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Australian Pesticides and Veterinary Medicines Authority

Reconsideration of Methiocarb: Supplementary Environmental Assessment

The reconsideration of the approvals of the active constituent methiocarb, registration of products containing methiocarb and their associated labels

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EXECUTIVE SUMMARY

Methiocarb is a carbamate, non-systemic pesticide that has been registered for use in Australia for over 30 years. It is used to kill insects, slugs and snails by interfering with the activity of acetylcholinesterase, an enzyme in the nervous system.

The Australian Pesticides and Veterinary Medicines Authority (APVMA) published the Toxicology, Occupational Health and Safety (OHS), Environment and Residues assessment reports along with the Preliminary Review Findings (PRF) in 2005. The environmental assessment of methiocarb was updated by the Department of the Environment (DotE) in 2006 to take into account the public comments received on the original environmental assessment. Additional environmental data were received after the finalisation of the report, which required additional assessment.

Since 2006, products with WP and SC formulations of methiocarb have not been registered. From 1 July 2015, there were only two registered methiocarb pelletised (BA) products to be reviewed: Baysol Snail & Slug Bait (product # 51851, a 20 g/kg pellet home garden product) and Mesurol Snail and Slug Bait (product # 33274, a 20 g/kg pellet commercial product).

The APVMA received additional data from 12 studies on the fate of methiocarb and its metabolites and 73 studies on the toxicity of methiocarb and its metabolites on non-target species. The studies have been assessed and compared to values relied on in previous assessments. This current report updates the previous environmental risk assessment of methiocarb where the new data require a revision of the end-points previously applied.

Currently in Australia, only snail and slug pellet formulations of methiocarb are registered. Additional data showed methiocarb residues in earthworms following field use of representative formulations. A revised assessment of secondary poisoning potential to Australian birds indicated a high risk from this exposure route. However, evidence provided in additional field tests in a wide range of cropping situations treated with methiocarb pellets indicated that, in reality, birds are unlikely to be impacted either lethally or sub-lethally.

Assessment of the additional data resulted in a revised (lower) aquatic end-point, which was then used in the re-assessment of runoff. The runoff assessment was undertaken using an evidence-based approach considering soil types, slopes, rainfall and stream flow characteristics in different regions of Australia. The assessments were undertaken on both a spatial and temporal scale, which identified potentially unacceptable risk specific to certain periods (seasons) in some Queensland horticultural regions. Results indicated that, if methiocarb is not applied during those specified periods, then the risk to aquatic organisms resulting from runoff would be considered as acceptable.

Additional toxicity data for methiocarb and metabolites to soil organisms necessitated an update to the soil organism risk assessment. It was demonstrated that the risk from methiocarb metabolites was no greater than that from the parent compound and the overall risk to soil organisms was considered acceptable.

1 INTRODUCTION

Methiocarb is a carbamate pesticide of high acute toxicity that has been used in both agricultural and home garden situations. In Australia, methiocarb products are currently registered to control snails and slugs in berry crops, cereals, gardens, nurseries, oilseed crops, orchards, pastures and vegetable crops. Methiocarb products are also registered to control slaters and millipedes in home gardens and as a control measure for false wireworm beetle in sunflowers.

Methiocarb has been registered for use in Australia since the early 1980s. Its mode of action is through inhibition of the enzyme acetylcholinesterase, an enzyme in the nervous system. This inhibition results in the over-stimulation of those parts of the nervous system that use acetylcholine to transmit nerve impulses. The APVMA began its review of the active constituent methiocarb, all products containing methiocarb, and their associated labels in 1995.

From 1 July 2015, there were only two registered methiocarb products to be reviewed: Baysol Snail & Slug Bait (product # 51851, a 20 g/kg pellet home garden product) and Mesurol Snail and Slug Bait (product # 33274, a 20 g/kg pellet commercial product). Consequently, the focus is on the environmental effects of this formulation only. Use patterns for both products are summarised below:

Crop/Situation	Pest	Rate
Berry crops (including grapevines), cereals, gardens, nurseries, oilseed crops, orchards, pastures, vegetable crops	Common garden snail, slugs	5.5 kg/ha (110 g ac/ha) or
	White Italian snail, white snail (not Qld)	11-22 kg/ha (220-440 g ac/ha)
Sunflowers (Qld, SA only)	False wireworm beetle	2.5 kg/ha (50 g ac/ha) (10 pellets/m^2)
Gardens	Snails, Slugs, Slaters, Millipedes	100 pellets/m^2 (500 g ac/ha equivalence)

