by the end of day 6. Gross pathological examination revealed abnormal findings in all 36 birds that died during the study, including gizzards and intestines void of feed, gas distended intestines, and coloured livers. No abnormalities were reported from the arbitrarily-selected 14-day survivors from all dose groups with the exception of a pale liver reported from the one remaining bird of the 1000 mg/kg bw group. Since the single mortality at 147 mg/kg was considered treatment-related, the NOEL could not be determined. The 14 day LD50 was calculated to be 437 mg/kg bw (95% CI 332–576 mg/kg).

Gallagher et al. (2001a): MB46136 (99.7% pure) was administered in gelatine capsules to bobwhite quail, 20 weeks old; initial body weight range 180–221 g/bird; fasted around 17 hours prior to dosing. Test groups consisted of a control and seven doses of 4.7, 9.4, 18.8, 37.5, 75, 150 and 300 mg/kg bw. Each test group consisted of five male and five females housed per sex. Daily observations were made over 14 days for mortalities or clinical signs of

intoxication. Individual bodyweight was determined on days -1, 3, 7 and 14. Food consumption was recorded per pen on test days 0–3, 4–7 and 8–14. All birds dying in the course of the test and all birds remaining at termination were subjected to gross necropsy. The LD50 was determined based on non-linear interpolation.

No mortalities were found in the control group, or test groups up to and including 18.8 mg/kg bw. Forty per cent mortality was found at 37.5 mg/kg bw, with 100% mortality in all groups higher than this. Clinical signs of toxicity including lethargy, loss of coordination and convulsions were reported at higher levels. Food consumption and body weight gain was temporarily reduced in birds dosed at 9.4 mg/kg bw and above. Recovery of food intake and body weight gain was visible 3 days after dosing at 9.4 mg/kg bw, and no difference to the control existed at the end of the test. Birds dosed at 18.8 mg/kg showed very high rates of compensatory body weight gain during the second week and after dosing and did nearly catch up to the controls until the end of the test. Birds found dead during the study largely had lost muscle mass or were considered thin or emaciated. Gross necropsy of 14-day survivors revealed no findings attributed to the test item. Based on reduced body weight over days 1–3 the NOEL was reported at 4.7 mg/kg bw. The acute oral LD50 was 41 mg.kg bw (95% CI 18.8–75 mg/kg bw).

Gallagher et al. (2001b): MB46136 (99.7% pure) was administered in gelatine capsules to mallard ducks, 20 weeks old; initial body weight range 995–1328 g/bird; fasted around 17 hours prior to dosing. Test groups consisted of a control and three doses of 500, 1000 and 2000 mg/kg bw. Each test group consisted of two pens with five male and five females housed per sex. Daily observations were made over 14 days for mortalities or clinical signs of intoxication. Individual bodyweight was determined on days -1, 3, 7 and 14. Food consumption was recorded per pen on test days 0–3, 4–7 and 8–14. All birds were subjected to gross necropsy.

There were no mortalities or overt signs of toxicity observed. All birds were normal in appearance and behaviour throughout the test. No treatment-related findings were reported from gross necropsy. No treatment-related effects on body weight or food consumption were observed. The NOEL was 2000 mg/kg bw and the acute oral LD50 was >2000 mg/kg bw.

2.2. Short-term

2.2.1. Active constituent

The following avian dietary toxicity studies were reviewed previously by DEWHA.

Table V3.5: Summary of avian dietary toxicity data previously reviewed by DEWHA

Species	Result	Reference	Notes
Mallard duck	LD50 >5000 mg/kg diet	Pedersen, 1990c	1
Bobwhite quail	LD50 = 48 mg/kg diet	Pedersen, 1990d	1

Dietary studies in mallard ducklings confirmed the practical absence of toxicity in this species. Birds were lethargic, anorexic and slow growing following administration in the feed at 2500 and 5000 mg/kg, but total remission of these symptoms was evident in survivors by the end of the 5-day exposure period. A single bird died after 4 days at the highest dose, with no abnormal pathological findings.

Dietary studies in quail indicated very high toxicity to this species. The onset of mortality occurred at 39 ppm, with no survivors at 156 ppm and above. Clinical symptoms (lethargy, diarrhoea and anorexia) were evident across this range, but with full remission in survivors within 24 hours following the 5-day treatment period. Body weights in survivors from the 39 ppm group remained depressed at the end of the 17-day recovery period.

2.2.2. Metabolites

DEWHA has not previously received any avian toxicity data related to fipronil metabolites.

The following regulatory studies are considered new data and are reported in the ESFA review:

Table V3.6: Summary of avian dietary toxicity data (metabolites) – new information

Metabolite	Species	Result (mg/kg diet)	Reference	Rating
MB46513	Bobwhite quail	8 d LC50 = 120	Gallagher et al. 1999a	4
MB46513	Bobwhite quail	8 d LC50 = 110	Gallagher et al. 2000	4
MB45950	Bobwhite quail	8 d LC50 = 114	Gallagher et al. 1999b	4
MB46136	Bobwhite quail	8 d LC50 = 84	Gallagher et al. 1999c	4

Gallagher et al. (1999a): MB46513 (97.8% pure) was mixed into standard bird diet (vehicle: corn oil and acetone). Homogeneity, stability and accuracy of the dietary concentrations were analytically confirmed. Test organisms were 10-day old bobwhite quail with initial body weight range of 15–24 g/bird. Test groups consisted of two pens with five birds each. The vehicle control groups consisted of six pens with five birds each. In a first run test diets offered over five consecutive days contained the test item at 0, 5.6, 10, 17.8, 31.6, 56.2 and 100 mg/kg diet. In a second phase with a new batch of birds, diets offered over 5 days contained 0, 178, 316 and 562 mg/kg diet. In both phases, the exposure phase was followed by a 3-day recovery period in which all surviving birds were offered untreated diet. Daily observations were made over 8 days for mortality and other signs of intoxication. Individual bodyweight was determined at days 0,

5 and 8. Average feed consumption was estimated by pen during the exposure period and post exposure period. All test birds that died during the course of the test and all birds remaining at the termination were subjected to a gross necropsy. The LC50 was based on binomial probability.

No mortalities were found in the controls or treatment groups up to 56.2 mg/kg diet. Twenty per cent mortality was observed at 100 mg/kg diet with 100% mortality at the three treatment groups above this. Signs of toxicity were observed at 31.6 and 56.2 mg/kg diet, including ruffled appearance, wing droop or convulsions, but recovered largely at the end of the post exposure period. Mean feed consumption during the exposure period appeared reduced at 56.2 and 100 mg/kg diet (7 g feed/bird/day vs 9–10 g/bird/day in the respective control and at lower doses). Dose-related reduction of body weight gain were observed at 31.6 mg/kg diet and above. Birds found dead during the test were classified in gross necropsy as being thin or emaciated with autolysis of tissues and empty crops. No treatment-related abnormalities were reported following gross necropsy from birds exposed to 31.6 mg/kg diet or lower. Based on signs of toxicity and reduced body weight gain, the NOEC was reported as 17.8 mg/kg diet. The 8 day LC50 was determined to be 120 mg/kg diet (95% CI 56.2–178 mg/kg diet).

Gallagher et al. (2000): MB46513 (97.8% pure) was mixed into standard bird diet (vehicle: corn oil and acetone). Homogeneity, stability and accuracy of the dietary concentrations were analytically confirmed. Test organisms were 10-day old bobwhite quail with initial body weight range of 17–22 g/bird. Test groups consisted of two pens with five birds each. The vehicle control groups consisted of six pens with five birds each. Test diets offered over five consecutive days contained the test item at 0, 17.8, 28.5, 45.6, 72.9, 117, 187 and 299 mg/kg diet. The exposure phase was followed by a 3-day recovery period in which all surviving birds were offered untreated diet. Daily observations were made over 8 days for mortality and other signs of intoxication. Individual bodyweight was determined at days 0, 5 and 8. Average feed consumption was estimated by pen during the exposure period and post exposure period. All test birds that died during the course of the test and all birds remaining at the termination were subjected to a gross necropsy. The LC50 was calculated with probit analysis.

No mortalities were found in the controls or treatment groups up to 45.6 mg/kg diet. 10% mortality was observed at 72.9 mg/kg diet, 50% mortality at 117 mg/kg diet with 100% mortality at the two treatment groups above this. Signs of toxicity were observed at 45.6 mg/kg diet and above, including ruffled appearance and lethargy. All surviving birds fully recovered by the morning of day 8. Mean feed consumption during the exposure period was 8, 9, 6, 7, 6 and 4 g/bird/day and the mean body weight at the end of the exposure period was 30, 26, 24, 23, 19 and 15 g/bird at 0, 17.8, 28.5, 45.6, 72.9 and 117 mg/kg diet respectively. Birds found dead during the test were characterised as being thin or emaciated with autolysis of tissues, loss of muscle mass and with empty crops. No remarkable necropsy findings were reported from surviving birds. The NOEC was determined to be 17.8 mg/kg diet and the 8 day LC50 was calculated at 110 mg/kg diet (95% CI 91–135 mg/kg diet).

Gallagher et al. (1999b): MB45950 (98.8% pure) was mixed into standard bird diet (vehicle: corn oil and acetone). Homogeneity, stability and accuracy of the dietary concentrations were analytically confirmed. Test organisms were 10-day old bobwhite quail with initial body weight range of 15–24 g/bird. Test groups consisted of two pens with five birds each. The vehicle control groups consisted of six pens with five birds each. Diets contained the test item at 0, 10, 17.8, 31.6, 56.2, 100 and 178 mg/kg diet. The exposure phase was followed by a 3-day recovery period in which all surviving birds were offered untreated diet. Daily observations were made over 8 days for mortality and other signs of intoxication. Individual bodyweight was determined at days 0, 5 and 8. Average feed consumption was estimated by pen during the exposure period and post exposure period. All test birds that died during the course of the test and all birds remaining at the termination were subjected to a gross necropsy. The LC50 was based on binomial probability.

No treatment-related mortalities or clinical signs of toxicity were noted at dietary concentrations of 10 and 17.8 mg/kg. Clinical signs of toxicity observed at 31.6 and 56.2 mg/kg included ruffled appearance and lethargy. All birds recovered largely by the end of the exposure period. Significant mortality was observed at 100 mg/kg (30%) and 178 mg/kg diet (100%). Mean feed consumption during the exposure period appeared reduced at the two highest test concentrations. Dose-related reductions of body weight gain were observed at 56.2 mg/kg diet and above. No treatment-related abnormalities were reported following gross necropsy from birds exposed to 31.6 mg/kg diet or below. Typical findings in gross necropsy of the birds dying during the study or surviving birds exposed to 56.2 and 100 mg/kg diet was thin condition. The NOEC was determined at 17.8 mg/kg diet and the 8 day LC50 was calculated at 114 mg/kg diet (95% CI 56.2–178 mg/kg diet).

Gallagher et al. (1999c): MB46136 (99.7% pure) was mixed into standard bird diet (vehicle: corn oil and acetone). Homogeneity, stability and accuracy of the dietary concentrations were analytically confirmed. Test organisms were 10-day old bobwhite quail with initial body weight range of 15–24 g/bird. Test groups consisted of two pens with five birds each. The vehicle control groups consisted of six pens with five birds each. Diets contained the test item at 0, 17.8, 31.6, 56.2, 100, 178 and 316 mg/kg diet. The exposure phase was followed by a 3-day recovery period in which all surviving birds were offered untreated diet. Daily observations were made over 8 days for mortality and other signs of intoxication. Individual bodyweight was determined at days 0, 5 and 8. Average feed consumption was estimated by pen during the exposure period and post exposure period. All test birds that died during the course of the test and all birds remaining at the termination were subjected to a gross necropsy. The LC50 was calculated with probit analysis.

There were no mortalities in the control group or the lowest two treatment groups. No overt signs of toxicity were noted at 17.8 mg/kg diet. At 31.6 mg/kg diet the only abnormality reported was one bird with transiently ruffled appearance. Mortalities and signs of toxicity were reported from birds exposed at 56.2 mg/kg diet and above. All surviving birds at these dietary levels

recovered and were normal in appearance and behaviour by the morning of day 8 at the latest. Compared with the control group, the food consumption during the exposure period appeared slightly reduced in birds at 56.2 mg/kg diet and was clearly reduced at 100 mg/kg diet and above. There were no effects on body weight among birds offered 17.8 or 31.6 mg/kg diet but the body weight gain of the surviving birds was reduced at 56.2 mg/kg diet and above. Gross necropsy of all birds that died during the test mainly reported thin to emaciated condition and pale organs. Necropsy of survivors did not reveal remarkable findings. The NOEC was determined at 17.8 mg/kg diet and the 8 day LC50 was calculated at 84 mg/kg diet (95% CI 66–106 mg/kg diet).

2.3. Reproduction

2.3.1. Active constituent

The following avian reproduction toxicity studies were reviewed previously by DEWHA.

Table V3.7: Summary of avian reproduction toxicity data previously reviewed by DEWHA

Species	Result	Reference	Notes
Bobwhite quail	NOEC = 10 mg/kg diet	Pedersen and DuCharme, 1992	1
Mallard duck	NOEC = 1000 mg/kg diet	Pedersen and Lesar, 1993	3

The reproduction study involved dietary administration for 20 weeks and 2 days at 2 and 10 ppm. No impacts on survival, food consumption or weight gain were noted in parent birds at this sub-lethal concentration, and parental reproductive success and viability of offspring remained unaffected.

A one-generation mallard duck reproduction study was conducted with all birds administered for 23 consecutive weeks at 100, 500 and 1000 ppm. Two deaths were recorded, one in the control group, and one at the highest tested level. No impacts on survival, food consumption or weight gain were noted in parent birds at this sub-lethal concentration, and parental reproductive success and viability of offspring remained unaffected.

2.3.2. Special studies

The following palatability studies were reviewed previously by DEWHA.

- Quail/fipronil granules (1996 assessment)

This study used groups of 10 birds offered a choice of unamended feed (chick crumbs which closely resembled the test granules), test granules containing 2% fipronil, and a 10% mix of granules in feed over a 14-day period. Food hoppers were rotated daily to eliminate positional bias. Birds were observed hourly for 7 hours after commencement, and then twice daily to the end of the study. No feeding was observed in the hoppers containing fipronil granules, and all birds remained in good health throughout the study. Daily feed consumption

(including spillages) exceeded 100 g from the untreated hopper, compared with 2 g from the 10% mixture and 1 g from the hopper containing only granules (Hakin & Rodgers 1992c).

- **Pheasant and partridge / granular soil treatment (1996 assessment)**

Palatability studies on ring-necked pheasant and red legged partridge were conducted in two large adjacent cages (25 x 100 m) containing 10 birds from each species (Anonymous 1992). Cages were planted with maize in three strips (7 x 80 m) separated by grassy borders planted with shrubs to provide shade. One of the cages was treated at planting with fipronil microgranules at 80 g per 100 m. Maize and wheat were provided as feed, initially ad libitum but reduced to half of daily consumption for 1 week following planting, and further to zero thereafter to force consumption of planted seed.

Birds (mostly pheasants) were observed feeding on seed left at or near the surface at planting, and began foraging for planted seed at the onset of emergence. Seedlings were also attacked. Birds would not be expected to be repelled from eating seeds and seedlings as fipronil is not systemic. A single male pheasant that died in the treated cage was considered to have succumbed to attack by another male rather than fipronil intoxication as it had many injuries. Two male pheasants in the other cage died similarly before testing began.

- **Grey partridge / treated seed (1996 assessment)**

A third study examined the palatability of treated maize seeds to grey partridge in a three-way choice test (Redgrave 1993). Groups of 10 birds were exposed for 1 week to untreated maize, treated maize containing 2200 mg/kg fipronil, or both in separate hoppers, rotated daily to avoid positional bias. Birds were fed on untreated maize before and after the exposure period, and weighed about 320 g at the start of the treatment phase. An LD50 of about 30 mg/kg equates to about 10 mg per bird, or around 4.5 g seed (30–50% of daily consumption) dressed at 2200 mg/kg.

Mean food consumption in controls over the treatment period was 9.2 g for males and 14.3 g for females. Consumption of untreated seeds (excluding spillages) in the birds offered both treated and untreated was similar (some reduction for females). Treated seeds were consumed by males and females at an average 0.6 g per day in both choice and no choice tests, but individual birds may have consumed more than others.

A single female bird that died in the choice test on the second day of treatment was found at post mortem to be in good condition with food in the gizzard but no intact maize grains in the crop. A male in the treatment only group exhibited signs of intoxication on the second day and died on the third. Post mortem examination revealed four intact seeds in the crop and food in the gizzard. Another male bird found dead in this group on the fourth day of treatment had food in the gizzard but no grains in the crop, and signs of congealed blood

around the heart. A female bird from this group was sacrificed on the sixth day as it was moribund, and very thin with reduced muscle and fat. Post mortem examination revealed no food in gizzard or crop. Surviving birds from this group were subdued for a further 5 days, with two more deaths (male and female) on the third and fourth days of the recovery period. Both these birds were very thin with reduced muscle and fat. Birds in the treatment group lost an average 25–30% of body weight over the treatment period.

Results indicate that the presence of 2200 mg/kg fipronil in feed almost completely suppresses food consumption in grey partridge in the absence of an alternative food source, and engenders an overwhelming preference for untreated feed where this is available. While consumption of treated seed may have contributed to mortality through intoxication, it appears that the main cause of death in exposed birds is starvation induced by anorexia.

- **Bird monitoring study (1998 assessment)**

In response to DEWHAs assessment of fipronil to rice as a seed treatment, Rhone-Poulenc Rural Australia commissioned a bird observation study to determine any detrimental effect on birds following the ingestion of Cosmos treated rice seed (Webster 1997).

Four sites were selected and intensive observations undertaken at each site. One site was located in the Coleambally Irrigation District, with three sites in the Wakool Irrigation District. Sites ranged in size from 40–100 ha. As each site consisted of a number of bays all of which could not be observed from a single location, a circuit around the bays was undertaken every 30–60 minutes. Observations were made at each site for 1 hour prior to sowing and then for the remainder of the day following sowing. Observations were continued for the first hour of darkness on the day following sowing to detect whether night foraging species were feeding upon rice seed.

Additionally, two airstrips were observed within the Deniliquin district. These could not be observed during observation periods on the bays due to distance between the two. Therefore, at the first airstrip a small amount (2 kg) of Cosmos-treated rice seed was placed on the airstrip near where the aircraft and loader would have operated. Observations on this grain were collected for the remainder of the day. At the second site a larger amount (4 kg) of rice seed was placed on the airstrip. Observations on this grain were made for the remainder of the day following the rice seeds placement and for 2 hours each morning during the peak foraging period for birds for the following 5 days.

Twenty-seven bird species were recorded during observations on bays. Fourteen bird species were observed on bays and in the immediate area prior to sowing. Of these, nine were observed foraging. After sowing, 22 species of the 27 recorded were observed foraging. The only Rallidae species observed was the Black-tailed Native Hen at the third site. This species was not recorded foraging during the observation period. The report states the habitat that exists

within the bays is generally unsuitable for Gallinule type birds, as it is usually devoid of vegetative cover which is preferred by these birds.

The species observed feeding (Crested Pigeon, Galah, Red-rumped Parrot, and Little Raven) did not appear to suffer any adverse effects from feeding on the treated rice. No individual was observed foraging on the rice for longer than 5 minutes. Of the remaining species observed foraging, it could not be determined what was being consumed. The Pacific Black Duck and Grey Teal were observed dabbling and up-ending to obtain the food they were foraging upon. The remaining species observed foraging except for the Whiskered and Gull-billed Terns all appeared to be gleaning food items from the soil or water surface. The two tern species were taking small invertebrates from the water surface.