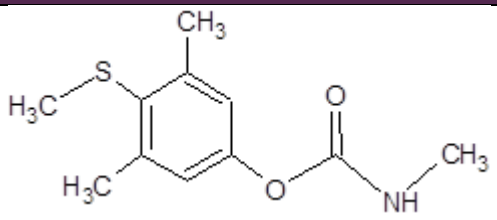
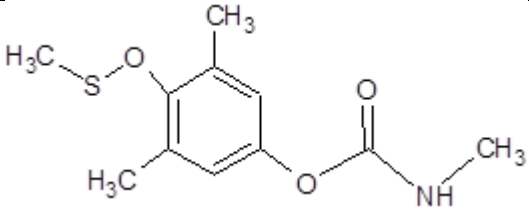
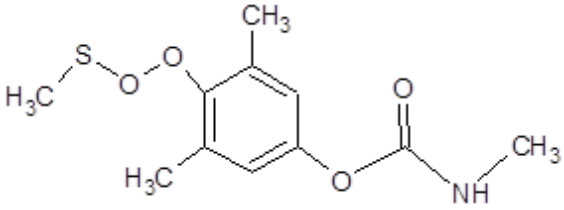
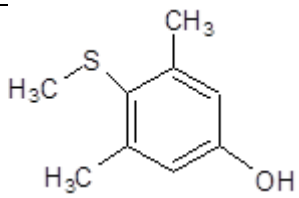
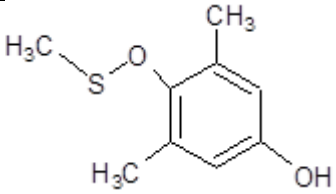
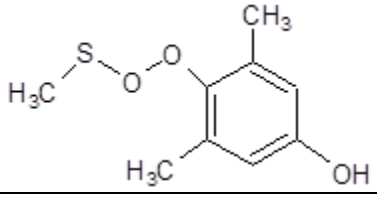
The APVMA received a submission of new fate and toxicity data from Bayer CropScience Pty Ltd, which included 12 studies on the fate of methiocarb and its metabolites and 73 studies on the toxicity of methiocarb and its metabolites on non-target species. These studies were assessed and compared to values relied on in the preliminary review findings and 2005 environmental assessment of methiocarb, including the document updated by the DotE in 2006. This included a revision of the runoff assessment for the parent compound due to a lowering of the aquatic toxicity end-point. Additionally, the new information on the parent compound and its main metabolites necessitated a consideration of the major metabolites and a revision of the soil organism risk assessment.

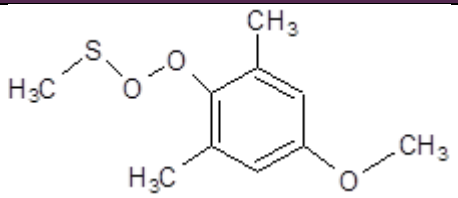
Study summaries of new data are provided in Appendix 3 (environmental fate) and 4 (environmental toxicity), and results only are summarised in this report.

2 METHIOCARB METABOLITES

The 2005 environmental assessment did not assess potential impacts of metabolite exposure. The DotE 2006 assessment appears to have considered metabolites in terms of their fate characteristics but not for toxicity or quantification of risk. A large body of information was submitted with the new data to consider potential formation fractions of different metabolites in terrestrial and aquatic environments, and considering the toxicity of these metabolites to the range of aquatic organisms. The following table shows structures and names of the major metabolites.

Table 1: Structures and names of methiocarb and major metabolites

Compound	Structure	Comments
Methiocarb		
M01 (Methiocarb sulfoxide)		Major soil (up to 60%) and water (up to 25%) metabolite.
M02		Intermediate.
M03 (Methiocarb phenol)		Major water (up to 83%) metabolite.
M04 (Methiocarb sulfoxide phenol)		Major soil (up to 36%) and water (up to 63%) metabolite.
M05 (Methiocarb sulfone phenol)		Major soil (up to 20%) metabolite.

Compound	Structure	Comments
M10 (Methiocarb methoxy sulphone)		Major soil (up to 13%) metabolite.

3 ENVIRONMENTAL FATE

The data assessment for the additional environmental fate studies is provided in Appendix 3, which can be consulted for further information and reference details. The following table summarises the additional studies and how they may impact the previous assessment.

Table 2: Additional methiocarb – parent environmental fate data considered in this assessment

Study type	New Result	Value applied previously	Comments/Recommendation	Reference
SOIL				
Soil photolysis	DT50 <1 d	DT50 = 21 d	The study provides new information on rate and route of degradation on soil surfaces. The relevance for the slug pellets is unclear but the overall indication is a faster photolysis half-life on soil than previously applied.	Stupp, H.-P. 2002
Soil aerobic degradation	4 soils, DT50 0.7–1.5 d	DT50 4-56 d	The new data did not test acidic soils, which were shown to increase persistence in the initial assessment. The soil half-life value should remain as it was in the initial assessment with no new data on more acidic soils. The new data do provide additional valuable information on the route of degradation for determination of metabolite fractions of formation.	Brumhard, B. 2002
Soil sorption	Not comparable. New data but only measured by HPLC.	Kd values of 4.3-9.0	No change required. A more detailed assessment of relationship between Kd and soil organic carbon may allow refinement in runoff modelling.	Sommer, H. 2000 & Moendel, M. 2002
Soil leaching	Column leaching study with slug pellets. Very limited leaching shown.		Directly relevant to slug pellets and may better inform the risk assessment for ground water contamination potential.	Babczinski, P. & Schramel, O. 2001
WATER				
Photolysis	31–48 days, summer	1-2 months	No change required.	Hellpointer, E. 2002
Water/sediment	Water DT50 1.3–4.3 d Whole DT50 2.1–8.7 d	None appear to be applied.	The new data can be used to inform a more refined aquatic risk assessment. The new study also provides valuable information on the route of degradation and therefore considering formation fractions of the main metabolites in aquatic systems.	Heinemann, O. 2005

Study type	New Result	Value applied previously	Comments/Recommendation	Reference
Bioconcentration	BCF: Edible49.6 Non-edible 262 Whole132	BCF: Whole 60–90	No change required.	Dorgerloh, M., Weber, E. & Eberhardt, R. 2002
AIR				
Modelling	DT50 = 9.5 h	Non used	Standard modelling study. Not relevant for current risk assessment.	Hellpointer, E. 2000

DT50 = Dissipation time 50%, Kd = Partition coefficient, BCF = Bioconcentration factor. HPLC = High-Performance Liquid Chromatography.

Table 3: Additional metabolites soil sorption data

Metabolite	Result	Comments/Recommendation	Reference
M01 (methiocarb sulfoxide)	HPLC result available. Koc = 31 L/kg	Not assessed previously. More mobile than the parent compound and will be considered in the risk assessment.	Sommer, H. 2000
M04 (Methiocarb phenol)		No test data were provided. However, there are toxicity data for this metabolite and it is a major metabolite in soil. Kd results available in the European Commisison DAR will be applied in the risk assessment.	
M05 (methiocarb sulfone phenol)	K_F 0.8–1.74 L/kg	Not assessed previously. More mobile than the parent compound and will be considered in the risk assessment.	Moendel, M. 2002
M10 (methiocarb methoxy sulphone	K_F 1.08–3.01 L/kg	Not assessed previously. More mobile than the parent compound and will be considered in the risk assessment.	Moendel, M. 2002

Koc = Adsorption coefficient, K_F = Adsorption constant, A column leaching study was also provided considering leaching of methiocarb from a slug pellet formulation. A hydrolysis study with M01 was provided with a DT50 up to 1.2 days. The study only measured hydrolysis at pH 5–7. No data were previously available for this metabolite.

4 ENVIRONMENTAL EFFECTS

The data assessments for the additional environmental toxicity studies are provided in Appendix 4, which can be consulted for further information and reference details.

4.1 Birds and mammals

Previously, the environmental assessment provided the lethal dose (LD_{50}) of 1 mg/kg, while the DotE 2006 assessment provided test values higher than this (LD_{50} of 5–15 mg/kg bw range).

The additional new data were directly relevant to the slug pellet formulations. They delivered updated information on palatability/acceptance of the pellets compared to standard food for different sized granivorous birds. In addition, there are several in-depth field studies that significantly increased the knowledge base of avian and mammalian exposure in the field. This included measurements of residues in avian food items and assessment of carcasses of birds and mammals to determine whether deaths were related to consumption of pellets.

The field data indicated that small mammals may be more susceptible to direct poisoning than birds. There was also new information relating to residue levels in earthworms that allowed a better assessment of secondary poisoning risks.

4.2 Aquatic organisms

The following tables summarises the additional studies and how they may impact the previous assessment. For Tables 4 to 11 (inclusive), methiocarb figures are for methiocarb parent only. Metabolite data are provided separately in Tables 12 to 14 (inclusive).

Table 4: Additional methiocarb toxicity data for fish

Species	Study type	New value	Value used in DotE 2006 assessment	Comments/Recommendation	Reference
Rainbow trout	Acute, 96 h	LC_{50} = 1.1 mg/L	LC_{50} = 0.44 mg/L	On its own the new data does not change the final value for rainbow trout previously used.	Peither, A. 2000
Bluegill sunfish	Acute, 96 h	LC_{50} = 0.65 mg/L	LC_{50} = 0.75 mg/L	The new data show a lower LC_{50} for this species, but the overall fish value applied in the previous risk assessment is lower still. On its own the new data does not change the final fish value applied in the previous risk assessment.	Peither, A. 2000

LC_{50} = lethal concentration 50%.