On the first airstrip, four species of bird (Crested Pigeon, Galah, Australian Magpie and Little Raven) were recorded foraging on the small pile of rice left on the strip. The Australian Magpie and Little Raven were observed searching through the rice seed and picking up material which could not be identified. As both species have been recorded taking plant material it is possible they were including treated rice seed in their diet. None of the feeding periods lasted longer than 7 minutes, and all four species flew away after foraging, apparently unaffected by any rice they had ingested, although it appears none were followed.

Two bird species (Galah and Little Raven) were recorded foraging on the rice pile left at the second airstrip. Food items included both rice seed and material which could not be identified. Again, foraging periods were short, with 8 minutes recorded as the longest, and the birds flew away after foraging, apparently unaffected by any rice they had ingested.

As the birds only feed on the treated rice for short periods of time, and rice remained on the ground for over a week, it is possible that the treated rice may be unpalatable to birds, which is supported by palatability studies above. No Galliform species were observed on either the bays or the airstrips during the sowing period.

- **APLC operations (1998 assessment)**

The Australian Plague Locust Commission (APLC) has conducted trials with fipronil being applied at rates of 1.25–5 g ac/ha. While no scientific study was performed on the effects on birds, many insectivorous and scavenger bird species were said to have been actively feeding in the trial area following spraying but did not appear to be affected as no signs of intoxication were encountered. It appeared locusts moved towards a water body, and were ingested by ducks without apparent effect (Spurgin 1997).

- **Other field or semi-field studies**

The following special studies are new information that **have not** previously been provided to DEWHA. They are all avoidance studies as to the level of attractiveness of treated seeds to birds where there is no food choice. They are reported in the EFSA review, and results along with study ratings are summarised:

Table V3.8: Summary of avian avoidance studies not previously reviewed by DEWHA

Species	Type of treated seed	Attractivity	Reference	Rating
Ring necked pheasant	Maize	Strongly repellent	Rodgers, 2001a	4
Pigeon	Maize	Low attractivity	Rodgers, 2001b	4
Ring necked pheasant	Haricot beans	Reduced attractivity	Rodgers, 2001c	4
Pigeon	Haricot beans	Reduced attractivity	Rodgers, 2001d	4
Ring necked pheasant	Wheat	Reduced attractivity	Rodgers, 2001e	4
Pigeon	Wheat	Reduced attractivity	Rodgers, 2001f	4
Pigeon	Sunflower	Strongly repellent	Grolleau, 1999	4

Rodgers (2001a): The study was conducted to estimate food consumption and risk of intoxication to birds allowed to feed on maize seeds treated with the fipronil formulation EXP80415A under laboratory conditions in a no-choice situation. Maize seeds (2.11 g ac/kg seeds) were presented scattered on the floor of the cages. Test birds were adult ring-necked pheasants (*Phasianus colchicus*), body weight range 739–1288 g/bird. Replication was six pens with one male and one female per test group. Test groups were 1) control; 2) no-choice group offered over 8-hour exposure after which untreated maize was provided. All birds were offered untreated maize during the pretreatment phase (days -7 to -1), after the 8-hour exposure phase, and during the post treatment phase (days 2–7). Birds were observed continuously during the first hour and frequently during the rest of the exposure phase for pecking at and ingestion of food. For the rest of the study, observations were made twice daily for behaviour, feeding and clinical signs of toxicity. Individual bodyweights were recorded on days -7, -1, and 7. Mean food consumption was estimated per cage daily over the whole study period. All birds were subjected to gross necropsy.

No mortalities, clinical signs of toxicity or abnormal findings in gross necropsy were reported from this study. The mean body weight over the study period did not significantly differ between control and test item group. Consumption of treated seeds was negligible (mean 0 g/bird). Intensive pecking of the birds at the treated seeds was observed during the exposure period. The avoidance factor as the proportion of consumed treated seed relative to the consumption of untreated diet is estimated as 0.0. Maize seeds treated with EXP80415A were strongly repellent to ring-necked pheasants.

Rodgers (2001b): The study was conducted to estimate food consumption and risk of intoxication to less sensitive, but starved birds allowed to feed on maize seeds treated with the fipronil formulation EXP80415A under laboratory conditions in a no-choice situation. Maize seeds (2.11 g ac/kg seeds) were presented scattered on a large feeding tray. Test birds were adult pigeons (*Columba livia*), body weight range 316–517 g/bird, starved the day prior to treatment. Replication was six pens with six males or six females per test group. Test groups were 1) control; 2) no-choice group offered over 8-hour exposure after which untreated maize was provided. All birds were offered untreated maize for 2 hours per day during the pretreatment phase (days -7 to -1), after the 8-hour exposure phase, and during the post treatment phase (days 2–7). Birds were observed continuously during the first hour and frequently during the rest of the exposure phase for pecking at and ingestion of food. For the rest of the study, observations were made twice daily for behaviour, feeding and clinical signs of toxicity. Individual bodyweights were recorded on days -7, -1, and 7. Mean food consumption was estimated per cage daily over the whole study period. All birds were subjected to gross necropsy.

No mortalities, clinical signs of toxicity or abnormal findings in gross necropsy were reported from this study. The mean body weight was slightly lower in the test item group compared with the control. Control food consumption was higher during the exposure period as all birds had been starved on day -1. Measured consumption of treated seeds was clearly reduced (mean avoidance factor 0.17), despite the observations of the starved birds feeding and pecking during the first hour of the exposure period. Maize seeds treated with EXP80415A were of very low attractivity even to starved pigeons.

Rodgers (2001c): The study was conducted to estimate food consumption and risk of intoxication to sensitive birds allowed to feed on haricot beans treated with the fipronil formulation EXP80415A under laboratory conditions in a no-choice situation. Haricot beans (0.44 g ac/kg seeds) were presented scattered on the floor of the cages. Test birds were adult ring-necked pheasants (*Phasianus colchicus*), body weight range 814–1399 g/bird. Replication was six pens with one male and one female per test group. Test groups were 1) control; 2) no-choice group offered over 8-hour exposure after which untreated maize was provided. All birds were offered untreated beans during the pretreatment phase (days -7 to -1), and untreated wheat during the post treatment phase (days 2–7). Birds were observed continuously during the first hour and frequently during the rest of the exposure phase for pecking at and ingestion of food. For the rest of the study, observations were made twice daily for behaviour, feeding and clinical signs of toxicity. Individual bodyweights were recorded on days -7, -1, and 7. Mean food consumption was estimated per cage daily over the whole study period. All birds were subjected to gross necropsy.

No mortalities or abnormal findings in gross necropsy were reported from this study. Transient clinical signs of toxicity were observed in two birds at the end of day 1 (unable to stand, flapping wings for approximately 10 minutes, then subdued) that fully recovered by the next morning. The mean body weight over

the study period did not significantly differ between control and test item group. Consumption of treated seeds was low (56% of control consumption), but intensive pecking of the birds at the treated beans was observed during the first hour of the exposure period. The avoidance factor as the proportion of consumed treated seed relative to the consumption of untreated diet was 0.56. Haricot bean-seeds treated with EXP80415A were of reduced attractivity to ring-necked pheasants.

Rodgers (2001d): The study was conducted to estimate food consumption and risk of intoxication to less sensitive birds allowed to feed on haricot bean seeds treated with the fipronil formulation EXP80415A under laboratory conditions in a no-choice situation. Haricot beans (0.44 g ac/kg seeds) were presented scattered on a large feeding tray. Test birds were adult pigeons (*Columba livia*), body weight range 291–467 g/bird. Since a large proportion of the pigeons appeared thin following the pretreatment phase on haricot beans only, the birds were not starved additionally prior to the treatment period. Replication was six pens with six males or six females per test group. Test groups were 1) control; 2) no-choice group offered over 8-hour exposure after which untreated wheat was provided. All birds were offered untreated beans for 2 hours per day during the pretreatment phase (days -7 to -1), and untreated wheat grains during the post treatment phase (days 2–7). Birds were observed continuously during the first hour and frequently during the rest of the exposure phase for pecking at and ingestion of food. For the rest of the study, observations were made twice daily for behaviour, feeding and clinical signs of toxicity. Individual bodyweights were recorded on days -7, -1, and 7. Mean food consumption was estimated per cage daily over the whole study period. All birds were subjected to gross necropsy.

No mortalities, clinical signs of toxicity or abnormal findings in gross necropsy were reported from this study. The mean body weight remained comparable to the control. Control food consumption was increased during the exposure period probably due to prolonged food availability. The increased feeding rate in the post treatment phase may indicate higher attractivity of the wheat grains supplied in this phase. Consumption of treated beans was 78% and 34% of male and female control food consumption respectively. Since results for both sexes differed significantly, an avoidance factor of 0.78 may serve as worst-case approach. The observed pattern of feeding and pecking during the first hour of the exposure period supports the conclusion of reduced attractivity of treated haricot beans to the pigeon.

Rodgers (2001e): The study was conducted to estimate food consumption and risk of intoxication to sensitive birds allowed to feed on wheat treated with the fipronil formulation EXP80415A under laboratory conditions in a no-choice situation. Wheat seeds (0.549 g ac/kg seeds) were presented scattered on the floor of the cages. Test birds were adult ring-necked pheasants (*Phasianus colchicus*), body weight range 754–1289 g/bird. Replication was six pens with one male and one female per test group. Test groups were 1) control; 2) no-choice group offered over 8-hour exposure after which untreated wheat was provided. All birds were offered untreated wheat during the pre-treatment phase

(days -7 to -1), and untreated wheat during the post treatment phase (days 2–7). Birds were observed continuously during the first hour and frequently during the rest of the exposure phase for pecking at and ingestion of food. For the rest of the study, observations were made twice daily for behaviour, feeding and clinical signs of toxicity. Individual bodyweights were recorded on days -7, -1, and 7. Mean food consumption was estimated per cage daily over the whole study period. All birds were subjected to gross necropsy.

Food consumption was clearly reduced in the test item group during the exposure phase (21% of control consumption = avoidance factor 0.21). Body weight decreased in both groups during the post treatment phase, with higher losses in the test item treated group. One bird died on day 2 in the test item group. Two birds that had lost large amounts of weight were considered subdued at the end of the post treatment period. All other birds remained in good health. No abnormal findings in gross necropsy were reported. No clear distinction was made between observed feeding or pecking bouts during the exposure period, but given the low food consumption in the test item treated group, these birds must have been mostly pecking at the seeds. Wheat seeds treated with EXP80415A were of clearly reduced attractiveness to ring-necked pheasants.

Rodgers (2001f): The study was conducted to estimate food consumption and risk of intoxication to less sensitive birds allowed to feed on wheat seeds treated with the fipronil formulation EXP80415A under laboratory conditions in a no-choice situation. Wheat seeds (0.549 g ac/kg seeds) were presented scattered on a large feeding tray. Test birds were adult pigeons (*Columba livia*), body weight range 365–544 g/bird, starved 24 hours prior to the treatment period. Replication was six pens with six males or six females per test group. Test groups were 1) control; 2) no-choice group offered over 8-hour exposure after which untreated wheat was provided. All birds were offered untreated wheat grains for 2 hours per day during the pre-treatment phase (days -7 to -1). Birds were observed continuously during the first hour and frequently during the rest of the exposure phase for pecking at and ingestion of food. For the rest of the study, observations were made twice daily for behaviour, feeding and clinical signs of toxicity. Individual bodyweights were recorded on days -7, -1, and 7. Mean food consumption was estimated per cage daily over the whole study period. All birds were subjected to gross necropsy.

No mortalities, clinical signs of toxicity or abnormal findings in gross necropsy were reported from this study. The amount of treated seeds consumed to approximately 67% of the seed consumption in the controls. Wheat seeds treated with EXP80415A were of reduced attractiveness to starved pigeons.

Grolleau (1999): This study was performed to address an alleged poisoning incident with sunflower seeds treated with EXP80415A that concerned 14 feral pigeons and two turtle doves of a private bird keeper in France. For the test, sunflower seeds treated at 4.9 mg ac/kg seeds were presented in hoppers to adult pigeons (*Columba livia*), wild caught and adapted over 3 weeks to captivity with a body weight range of 286–414 g/bird. Replication was 10

individually-caged birds (not sexed) each per test group. Test groups were 1) no-choice group (treated sunflower seeds), 24-hour exposure (day 4); 2) no-choice group (treated sunflower seeds), 72-hour exposure (days 4–6). All birds were offered 50 g untreated sunflower seeds per bird for 3 days during the pre-treatment phase and untreated mixed diet during a 3-day post treatment phase. Individual body weights were recorded on days 1, 4 and at the end of the post treatment phase. Mean food consumption was estimated per bird daily during the pre-treatment and treatment phase.

Compared with the mean food consumption during the pre-treatment phase, food consumption was significantly reduced during the treatment period. Overall avoidance factors were calculated at 0.088 (test group 1) and 0.073 (test group 2). Sunflower seeds treated with EXP80415A were strongly repellent to pigeons.

Further avoidance data is available in the literature:

Table V3.9: Summary of avian avoidance literature data not previously reviewed by DEWHA

Species	Type of treated seed	Findings	Reference	Rating
Red-wing blackbirds Brown-headed cowbird Boat-tailed grackles	Rice	Fipronil treated seeds (350 and 500 mg/kg) did not affect birds' response to the seeds. Birds avoided the seeds when a dye was present.	Avery et al. 1998	4

3. Fish

3.1.1. Acute exposure

3.1.1.1. Active constituent

The following acute fish toxicity studies were reviewed previously by DEWHA.

Table V3.10: Summary of fish acute toxicity data previously reviewed by DEWHA

Species	Result	Reference	Notes
Carp (<i>Cyprinus carpio</i>)	96 h LC50 = 430 µg/L	Handley et al. 1991a	1
Rainbow trout (<i>O. mykiss</i>)	96 h LC50 = 250 µg/L	Scott-Ward, 1991a	1
Bluegill sunfish (<i>L. macochirus</i>)	96 h LC50 = 85 µg/L	Scott-Ward, 1991a	1
Sheepshead minnow (<i>C. variegatus</i>)	96 h LC50 = 130 µg/L	Machado, 1993	3

Tests on fish were generally conducted under flow-through conditions, with results expressed as mean measured concentrations. Symptoms of intoxication included loss of equilibrium and lethargy. Mortality of carp occurred within hours at concentrations above 1 mg/L. Acute no effect concentrations for carp, trout and bluegill were 73, 34 and 43 µg/L, respectively.

The following regulatory studies are considered new data and are reported in the ESFA review:

Table V3.10a: Summary of new regulatory fish acute toxicity studies not reviewed by DEWHA

Species	Result	Reference	Rating
Channel catfish (<i>Ictalurus punctatus</i>)	96 h LC50 = 560 µg/L	Dionne, 1997.	4

Dionne (1997): The test was performed to US EPA guidelines and GLP. The test organisms were juvenile channel catfish, mean total length 59 mm, mean wet weight 1.7 g, starved over 72 hours prior to and during 96 hours of exposure. Dilution water was well water (hardness 38 mg/L as CaCO₃), temperature 22–23°C, oxygen content at least 6.9 mg/L, pH range 7.1–7.4. Exposure was 96 hours under flow-through conditions. Biological loading was around 0.17 g/L. Test groups consisted of two replicate aquaria with 10 fish each per concentration. Nominal test concentrations were 0 (control), 0 (solvent control, acetone), 0.094, 0.19, 0.38, 0.75 and 1.5 mg ac/L. Mortality or abnormalities among the fish were monitored daily. The exposure concentrations were analysed at the beginning and end of the exposure period. The LC50 was calculated by non-linear interpolation, the 95% confidence intervals by binomial probability.

Mean measured concentrations were 0.089, 0.17, 0.32, 0.61 and 1.2 mg ac/L. After 96 hours, mortality of 60% and 100% was found at 0.61 and 1.2 mg ac/L respectively, with no other mortality observed. Sublethal effects including lethargy and loss of equilibrium were observed at all test concentrations. Undissolved material was observed at the top concentration between 24 and 96 hours. The LC50 after 96 hours of exposure was calculated at 0.56 mg ac/L (95% CI 0.32–1.2 mg ac/L). The NOEC was not determined due to sublethal effects at all test concentrations.

Amphibians

The following literature result is available for the frog, *Xenopus laevis*, tested on tadpoles.

Table V3.10b: Summary of new literature tadpole acute toxicity studies not previously reviewed by DEWHA

Species	Result	Reference	Rating
Frog (<i>X. laevis</i>) – tadpole	96 h LC50 = 850 µg/L	Overmyer et al. 2007.	4

A relatively standard toxicity test method was used for tadpoles. Organisms were exposed to four concentrations of the racemate and each enantiomer along with a control. Five tadpoles were added to each of the test solutions with three replicates each. Fresh solutions were made after 48 hours for a total of 96 hours exposure. The individual enantiomers were less toxic than the racemate

with 96 hours LC50s of 910 and 1140 µg/L for the S+ and R- enantiomer, respectively, compared with 850 µg/L for the racemate.

3.1.1.2. Formulation

The following fish acute toxicity formulation studies were reviewed previously by DEWHA.

Table V3.11: Summary of fish acute (formulation) toxicity data previously reviewed by DEWHA

Species	Result (µg ac/L)	Reference	Notes
Carp (<i>Cyprinus carpio</i>)	96 h LC50 = 800 µg/L	Suteau, 1994a	2
Carp (<i>Cyprinus carpio</i>)	96 h LC50 = 390 µg/L	Suteau, 1994b	3
Rainbow trout (<i>O. mykiss</i>)	96 h LC50 = 233 µg/L	Suteau, 1996a	3

This result for carp (Suteau 1994a) was obtained under static conditions using a 0.3% sand granule formulation. The end point is expressed as active constituent based on nominal concentrations of the granule.

Suteau (1996a) indicated sub-lethal effects to rainbow trout at low concentrations. After 96 hours of exposure, significant sub-lethal effects such as muscular contraction, lethargy, moribundity, respiratory problems, erratic swimming of darkened pigmentation were observed among the fish exposed to the concentrations of 63, 134 and 254 µg ac/L. Minor sub-lethal effects (lethargy) were also observed at the lowest concentration of 35 µg ac/L, so the NOEC is likely to be less than this value.

3.1.1.3. Metabolites

The following fish acute toxicity fipronil metabolite studies were reviewed previously by DEWHA.