Table 5: Additional methiocarb toxicity data for aquatic invertebrates

Species	Study type	New value	Value used in DotE 2006 assessment	Comments/Recommendation	Reference
Daphnia magna	Acute, 48 h	EC_{50} = 0.0077 mg/L	EC_{50} = 0.019 mg/L	On its own, the new data require a significant change in the aquatic invertebrate end point from the previous risk assessment. This new study reports a result that was previously known, but did not have the study to assess it. Consequently, the assessment needed to apply a higher value. The *Daphnia magna* acute endpoint was the lowest aquatic value used in the risk assessment so adoption of this new value will result in increased risk if the same exposure arguments from the previous assessment are applied.	Peither, A. 2000
Daphnia magna	Chronic 21 d, water/sed	NOEC > 3 X 0.008 mg/L	NOEC = 0.0001 mg/L	This value may be used to refine the risk assessment but a full analysis of the study would be required. It indicates a more realistic exposure situation through the inclusion of sediment in the test system helps reduce toxicity. The preliminary environmental assessment does not address chronic risk.	Dorgerloh, M. 2007

EC_{50} = Effective concentration 50%. NOEC = No Observable Effect Level.

Table 6: Additional methiocarb toxicity data for algae

Species	Study type	New value	Value used in DotE 2006 assessment	Comments/Recommendation	Reference
Scenedesmus subspicatus	Chronic, 72 h	EC_{50} = 2.2 mg/L (growth rate) EC_{50} = 0.82 mg/L (biomass)	96 h EC_{50} = 1.15 mg/L	The preferred assessment end-point is growth rate, but with limited data, the biomass value can be applied for increased conservatism. This study on its own can be used to apply a lower algae endpoint than used previously, but it will not be the driver for the aquatic risk assessment.	Peither, A. 2000

Table 7: Additional methiocarb toxicity data for sediment organisms

Species	Study type	New value	Value used in DotE 2006 assessment	Comments/Recommendation	Reference
Chironomus riparius	Acute, 48 h	EC_{50} = 0.103 mg/L	None	This study on its own can be used to apply a sediment toxicity endpoint as none was previously used, but it will not be the driver for the aquatic risk assessment.	Silke, G. 2014
Chironomus riparius	Chronic, 28 d, spiked water	EC_{50} = 0.275 mg/L NOEC = 0.160 mg/L	None	This study on its own can be used to apply a sediment toxicity endpoint as none was previously used, but it will not be the driver for the aquatic risk assessment.	Bruns, E. 2006

4.3 Other terrestrial organisms

Table 8: Additional methiocarb toxicity data for bees

Species	Study type	New value	Value used in DotE 2006 assessment	Comments/Recommendation	Reference
Apis mellifera	Acute, 48 h	LD_{50} = 0.08 µg/bee (Oral)	LD_{50} = 0.47 µg/bee	The new study lowers the oral LD50 value. However, exposure to bees from slug pellets is not likely so the risk assessment will not change.	Schmitzer, S. 2008
Apis mellifera	Acute, 48 h	LD_{50} = 0.43 µg/bee (Contact)	LD_{50} = 0.43 µg/bee	The new study does not change the previous value. Exposure to bees from slug pellets is not likely.	Schmitzer, S. 2008
Apis mellifera	72 h larval toxicity	Oral LD_{50} = 0.547 µg/bee	None	This new study now provides a larval oral toxicity result previously not	Ehmke, A. & Muenz, J. 2015

Species	Study type	New value	Value used in DotE 2006 assessment	Comments/Recommendation	Reference
				available. However, exposure to bees from slug pellets is not likely so the risk assessment will not change.	

Table 9: Additional methiocarb toxicity data for non-target terrestrial arthropods

Species	Study type	New value	Value used in DotE 2006 assessment	Comments/Recommendation	Reference
Pardosa spp.	Extended laboratory	No effects up to 240 g ac/ha	None	This provides new information on slug pellets and their toxicity to spiders that was not previously available.	Hermann, P. 2001
Aleochara bilineata (beetle)	Extended laboratory	No effects up to 240 g ac/ha	None	This provides new information on slug pellets and their toxicity to ground beetles that was not previously available.	Hermann, P. 2001
Folsomia candida (Collembola)	Chronic, reproduction	EC_{10} = 1358 mg/kg dw	None	This provides new information on slug pellets and their toxicity to Collembola that was not previously available.	Friedrich, S. 2014

Table 10: Additional methiocarb toxicity data for earthworms

Species	Study type	New value	Value used in DotE 2006 assessment	Comments/Recommendation	Reference
Eisenia fetida	Acute, 14 d	LC_{50} = 1322 mg/kg dw (10% peat)	LC_{50} = 129.2 mg/kg dw (10% peat)	The new study does not change the earthworm acute toxicity value previously applied.	Meisner, P. 2000
Eisenia fetida	Chronic, 56 d	NOEC >333 mg/kg dw	NOEC not defined.	The new study assessed toxicity of slug pellets pressed into the soil surface, so is relevant to the use pattern. However, there are new field studies available also with the slug pellets that will allow a more refined assessment to earthworms.	Friedrich, S. 2014
Field populations	Long term field tests	-----	-----	Several additional long term field tests applying slug pellets in different agricultural systems are available. These can be used to refine the earthworm risk assessment, and also provide in cases	Kunze, C.L. 2003, Heimbach, F. 2000 & Schulz, L. 2011

Species	Study type	New value	Value used in DotE 2006 assessment	Comments/Recommendation	Reference
				valuable results with respect to residue levels in worms that can be considered in a secondary poisoning assessment.	