Table V3.12: Summary of fish acute (metabolite) toxicity data previously reviewed by DEWHA

Metabolite	Species	Result (µg ac/L)	Reference	Notes
RPA20076 6	Rainbow trout (<i>O. mykiss</i>)	96 h LC50 >20000 µg/L	Suteau, 1992a	3
RPA10461 5		96 h LC50 >10000 µg/L	Collins, 1993a	3
MB46513		96 h LC50 = 31 µg/L	Collins, 1993b	3
MB46136		96 h LC50 = 39 µg/L	Bettencourt, 1992a	3
MB45950		96 h LC50 = 31 µg/L	Jenkins & Jenkins, 1989	3
MB46513 MB46136	Bluegill sunfish (<i>L. macrochirus</i>)	96 h LC50 = 20 µg/L	Collins, 1993c	3
		96 h LC50 = 25 µg/L	Bettencourt, 1992b	3

The following regulatory studies are considered new data and are reported in the ESFA review:

Table V3.13: Summary of fish acute (metabolite) toxicity data not previously reviewed by DEWHA

Metabolite	Species	Result (µg ac/L)	Reference	Rating
MB45950	Rainbow trout (<i>O. mykiss</i>)	96 h LC50 29.5 µg/L	Suteau, 1996b	4
RPA20076 6		96 h LC50 >17000 µg/L	Machado, 2001a	4
RPA20076 1		96 h LC50 >100000 µg/L	Wetton and Mullee, 1999a	4

Suteau (1996b): This standard fish test was conducted to OECD TG 203 and GLP. Juvenile rainbow trout were exposed to a control, solvent control (DMF) and test concentrations of MB45950 (95.4% purity) of 4, 9, 21, 45 and 100 µg ac/L in a 96-hour flow-through test with each test group being one replicate aquarium with 10 fish per concentration. Mortality or abnormalities among the fish were monitored at least daily. The exposure concentrations were analysed at all concentrations at the start and end of the test and after 24 hours in the top concentration. The LC50 was estimated using the method of Dragstedt and Lang (citation in the report, but not available here).

Mean measured concentrations were 3.5, 7.9, 16.6, 37.2 and 68.9 µg/L. Mortality was observed at the top two concentrations (70% and 100% at 37.2 and 68.9 µg/L respectively after 96 hours). Sublethal effects were found at all concentrations and included accelerated respiration, erratic swimming, lethargy and dark pigmentation. The LC50 was calculated to be 29.5 µg/L. 95% confidence limits and the NOEC could not be determined. The result confirms that of Jenkins and Jenkins (1989 – see Table V3.12) and shows this metabolite to be more toxic than the parent compound by almost an order of magnitude to this species. However, it is formed in the water column at <10% parent. Consequently, this test is not being requested.

Machado (2001a): This standard fish test was conducted to OECD TG 203 and GLP. Juvenile rainbow trout were exposed to a control, solvent control (DMF) and test concentrations of RPA200766 (99.8% purity) of 1.3, 2.5, 5.0, 10 and 20 mg ac/L in a 96-hour static renewal test with each test group being one replicate aquarium with 10 fish per concentration. Observations were made at 0, 2, 3, 6, 24, 48, 72 and 96 hours for mortalities and sublethal effects. Exposure concentrations were analytically verified in fresh solutions at test initiation and after 48 hours. Aged solutions were sampled at test termination.

Mean measured concentrations were 0.95, 1.9, 3.6, 7.9 and 17 mg/L. No mortalities were observed in any test group or the controls. No behavioural abnormalities or other sublethal effects were observed in any test group or controls except the highest test group where loss of equilibrium, loss of pigmentation and lethargy were observed. The study NOEC was therefore 7.9 mg/L and the 96-hour LC50 was >17 mg/L.

Wetton and Mullee (1999a): This limit test was conducted to OECD TG 203 and GLP. Juvenile rainbow trout were exposed to a control, and single test concentrations of RPA200761 (94.5% purity) of 100 mg ac/L in a 96-hour semi-static test with each test group being three replicates with 10 fish each (two replicates for the control). Mortality or abnormalities were monitored at least daily. Chemical analysis was performed in fresh solutions at 0 hours and in 24-hour aged solutions after 24 and 96 hours of exposure.

Measured concentrations were 102–105% nominal. No treatment-related mortality or sublethal effects were observed at the single tested concentration of 100 mg/L. The LC50 was therefore >100 mg ac/L and the study NOEC was 100 mg/L.

3.1.2. Reproductive / chronic exposure

3.1.2.1. Active constituent

The following fish chronic toxicity studies were reviewed previously by DEWHA.

Table V3.14: Summary of fish chronic toxicity data previously reviewed by DEWHA

Species	Result (µg ac/L)	Reference	Notes
Rainbow trout (<i>O. mykiss</i>)	90 d ELS NOEC = 15 µg/L	Machado, 1992	1

Larval survival at 60 days post hatch was the most sensitive indicator of toxicity in the early life stage test, where the lowest effect concentration was 26 µg/L. Embryos were viable and hatchlings survived to the completion of the hatching period (30 days of exposure) at all concentrations to 60 µg/L.

The following regulatory studies are considered new data and are reported in the ESFA review:

Table V3.15: Summary of new regulatory fish chronic toxicity studies not reviewed by DEWHA

Species	Result	Reference	Rating
Sheepshead minnow (<i>Cyprinodon variegatus</i>)	34 d NOEC = 1.6 µg/L	Sousa, 1998a	4
	35 d NOEC = 2.8 µg/L	Sousa, 1998b	4 ¹
	88 d NOEC = 6 µg/L	Dionne, 2000	4 ¹

¹ These studies provide new information for the chronic toxicity of fipronil to fish, and should be provided for review.

The result from Sousa (1998a) is rated as '4'. While in the EFSA review the study was acceptable, the dose-response was unclear, and the study was considered to be superseded by the two other results in the above table for the same test species.

Sousa (1998a): This GLP study was performed to US EPA guideline 72–4. Fipronil (97.1% pure) was exposed to the marine fish sheepshead minnow and exposure started with 6 days of exposure to fertilised eggs followed by 28 days exposure of newly-hatched larva under continuous flow-through conditions. The test concentrations were 0 (control), 0 (solvent control, acetone), 1.6, 3.1, 6.3, 13 and 25 µg/L. Replication in the egg exposure phase was 60 eggs per concentration in two replicate cups for determination of hatch success. Replication in the larvae exposure phase was successful hatched larvae in two replicate aquaria (up to 30 larvae in each replicate). Behaviour and appearance were monitored daily and survival was estimated at least twice weekly. At 28 days post hatch, the final survival rate was determined and individual larval length, dry weight and wet weight were measured. Exposure concentrations were verified by chemical analysis at exposure initiation and at least weekly thereafter. Statistical significant differences were evaluated in comparison to pooled controls with the Williams test.

Mean measured concentrations were 1.6, 2.7, 5.7, 10 and 22 µg/L and clear effects of exposure were observed in the top concentration where hatch success, larval survival and all of the growth parameters were substantially reduced compared with pooled control and compared with lower exposure levels. Between 1.6 and 10 µg/L, growth parameters were statistically significantly different from the controls, but without a clear dose-response for wet and dry weights. The overall NOEC was reported to be <1.6 µg/L. At this level, the total length was statistically similar to the pooled control, but wet weight and dry weight were reduced by ~15% and ~14% respectively.

A non-standard test is described in the literature:

Table V3.16: Summary of new literature fish chronic toxicity studies not reviewed by DEWHA

Species	Result	Reference	Rating
Zebrafish (<i>Danio rerio</i>)	72 h EC50 = 162 µg/L	Stehr et al. 2006	4

This study is reported in the chronic section of this report as exposure was to embryos. Exposure to fipronil was through the water with seven concentrations ranging from 3–5000 µg/L. All embryos exposed to a fipronil pulse from 24–32 hours post fertilisation (n = 42) and >75% exposed at 32–40 hours post fertilisation (n = 44) or 40–48 hours post fertilisation (n = 45) showed extensive notochord degeneration, shortened body length and disrupted muscle morphology when examined at 48 hours post fertilisation. When animals were transferred to clean water for 24 hours and examined again at 72 hours post fertilisation, notochord degeneration and shortened body length were still present. Shortened body length was dose dependent with an EC50 of 162 µg/L. Significant lethality was only observed at the highest concentration (~5000 µg/L). For this exposure, viability was only 7% (n = 44) at 5 days post fertilisation. Survival at all other concentrations ranged from 73–100% and were not significantly different to the control. In other results from this study, it

appeared that fipronil impaired the development of spinal locomotor pathways by inhibiting a structurally-related glycine receptor sub-type.

4. Aquatic invertebrates

4.1.1. Acute exposure

4.1.1.1. Active constituent

The following acute aquatic invertebrate toxicity studies were reviewed previously by DEWHA.

Table V3.17: Summary of acute aquatic invertebrate toxicity data previously reviewed by DEWHA

Species	Result ($\mu\text{g ac/L}$)	Reference	Notes
<i>Daphnia magna</i>	48 h EC50 = 190 $\mu\text{g/L}$	McNamara, 1990a	1
Mysid shrimp (<i>Mysidopsis bahia</i>)	96 h LC50 = 0.14 $\mu\text{g/L}$	Machado, 1994	3
Eastern oyster (<i>C. virginica</i>)	48 h EC50 = 770 $\mu\text{g/L}$	Dionne, 1993	3
Daphniidae (<i>Simocephalus elizabethae</i>)	48 h EC50 = 5.3 $\mu\text{g/L}$	Stevens, 2001	5

The test on *Daphnia magna* was performed under flow-through conditions and results reported as mean measured concentrations (note that these are expressed as $\mu\text{g/L}$ in the text of the report and ng/L in attached tables). The acute no effect level was 50 $\mu\text{g/L}$.

A test of the parent compound fipronil on mysid shrimp shows this species to be particularly susceptible, with an LC50 = 0.14 $\mu\text{g/L}$ (ppb), and NOEC = 0.062 ppb. While there were no deaths at this lower level, several organisms were observed to exhibit darkened pigmentation and two mysids were also noted to be swimming erratically.

The end point for testing on the Eastern oyster was a 50% reduction of shell deposition. This test showed the oyster to be less sensitive than fish and *Daphnia*, although fipronil can still be described as highly toxic to the species.

The following regulatory studies are considered new data and are reported in the ESFA review:

Table V3.18: Summary of new regulatory aquatic invertebrates acute toxicity studies not reviewed by DEWHA

Species	Result ($\mu\text{g/L}$)	Reference	Rating
<i>Daphnia magna</i>	96 h EC50 = 12.9 $\mu\text{g/L}$	Ward and Rabe, 1989	4

Ward and Rabe (1989): Details of this test are sparsely reported in the EFSA review. The GLP study was conducted following US EPA guideline 72–2. Nominal concentrations were 2.6, 4.3, 7.2, 12 and 20 µg/L, along with a control and solvent control, for 96 hours under flow-through conditions. Mean measured concentrations were 2.36, 3.98, 6.27, 11 and 16 µg/L, and at these concentrations, mortality after 96 hours was 0%, 0%, 5%, 5% and 100% respectively (5% in the control and 10% in the solvent control). The 96-hour EC50 was 12.9 µg/L (95% CI 11–16 µg/L) and the 96-hour NOEC was 3.98 µg/L. The study is not being requested as there are more sensitive aquatic invertebrate toxicity results available that are used in the risk assessment.

The following literature studies are considered new data:

Table V3.19: Summary of new literature aquatic invertebrates acute toxicity studies not previously reviewed by DEWHA

Species	Result (µg/L)	Reference	Rating
<i>Ceriodaphnia dubia</i>	LC50 = 20.3 (racemate) LC50 = 11.7 (+ enantiomer) LC50 = 38.9 (- enantiomer)	Konwick et al. 2005	2*
Black fly (<i>Simulium vittatum</i>) – larvae	48 h LC50 = 0.19; 0.19; 0.29 (3 tests, measured)	Overmyer et al. 2005	2*
	LC50 = 0.65 (racemate) LC50 = 0.72 (+ enantiomer) LC50 = 0.74 (- enantiomer)	Overmyer et al. 2007	2*
Shrimp (<i>Macrobrachium rosenbergii</i>)	96 h LC50 = 0.98	Shan et al. 2003	4
Shrimp (<i>Macrobrachium nipponensis</i>)	96 h LC50 = 4.32		
Crab (<i>Eriocheir sinensis</i>)	96 h LC50 = 8.56		
Red swamp crayfish (<i>P. clarkia</i>)	LC50 = 125 (racemate) LC50 = 81.7 (+ enantiomer) LC50 = 163 (- enantiomer)	Overmyer et al. 2007	2*
	96 h LC50 = 14.3	Schlenk et al. 2001	2*
White river crayfish (<i>P. zonangulus</i>)	96 h LC50 = 19.5		
Estuarine copepod (<i>Amphiascus tenuiremis</i>)	96 h LC50 = 6.8	Chandler et al. 2004a	2*
Estuarine copepod (<i>A. tenuiremis</i>) – male	96 h LC50 = 3.5		
Estuarine copepod (<i>A. tenuiremis</i>) – female	96 h LC50 = 13.0		
Grass shrimp (<i>P. pugio</i>) Adult	96 h LC50 = 0.32	Key et al. 2003	4
Grass shrimp (<i>P. pugio</i>) Larvae	96 h LC50 = 0.68		
Grass shrimp (<i>P. pugio</i>) Embryo	96 h LC50 >512		
Grass shrimp (<i>P. pugio</i>) Adult	LC50 = 0.32 (racemate) LC50 = 0.37 (+ enantiomer) LC50 = 0.32 (- enantiomer)	Overmyer et al. 2007	2*
Grass shrimp (<i>P. pugio</i>) Larvae	LC50 = 0.68 (racemate) LC50 = 0.54 (+ enantiomer) LC50 = 0.35 (- enantiomer)	Overmyer et al. 2007	2*
Clam (<i>Mercenaria mercenaria</i>)	LC50 = 177 (racemate) LC50 = 208 (+ enantiomer) LC50 = 187 (- enantiomer)	Overmyer et al. 2007	2*

Konwick et al. (2005): This study was undertaken to evaluate the differences in toxicity of the two fipronil enantiomers and the racemate to *Ceriodaphnia dubia*. The racemate results are considered here as they will be applied in the risk assessment. The test method was based on the US EPA method EPA/600/4-90/027F for measuring the acute toxicity of effluents and receiving water to freshwater and marine organisms. Briefly, 5 *C. dubia* neonates (<24 hours) were pipetted into 30 mL vessels containing 15 mL test solutions. For each test, three replicates were used (total 15 neonates per test) along with a control and solvent control (acetone). For the racemate, average measured exposure concentrations were 4.7, 9.3, 18.6, 37.2 and 74.4 µg/L. Two series of tests were conducted, namely under normal culture photoperiod (16 hours:8-hours light:dark) or under dark conditions. After 48 hours, dissolved oxygen and pH were measured and reported at 7.8–8.38 mg/L and 8.3–8.46 respectively. These were stated as within test guidelines. The mortality results are only provided graphically. However, mortality increased with increasing concentration. When comparing LC50 values exposure conditions (light and dark) did not significantly influence fipronil toxicity. Combined data were used to determine the final LC50, which was calculated to be 20.3 µg/L calculated by logistic regression. By comparison, the (+) enantiomer was around twice as toxic as the racemate and the (-) enantiomer was around half as toxic.

Overmyer et al. (2005): This study was performed to establish baseline toxicity data for fipronil using laboratory reared black fly larvae (*Simulium vittatum*). Moderately hard reconstituted test water was used with the test conducted at 20°C and a 18:6-hour light:dark photoperiod. An orbital shaker toxicity test was used to determine the 48-hour LC50 values. Briefly, six concentrations (actual concentrations not reported) were prepared in 200 mL flasks by spiking test water with the stock solution. Test concentrations were measured before and after the test. Along with the six test concentrations a water control and solvent control (acetone) were maintained. Each treatment was replicated five times, and it appears 15 larvae per replicate were used. The test was replicated three times. All data were adjusted for control mortality before statistical analysis using Abbott's formula. Mortality in the controls was <4% for all tests. Data were analysed using the maximum likelihood method fitting normal (probit) and logistic models to the data. The model with the best fit across all repetitions was used for determining the LC50. From the three replications of the test, the 48-hour LC50s were calculated, based on measured concentrations, to be 0.19, 0.19 and 0.29 µg/L (probit analysis). Although not quantified, abnormal behaviour and muscle control were observed in larvae exposed to fipronil at all concentrations, and the NOEC for fipronil was therefore considered to be <0.05 µg/L.

Overmyer et al. (2007): The toxicity of fipronil and its enantiomers was assessed using several species of aquatic invertebrates, a tadpole and a plankton using similar methodology as described in Overmyer et al. (2005) above. Several of the aquatic invertebrate results are considered further in the risk assessment, and these results are reported here. Results discussed are restricted to the racemate. The following table summarises the test conditions

for the species of interest:

Species	Black fly	Crayfish	Grass shrimp	Grass shrimp	Clam
Life stage	4 th /5 th instar larvae	Adult	Adult	1–2 d old larvae	Juvenile
Exposure duration	48 h non-renewal	96 h static	96 h static renewal	96 h static renewal	96 h static renewal
Test endpoint	Mortality	Mortality	Mortality	Mortality	Mortality
Test media	Moderately hard water		20 ppt seawater	20 ppt seawater	30 ppt seawater
Photoperiod	16:8 h light:dark	16:8 h light:dark	16:8 h light:dark	16:8 h light:dark	12:12 h light:dark
Temperature	20°C	20°C	25°C	25°C	20°C
LC50 (µg/L)	0.65	124.89	0.32	0.68	177.0
95% CI (µg/L)	0.60–0.70	87.20–179.24	0.24–0.41	0.57–0.80	46.0–674.0

Black fly larvae exposed to 0.06 µg/L had mortality <2%. However, the majority of the larvae still alive were impaired such that they could not attach to the bottom of the test vessel and showed minimal movement. After 48 hours in clean water, additional mortality was observed and increased to 57.1%. This indicates a lag time in mortality and the true LC50 for this species could well be below 0.06 µg/L. Recovery at this low rate was <20%. In the crayfish experiment, no recovery was observed in impaired crayfish, however, an increase in mortality was observed. Per cent mortality in crayfish previously exposed was 60%, but the concentration in question is not reported. For adult grass shrimp, the mortality resulting from exposure at 2.0 µg/L was 43%. Mortality continued to occur in all fipronil exposures after the shrimp were moved to clean water. There was no recovery of impaired shrimp and the total mortality rates were similar after the recovery phase.

Schlenk et al. (2001): This study measured the toxicity of fipronil to two species of crayfish, namely, the red swamp crayfish (*Procambarus clarkia*) and the white river crayfish (*P. zonangulus*). The tests followed standard methods for examination of water and wastewater published by the American Public Health Association. Briefly, static exposures of 96 hours were used with exposure concentrations of 1, 25, 50, 100, 200 or 500 µg/L nominal. Six litres of exposure water was placed into a 40 L glass aquaria at which five crayfish were added to each of three replicate aquaria. Deionized reconstituted water (pH 8.1; 135 mg/L hardness CaCO₃; 90 mg/L CaCO₃ alkalinity; 25°C) was used. At the end of the exposure period the number of live organisms was assessed. Water concentrations were measured at three levels, and based on these, the estimated actual concentrations of exposure were 0.3, 7.5, 15, 30 and 150 µg/L. The 96-hour LC50 for red swamp crayfish was calculated to be 14.3 µg/L and for white river crayfish, was calculated to be 19.5 µg/L.

Chandler et al. (2004): The acute toxicity of fipronil to adult male and female copepods *A. tenuiremis* was performed in a 96-hour test. Four nominal concentrations (4.3, 7.2, 12.0 and 20.0 µg/L) along with an acetone control were

tested. Artificial seawater (30% salinity) was aerated until dissolved oxygen exceeded 90% saturation and then filtered. Control and treatment solutions were transferred to 250 mL glass beakers and homogenised for 1 hour in the dark. After mixing, 30 mL of control or treatment solution was added to a 50 mL glass dish. Each treatment employed four replicates. Ten adult males and 10 nongravid females were transferred to each dish. The chambers were incubated under static conditions at 20°C for 96 hours with a 12:12-hour light:dark photoperiod. The measured fipronil concentrations were 2.77, 5.44, 10.84 and 19.64 µg/L and water quality parameters were within guideline (ASTM standards). Fipronil had an overall 96-hour LC50 of 6.8 µg/L. However, toxicity was higher to males (96-hour LC50 = 3.5 µg/L) than females (96-hour LC50 = 13.0 µg/L).

4.1.1.2. Formulation

The following acute aquatic invertebrate (formulation) toxicity studies were reviewed previously by DEWHA.

Table V3.20: Summary of acute aquatic invertebrate (formulation) toxicity data previously reviewed by DEWHA

Species	Result (µg ac/L)	Reference	Notes
<i>Daphnia magna</i>	48 h EC50 = 19 µg/L	Suteau, 1994c	2
<i>Daphnia magna</i>	48 h EC50 = 36 µg/L	Suteau, 1994d	3
<i>Daphnia magna</i>	48 h EC50 = 178 µg/L	Suteau, 1996c	3
Daphniidae (<i>Simocephalus elizabethae</i>)	48 h EC50 = 15.7 µg/L	Stevens, 2001	5
Prawn (<i>Macrobrachium australiense</i>)	24 h EC50 = 24.6 µg/L	Stevens, 2001	5

This study was performed under static conditions using a 0.3% sand granule introduced 24 hours before the test organisms. The end point is expressed as active constituent based on nominal concentrations of the granule. The company advised with respect to this study that no analytical verification of test concentrations were carried out and suggested there may have been a dilution or weighing error. Further, it appears non-standardised equipment were used to conduct the study.

Suteau (1994d) also indicates a higher sensitivity to *Daphnia* than the TGAC study. A 5% suspension concentrate (EXP 60658) was tested under static conditions, with concentrations stated as nominal values of the test substance (5% fipronil). This test determined the EC50 to be 36 µg/L. The company explained this apparent increase in sensitivity over the TGAC study as a result of the oil used in formulation.

This argument may be supported in Suteau (1996b) where an 800WG formulation showed sensitivity to *Daphnia* approaching that of the TGAC study with an EC50 = 178 µg/L. However, within this test, a NOEC could not be

determined as lethargy was determined at all concentrations, including the lowest concentration tested of 34 µg ac/L.

From Stevens (2001), following collection from a recently flooded rice field at Yanco, New South Wales, and laboratory culturing, eight neonate (<72 hours old) *S elizabethae* were subjected to five concentrations of technical and formulated fipronil, prepared by serial dilution, in 150 mL glass crystallising dishes. These were maintained at 25°C with a 15-hour light:9-hour dark photoperiod. Ten replicates were conducted for each bioassay, with and without food. Neonates were classed as dead if they failed to respond when touched with a fine metal probe. Forty-eight hour LC50 values ranged from 5.3 µg/L (technical grade/no food) to 15.7 µg/L (Regent 300 EC, note this formulation has been assessed but is not marketed in Australia), also with no food provided. Field collected (from the main irrigation channel, Yanco) *M australiense* were acclimatised for 2 hours and then subjected to five concentrations of formulated fipronil prepared by serial dilution in 2 L glass beakers (four shrimp per container), which were maintained at 25°C with a 15-hour light:9-hour dark photoperiod for 24 hours. They were then transferred to control solution for a 48-hour recovery period, when mortality was again assessed. No food was provided and only eight replicates have been conducted to date (the test is continuing). Preliminary results show these prawns are less susceptible than the daphnids with a 24-hour LC50 of 24.6 µg/L, and an LC50 of 188.6 µg/L after a further 48-hour recovery period.

The following results from literature studies are considered new data:

Table V3.21: Summary of aquatic invertebrate acute (formulation) toxicity data from the literature not previously reviewed by DEWHA

Species	Result (µg ac/L)	Reference	Rating
<i>Daphnia pulex</i>	48 h LC50 = 15.6 µg ac/L	Stark and Vargas, 2005	4
Fairy shrimp (<i>Streptocephalus sudanicus</i>)	48 h EC50 = 9.94 µg ac/L	Lahr et al. 2001	4
Backswimmer (<i>Anisops sardeus</i>)	48 h EC50 = 9.06 µg ac/L		
Red swamp crayfish (<i>P. clarkia</i>)	96 h LC50 = 180 µg ac/L	Biever et al. 2003	4

4.1.1.3. Metabolites

The following aquatic invertebrate acute toxicity fipronil metabolite studies were reviewed previously by DEWHA.

Table V3.22: Summary of aquatic invertebrate acute (metabolite) toxicity data previously reviewed by DEWHA

Metabolite	Species	Result ($\mu\text{g ac/L}$)	Reference	Notes
RPA20076 6	<i>Daphnia magna</i>	48 h EC50 >20000 $\mu\text{g/L}$	Suteau, 1992b	3
RPA10461 5		48 h EC50 >10000 $\mu\text{g/L}$	Collins, 1993d	3
MB46136		48 h EC50 = 29 $\mu\text{g/L}$	McNamara, 1990c	3
MB45950		48 h EC50 = 14 $\mu\text{g/L}$	Jenkins, 1989	3
MB45950		48 h EC50 = 100 $\mu\text{g/L}$	McNamara, 1990d	3

The sulfide metabolite (MB45950) was tested for acute toxicity twice on *Daphnia*, once under static conditions, which was a preliminary toxicity screen test (EC50 = 14 ppb), and secondly under flow-through conditions as a definitive test (EC50 = 100 ppb). The difference in toxicity is difficult to explain, when considering that the flow-through experiment using measured concentrations gave a toxicity an order of magnitude less than the static test using nominal concentrations. Nonetheless, the definitive test is acceptable, and both results indicate that MB45950 is very highly toxic to *Daphnia*.

The following regulatory studies are considered new data and are reported in the ESFA review:

Table V3.23: Summary of aquatic invertebrate acute (metabolite) toxicity data not previously reviewed by DEWHA

Metabolite	Species	Result ($\mu\text{g ac/L}$)	Reference	Rating
MB45950	<i>Mysid shrimp (Mysidopsis bahia)</i>	96 h LC50 = 0.077 $\mu\text{g/L}$	Putt, 2000a	4 ¹
MB46136		96 h LC50 = 0.056 $\mu\text{g/L}$	Putt, 2000b	4 ¹
MB46513		96 h LC50 = 1.5 $\mu\text{g/L}$	Putt, 2000c	4 ¹
RPA20076 1	<i>Daphnia magna</i>	48 h EC50 >20000 $\mu\text{g/L}$	Machado, 2001b	4
RPA20076 6		48 h EC50 >100000 $\mu\text{g/L}$	Wetton and Mullee, 1999b	4

¹ These studies are considered significant new data and should be provided for review.

The results for MB45950 and MB46136 (Putt 2000a & 2000b) are notable. These show mysid shrimp to be very sensitive to both these compounds and results are in terms of measured concentrations (radiolabelled material used for the studies). The 96-hour NOEC in these studies are very consistent and were 0.033 and 0.031 $\mu\text{g/L}$ respectively. It is interesting to note that toxicity of these two metabolites to *Daphnia magna* were not dissimilar to the parent compound. Further, toxicity of parent fipronil to mysid shrimp was much higher than that to *Daphnia magna*. The two results for MB45950 and MB46136 suggest these metabolites are around twice as toxic to mysid shrimp than the parent compound.

Machado (2001b): Acute toxicity of RPA200766 (purity 99.8%) was tested to *Daphnia magna* (<24 hours old) under static conditions for 48 hours. Nominal test concentrations were 1.3, 2.5, 5.0, 10 and 20 mg/L along with a control and solvent control (DMF). The test concentrations were prepared after filtration of the stock solution by dilution with the dilution water. Test conditions included temperature of 20–21°C, pH 8.0–8.1 and oxygen 97–100% saturation. Four replicates with five daphnids per treatment were used and observations were made at 0, 24 and 48 hours for immobilisation and abnormal appearance. Exposure concentrations were analytically verified in fresh solutions at test initiation and in aged solutions at the end of the 48-hour exposure period.

The mean measured concentrations were 1.1, 2.4, 5.1, 9.0 and 20 mg/L. No immobilisation after 48 hours was found in either control or the two lowest test concentrations. At 5.1, 9.0 and 20 mg/L, immobilisation after 48 hours was 10%, 20% and 30% respectively. The 48 hours EC50 was >20 mg/L and the NOEC was 2.4 mg/L.

Wetton and Mullee (1999b): In this limit test, the acute toxicity of RPA 200761 (94.5% pure) was tested on *Daphnia magna* (<24 hours old). The GLP study was performed to OECD TG 202. The static, 48-hour exposure was conducted using four replicate vessels with 10 daphnids each for a single test concentration of 100 mg/L along with two replicates with 10 daphnids each for a dilution water control. Immobilisation and any adverse reactions to exposure were recorded after 24 and 48 hours. Chemical analysis of the exposure concentrations was performed at test initiation and termination.

The measured concentrations ranged from 97–103% nominal. No immobilisation or other adverse reactions to exposure were observed at the single test concentration of 100 mg/L, or in the control. The 48-hour EC50 was >100 mg/L and the NOEC was 100 mg/L.

The following literature studies are considered new data:

Table V3.24: Summary of aquatic invertebrate acute (metabolite) toxicity data from the literature not previously reviewed by DEWHA

Metabolite	Species	Result (µg ac/L)	Reference	Rating
MB46513	<i>Ceriodaphnia dubia</i>	48 h EC50 = 355 µg/L	Konwick et al. 2005	2*
MB45950	Red swamp crayfish (<i>Procambarus clarkia</i>)	96 h LC50 = 15.5 µg/L	Schlenk et al. 2001	2*
MB46513		96 h LC50 = 68.6 µg/L		
MB46136		96 h LC50 = 11.2 µg/L		

For information on the test methodology used by these authors, see Section V.3.4.1.1.1.

4.1.2. Reproductive / chronic exposure

4.1.2.1. Active constituent

The following chronic aquatic invertebrate toxicity studies were reviewed previously by DEWHA.

Table V3.25: Summary of chronic aquatic invertebrate toxicity data previously reviewed by DEWHA

Species	Result	Reference	Notes
<i>Daphnia magna</i>	21 d NOEC = 10 µg/L	McNamara, 1990b	1

The test was performed under flow-through conditions and results reported in terms of mean measured concentrations. Growth was the most sensitive indicator of toxicity in the chronic test, with a lowest effect concentration of 20 µg/L. The EC50 was 40 µg/L. No effects on reproductive performance were observed at sub-lethal concentrations (20 µg/L and below).

The following regulatory studies are considered new data and are reported in the ESFA review:

Table V3.26: Summary of new regulatory aquatic invertebrates chronic toxicity studies not reviewed by DEWHA

Species	Result (µg/L)	Reference	Rating
Mysid shrimp (<i>Mysidopsis bahia</i>)	28 d NOEC = 0.0077 µg/L	Machado, 1995	4 ¹

¹ This study is considered significant new data and should be provided for review.

Mysid shrimp was very sensitive to fipronil exposure in this study. Results were in terms of measured concentrations (based on the use of radiolabelled material). The overall NOEC was based on length of males, which were considered statistically significantly different at the next highest test concentration of 15 ng/L. Other results from this study included a decrease in survival of adults at 28 days of 38% compared with controls at 57 ng/L (statistically significantly different). Reproductive success at the test concentrations of 5, 7.7, 15, 28 and 57 ng/L compared with the pooled control was +2.8%, -8.6%, -31.4%, -54% and -92% respectively. Given this study was available prior to fipronil first being registered for cropping situations in Australia, it is unclear why this important test was never provided to the APVMA.

The following results from literature studies are considered new data:

Table V3.27: Summary of aquatic invertebrate chronic toxicity data from the literature not previously reviewed by DEWHA

Species	Result (µg ac/L)	Reference	Rating
<i>Ceriodaphnia dubia</i> (8-day study)	LOEC = 15 µg/L (racemate) LOEC = 2 µg/L (+ enantiomer) LOEC = 30 µg/L (- enantiomer) LC50 ¹ = 30.3 µg/L (racemate) LC50 ¹ = 10.3 µg/L (+ enantiomer) LC50 ¹ = 50.1 µg/L (- enantiomer)	Wilson et al. 2008	4

¹ LC50 to 48 hour-old neonates born during the course of the experiment.

4.1.2.2. Metabolites

The following chronic invertebrate fipronil metabolite toxicity studies were reviewed previously by DEWHA.

Table V3.28: Summary of chronic invertebrate (metabolite) toxicity data previously reviewed by DEWHA

Metabolite	Species	Result (µg ac/L)	Reference	Notes
MB46513	<i>Daphnia magna</i>	21 d NOEC = 41 µg/L	Putt, 1992	3
MB46136		21 d NOEC = 6.3 µg/L	McNamara, 1992	3
MB45950		21 d NOEC = 13 µg/L	McNamara, 1990e	3

The following regulatory studies are considered new data and are reported in the ESFA review:

Table V3.29: Summary of chronic invertebrate (metabolite) toxicity data not previously reviewed by DEWHA

Metabolite	Species	Result (µg ac/L)	Reference	Rating
MB45950	Mysid shrimp (<i>M. bahia</i>)	28 d NOEC = 0.0046 µg/L	Lima, 2000a	4 ¹
MB46136		28 d NOEC = 0.0051 µg/L	Lima, 2000b	4 ¹

¹ These studies are considered significant new data and should be provided for review.

These two studies, performed using radiolabelled test material with results in terms of measured concentrations, confirm the sensitivity of mysid shrimp to fipronil and the results are similar to those reported in Machado (1995) for the

parent compound (see Table V3.26). The NOEC for MB45950 was based on male dry weight while that for MB46136 was based on both male and female dry weight. Reproductive success was statistically unaffected up to and including 18 ng/L and 9.3 ng/L for MB45950 and MB46136 respectively.

4.1.3. Endocrine disruption potential / reproduction

A number of literature studies are available that DEWHA has not previously considered in assessments of fipronil. Due to the lack of recognised regulatory testing procedures, these studies are 'non-standard' from a regulatory perspective. However, they can provide important information for the risk assessment. The literature papers are rated separately below with a brief description of the findings.

Title	Phenylpyrazole Insecticide Fipronil Induces Male Infertility in the Estuarine Meiobenthic Crustacean <i>Amphiascus tenuiremis</i>
Authors	Cary et al.
Date	2004
Test guideline	None identified.
Data validity	2* - reliable with restrictions
Data relied on	Yes

The study was performed using a 96-well microplate lifecycle bioassay. More than 700 individual Stage-1 copepod *A. tenuiremis* juveniles were reared to adulthood in 200 µL of control solution (C), or 0.63 µg fipronil/L (F) seawater solution. Individual virgin male:female pairs were then cross-mated for all possible combinations within and across rearing treatments and allowed to mate for an additional 12 days in either C or F (0.63 µg/L) solutions. Fipronil caused no significant lethality to any mating combinations, but evoked 73% and 89% inhibition of reproduction when F-reared males were mated with either C or F-reared females respectively in the fipronil solution. In contrast, when C-reared males were mated with F-reared females in the fipronil solution there was no difference in reproductive success compared with the controls. When F-reared males were mated with either female group in the control solution, these mating pairs displayed a 3-day delay in time to brood sac extrusion, but ultimately did reproduce.

Title	Fipronil Effects on Estuarine Copepod (<i>A. tenuiremis</i>) Development, Fertility and Reproduction: A rapid Life-Cycle Assay in 96-Well Microplate Format
Authors	Chandler et al.
Date	2004a
Test guideline	None identified
Data validity	2* – reliable with restrictions
Data relied on	Yes

The study was performed using a 96-well microplate lifecycle bioassay. Single individuals of the estuarine copepod *A. tenuiremis* were reared to adulthood in 200 µL microwells and concurrently assessed for developmental and reproductive effects (after paired virginal matings) of aqueous fipronil concentrations of 0.16, 0.22 and 0.42 µg/L. Throughout the entire life cycle, copepod survival in all treatments was >90%. However, fipronil at 0.22 µg/L and

higher significantly delayed male and female development from stage 1 copepodite to adult by approximately 2 days. Additionally, fipronil significantly halted female egg extrusion by 71% at 0.22 µg/L and nearly eliminated reproduction (94% failure) in the 0.42 µg/L treatment. Even at 0.16 µg/L there was a 58% reduction in gravid females.

Title	Population Consequences of Fipronil and Degradates to Copepods at Field Concentrations: An Integration of Life Cycle Testing with Leslie Matrix Population Modelling
Authors	Chandler et al.
Date	2004b
Test guideline	None identified
Data validity	2* – reliable with restrictions
Data relied on	Yes

Larvae of the estuarine copepod *Amphiascus tenuiremis* (<24 hours) were reared individually in 96-well microplate exposure to fipronil (0.25 and 0.5 µg/L), desethionyl fipronil (assumed to be MB46513, 0.25 and 0.5 µg/L) and fipronil sulphide (assumed to be MB45950, 0.075 and 0.15 µg/L). Survival, development rates, sex ratio change, fertility, fecundity and hatching success were tracked daily for 32 days through mating and production of three broods in spiked seawater. These data were then inserted in a Leslie matrix stage-based population growth model to predict relative rates of population increase and changes in net population growth with time and toxicant concentration. Strong reproductive (52–88%) and net production (40–80%) depressions for fipronil and MB46513 at both concentrations and MB45950 at 0.15 µg/L were found compared with controls. Spiked sediment exposures of 65–300 µg/kg of fipronil yielded significantly reduced production rates per female that were 50–67% of control production.

Title	Effects of Fipronil and Chlorpyrifos on Endocrine-Related Endpoints in Female Grass Shrimp (<i>Palaemonetes pugio</i>)
Authors	Volz et al.
Date	2003
Test guideline	None identified
Data validity	4
Data relied on	No – information only

The potential sub-lethal effects of fipronil on endocrine-mediated process in female grass shrimp (*P. pugio*) was evaluated. The occurrence of gravid females, body weight and length, cholesterol, ecdysteroids and vitellogenin in gravid females were determined. Adult shrimp (both male and female) were exposed to 0, 0.1 and 0.2 µg/L nominal fipronil concentrations for 45 days. Survival was assessed daily. Females were immediately removed without replacement once identified as holding a newly extruded clutch. Adult survival was significantly decreased (19.6%) at 0.2 µg/L. However, no biologically significant effects on egg production and related reproductive parameters were observed.

Title	An Enzyme-Linked Immunosorbent Assay for Lipovitellin Quantification in Copepods: A Screening Tool for Endocrine Toxicity
Authors	Volz and Chandler
Date	2004
Test guideline	None identified
Data validity	4
Data relied on	No – information only

The authors developed a fluorescence-based vitellin enzyme-linked immunosorbent assay (ELISA) to quantify microquantities of vitellin in the estuarine copepod *Amphiascus tenuiremis*. To validate this system, stage-I juveniles were reared individually in isolation to sexual maturity in 96-well microplate volumes (200 µL) in either a control solution, or one containing 0.6 µg/L fipronil. Control and treatment solutions were 90% renewed every 3 days. After developing copepods reached adult stage, they were assayed for vitellin. The results showed that fipronil exposed virgin adult females (but not males) exhibited significantly higher levels of vitellin relative to control males and females.

5. Benthic invertebrates

5.1.1. Acute exposure

5.1.1.1. Active constituent

No regulatory studies for effects on sediment dwelling organisms have previously been supplied to the APVMA.

The EFSA review does not report any sediment dwelling toxicity data for parent fipronil, although some metabolite data are summarised below.