Table 11: Additional methiocarb toxicity data for soil microorganisms

Study type	New value	Value used in DotE 2006 assessment	Comments/Recommendation	Reference
Nitrogen transformation	<25% effects up to 66.67 mg/kg dw	<25% effects up to 22.5 mg/kg dw	This study allows for the previous end-point to be relaxed, but will not change the risk assessment outcomes for soil microorganisms.	Schulz, L. 2013

4.4 Additional metabolite toxicity data

The following table summarises results for the additional data provided with respect to metabolite toxicity data. A fraction of metabolite toxicity to parent toxicity is provided (e.g. metabolite LC_{50} ÷ parent LC_{50}), where a fraction <1 indicates the metabolite has lower toxicity than the parent and >1 indicates toxicity higher than the parent.

Table 12: Additional methiocarb toxicity data for soil microorganisms

Species	Metabolite	Result	Parent:Metabolite	Reference
Fish				
Rainbow trout	M01 (methiocarb sulfoxide)	Acute, LC_{50} = 6.6 mg/L	0.17	Dorgerloh, M. 2000
	M03 (methiocarb phenol)	Acute LC_{50} = 3.2 mg/L	0.34	Peither, A. 1999
	M04 (methiocarb sulfoxide phenol)	Acute, LC_{50} >106 mg/L	<0.01	Dorgerloh, M. & Sommer, H. 2001
	M05 (methiocarb sulfone phenol)	Acute, LC_{50} = 68.7 mg/L	0.016	Dorgerloh, M. & Sommer, H. 2001
	M10 (methiocarb methoxy sulphone	Acute LC_{50} = 26.8 mg/L	0.04	Dorgerloh, M. & Sommer, H. 2001
Aquatic invertebrates				
Daphnia magna	M01 (methiocarb sulfoxide)[1]	Acute EC_{50} = 0.056 mg/L	0.14	Hendel, B. & Sommer, H. 2001

Species	Metabolite	Result	Parent:Metabolite	Reference
		Chronic NOEC = 0.0065 mg/L	0.022	Matlock, D. & Lam, C.V. 2008
	M03 (methiocarb phenol)	Acute EC_{50} = 6.8 mg/L	<0.01	Peither, A. 1999
	M04 (methiocarb sulfoxide phenol)	Acute EC_{50} = 157 mg/L	<0.01	Hendel, B. 2001
	M05 (methiocarb sulfone phenol)	Acute EC_{50} = 54 mg/L	<0.01	Hendel, B. 2001
	M10 (methiocarb methoxy sulphone	Acute EC_{50} = >180 mg/L	<0.01	Hendel, B. 2000
Algae				
Scenedesmus subspicatus	M01 (methiocarb sulfoxide)	ErC_{50} = 1.31 mg/L EbC_{50} = 2.75 mg/L	0.8 0.63	Dorgerloh, M. & Sommer, H. 2001
	M03 (methiocarb phenol)	ErC_{50} = 11 mg/L EbC_{50} = 6.0 mg/L	0.20 0.14	Peither, A. 1999
	M04 (methiocarb sulfoxide phenol)	ErC_{50} = >100 mg/L EbC_{50} = >100 mg/L	<0.02 <0.01	Dorgerloh, M. & Sommer, H. 2001
	M05 (methiocarb sulfone phenol)	ErC_{50} = 120 mg/L EbC_{50} = 105 mg/L	0.02 <0.01	Dorgerloh, M. & Sommer, H. 2001
	M10 (methiocarb methoxy sulphone	ErC_{50} = 137 mg/L EbC_{50} = 97.7 mg/L	0.02 <0.01	Dorgerloh, M. & Sommer, H. 2001

[1] *Results are also available for this metabolite to Daphnia magna in a water/sediment system. The chronic ratio was based on DotE water only 21 d NOEC (0.0001 mg/L for parent substance).*

Metabolite toxicity was available for effects on soil arthropods. These results were not previously available and allowed for a risk assessment of non-target soil arthropods from breakdown and exposure to metabolites:

Table 13: Metabolite toxicity to soil organisms

Soil organism	Metabolite	Result	Metabolite:Parent	Reference
Eisenia fetida (earthworms)	M01 (methiocarb sulfoxide)	LC_{50} = 78 mg/kg dw	17	Batscher, R. 2000
		Chronic NOEC >2 mg/kg dw	Not determined	Witte, B. 2013
	M04 (methiocarb sulfoxide phenol)	LC_{50} > 1000 mg/kg dw	Toxicity ratios not determined—no toxicity exhibited by metabolites.	Batscher, R. 2001
		Chronic NOEC >100 mg/kg dw		Witte, B. 2013
	M05 (methiocarb sulfone phenol)	LC_{50} > 1000 mg/kg dw		Meisner, P. 2001
		Chronic NOEC >100 mg/kg dw		Witte, B. 2013
	M10 (methiocarb methoxy sulphone	LC_{50} = 562 mg/kg dw	2.4	Batscher, R. 2000
		Chronic NOEC >100 mg/kg dw	Not determined	Witte, B. 2013
Hypoaspis aculeifer (predatory soil mite)	M01 (methiocarb sulfoxide)	EC_{10} = 10.3 mg/kg dw NOEC= 10 mg/kg dw	No parent toxicity data available. Toxicity ratios not determined.	Witte, B. 2013
	M04 (methiocarb sulfoxide phenol)	EC50 >100 mg/kg dw NOEC >100 mg/kg dw		Witte, B. 2013
	M05 (methiocarb sulfone phenol)	EC50 >100 mg/kg dw NOEC >100 mg/kg dw		Witte, B. 2013
	M10 (methiocarb methoxy sulphone	EC_{50} >100 mg/kg dw NOEC >100 mg/kg dw		Witte, B. 2013
Folsomia candida (Collembola)	M01 (methiocarb sulfoxide)	NOEC = 50 mg/kg dw	No parent toxicity data available. Toxicity ratios not determined.	Moser, Th. 2001
	M04 (methiocarb sulfoxide phenol)	NOEC = 100 mg/kg dw		Moser, Th. 2001
	M05 (methiocarb sulfone phenol)	NOEC = 1000 mg/kg dw		Moser, Th. 2001
	M10 (methiocarb methoxy sulphone	NOEC = 10 mg/kg dw		Moser, Th. 2001

Table 14: Metabolite toxicity to soil microorganisms

Soil microorganisms	Metabolite	Results	Metabolite:Parent	Reference
Nitrogen turnover	M01 (methiocarb sulfoxide)	<25% effects up to 1.47 mg/kg dw	Toxicity ratio not determined. Tests performed to different concentrations and	Anderson, J.P.E, 2000
	M04 (methiocarb sulfoxide phenol)	<25% effects up to 1.09 mg/kg dw		Anderson, J.P.E, 2000

Soil microorganisms	Metabolite	Results	Metabolite:Parent	Reference
	M05 (methiocarb sulfone phenol)	<25% effects up to 1.20 mg/kg dw	not directly comparable due to no defined values.	Anderson, J.P.E, 2001
	M10 (methiocarb methoxy sulphone	<25% effects up to 1.33 mg/kg dw		Anderson, J.P.E, 2000

5 AMENDED RISK ASSESSMENT

5.1 Birds and mammals

The risk assessment for direct exposure to birds and mammals has not been updated since the DotE 2006 assessment. The additional field data indicated small mammals may be more susceptible to direct poisoning than birds. These additional studies were considered in the European Food Safety Authority (EFSA) (2006) report conclusions.

5.1.1 Secondary poisoning

There was also new information relating to residue levels in earthworms that allowed for better assessment of secondary poisoning risks.

General methodology exists for considering exposure through the food chain, for example, from earthworms to earthworm eating birds and mammals. That methodology involves predicting concentrations based on soil residues and considering the bioconcentration factor. There is no standardised scheme available for assessing the risk of residues of granular formulations in food items such as earthworms.

For methiocarb, however, there are measured residues in earthworms and slugs from two long term field trials that were provided to the APVMA. In these studies (Wolf and Wilkens, 2003; Wolf et al, 2003 – see Appendix 3), residues of methiocarb and its main metabolites were measured in earthworms and slugs from two fields per study. Earthworm residues were measured at regular intervals up to 28 days following the initial application with a second application occurring ~14 days after the first. A more detailed analysis of the studies with respect to earthworm sampling and residues is found in Appendix 3.

Residues were remarkably similar between fields within studies, and between the different studies with mean total methiocarb concentrations of 30.1 to 31.06 mg/kg freshweight. One study applied a 4% pellet formulation followed by a 2% pellet formulation. It was apparent that residues in earthworms were approximately half those with the 2% formulation than measured from the 4% formulation. In terms of total residues (methiocarb + M01), an earthworm residue level of 22 mg/kg fw will be applied for the secondary poisoning assessment. This value represents the mean total residues from both fields following application of 2% pellet formulation with measurements undertaken over a 2 week period after that application. The residues in earthworms are based on their consumption of pellets, and increased worms at the soil surface in the studies increased with rainfall. The residue levels are therefore a function of the concentration of methiocarb in the pellet, not the overall application rate of the product.

This level was applied as a surrogate to predict exposure concentration to native birds that consume soil arthropods within their diets.

The DotE currently applies the birds allometric equation based on the work of Nagy (1987) and applied by the United States Environmental Protection Agency (US EPA) as reported in US EPA (1993).