The following literature studies are considered new data:

Table V3.30: Summary of (metabolite) toxicity data to sediment dwelling organisms not previously reviewed by DEWHA

Species	Result (µg ac/kg)	Reference	Rating
Midge (<i>Chironomus tentans</i>)	10 d LC50 = 0.90 µg/kg 10 d EC50 = 0.69 µg/kg	Maul et al. 2008	2*

Maul et al. (2008): The instantaneous growth rate (IGR), body mass, body condition index (BCI), immobilisation and survival of the chironomid *C. tentans* was evaluated. Sediment (0.69% OC) was prepared in bulk and stock solutions were added dropwise to the slurry, then stirred for 1 hour. For each concentration, the bulk sediment received acetone carrier. Sediment was aged in the dark for 14 days after which ~50 g dw was distributed to experimental chambers. Standard 10-day bioassays were used. Early to mid-4th instar larvae were used. Experimental units consisted of an 800 mL jar with the 50 g sediment dw and around 700 mL overlying reconstituted moderately hard water. Five replicate experiment units were used at each of the six concentrations,

negative control and solvent control. Ten organisms were distributed to each experimental unit. Test units were incubated at 23°C with a 16:8-hour light:dark photoperiod. Each unit had 75% of overlying water renewed. Chironomids were retrieved after 10 days of exposure. For surviving animals, the sublethal responses of BCI, IGR and immobilisation were evaluated. The test concentrations were 0.03, 0.04, 0.07, 0.11, 0.15 and 0.18 mg/kg OC. The 10-day LC50 was calculated to be 0.13 mg/kg OC (95% CI 0.12–0.14 mg/kg OC). The 10-day EC50 (immobilisation) was 0.1 mg/kg OC while the IC50 (IGR) was 0.12 mg/kg OC.

The results were provided in terms of µg/g OC. The level of organic carbon was stated as 0.69%. The results in Table V3.30 were therefore converted to µg/kg sediment through the following equation:

$$\text{LC/EC50 } (\mu\text{g/g OC}) \times 1000 (\mu\text{g/kg}) \times 0.69\% = \text{Result } (\mu\text{g/kg sediment})$$

5.1.1.2. Metabolites

The following regulatory studies are considered new data and are reported in the ESFA review:

Table V3.31: Summary of (metabolite) toxicity data to sediment dwelling organisms not previously reviewed by DEWHA

Metabolite	Species	Result (µg ac/kg)	Reference	Rating
MB45950	Midge (<i>Chironomus tentans</i>)	10 d LC50 = 140 µg/kg 10 d EC50 = 46 µg/kg	Putt, 2000d	4 ¹
MB46136		10 d LC50 = 43 µg/kg 10 d EC50 = 47 µg/kg	Putt, 2000e	4 ¹
MB46513		10 d LC50 = 1300 µg/kg 10 d EC50 = 640 µg/kg	Putt, 2001	4 ¹

¹ This study is considered significant new data and should be provided for review.

Putt (2000d): ¹⁴C-MB45950 was tested for toxicity to midge (*Chironomus tentans*) during a 10-day sediment exposure study following the draft OPPTS guideline 850.1735 (GLP). Midge larvae, 8–9 days old (3rd instar) at test initiation were tested in 300 mL vessels with around 2 cm depth (100 mL) sediment and 175 mL overlying well water (hardness 44–56 mg/L as CaCO₃; temperature 23–24°C and pH 6.6–6.8). The sediment was natural pond sediment sieved (1 mm) with 2.9% OC and composed of 97% sand, 2% silt and 1% clay with a pH of 4.2. The test substance was spiked into the sediment and aged for 30 days. The test concentrations were 0 (control), 0 (solvent control, acetone), 13, 25, 50, 100 and 200 µg/kg sediment. Eight vessels with 10 larvae each per test group were used along with an additional four vessels per test group for radiochemical analysis of sediment, pore water and overlying water. Observations of mortality and abnormal behaviour of the larvae were made daily. At test termination survival and dry weight of survivors were determined. LC50 and EC50 values were calculated by regression analysis based on comparison with the solvent control.

Mean measured concentrations in the sediment were 15, 29, 54, 100 and 200 µg/kg with corresponding mean measured pore water concentrations of 0.12, 0.30, 0.70, 1.55 and 3.55 µg/L. Survival and dry weight were statistically different from the control in the top three test concentrations with survival at the highest test concentration being 5% after 10 days (compared with 90% in the solvent control). The 10-day LC50 was calculated at 140 µg/kg (95% CI 130–>140 µg/kg) while the 10-day EC50, based on dry weight, was 46 µg/kg (95% CI 43–47 µg/kg). The study NOEC was 29 µg/kg.

The EU did not accept the results of this study, although the reason for rejection appears to be based on the use of the US EPA guideline, which doesn't fulfil the EU requirements according to directives 91/414/EEC and 96/12/EEC, and the guidance document Sanco/3286/2001 rev 4.

Putt (2000e): ¹⁴C-MB46136 was tested for toxicity to midge (*Chironomus tentans*) during a 10-day sediment exposure study following the draft OPPTS guideline 850.1735 (GLP). Midge larvae, 10 days old (3rd instar) at test initiation were tested in the same manner and system as described in Putt (2000d).

Mean measured concentrations in the sediment were 9.1, 14, 33, 69 and 140 µg/kg with corresponding mean measured pore water concentrations of 0.13, 0.33, 0.50, 0.72 and 1.41 µg/L. Survival and dry weight were statistically different from the control in the top three and top four test concentrations, respectively. At 69 and 140 µg/kg, survival was reduced to 16% and 11% respectively, compared with 95% in the solvent control. The 10-day LC50 was calculated at 43 µg/kg (95% CI 35–49 µg/kg) while the 10-day EC50, based on dry weight, was 47 µg/kg (95% CI 43–50 µg/kg). The study NOEC was 9.1 µg/kg.

The EU did not accept the results of this study, although the reason for rejection again appears to be based on the use of the US EPA guideline, which doesn't fulfil the EU requirements according to directives 91/414/EEC and 96/12/EEC, and the guidance document Sanco/3286/2001 rev 4.

Putt (2001): ¹⁴C-MB46513 was tested for toxicity to midge (*Chironomus tentans*) during a 10-day sediment exposure study following the draft OPPTS guideline 850.1735 (GLP). Midge larvae, 9 days old (3rd instar) at test initiation were tested in the same manner and system as described in Putt (2000d). In this study, the sediment consisted of 94% sand and 6% silt with no clay. Nominal test concentrations were 200, 400, 800, 1600 and 3200 µg/kg sediment along with a control and solvent control (acetone). This study included an earlier definitive exposure period where pore water concentrations were not measured, and in this phase, test concentrations were 9.3, 20, 36, 75 and 160 µg/kg.

Mean measured concentrations in the sediment (second definitive exposure test only) were 200, 380, 790, 1500 and 3200 µg/kg with corresponding mean measured pore water concentrations of 3.25, 11.5, 12.5, 44 µg/L. Survival was statistically reduced at 380 µg/kg and higher, while dry weight was statistically reduced at all treatment levels. The 10 day LC50 was calculated at 1300 µg/kg

(95% CI 710–2300 µg/kg) while the 10 day EC50, based on dry weight, was 640 µg/kg (95% CI 560–720 µg/kg). The NOEC was determined to be 160 µg/kg, which was based on the first definitive exposure test.

The EU did not accept the results of this study, although the reason for rejection too appears to be based on the use of the US EPA guideline, which doesn't fulfil the EU requirements according to directives 91/414/EEC and 96/12/EEC, and the guidance document Sanco/3286/2001 rev 4.

The following literature studies are considered new data:

Table V3.32: Summary of (metabolite) toxicity literature data to sediment dwelling organisms not previously reviewed by DEWHA

Metabolite	Species	Result (µg ac/kg)	Reference	Rating
MB45950	Midge (<i>Chironomus tentans</i>)	10 d LC50 = 1.1 µg/kg	Maul et al. 2008	2*
MB46136		10 d EC50 = 0.41 µg/kg		
		10 d LC50 = 0.83 µg/kg		
		10 d EC50 = 0.28 µg/kg		

The test method for these experiments is described above under Table V3.30. Test concentrations for MB45950 were 0.07, 0.11, 0.13, 0.23 and 0.33 mg/kg OC while those for MB46136 were 0.03, 0.06, 0.1 and 0.2 mg/kg OC.

It is unclear why these results are much more sensitive than the regulatory studies described above. The results were provided in terms of µg/g OC. The level of organic carbon was stated as 0.69%. The results in Table V3.32 were therefore converted to µg/kg sediment through the following equation:

$$\text{LC/EC50 (µg/g OC)} \times 1000 \text{ (µg/kg)} \times 0.69\% = \text{Result (µg/kg sediment)}$$

6. Algae, diatoms and aquatic plants

6.1.1. Active constituent

The following algae/aquatic plant toxicity studies were reviewed previously by DEWHA.

Table V3.33: Summary of algae/aquatic plant toxicity data previously reviewed by DEWHA

Species	Result	Reference	Notes
<i>Scenedesmus subspicatus</i>	96 h EC50 = 68 µg/L	Handley et al. 1991b	1
<i>Selenastrum capricornutum</i>	5 d NOEC = 140 µg/L	Hoberg, 1993a	1
<i>Anabaena flos-aquae</i>	5 d NOEC = 170 µg/L	Hoberg, 1993b	1
<i>Skeletonema costatum</i>	5 d NOEC = 140 µg/L	Hoberg, 1993c	1
<i>Navicula pelliculosa</i>	5 d NOEC = 120 µg/L	Hoberg, 1993d	1
Duckweed (<i>Lemna gibba</i>)	14 d NOEC = 160 µg/L	Hoberg, 1993e	1

The 96-hour algal test (*Scenedesmus subspicatus*) was conducted under static conditions on an orbital shaker, with the result expressed as nominal concentration. The no effect level, based on clumping of cells, was 40 µg/L.

Results for the remaining algal species are expressed in terms of mean measured concentrations at initiation and termination, which were generally 60–90% of nominal, declining slightly over the course of the test. Algal growth was monitored by direct cell counts, and found not to be significantly affected (a slight stimulation of growth was noted in some species) at the test concentrations chosen. Reasons for the anomalous sensitivity observed in the earlier test are unclear, but it may be noted that this test relied on absorbance at 665 nm rather than direct cell counts. A possible explanation would therefore be depression of chlorophyll production rather than reduction in algal growth.

The result for duckweed is based on initial measured concentrations as final concentrations had declined markedly in the 14 day test. No effects were observed at the concentration selected, based on measurement of biomass. However, slight chlorosis was observed, consistent with the hypothesis that exposure to fipronil reduces chlorophyll levels in aquatic plants, and frond density was reduced by 8% relative to pooled blank and solvent controls.

The following literature data are considered new and have not been reviewed previously by DEWHA.

Table V3.34: Summary of literature algae/aquatic plant toxicity data not previously reviewed by DEWHA

Species	Result	Reference	Rating
Phytoplankton (<i>Dunaliella tertiolecta</i>)	96 h EC50 = 631 µg/L	Overmyer et al. 2007	4

For information on the test methodology used by this author, see Section 3.4.1.1.1.

6.1.2. Formulation

The following algae/aquatic plant toxicity studies (formulations) were reviewed previously by DEWHA.

Table V3.35: Summary of algae/aquatic plant toxicity data (formulations) previously reviewed by DEWHA

Species	Result	Reference	Notes
<i>Scenedesmus subspicatus</i>	72 h EC50 = 169 µg/L	Suteau, 1996d	4

After 48 hours of exposure, the observed inhibition percentage was 60.3% at the concentration of 98 µg/L but no statistical correlation was shown between test concentrations and growth inhibition. After 72 hours of exposure, the calculated biomass EC50 (based on growth curve area) was 169 µg/L. However, the reproduction EC50 (based on growth rates) could not be calculated as less than 50% inhibition of growth was observed even in the highest measured test level of 169 µg/L.

6.1.3. Metabolites

The following algae/aquatic plant toxicity fipronil metabolite studies were reviewed previously by DEWHA.

Table V3.36: Summary of algae/aquatic plant (metabolite) toxicity data previously reviewed by DEWHA

Metabolite	Species	Result (µg ac/L)	Reference	Notes
MB46513	<i>Selenastrum capricornutum</i>	120 h EC50 = 65 µg/L	Hoberg, 1993f	3

The concentration of the test material in each treatment level was generally consistent between sampling intervals and averaged 86% of nominal concentrations throughout the study period. Based on the average of the initial and final measured concentrations, the treatment levels were defined as 330, 140, 72, 45, 27 and 12 ppb.

The 120-hour EC50 value, based on cell density, was calculated to be 65 ppb, indicating the metabolite is highly toxic to aquatic flora. Bloated cells and cell fragments were observed in cultures exposed to treatment levels of 45 ppb and higher, while cells exposed to the two lowest concentrations were observed to be normal.

The following regulatory studies are considered new data and are reported in the ESFA review:

Table V3.37: Summary of (metabolite) toxicity data to algae/aquatic plants not previously reviewed by DEWHA

Metabolite	Species	Result (µg ac/L)	Reference	Rating
MB45950	<i>Scenedesmus subspicatus</i>	72 h EbC50 = 450 µg/L 72 h ErC50 = 1300 µg/L NOEC = 260 µg/L	McElligott, 1999a	4
MB46136		72 h EbC50 >510 µg/L 72 h ErC50 >510 µg/L NOEC = 510 µg/L	Odin-Feurtet, 1999a	4
RPA20076 6		72 h EbC50 >7500 µg/L 72 h ErC50 >7500 µg/L NOEC = 7500 µg/L	Hoberg 2001	4
RPA20076 1		72 h EbC50 >100000 µg/L 72 h ErC50 >100000 µg/L NOEC = 56000 µg/L	Mead and Mullee, 1999	4

McElligott (1999a): MB45950 (98.8% pure) was tested on the freshwater alga *Scenedesmus subspicatus* in a GLP test following OECD 201. Initial cell density was $\sim 2 \times 10^4$ cells/mL. Test conditions included temperature around 23°C, pH range 8.0–9.1, agitation at approximately 100 rpm and continuous illumination at around 7400–7700 lux. The test was conducted under static conditions over 72 hours. Cell density was counted at 0, 24, 48 and 72 hours. Effects were evaluated based on cell density and on inhibition of the area under the growth curve and of the growth rate. Nominal test concentrations were 0 (control), 0 (solvent control, DMF), 60, 140, 310, 680 and 1500 µg/L. Precipitation was observed at the nominal concentration of 1500 µg/L. Each test item concentration and the solvent control consisted of three replicate vessels, the untreated control consisted of six replicate vessels. Chemical analysis of exposure concentrations was performed at 0 and 72 hours. The EC50 values were determined by linear regression while the NOEC was estimated empirically.

Mean measured concentrations were 50, 120, 260, 540 and 1300 µg/L. Significant effects on cell culture growth were observed at 540 µg/L and above. The 72-hour EbC50 was 450 µg/L and the 72-hour ErC50 was 1300 µg/L. The NOEC was determined to be 260 µg/L.

Odin-Feurtet (1999a): MB46136 (99.7% pure) was tested on the freshwater alga *Scenedesmus subspicatus* in a GLP test following OECD 201. Initial cell density was $\sim 2 \times 10^4$ cells/mL. Test conditions included temperature around 23°C, pH range 7.79–9.63, agitation at approximately 100 rpm and continuous illumination at around 7470–7700 lux. The test was conducted under static conditions over 72 hours. Cell density was counted at 0, 24, 48 and 72 hours. Effects were evaluated based on cell density and on inhibition of the area under the growth curve and of the growth rate. Nominal test concentrations were 0 (control), 0 (solvent control, DMF), 100, 170, 310, 560 and 1000 µg/L.

Precipitation was observed at the nominal concentration of 1000 µg/L. Each test item concentration and the solvent control consisted of three replicate vessels, the untreated control consisted of six replicate vessels. Chemical analysis of exposure concentrations was performed at 0 and 72 hours. The EC50 values and the NOEC were estimated empirically.

Mean measured concentrations were 70, 130, 190, 220 and 510 µg/L. No significant effects were observed up to the top concentration. The 72-hour EbC50 and the 72-hour ErC50 were therefore >510 µg/L. The NOEC was determined to be 510 µg/L.

Hoberg (2000): RPA200766 (99.8% pure) was tested on the freshwater alga *Scenedesmus subspicatus* in a GLP test following OECD 201. Initial cell density was 10⁴ cells/mL. Test conditions included temperature around 23°C, pH range 7.4–7.5 (initial), 9.9–10.3 (96 hours), agitation at approximately 100 rpm and continuous illumination at around 4600–5400 lux. The test was conducted under static conditions over 96 hours. Cell density was counted at 24, 48, 72 and 96 hours. Effects were evaluated based on cell density and on inhibition of the area under the growth curve and of the growth rate at 72 hours. Nominal test concentrations were 0 (control), 0 (solvent control, DMF), 0.63, 1.3, 2.5, 5.0, 10 and 20 mg/L. Each test item concentration and the controls consisted of three replicate vessels. Chemical analysis of exposure concentrations was performed at 0 and 96 hours. The EC50 values and the NOEC was estimated empirically.

Mean measured concentrations were 0.33, 0.62, 1.3, 2.3, 3.9 and 7.5 mg/L. At the two highest concentrations, small but statistically significant differences in the 0–72 hour growth rate were observed. Due to the questionable biological significance and the absence of a clear dose response, these differences were not considered relevant, and the overall NOEC was reported at 7.5 mg/L. There was no inhibition of any growth parameter above 50%, and the 72-hour ErC50 and 72-hour EbC50 were both >7.5 mg/L.

Mead and Mullee (1999): RPA200761 (94.5% pure) was tested on the freshwater alga *Scenedesmus subspicatus* in a GLP test following OECD 201. Initial cell density was ~1X10⁴ cells/mL. Test conditions included temperature around 24°C, pH range 5.7–8.4 (initial pH decreased with increasing test item concentration), agitation at approximately 100 rpm and continuous illumination at around 7000 lux. The test was conducted under static conditions over 72 hours. Cell density was counted at 0, 24, 48 and 72 hours. Effects were evaluated based on cell density and on inhibition of the area under the growth curve and of the growth rate. Nominal test concentrations were 0 (control), 10, 18, 32, 56 and 100 mg/L. Each test item concentration and the control consisted of three replicate vessels. Chemical analysis of exposure concentrations was performed at 0 and 72 hours. The EC50 values and the NOEC were estimated empirically.

Mean measured concentrations were 96–100% of nominal. No significant effects were observed up to 56 mg/L. At the highest level tested (100 mg/L), temporary growth inhibition was observed after 24 and 48 hours, however,

no significant difference existed after 72 hours. The 72-hour EbC50 and the 72-hour ErC50 were therefore >100 mg/L. The NOEC was determined to be 56 mg/L.

7. Mesocosm / microcosm studies

A microcosm study was provided and assessed as part of the 1998 assessment of fipronil (extension to cotton, sugarcane, sorghum etc). Fipronil was included in a microcosm assessment of potential molluscicides for the control of the rice snail (*Isidorella newcombi*), conducted by Yanco Agricultural Institute in New South Wales (Stevens et al. 1996). In this study, 27 compounds were screened, all at a uniform rate of 3 mg ac/L. Microcosms were treated with the appropriate amount of formulated or laboratory grade chemical, and 15 mature rice snails collected from rice fields or irrigation channels were added. Each test microcosm was run against a control microcosm containing snails from the same population. Snails were recovered after 24 hours of exposure and transferred to 1 L beakers containing 800 mL of clean irrigation water. In the cases of formulated products, mortality was assessed 24 hours after removal from the treated microcosms. Snails were considered dead if they did not move when a sharp probe was applied to the edge of the mantle. No mortality in this mollusc was apparent when exposed to fipronil at 3 mg ac/L for 24 hours.

A further literature study describing a mesocosm study is available that DEWHA has not previously considered in assessments of fipronil. This paper is rated separately below with a brief description of the findings.

Title	The Effects of the Contemporary use Insecticide (Fipronil) in an Estuarine Mesocosm
Authors	Wirth et al.
Date	2004
Test guideline	None identified
Data validity	4
Data relied on	No – information only

To examine the effects of fipronil concentrations on estuarine ecosystems, replicated mesocosms containing intact marsh plots and seawater were exposed to three treatments of fipronil (0.15, 0.355 and 5.0 µg/L) and a control. Juvenile fish (*Cyprinidon variegatus*), juvenile clams (*Mercenaria mercenuria*), oysters (*Crassostrea virginica*) and grass shrimp (*Palaemonetes pugio*) were added prior to fipronil in an effort to quantify survival, growth and the persistence of toxicity during the 28-day exposure period. Results indicated there were no fipronil-associated effects on the clams, oysters or fish. Shrimp were sensitive to the highest two concentrations (40% and 0% survival at 0.355 and 5.0 µg/L respectively). Additionally, the highest fipronil treatment was toxic to shrimp for 6 weeks post dose.

8. Terrestrial Invertebrates

8.1.1. Bees

In the original data package assessed by DEWHA (1996 assessment), fipronil was found to be very highly toxic to bees exposed via oral or contact routes, with respective 48-hour LD50s of 0.00417 µg and 0.00593 µg per bee (Cole 1991).

8.1.1.1. Acute oral / contact exposure

The following literature data are considered new and have not been reviewed previously by DEWHA.

Table V3.38: Summary of literature acute bee (LD50) toxicity data not previously reviewed by DEWHA

Species	Result	Reference	Rating
Honey bee (<i>Apis mellifera</i>)	Contact LD50 = 0.013 µg/bee	Mayer and Lunden, 1999	2*
Alkali bee (<i>Nomia melanderi</i>)	Contact LD50 = 0.004 µg/bee		
Leafcutter bee (<i>M. rotundata</i>)	Contact LD50 = 1.13 µg/bee		
Honey bee (<i>Apis mellifera</i>)	0.0001 µg/bee – 100% mortality ¹	Aliouane et al. 2009	2*
Honey bee (<i>A. mellifera ligustica</i>)	0.000075 µg/bee – 40.6% mortality 0.00015 µg/bee – 87.3% mortality 0.0003 µg/bee – 91.1% mortality NOEC = 0.000075 µg/bee ²	Decourtye et al. 2005	2*

¹ A 100% mortality from both topical and oral exposure at around 7 and 10 days following exposure respectively.

² NOEC based on learning behaviour, not mortality.

Mayer and Lunden (1999): Fipronil was dissolved in acetone to obtain a series of six concentrations. Thirty adult female bees of three species, *Apis mellifera*, *Megachile rotundata* and *Nomia melanderi* were treated topically with 2 µL of each solution, applied to the mesoscutum. For each test a control group was treated with 2 µL of acetone only. After treatment, bees were kept in cages for 24-hour mortality counts at 26–29°C and 50% relative humidity. The LD50 values were calculated by probit and logit analysis. The LD50s and associated 95% confidence intervals were 0.013 (0.02–0.008); 0.004 (0.007–0.001) and 1.13 (1.937–0.658) µg/bee for *Apis mellifera*, *Megachile rotundata* and *Nomia melanderi* respectively.

Aliouane et al. (2009): These results demonstrate the highly toxic nature of fipronil to bees, and suggest the more standard studies (48 hours) will not adequately capture this toxicity. Bees were exposed at 0.1 and 0.01 ng/bee

(0.0001 and 0.00001 µg/bee) through both topical and oral routes with an 11-day exposure period. After 48 hours, mortality was around 25% (values read from a graph) following oral exposure, and 0% following topical exposure. However, mortality increased significantly compared with controls from days 3 and 5 for oral and topical exposures, respectively at 0.1 ng/bee, and all animals were dead after 7 and 10 days for oral and topical exposures respectively. Mortality in the 0.01 ng/bee exposure groups were not statistically different to controls at any time. This suggests the LD50 to honey bees lies somewhere between 0.1 and 0.01 ng/bee for this study.

Decourtye et al. (2005): These authors aimed to compare the effects of sublethal exposure on the olfactory learning performances of worker bees using a proboscis extension response (PER) assay. Fipronil was one of the tested pesticides, and was tested at three concentrations. The highest concentration corresponded to the 48-hour oral LD50, received per bee per day, divided by 20. The three tested rates for fipronil were 0.075, 0.15 and 0.3 ng/bee/day (equating to 2.2, 4.5 and 9.0 µg/L in the sucrose solution used for exposure). Stock solutions with a given concentration were prepared in acetone and aliquots added to a 500 g/L sucrose solution. Experiments were carried out with worker bees of *Apis mellifera ligustica* L. Emerging worker bees were caged in groups of 60 individuals. They were provided with sugar food and water during the first 2 days and then with pollen for the next 8 days. After 2 days, bees were continuously fed with sucrose solution contaminated or not during 11 consecutive days. The feeders were changed daily. Bees were kept in an incubator (~33°C, 40% relative humidity) until 14–15 days old, and were used in the PER assay. During the treatment period, the volumes of syrup consumed was in the order of 24–45 µL/bee/day, and was not statistically different to control bee consumption. There was significant mortality found in the fipronil test, with 40.6%, 87.3% and 91.1% mortality at 0.075, 0.15 and 0.3 ng/bee/day respectively. Mortality in the control group was 6.6%. This high mortality, particularly in the two top treatments, makes interpretation of the PER assay outcomes difficult. However, of the surviving bees in the 0.075 and 0.15 ng/bee/day treatment groups, reflex responses in the lowest group were similar to those in the control bees. The NOEC therefore was established to be 0.075 ng/bee/day.

8.1.1.2. Other laboratory studies

The following literature studies are considered new data. These papers are rated separately below with a brief description of the findings.

Title	Subchronic Exposure of Honeybees to Sub-lethal Doses of Pesticides: Effects on Behaviour
Authors	Aliouane et al.
Date	2009
Test guideline	None identified
Data validity	2*
Data relied on	Yes

Mortality results from this study are described in Section V3.8.1.1.1 above. Due to complete mortality at 0.1 ng/bee, only behavioural findings at the treatment of 0.01 ng/bee are considered. Following the end of the 11-day exposure period, bees were individually tested for locomotor activity, water consumption, sucrose responsiveness and learning abilities. Bees exposed orally at 0.01 ng/bee demonstrated no effect in terms of locomotor activity, water consumption or learning performance. However, a decrease in sucrose responsiveness was found. Bees exposed topically spent more time in an immobile state and demonstrated a significant increase in the volume of water consumed. There was no effect on learning performance or sucrose responsiveness for bees exposed topically. Bees exposed through both routes failed to discriminate between a known and an unknown odorant 2 days after exposure ceased.

Title	Field and Laboratory Tests of the Effects of Fipronil on Adult Female Bees of <i>Apis mellifera</i> , <i>Megachile rotundata</i> and <i>Nomia melanderi</i>
Authors	Mayer and Lunden
Date	1999
Test guideline	None identified
Data validity	4
Data relied on	No

In what is essentially a dose / response study, two different formulations of fipronil were applied to alfalfa plants at rates of 0.014 kg ac/ha to 0.11 kg ac/ha (80 WG formulation) and 0.014 kg ac/ha to 0.22 kg ac/ha (20% SC formulation). Foliage samples were collected at either 2 hours or 8 hours post application and placed in cages. Adult bees were exposed to the foliage for 24 hours after which time mortality was assessed. Each treatment was replicated four times using four groups of 30 worker honey bees, 20 female leafcutter bees or 20 female alkali bees. Bee mortality increased as fipronil rates increased, but mortality generally reduced when caged with foliage collected 8 hours after spraying compared with 2 hours after spraying. Honey bees and leafcutter bees were of similar sensitivity. At 0.22 kg ac/ha (20% SC formulation), on foliage collected 2 hours after application, mortality after 24 hours for both these species was 76% and 84% respectively. When exposed to the foliage collected after 8 hours following application, 24-hour mortality for these two species was 46% and 48% respectively. No field rate LD50 was calculated.

8.1.1.3. Specific field / semi-field studies

The following regulatory studies are considered new data and are reported in the ESFA review:

Table V3.39: Summary of field and semi-field bee toxicity data not previously reviewed by DEWHA

Test type	Reference	Rating
Tunnel tests	Maurin, 1999a	4 ¹
Field study (impact assessment on bees)	Maurin, 1999b	4 ¹
Tunnel tests	Giffard, 2001	4 ¹
Tunnel tests	Maurin, 2001	4 ¹

¹ Given the concern expressed in the literature about the toxicity of fipronil to bees in the field, these studies are considered significant new data and should be provided for review.

The first two tests were based around exposure following treatment of sunflower seeds with a commercial REGENT formulation. Maurin (1999a) evaluated effects on bees during sunflower bloom, following growth of sunflowers using the treated seed in pots for the tunnel test, while Maurin (1999b) considered impacts during sunflower bloom in an experimental field plot of 4 ha sown with 73000 seeds/ha. In the tunnel test, observations were made for foraging activity (three times daily), mortality and abnormal behaviour. In the field study, observations included mortality, brood development and colony strength. Honey production was estimated.

In the tunnel test there were no adverse effects on mortality, behaviour and colony development observed in bees foraging on sunflowers grown with fipronil-treated seeds, while in the field test, there were no effects on colony mortality, behaviour or honey production.

During these studies, samples were taken from leaf stalks, dead bees, pollen, flowers, honey and bumble bee nectar (from control nests beside hives in Maurin, 1999b). This is described in Maurin (1999c). These samples were assayed for fipronil and three metabolites, being MB45950, MB46136 and MB46513 (Goller 1999). The limit of quantification is given as 2 µg/kg, and no traces of fipronil or the metabolites were found in any substrate from the studies with sunflower grown from fipronil-treated seeds.

Giffard (2001) evaluated effects on honey bees of a soil treatment with a fipronil formulation applied as a soil spray at 200 g/ha with soil incorporation 1 week prior to sowing seeds. Several test groups were used involving a mixture of treatments being either surface applied and sown with untreated sunflower seeds, no surface application but sown with fipronil-treated sunflower seeds, or both surface applied and sown with fipronil-treated sunflower seeds. In addition, a control group was maintained (no surface application and sown with untreated seed). Observations were made for foraging activity, mortality and any abnormal behaviour over 10 days. Colony status, brood strength and status of the storage combs was determined the day of introduction of the hives in the tunnels and 10 days later. No adverse effects were observed from any of the treatment groups in this experiment.

A similar tunnel test (Maurin 2001) also tested different treatments including one tunnel with sunflowers from fipronil-treated seeds, four tunnels with a fipronil formulation applied at 5.18 kg ac/ha in furrows and untreated sunflower seeds, and a control treatment (one tunnel). Hives were introduced to tunnels shortly before ~40% of flowerets were considered attractive to bees. Observations

were made for foraging activity, mortality and abnormal behaviour over 11 days. Colony status, brood strength and status of storage combs were assessed at the day of introduction of the hives and on the day of removal. Again, no adverse effects were found in any treatment for any of the end points observed.

The following data available from the literature are considered new data:

Title	A Method To Quantify and Analyze the Foraging Activity of Honey Bees: Relevance to the Sub-lethal Effects Induced by Systemic Insecticides
Authors	Colin et al.
Date	2004
Test guideline	None identified
Data validity	4
Data relied on	No – for information only

The test was undertaken to quantify the foraging activity of small colonies of honey bees confined in insect-proof tunnels. The basis of the study was not the colony itself, but the change in each colony on a specific day and between days. Attendance at feeding sources were established by observing eight control colonies at different times of the season during 5 days. Secondly, feeding activity was assessed on three different colonies (with a further colony serving as a control) by determining the ratio of 'active' vs 'inactive' feeders. Fipronil was present in sucrose at 2 µg/kg. Fipronil induced a drastic decrease in the number of foragers (attendance) coupled with an increase in active bees at the feeder. In addition, the authors noticed the presence of bees showing evident clinical signs of intoxication.

Title	Destroying managed and feral honey bee (<i>Apis mellifera</i>) colonies to eradicate honey bee pests
Authors	Taylor et al.
Date	2007
Test guideline	None identified
Data validity	2*
Data relied on	Yes

Combined analyses of attractiveness, toxicity and lethal time trials identified fipronil (as Ascend 200 SC formulation) as effective for depopulating feral honey bee colonies in New Zealand using poisoned baits. Nucleus colonies were placed between bait stations set out in a 4 km² grid. The stations were baited with sugar syrup containing fipronil (0.05 mL/L). In the autumn trial, all 20 colonies died within 13 days of poisoning. After 6 weeks, the effect of poisoned hives on the survival of newly introduced colonies was assessed. Five colonies were placed next to 10 poisoned hives in the original eradication area, and five colonies were placed with 10 poisoned hives at least 4 km from the original area. An additional 10 colonies were placed at least 4 km from the original area and 4 km apart. All five colonies brought into the eradication area died within the first week. Of the five colonies positioned next to poisoned hives that were 4 km from the eradication area, ~50% of bees in one colony died while ~25% in the other four died. The 10 healthy colonies that were individually positioned at least 4 km from the eradication area showed no signs of reduced bee strength. No honey remained in the poisoned nucleus hives after 3 weeks as they had all been 'robbed out'.

In a separate component to this study, toxicity of fipronil (again as Ascend 200 SC) was tested to bees at concentrations ranging from 0.00–0.015% (20 bees per treatment, fed through a gravity feeder containing 40% sucrose solution and treatment concentration). Fipronil was toxic to all bees at all concentrations. While there was around a 3-hour delay time from feeding to death commencing, by 20 hours after treatment, 100% of bees at all concentrations were dead.

A final component of this study considered the length of time contaminated honey remained toxic. Ascend 200 SC was mixed at 0.05 mL/L (presumably equating to 0.01 mg/L fipronil) with one-third stored at 20°C, one-third at 5°C and one-third frozen. Adult honey bees were exposed to this contaminated honey at regular intervals up to 26 months (n = 50 per exposure time). All caged bees that were fed this contaminated honey died within 24 hours regardless of the temperature of storage. This compared with at least 98% survival in control bees and shows that contaminated honey can remain toxic to bees for at least 26 months.

8.1.2. Earthworms

In the original data package assessed by DEWHA (1996 assessment), fipronil was found to be practically non-toxic to earthworms (*Eisenia foetida*), with no adverse effects apparent at 1000 ppm fipronil in a 14-day artificial soil test (Handley & Wetton 1991).

The following regulatory studies are considered new data and are reported in the ESFA review:

Table V3.40: Summary of earthworm (*Eisenia foetida*) toxicity data not previously reviewed by DEWHA

Material	Test type	Result	Reference	Rating
Fipronil	Acute toxicity	14 d LC50 >1000 mg/kg soil 14 d NOEC = 1000 mg/kg soil	Hamon and Lacy, 1989	4
MB45950	Acute toxicity	14 d LC50 >1000 mg/kg soil 14 d NOEC = 556 mg/kg soil	McElligott, 1999b	4
MB46136	Acute toxicity	14 d LC50 >1000 mg/kg soil 14 d NOEC = 1000 mg/kg soil	Odin-Feurtet, 1999b	4
RPA200766	Acute toxicity	14 d LC50 >1000 mg/kg soil 14 d NOEC = 1000 mg/kg soil	McElligott, 1999c	4
EXP61829A ¹	Acute toxicity	14 d LC50 >1000 mg/kg soil 14 d NOEC = 560 mg/kg soil	Ebeling and Nguyen, 2001	4
Fipronil	Reproduction	56 d NOEC >1000 mg/kg soil	McElligott, 1999d	4
MB46136	Reproduction	56 d NOEC >1000 mg/kg soil	Lührs, 2001	4

¹ Insecticidal granular bait formulation containing fipronil at 5.87 g/kg.

Hamon and Lacy (1998): The EFSA review does not summarise this study in detail as it was considered less relevant than studies conducted under GLP with the parent compound. In this study, 100% survival of adult earthworms exposed over 14 days at 1000 mg ac/kg artificial soil is reported. The test was conducted following OECD 207 but not to GLP.

McElligott (1999b): This acute toxicity to earthworms (artificial soil method) was undertaken according to OECD TG 207 and GLP. MB45950 (98.8% pure) was incorporated in artificial soil at 0, 95, 171, 309, 556 and 1000 mg/kg soil dw. The test was undertaken at around 22°C, continuous lighting (400–800 lux), initial soil pH 5.7–5.9, soil moisture around 37–39% dw. Test organisms were clitellated earthworms (*Eisenia foetida*) at least 2 months old with an initial weight range of 300–600 mg/worm. Each treatment was replicated four times with 10 worms each. Observations for mortality and abnormal behaviour or appearance were made after 7 and 14 days of exposure. Worms were weighed in groups of 10 at the start and end of the test. Worm weight was compared with the control using the Kruskal-Wallis test.

No mortality occurred in either the control or any of the test concentrations apart from a single worm that died at 556 mg/kg (not treatment-related). The acute LC50 was >1000 mg/kg. No effect on the mean body weight was recorded among the exposed worms at any concentration and no sublethal effects were observed with the exception of partial paralysis noted after 14 days at 1000 mg/kg. The 14 day NOEC was determined to be 556 mg/kg dw.

Odin-Feurtet (1999b): This acute toxicity to earthworms (artificial soil method) was undertaken according to OECD TG 207 and GLP. MB46136 (99.7% pure) was incorporated in artificial soil at 0, 95, 171, 309, 556 and 1000 mg/kg soil dw. The test was undertaken at around 22°C, continuous lighting (400–800 lux), initial soil pH 5.7–5.9, soil moisture around 35–39% dw. Test organisms were clitellated earthworms (*Eisenia foetida*) at least 2 months old with an initial weight range of 300–600 mg/worm. Each treatment was replicated four times with 10 worms each. Observations for mortality and abnormal behaviour or appearance were made after 7 and 14 days of exposure. Worms were weighed in groups of 10 at the start and end of the test. Worm weight was compared with the control using the Dunnett test.

No mortality occurred in either the control or any of the test concentrations. The acute LC50 was >1000 mg/kg. Worms exposed at concentrations of 309 mg/kg and above lost significantly less weight than worms exposed to lower levels and control. This was not considered to indicate a treatment-related adverse effect. No behavioural abnormalities were observed in the worms exposed to any concentration. The NOEC was 1000 mg/kg dw.

McElligott (1999c): This acute toxicity to earthworms (artificial soil method) was undertaken according to OECD TG 207 and GLP. RPA200766 (97.7% pure) was incorporated in artificial soil at 0, 63, 125, 250, 500 and 1000 mg/kg soil dw. The test was undertaken at around 22°C, continuous lighting (400–800 lux), initial soil pH 5.9–6.1, soil moisture around 36–38% dw. Test organisms were

clitellated earthworms (*Eisenia foetida*) at least 2 months old with an initial weight range of 300–600 mg/worm. Each treatment was replicated four times with 10 worms each. Observations for mortality and abnormal behaviour or appearance were made after 7 and 14 days of exposure. Worms were weighed in groups of 10 at the start and end of the test.