Nagy (1987) determined a relationship for “all birds” where the FMR (field metabolic rate, also known as Daily Energy Expenditure or DEE) was derived. This equation was:

DEE = 10.9 X BW^0.64 (kJ/day)

In order to determine the food intake rate (g dw/d), Nagy applied mean metabolisable energy contents in a broad way such that it was assume ME (metabolisable energy) was 18.0 kJ/g for insects and 14.0 kJ/d for omnivorous birds. These metabolisable energy values were applied such that:

FI (g/d dw) = DEE/ME

And the resulting food intake rate was:

FI (g/d dw) = 0.648 X bw^0.651

Where FI = food intake rate; bw = body weight (g).

While separate equations were developed for different bird groups (passerines, non-passerines, seabirds), it is the above equation that is currently applied by the DotE.

As noted by both Nagy (1987) and the US EPA (1993), a more accurate estimate of food requirements can be made from the estimated FMR, dietary composition and assimilation efficiency for the species of interest. To better assess this, assimilation efficiency, moisture content in different food items and dry weight energy of different food items have been obtained from EFSA (2009). The FMR (DEE) equation is still applied as:

DEE = 10.9 X BW^0.64 (kJ/day)

For soil invertebrates, EFSA (2009) suggests an energy level of 22.7 kJ/g dry weight, 84.3% moisture content and an assimilation efficiency (AE) of 0.76 for passerine birds.

The estimates of food intake are based on means of daily energy expenditure for free-ranging animals, energy and moisture content and assimilation efficiencies. The food intake rate (FIR) is calculated as follows (EFSA, 2009):

$$FIR = \left(\frac{DEE}{FE\ X\ \left(1 - \frac{MC}{100}\right)\ X\ \left(\frac{AE}{100}\right)} \right)$$

Where:
FIR = Food Intake Rate (g/d, fresh weight)
DEE = Daily energy expenditure of the indicator species (kJ/d);
FE = Food energy (kJ/dry g);
MC = Moisture content (%); and
AE = Assimilation efficiency (%)

While Australia has a large number of native birds that consume mixed diets (fruits, nectar, insects, seeds), the smallest birds can be primarily insectivorous (< 80 g body weights). Birds that may consume earthworms are expected to be larger than the small insectivorous species, and for the secondary poisoning assessment, birds with the 90 g and 300 g body weights will be assessed:

Diet	Body weight (g)	Example species
Insectivore (assumed 100% earthworms)	90 g	Magpie lark
	300 g	Kookaburra

It is understood that the example species are not representative for every growing region or scenario that will be considered. However, at this stage they are considered as a suitable surrogate for other birds where data have not been obtained.

Based on the above formulae and bird body weights, and applying a mean residue level of 30 mg/kg freshweight in soil arthropods, the following exposure concentrations and risk quotients for secondary poisoning were calculated. The estimated theoretical exposure is determined from the calculation:

ETE = FIR/bw X C

Where ETE = estimated theoretical exposure (mg/kg bw/d) and C = concentration of methiocarb in the fresh diet (mg/kg). The risk quotient is then determined by:

RQ = ETE/LD50

Where the LD50 = 1 mg/kg as previously determined by the DotE.

Diet	Body weight (g)	Food intake rate, g/d, freshweight	FIR/BW	ETE (mg/kg bw/d)	RQ
Insectivore (assumed 100% earthworms)	90 g	83.9	0.93	20.5	20.5
	300 g	180	0.60	13.2	13.2

These risk quotients for secondary poisoning are very high in indicator birds with a high dietary intake of earthworm/soil arthropod that forage in crops after application of snail and slug bait may be at risk.

5.1.2 Field observations

A detailed assessment of birds on oilseed rape fields was conducted when measuring earthworm residues (Wolf and Wilkens, 2003). Despite the prolonged periods (>4 weeks following the initial application) of residues in worms being at potentially lethal concentrations, no bird mortalities were caused by methiocarb poisoning. There was definite feeding activity of earthworm eating birds in the treated fields. Several species were observed ingesting potentially contaminated earthworms but none showed signs of intoxication. The authors concluded that application of Mesurol Snail and Slug Bait on winter rape fields had no impact on birds feeding on the fields and earthworms were ingested in low amounts without affecting bird individuals sub-lethally or lethally. In general, feeding activity of birds decreased after application of slug pellets on the study fields due to a generally reduced food availability on the fields.

A number of additional field studies were provided to the APVMA and reported in Appendix 4 providing results of field monitoring of birds in different cropping systems in Europe following application Mesurol RB4. The exposure of birds to Mesurol RB4 slug pellets applied on maize, sugar beet and sunflower fields was shown to be low.

Freshly treated fields were not especially attractive to birds and the abundance of foraging birds was low and no impacts of Mesurol RB4 on birds could be detected.

In artichoke fields, overall the application of Mesurol RB4 had no impact on birds, while some wood mice individuals were at risk, appearing to be lethally intoxicated.