No mortality occurred in either the control or any of the test concentrations. The acute LC50 was >1000 mg/kg. No effect on the mean body weight was recorded among the exposed worms at any concentration and no sublethal effects were observed. The 14-day NOEC was determined to be 1000 mg/kg dw.

Ebeling and Nguyen (2001): This acute toxicity test to earthworms was undertaken to OECD TG 207 and GLP. The test material was EXP61829A, a granular bait formulation with fipronil at 5.87 g/kg. The test substance was incorporated into artificial soil (prepared as per the guideline) at nominal concentrations of 100, 180, 320, 560 and 1000 mg product/kg soil dw. Four replicate vessels each with 10 worms were used for each test concentration and the control. Adult clitellated *Eisenia fetida andrei*, initial weight per 10 worms of 4.4–5.0 g, were exposed for 14 days. Mortality and abnormal behaviour were investigated after 7 and 14 days. The worm wet weight was determined per vessel at the start and end of the study and statistical significance of body weight changes assessed using Duncan's test.

No mortality was observed among the worms in any of the test groups. No effects on the body weight change nor abnormal behaviour were observed during the test with the exception of the worms exposed to the highest test rate, that were considered after 14 days to be hyperactive compared with controls. The LC50 was >1000 mg product/kg and the NOEC of 560 mg product/kg.

McElligott (1999d): This chronic (reproduction and growth) test with earthworms in artificial soil was undertaken following ISO 11268 part II (draft) to GLP. Fipronil (96% pure) was incorporated in artificial soil at 0, 63, 125, 250, 500 and 1000 mg/kg soil dw. A toxic standard (carbendazim at 3 mg/kg) was run in parallel. The artificial soil was prepared according to OECD TG 207 and received an additional 10 g of dried horse manure per 500 g soil. Test conditions included temperature around 20°C, continuous lighting (400–800 lux), initial soil pH 5.6–5.7, soil moisture around 60% dry weight. The test organisms were adult earthworms, *Eisenia foetida andrei*, at least 2 months of age, mean weight range 250–600 mg at test initiation. Each treatment and the control was replicated four times with 10 worms each. Survival and body weight gain of the adult worms were determined after 28 days of exposure. The number of offspring produced was determined after 56 days and compared with the control (ANOVA for test substance, t-test for toxic reference).

No adverse effects on survival, growth or reproduction were observed even at the highest concentration tested. Food consumption was comparable between the different test concentrations and the controls. The NOEC was 1000 mg ac/kg soil.

Lührs (2001): This chronic (reproduction and growth) test with earthworms in artificial soil was undertaken following ISO 11268 part II (draft) to GLP. MB46136 (99.7% pure) was incorporated in artificial soil at 0, 62.5, 125, 250, 500 and 1000 mg/kg soil dw. A toxic standard (carbendazim at 3 mg/kg) was run in parallel. The artificial soil was prepared according to OECD TG 207 and received an additional 10 g of dried cattle manure per 500 g soil. Test conditions included temperature around 20°C, continuous lighting (400–800 lux), initial soil pH 5.3, soil moisture around 27–31% dry weight. The test organisms were adult earthworms, *Eisenia foetida andrei*, around 9–10 months of age, mean weight range 300–491 mg at test initiation. Each treatment and the control was replicated four times with 10 worms each. Survival and body weight gain of the adult worms were determined after 28 days of exposure. Body weight gain and reproductive performance were compared with the control with the help of the Dunnett test.

No adverse effects on survival, growth or reproduction were observed even at the highest concentration tested. Food consumption was comparable between the different test concentrations and the controls. The NOEC was 1000 mg ac/kg soil.

8.1.3. Beneficial terrestrial invertebrates

No test data for toxicity of fipronil to beneficial terrestrial invertebrates were reviewed by DEWHA in the original fipronil assessment (1996 assessment), although an unreferenced laboratory bioassay result of an LD50 of fipronil for banana weevil borers was reported as 1.9 µg per beetle when topically applied.

The following regulatory studies are considered new data and are reported in the ESFA review:

Table V3.41: Summary of non-target terrestrial arthropod toxicity data not previously reviewed by DEWHA (80% WG formulation)

Species / test system	Findings	Reference	Rating
Data obtained with EXP60720A (80% WG formulation)			
Parasitic wasp <i>Aphidius rhopalosiphii</i> Laboratory Extended laboratory	LR50 = 10 mg ac/ha LR50 = 106 mg ac/ha	Moll and Bützler, 2001a Moll and Bützler, 2001b	4 ¹ 4 ¹
Predatory mite <i>Typhlodromus pyri</i> Laboratory Extended laboratory	LR50 = 101 mg ac/ha LR50 = 224 mg ac/ha	Gossmann, 2001a Gossmann, 2001b	4 ¹ 4 ¹
Ladybird <i>Coccinella septempunctata</i> Laboratory, 25 & 100 mg ac/ha	100% mortality	Vinall and Mead-Briggs, 1997	4 ¹
Rove beetle <i>Aleochara bilineata</i> Laboratory Extended laboratory	EC50 = 27 g ac/ha; 0.078 mg ac/kg Reproduction (% decrease)	Drexler, 2001a	4 ¹
Field soil spiked at 1 mg ac/kg 160 DAT; 100 g/ha, in-furrow	97.8% 24.7%	Drexler, 2002	4 ¹
Collembola <i>Folsomia candida</i> Laboratory	LC50 2.13 mg/kg EC50 0.32 mg/kg NOEC = 0.04 mg/kg	Meister, 2002	4 ¹
Carabid beetle <i>Poecilus cupreus</i> Laboratory Extended laboratory	LC50 <0.34 mg ac/kg	Waltersdorfer, 2002a	4 ¹
Field soil spiked at 1.15 mg ac/kg 28 DAT; 100 g/ha, in-furrow 28 DAT; between furrows 119 DAT; 100 g/ha, in-furrow	77% mortality 20% mortality 0% mortality 0% mortality	Waltersdorfer, 2002b	4 ¹
Spiders <i>Pardosa</i> sp Laboratory, 25 g ac/ha Laboratory, 100 g ac/ha	54.5% mortality 70.5% mortality	Mead-Briggs, 1996	4 ¹

¹ These studies are considered significant new data and should be provided for review.

Two studies were conducted with baits incorporated in the sand in two cross lines near the surface, intended to mimic good agricultural practice. The results are summarised as follows:

Table V3.42: Summary of non-target terrestrial arthropod toxicity data not previously reviewed by DEWHA (bait)

Species / test system	Findings	Reference	Rating
Data obtained with EXP61829A (granular bait 0.5%)			
Rove beetle <i>Aleochara bilineata</i> Laboratory, 19 g ac/ha Laboratory, 38 g ac/ha	% reproduction reduction 2.8% 22.6%	Drexler, 2001b	4
Carabid beetle <i>Poecilus cupreus</i> Laboratory, 18 g ac/ha Laboratory, 35 g ac/ha	Mortality 27% Mortality 33%	Waltersdorfer, 2001	4

These studies are not being requested as granular baits are not registered in Australia.

Drexler (2001b): Effects of the granular bait EXP61829A (5.87 g fipronil/kg) on the reproduction of rove beetles *Aleochara bilineata* Gyll. was tested in the laboratory following IOBC methods and GLP. The test substance was incorporated in a diagonal line over the test unit 2–3 cm deep in quartz sand at 9.25 and 18.5 mg product/test unit filled with quartz sand (850 g). The untreated control and the toxic standard (dimethoate) were sprayed with 400 L/ha water. Adult beetles (1–3 days old) were exposed in four replicates with 10 males and 10 females each per test group over 28 days. On day 7, 14 and 21, around 750 fly pupae were added to each replicate as hosts for parasitism by the juveniles produced during the test. After 28 days the adults were removed. After 35 days all fly pupae were sieved from the sand and transferred into separate emergence containers. After 91 days the reproductive performance of the exposed beetles per replicate was assessed as the total number of juvenile beetles hatching from the parasitised fly pupae. The statistical significance of differences in the reproductive performance between treatment groups and the controls was evaluated with the Student-T-test.

The reduction in reproduction compared with the controls was 2,8% at 9.25 mg/box (19 g ac/ha) and 22.6% at 18.5 mg/box (38 g ac/ha). These were not considered significantly different to the control.

Waltersdorfer (2001): Effects of the granular bait EXP61829A (5.87 g fipronil/kg) on the ground dwelling predator *Poecilus cupreus* L. was tested in the laboratory following BBA Guideline VI, 23-2.1.8 and GLP. The test substance was incorporated into quartz sand (dry weight) close to the surface in the form of a cross diagonally over the rectangular test unit at concentrations of 8.6 and 17.2 mg product/box in 250 g quartz sand. The untreated control and the toxic standard (pyrazophos) were sprayed with 400 L/ha water. Adult *P. cupreus* around 7 weeks old at test initiation were exposed in five replicates with three males and three females each per test group over 14 days. On days 0, 2, 4, 7 and 11 after test initiation, one punctured fly pupa per beetle alive was added to each replicate as food and partly consumed pupae were removed from the vessels. Observations for mortality and sublethal effects were made 2, 4, 6

hours and 1, 2, 4, 7, 11 and 14 days after test initiation. Food consumption was recorded as number of pupae consumed (total or partly) after 2, 4, 7, 11 and 14 days and reported as average feeding activity.

No mortality or sublethal effects were observed in the control. Mortality in the exposure groups averaged 27% and 33% in the 8.6 (18 g ac/ha) and 17.2 (35 g ac/ha) mg/box groups respectively. Sublethal effects were observed in beetles prior to death. Average feeding activity was similar to control and showed no dose response.

Two laboratory studies were conducted with treated maize seeds. The results are summarised below:

Table V3.43: Summary of non-target terrestrial arthropod toxicity data not previously reviewed by DEWHA (treated maize seeds)

Species / test system	Findings	Reference	Rating
Data obtained with EXP80415A (treated maize seeds)			
Rove beetle <i>Aleochara bilineata</i> Laboratory, 396 g ac/ha	Mortality 91.6%	Goßmann, 1997	4 ¹
Extended Laboratory, 75 g ac/ha	Mortality 50% Reproduction decrease 89%	Goßmann, 1998	4 ¹
Carabid beetle <i>Poecilus cupreus</i> Laboratory, 246 g ac/ha	Mortality 10%	Klepka and Groer, 1997	4 ¹

¹ These studies are considered significant new data and should be provided for review.

The following results reported in the literature are considered new data:

Table V3.44: Summary of non-target terrestrial arthropod toxicity data (literature) not previously reviewed by DEWHA (treated maize seeds)

Species / test system	Findings	Reference	Rating
Parasitoid (<i>Cotesia marginiventris</i>)	100% mortality @ 35 g ac/ha	Tillman and Scott, 1997.	4
Springtail <i>Folsomia candida</i> (repro.)	NOEC = 250 µg/kg	San Miguel et al. 2008.	4
Lacewing (<i>Chrysoperla carnea</i>) ¹ 0 g ac/ha 0.7 g ac/ha 3.6 g ac/ha 7.2 g ac/ha 14.4 g ac/ha 21.5 g ac/ha	Mortality (%) 48 h/5 days 2.3/11.6 0.0/28.5 2.3/35.8 2.3/33.5 0.0/84.8 0.0/92.6	Medina et al. 2004	4

Species / test system	Findings	Reference	Rating
Lacewing (<i>Chrysoperla carnea</i>) Larvae, topical contact (24 h) Adult, topical contact (24 h) Ingestion (48 h)	LD50 = 0.5 µg ac/g insect LD50 = 0.05 µg ac/g insect LD50 = 4.28 mg/L	Medina et al. 2004	4
Endoparasitoid (<i>Hyposoter diymator</i>) Host @ 0.023 g/kg diet Host @ 0.03 µg/insect Pupae @ 0.03 µg/insect	Host mortality 100% at 3 days Host mortality 20% at 7 days Adult emergence 0% Adult mortality 100% at 7 days	Medina et al. 2007	4
Lady beetle (<i>Hippodamia convergens</i>)	Adult 72 h LD50 2.6 µg/g insect	Kaakeh et al. 1996	4
Flower bug (<i>Orius insidiosus</i>) Field Male 0.042 kg/ha Female 0.056 kg/ha Nymph 0.042 kg/ha	Mortality 100% Mortality 96.3% Mortality 100%	Studebaker and Kring, 2003	4

¹ Rates calculated by DEWHA based on information provided in the paper being 1 mL test substance at rates of 0–30 mg ac/L applied to glass plates 11.8 cm X 11.8 cm.

In addition to the mortality results described in Medina et al. (2004), lacewing eggs were dipped in an aqueous range of concentrations, up to 20 mg ac/L (stated to equate to an application rate of 24 g ac/ha in a tank mix of 800 L/ha) and no effects were recorded, except at the highest concentration. Pupae treated topically on the silk cocoon moulted to healthy adults without any deleterious effects on their reproduction.

8.1.4. General field studies

Several additional field studies for terrestrial non-target arthropods were provided and assessed as part of the 1998 assessment of fipronil (extension to cotton, sugarcane, sorghum etc).

Lucerne

The test conducted on lucerne (Thompson 1997) was originally intended for cotton, but the lack of suitable infestations of green mirid necessitated transferring the trial to lucerne in the Lockyer valley. Within this study, Regent 200 SC was applied at 25, 30, 35 and 50 g ac/ha, and assessments of insect populations were made at 0, 1, 4 and 8 days after application. The crop had flowered prior to spraying. Identification of captured specimens was attempted by the Queensland Department of Primary Industry. Beneficial species included flies (Diptera), small wasps (Microhymenoptera), a Trichogrammid which was clearly identified as a parasite of leaf hoppers, bees, assassin bugs

(Reduviidae), both transverse (*Coccinella transversalis*) and three-banded ladybirds (*Harmonia octomaculata*) and spiders.

Fipronil provided 100% knockdown of ladybirds at all doses, but numbers appeared to recover within 4 days of application when compared with control plots. Fipronil significantly reduced the population of assassin bugs, which are a predator of Lepidopteran larvae, and numbers had not recovered within the 8-day testing period.

All fipronil treated plots ultimately resulted in significantly greater numbers of thrips, which suggests that the chemical is controlling an unidentified predator of this pest.

There was no apparent effect on the number of bees foraging at 11 am 1 day after treatment, although the trial design was not adequate to clarify the effect of the treatments upon bees since hives were not monitored. The chemical appeared ineffective against flies, although the activities of small predatory wasps (including *Trichogramma* spp.) were disrupted. The results differ slightly with respect to spider observations from APLC testing, in that lower doses did not significantly affect these animals, giving approximately 40–50% control. However, at doses at and above 35 g ac/ha, fipronil provided significant control of spiders.

Cotton

A further study applied Regent 25UL at rates of 25, 50 and 100 g ac/ha to pre-flowering cotton to evaluate its activity on green mirids and other insect species (Thompson 1995). Counts of all insects in each plot were made at 0, 1, 3 and 8 days following treatment. All doses provided 82–89% knockdown of mirids, although by 8 days after treatment, all plots were clearly being reinfested.

The chemical exhibited varying degrees of control against other species. Members of the Order Hemiptera (sucking bugs) were the most abundant. Jassids and leafhoppers appeared not to be effected at any treatment level, and there was little effect on Lygaeidae (family of Hemiptera).

Treatment effects against some beneficial species were not entirely clear due to low population levels. Predatory Shield Bug (Pentatomidae) appeared to have been affected although this was not statistically shown. Numbers of spiders visibly declined following application, but by the eighth day after treatment, numbers had started to increase again, although remaining well below pre-treatment levels.

There was no apparent residual control of thrips, ladybirds, flea beetle or weevils. The data suggested some effect on Ichneumonidae (wasp), as well as a knockdown effect but no residual control of Braconids (Microhymenoptera).

The study did demonstrate a general resurgence in the number of beneficial insects following initial knockdown. Within 8 days following treatment, numbers of beneficials had recovered to 60–80% of post application levels.

Other cotton

The studies conducted by Litzow (1997) and Price (1995) concentrated more on the efficacy of the chemical to targets, with little information available on non-target effects.

Litzow treated different plots at 12.5, 25, 30, 35 and 50 g ac/ha, and showed that fipronil provided no control of the jassid population, while it did reduce the number of heliothis. By 9 days after treatment, re-infestation had occurred, although the residual effect appeared to be greater in fipronil than another treatment used in the experiment.

Fipronil appeared to have an adverse effect on predatory bugs (it is not stated what these insects were). An average of 0.53 predatory bugs was found per metre of row prior to spraying. At 9 DAT, populations appeared similar at all treatment levels (although none were found in the 25 g ac/ha treatment), with between 0.03 and 0.05 bugs being found per metre.

With spiders, approximately 0.46 were found per metre prior to treatment. After 9 DAT, populations remained significantly lower, with 0.23 spiders found per metre in the 12.5, 25 and 50 g ac/ha plots, 0.3 spiders per metre in the 30 g ac/ha plot, but only 0.05 spiders per metre in the 35 g ac/ha plot.

There appears to have been limited effect to predatory beetles (again, not stated what species), and no phytotoxicity symptoms were observed as a result of the treatments applied.

The following studies obtained from the literature are considered new data:

Title	Impacts on Nontarget Insects of a New Insecticide Compound used Against the Desert Locust [<i>Schistocerca gregaria</i> (Forskål 1775)]
Authors	Balança and de Visscher
Date	1997a
Test guideline	None identified
Data validity	4
Data relied on	No – information only

At the end of 1994 the effects of fipronil were tested against desert locusts during a field experiment in Mauritania under Saharan conditions. The impacts of these spray treatments on nontarget Coleoptera and Hymenoptera were also studied. Two plot sizes and treatment rates were used (28 hectares @ 13.4 g ac/ha; 8 hectares @ 4.2 g ac/ha). Population size variations of non-target insects before and after treatment were studied. For Carabidae and Tenebrionidae, regardless of the dose, fipronil caused >90% mortality in 2 days with very poor recolonisation in at least 4 weeks (13.4 g ac/ha) or 2 weeks (4.2 g ac/ha). Generally, for Hymenoptera the impacts of fipronil at 13.4 g ac/ha seemed less severe and persistent than for Coleoptera, possibly partially due to

the higher mobility of the former. However, for the three studied families, this was only true for Apoidea. Fipronil caused almost 100% mortality of Scelionidae and Sphecidae with very poor recolonisation in 4 weeks.

Title	Effects of very low doses of fipronil on grasshoppers and non-target insects following field trials for grasshopper control
Authors	Balança and de Visscher
Date	1997b
Test guideline	None identified
Data validity	4
Data relied on	No – information only

The high activity found in Balança and de Visscher (1997a) prompted assessment of even lower doses of fipronil. In this experiment, four square plots were sprayed with fipronil at 1 and 2 g ac/ha, and 0.6 and 1.2 g ac/ha (four plots, grasshopper mortality assessed only). The effects on non-target insect populations were evaluated using pitfall traps for ground-dwelling Coleoptera (Carabidae, Scarabaeidae, Tenebrionidae) while flying insects (Diptera, Hymenoptera) were sampled with Malaise traps. At 0.6 g ac/ha spraying treatment was effective against grasshopper outbreaks, with 47% mortality after 2 days and 91% after 10 days. Very low doses had an immediate impact on Coleoptera, Hymenoptera and Diptera. However, in most groups, the relative abundance of collected insects increased again in the last two survey samples, 20 and 32 days after treatment.

Title	Non-target effects of Carbaryl baited trench and fipronil barrier spray treatment on the ant <i>Anoplolepis custodiens</i> (Hymenoptera: Formicidae)
Authors	Mosupi
Date	2003
Test guideline	None identified
Data validity	4
Data relied on	No – Information only

One objective of this study was to determine the immediate and possible long-term impact of fipronil barrier sprays on selected non-target invertebrates. The fipronil spray rate was applied at 29 g ac/ha as a low volume spray around the periphery of test plots. The impact of the treatment was monitored using the pugnacious ant as a bioindicator. Sampling for active ant burrows took place 48 hours prior to treatment and on nine weekly intervals after treatment. The experiment was terminated when the numbers of ant burrows in the treated area was similar to control levels. A return to active burrow levels similar to that of the control group was observed 4–6 weeks after application.

8.1.5. Special studies

Title	Horizontal and Trophic Transfer of Diflubenzuron and Fipronil Among Grasshoppers (<i>Melanoplus sanguinipes</i>) and Between Grasshoppers and Darkling Beetles (Tenebrionidae)
Authors	Smith and Lockwood
Date	2003
Test guideline	None identified
Data validity	4
Data relied on	No – information only

This non-standard study provides important information on toxicity of dead insects when considering horizontal transfer. In laboratory assays, fipronil applied at 4, 100 and 1000 g ac/ha to barley plant leaves resulted in complete mortality to grasshopper nymphs within 4–12 hours of exposure. These contaminated nymphs were used as a food source for the first passage. Fourth instar nymphs (n = 11 or 12 per treatment level) were each exposed to a cadaver. In this first passage, death usually occurred within 12 hours after consuming >25% of the cadaver. Complete mortality of nymphs was found in the 100 and 1000 g ac/ha group, and 91% in the 4 g ac/ha. In the second passage, the dead nymphs from the first passage were again used as a food source. Complete mortality was found in the 1000 g ac/ha group (n = 12), while 50% mortality was found in the other two groups (n = 6). In the third passage, no mortality was found at 4 g ac/ha (n = 3). While 50% mortality was still found at 100 g ac/ha, the sample size was only two.

In field assays, fipronil was applied at 12.5 g ac/ha to mixed grass prairie. Grasshopper nymphs were placed within a cage over the treated grass. Dead nymphs (*A. deorum* and *A. coloradus*) were collected. Fourth instar grasshoppers were exposed to these cadavers as the first passage while any dead grasshoppers resulting from this passage were then used for subsequent passages. In the first passage (n = 20), complete mortality of grasshopper nymphs was observed, while 30% mortality was found in the second passage. No mortality was observed in the third passage.

Title	Impact of locust control on harvester termites and endemic vertebrate predators in Madagascar
Authors	Peveling et al.
Date	2003
Test guideline	None identified
Data validity	4
Data relied on	No – information only

This study focussed on the harvester termite (*Coarctotermes clepsydra*) and its vertebrate predators following locust control activities at two sites in Madagascar. Effects on the termite was studied at Ankazoabo in 1998 (rainy season barrier treatments with fipronil at 7.5 g ac/ha and triflumuron). At Malaimbandy monitoring was undertaken for effects on the termite along with those on lizards (*Chalarodon madagascariensis* and *Mabuya elegans*), lesser hedgehog tenrec (*Echinops telfairi*) and their non-termite arthropod prey following early dry season, full cover sprays with fipronil (3.2–4.0 g ac/ha) and deltamethrin.

At both sites, fipronil caused a strong reduction of termite activity, culminating in high mortality. At Ankazoabo, 10 months post spray colony, mortality was 44.9% at fipronil sites compared with unsprayed sites. Mortality within spray barriers was 90.7% for fipronil. Similar results were found at Malaimbandy 6 months post spray where fipronil caused 80.5% colony mortality compared with 3.5% in the unsprayed plots.

There was a significant decline in the relative abundance of the two lizard species in fipronil plots at Malaimbandy with effective reductions of 45.2–52.7%. *E. telfairi* was not found in fipronil plots while being frequent in unsprayed plots.

Termites proved to be important dietary components of all vertebrates studied. The abundance of lizards and the lesser hedgehog tenrec was positively correlated with the density of live termite colonies.

9. Soil macroorganisms

The following studies, assessing impacts to soil macroorganisms by considering organic matter breakdown, are considered new data and are reported in the ESFA review:

Table V3.45: Summary of soil macroorganism (organic matter breakdown) toxicity data not previously reviewed by DEWHA

Study title	Reference	Rating
Effects of EXP60720A on the Decomposition of Organic Material enclosed in Litter Bags in the Field	Meister and Schwiening, 2002	4
Impact of phytopharmaceutical products on soil microarthropods in an irrigated maize field: the use of the litter bag method	Cortet and Poinso-Balaguer, 1999	4

Both studies used the litter bag method to assess impacts. Meister and Schwiening (2002) appears to be a regulatory study (GLP) and found there was no treatment-related effects on the organic matter breakdown under field conditions. The risk to soil organisms involved in this functional endpoint was considered low, even if fipronil as the applied 80WG formulation is applied every year on the same field at rates up to 100 g ac/ha.

The second study (Cortet & Poinso-Balaguer 2000) was a literature paper submitted for the EFSA review. Treatment with fipronil at 200 g ac/ha did not adversely affect organic matter decomposition. The Rapporteur Member State for the EFSA review concluded this study was of limited validity due to the presence of fipronil in the control plot. This test was not considered valid.

10. Microorganisms

10.1.1. Soil microorganisms

In the original data package assessed by DEWHA (1996 assessment), no significant impacts on carbon mineralisation and nitrogen turnover were observed in glucose amended clay loam and sandy loam soils 28 days after application at 1 kg/ha fipronil (Alfred & Seal 1992).

The following regulatory studies, assessing impacts on soil respiration and nitrogen transformation, are considered new data and are reported in the ESFA review:

Table V3.46: Summary of soil microorganism (soil respiration and nitrogen transformation) toxicity data not previously reviewed by DEWHA

Material	Exposure (mg/kg soil)	Result	Reference	Rating
Fipronil	0.133 and 0.667	No treatment-related effects $\geq 25\%$ at day 28	Reis, 2002a	4
MB45950	0.12 and 0.60		Reis, 2002b	4
MB46136	0.027 and 0.133		Reis, 2002c	4
RPA200766	0.053 and 0.267		Reis, 2002d	4

Reis (2002a): Effects of fipronil (96.8% pure) on the activity of the soil microflora in the laboratory was tested following OECD TG 216 and 217 and GLP. Fipronil was applied with quartz sand to soil at 0.133 and 0.667 mg ac/kg dw, corresponding to initial soil concentrations after application of 100 and 500 g ac/ha respectively is distributed in the top 5 cm layer and soil density of 1.5 kg/L. Natural microflora communities in field soil were used for the test and test units were plastic boxes containing around 1000 g (respiration test) or 500 g soil (nitrogen transformation test) with sufficient headspace to allow air exchange. The glucose-induced respiration rate was determined in each sample of treated and control soils (three replicates) after 0, 7, 14 and 28 days, reading the CO₂ release from the linear part of the respiration curve. The nitrogen content was determined in each sample of lucerne-amended test item treated and control soils (four replicates) by determination of NH₄⁺, NO₃⁻ and NO₂⁻ following extraction from the soils with KCl solution after 0, 7, 14 and 28 days. The respiration rates and nitrate concentrations measured after 28 days were statistically compared with the control with the Students-T-test.

For respiration, with both test item concentrations, an initial increase of the respiration rate was recorded on day 0 but no further effect on this parameter was observed on any of the following sampling days. After 28 days, deviation from the control were -2.11 and +6.82% at 0.133 and 0.667 mg/kg respectively. These differences were not statistically significant and did not exceed the 25% trigger value in the guideline. For nitrogen transformation, no effect was observed on any of the sampling days.

Reis (2002b): Effects of MB45950 (98.8% pure) on the activity of the soil microflora in the laboratory was tested following OECD TG 216 and 217 and GLP. The test followed the same methodology described in Reis (2002a). Application assumed 20% conversion of fipronil to MB45950, so two test concentrations of 0.027 and 0.133 mg/kg soil were tested. The respiration rate recorded in both test concentrations was lower than the control, but the differences never exceeded 25%. After 28 days, the deviations were -19.7% and -20.5% at 0.027 and 0.133 mg/kg respectively. These differences were statistically significantly different but did not exceed the 25% trigger value in the guideline. No effect on the nitrogen transformation was observed at any of the sampling days.

Reis (2002c): Effects of MB46136 (99.7% pure) on the activity of the soil microflora in the laboratory was tested following OECD TG 216 and 217 and GLP. The test followed the same methodology described in Reis (2002a). Application assumed 90% conversion of fipronil to MB46136, so two test concentrations of 0.12 and 0.60 mg/kg soil were tested. In all measurements the respiration rate recorded in both test concentrations exceeded the control respiration, but at levels <25% and without dose response. After 28 days the deviations from the control were +14.0% and +9.66% at 0.12 and 0.60 mg/kg respectively. These results were not considered statistically significant. No effect on the nitrogen transformation was observed at any of the sampling days.

Reis (2002d): Effects of RPA200766 (99.8% pure) on the activity of the soil microflora in the laboratory was tested following OECD TG 216 and 217 and GLP. The test followed the same methodology described in Reis (2002a). Application assumed 40% conversion of fipronil to RPA200766, so two test concentrations of 0.053 and 0.267 mg/kg soil were tested. In all but one measurement, the respiration rate recorded in both test concentrations exceeded the control respiration but at low levels (<10%) and without dose response. No effect on the nitrogen transformation was observed at any of the sampling days.

10.1.2. Microbial toxicity studies

The following regulatory studies are considered new data and are reported in the ESFA review:

Table V3.47: Summary of activated sludge respiration inhibition data not previously reviewed by DEWHA

Material	Test type	Result	Reference	Rating
EXP60720A ¹	Activated sludge Respiration inhibition test	3 h EC50 >1000 mg ac/L NOEC = 1000 mg ac/L	Hertl, 2001	4

¹ A WG formulation containing fipronil at a nominal 800 g/kg.

Hertl (2001): Toxicity of fipronil as an 800 g/kg formulation (EXP60720A) to activated sludge in a respiration inhibition test was performed following OECD TG 209 and GLP. The test system consisted of flasks containing sewage sludge, synthetic sewage feed and the test substance at nominal concentrations of 10, 32, 100, 320 and 1000 mg ac/L. A toxic reference (3,5-dichlorophenol at 3.2, 10 and 32 mg/L) and two inoculums controls were included. The sewage sludge was obtained from a domestic waste water treatment plant. The inoculums corresponded to 3 g/L dry material of microorganisms. 50 mL/L synthetic sewage feed were added 1 day prior to use and the sludge was kept at room temperature and was aerated until use. Environmental conditions included initial pH of 7.5 and temperature of 21–22°C. After 3 hours of incubation the oxygen consumption was measured over approximately 10 minutes in the absence of oxygenation. The effects of exposure were determined by comparison of the respiration rate to the controls and toxic reference. Probit analysis was used to calculate the 3-hour EC50 values where possible.

No significant (>15% deviation) or dose-related effect of the test substance on the respiration rate of activated sludge was observed at any test concentration while the 3-hour EC50 of the toxic reference was calculated as 5.1 mg/L. The EC50 of fipronil as the tested formulation rate was empirically estimated to be >1000 mg ac/L.

11. Mammals

In the original data package assessed by DEWHA (1996 assessment), it was stated that fipronil is moderately toxic following a single oral administration in the rat (LD50 = 97 mg/kg bw) with symptoms of intoxication (abnormal gait and posture, piloerection, lethargy, tremors and convulsions) consistent with a chemical acting at a neurotransmitter. An efficacy review (Urquhart 1993) indicates that the affinity for fipronil at the vertebrate binding site is weak, as opposed to invertebrates where the binding is very tight. This weak binding presumably accounts for the ability of birds to recover from fipronil intoxication, provided that the initial intake is not too large.

12. Reptiles

The following information was available at the time DEWHA assessed fipronil for use as a termiticide (2002 assessment):

In the laboratory, wild caught lizards *Acanthodactylus dumerili* were acclimatised for at least 3 weeks in 1.5 L plastic mineral water bottles with 320 mL of sand (for ballast and a natural substrate for digging and burrowing) placed horizontally in a room with a 12:12-hour light:dark cycle, a temperature range of 25–32.6°C and 22.4–28.0% RH (Peveling & Demba 1997). House flies were captured and treated with fipronil by injection of up to 1.5 µL of Adonis 10 UL into the thorax, and fed to the lizards on a single occasion to arrive at a final

dose of 30 µg/g bodyweight. The total number of flies and its active ingredient content were individually adjusted to the actual bodyweight of each lizard. In this way 3 g lizards, which had been starved for 36 hours, received a meal of six flies. About 67% of lizards consumed all the flies within 2 minutes, though the rest became repelled over time and refused a complete meal. For these four (out of 12) lizards the final doses ranged from 10.3–25.4 µg fipronil/g bodyweight. Another 12 lizards were similarly treated with chlorpyrifos.

The effects of fipronil were measured over 4 weeks through the following end points: mortality, activity (such as digging, climbing) and feeding activity at least daily, and food consumption and body weight at weekly intervals. Gross necropsy was carried out for all surviving treated lizards. The mortality of lizards treated at the maximum rate was 50%, indicative of an LD50 of about 30 µg/g bodyweight. However, compared with chlorpyrifos, where all lizards dosed with >26 µg/g bodyweight died within 6 hours, the lethal effects of fipronil showed up only after a delay, with the first dead lizard recorded after 3 days of treatment, and the other three on days 14, 18 and 26, respectively. All underdosed lizards as well as controls survived until the end of the test.

In survivors, overall activity was depressed for 12 days, but was higher than controls thereafter. Feeding activity was significantly depressed for 21 days, and after a brief return to the level of controls remained lower until 28 days. Food consumption was significantly reduced for the full 4 weeks, with the average consumed over this period about 50% of controls. This was attributed to learned aversion, with treated lizards also leaving alternative non-treated locust food. Body weight declined to 75% of normal by 14 days, still remaining lower than normal after 28 days, and liver weight decreased in lizards whether fully dosed or not. As survivors still had not fully recovered after 28 days, fipronil could be considered to be highly or very highly toxic to *A. dumerili* on an acute oral or subacute dietary basis respectively, and the authors concluded only the latter need now be tested over a longer time period to “better reflect the real exposure to this persistent pesticide”.

In a later test with the same species of lizard a single dose of 30 µg fipronil/g body weight was administered via contaminated prey or stomach instillation (Peveling & Demba 2003). For contaminated prey, fipronil was introduced through the formulation by injecting up to 1.5 µL of Adonis (15 µg ac) into the ventral thorax of live flies. To attain a dose of 30 µg ac/g, a lizard of 3 g body weight would receive a meal of six flies that had been injected a volume of 1.5 µL Adonis each. For stomach instillation, the dose was the same as that through contaminated prey. Adonis was diluted with sesame oil to get a test concentration of 7.5 µg ac/L. Lizards were immobilized and administered with 4 µL/g body weight of the test concentrate into the stomach. The percentage of dead or moribund lizards at 4 weeks post treatment was 62.5% in animals fed contaminated prey and 42.0% in gavaged animals. In both tests, survivors showed significantly reduced feeding activity, food consumption, body weight, and organ-to-body-weight ratios (liver and/or fat body). The results also indicate an LD50 in the order of 30 µg fipronil/g bw to this species of lizard.

13. Terrestrial plants

No tests on fipronil effects to non-target terrestrial plants were reviewed by DEWHA for previous fipronil assessments.

No additional data are available in international regulatory assessments or in the literature.

14. Conclusions on environmental toxicity

Significant new environmental toxicity data have become available that were not available or provided to the APVMA at the time of fipronil assessments for Australian use. These data were reported above, and their use will result in downwards revision of ecotoxicity thresholds used in the risk assessment. The revised environmental toxicity end points that will be used in the risk assessment are provided in the following table. Highlighted rows report new, more sensitive results that will lead to changes in the risk characterisation for Australian use patterns.

For sediment organisms, there are no regulatory test data available for the parent compound to sediment organisms exposed through the sediment. Literature data was therefore relied on. The corresponding literature values for metabolites were also used for these sediment organisms.

For bird toxicity, there are some dietary metabolite toxicity data. However, these metabolites were less toxic than the parent compound (in one case more toxic but formed at <10%), so there are no avian metabolite toxicity end points proposed for use in the risk assessment.

Table V3.48: Ecotoxicity data end points for risk characterisation – based on updated information. Highlighted cell show changed end points.

Aquatic organisms (parent fipronil)				
Fish – acute	Bluegill sunfish	LC50	85	µg/L
Fish – chronic	Sheepshead minnow ¹	NOEC	2.8	µg/L
Aq. invertebrates acute	Mysid shrimp	LC50	0.14	µg/L
Aq. invertebrates chronic	Mysid shrimp ¹	NOEC	0.0077	µg/L
Algae/aquatic plants acute	Green algae	EC50	68	µg/L
Algae/aquatic plants chronic	Green algae	NOEC	40	µg/L
Sediment organisms	<i>Chironomus tentans</i> ¹	10 d LC50	0.90	µg/kg
Aquatic organisms (metabolites)				
Fish – acute	MB45950, Rainbow trout ¹	LC50	29.5	µg/L
	MB46513, Bluegill sunfish ²	LC50	20	µg/L
	MB46136, Bluegill sunfish ²	LC50	25	µg/L
Aq. invertebrates – acute	MB45950, Mysid shrimp ¹	LC50	0.077	µg/L
	MB46513, Mysid shrimp ¹	LC50	1.5	µg/L
	MB46136, Mysid shrimp ¹	LC50	0.056	µg/L
Aq. invertebrates chronic	MB45950, Mysid shrimp ¹	NOEC	0.0046	µg/L
	MB46513, <i>Daphnia magna</i> ²	NOEC	6.3	µg/L
	MB46136, Mysid shrimp ¹	NOEC	0.0051	µg/L
Sediment organisms	MB45950, <i>Chironomus tentans</i> ¹	10 d LC50	1.1	µg/kg
	MB46136, <i>Chironomus tentans</i> ¹	10 d LC50	0.83	µg/kg
Terrestrial organisms				
Birds, acute oral	Bobwhite quail	LD50	11.3	mg/kg bw
Birds, short term, dietary	Bobwhite quail	LC50	48	ppm diet
Birds, chronic, reproduction	Bobwhite quail	NOEC	10	ppm diet
Bees (oral toxicity)	New data are available showing the highly sensitive nature of bees to fipronil. A higher tier risk assess for bees is required based on these data. ¹			
Bees (contact)				
Non-target terrestrial arthropods (Exposure through spray)		LR50 ¹	0.106	g ac/ha
Non-target terrestrial arthropods (Exposure through soil) - collembola		NOEC ¹	0.04	mg/kg dw
Earthworms (based on soil concentration)		NOEC	1000	mg/kg dw
Soil microorganisms	(<25% effects at day 28)	NOEC ¹	0.667	mg/kg dw
Non-target terrestrial plants (end point based on plant height data)		ER25	-	g ac/ha

¹ New data not previously available to DEWHA.

² Available for fipronil assessments by DEWHA from 1998, therefore leading to a revision of assessments prior to this.

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