The five most abundant bird species were black-headed gull, magpie, herring gull, wood pigeon and lesser black-backed gull in freshly planted cabbage fields, and robin, blackbird, dunnock, wood pigeon and great tit in pre-harvest potato fields. Residue analysis of recorded carcasses indicate that 'methiocarb RB4 slug pellets (Mesurol Pro)' was involved in the death of wood mice and shrews in most cases (92% and 50%, respectively). Additionally, methiocarb-contaminated insects cannot be excluded as a reason for the exceptional death of one robin.

The evidence from these field studies suggests that, despite potentially high concentrations of methiocarb residues in earthworms (and possibly other soil invertebrates), an overall exposure to birds does not appear to be high and the calculated high risk of secondary poisoning may not be reflected in reality.

5.2 Aquatic organisms

The risk assessment to aquatic organisms was updated with respect to exposure through runoff as more sensitive toxicity value and additional information on metabolite formation and toxicity were available.

In the DotE 2006 assessment, the risk from runoff was considered acceptable based on a simple model and applying a regulatory acceptable concentration (RAC) of 19 µg/L (acute *Daphnia* EC_{50}). The new data submitted to the review have resulted in an amendment of the RAC to 7.7 µg/L. In addition, information is now available on major metabolites that are more mobile than the parent compound and may exhibit high toxicity also to Daphnia magna.

Despite the European Commission Draft Assessment Report (EC DAR) and EFSA (2006) reports modelling small concentrations of methiocarb in receiving water, the runoff potential was recognised with EFSA (2006) stating that a comprehensive surface water risk assessment including runoff will be necessary at Member State level to complete the risk assessment of the pellets formulation.

For Australian agricultural uses, a comprehensive runoff assessment was undertaken with the PERAMA (Pesticide Environmental Risk Assessment Model for Australia) software[1]. A description of the methodology is provided in Appendix 1.

For the metabolites, the rate of degradation of the main soil metabolites was used from the time of peak formation based on the data in Brumhard (2002), reported in Appendix 3. The data from the time of peak formation to the end of the study were fitted with a 2-phase exponential decay model (Double First Order in Parallel, DFOP model), a 1 phase exponential decay model or a linear decay model depending on the number of data points and which approach resulted in the best fit.

[1] PERAMA beta V1.0, © Australian Environment Agency Pty Ltd, 2017

The fraction of formation that was applied to each metabolite is based on the maximum level (% applied radioactivity (AR)) found in any of the four soils tested in Brumhard (2002) and corrected for molecular weight of the metabolite.

There was no great variation between partition coefficient (Kd) results for individual substances with test results for up to six soils (parent compound, results reported in the DotE 2006 assessment) and four soils for each metabolite. The values for the four main soil metabolites are reported in Appendix 3. These included values for sand as there are only limited data available and the sand Kd is likely to give a more conservative outcome. With respect to M04, the study for adsorption/desorption testing of this metabolite was not included in the new data submitted to the review. The value was obtained from the EC DAR for use in the metabolite runoff modelling**.** The geometric mean Kd for each substance will be applied in the modelling. M01 only has an adsorption coefficient (Koc) based on HPLC = 31 L/kg. A Kd = 0.31 L/kg assuming 1% soil organic carbon will be used for this metabolite.

The most sensitive aquatic organism for the parent compound was toxicity to *Daphnia magna*. New data submitted to the review resulted in a revision of end-point applied in the previous runoff modelling of 19 µg/L to 7.7 µg/L. NOTE: the end-point is based on the mean measured concentration. The runoff assessment is based on peak concentrations so it may be more appropriate to apply the EC_{50} calculated from initial concentrations. The study design (semi static) does not allow for initial measured concentrations to be applied due to renewal of test solutions through the study. Applying a level of concern of 10 (use of acute EC_{50} data), the regulatory acceptable concentration (RAC) for the parent compound is 0.77 µg/L (770 ng/L). There are equivalent acute toxicity data for the main soil metabolites. These are reported in Appendix 4. An equivalent RAC for methiocarb and its main metabolites is derived by taking the EC_{50} and applying a level of concern of 1.0. The most toxic of the metabolites was M01 with an EC50 = 56 µg/L. This metabolite was also tested in a flow through system for 21 days and a reproduction NOEC of 6.52 µg/L was determined, which will be initially applied as the RAC for M01.

The following table summarises the input variables for each substance that will be modelled in the runoff assessment:

Table 15: Summary of values applied in runoff modelling

Substance	Kd (L/kg)	DT50 (d)	Fraction of formation	RAC (µg/L)
Methiocarb	5.63	14.3	1	0.77
M01	0.31	9.24	0.63	6.52
M04	0.43	18.0	0.293	15700
M05	1.20	65.0	0.176	5400
M10	1.86	62.0	0.126	18000

The new data submitted to the review included an aerobic soil metabolism study in four different soil types showing rapid laboratory degradation of methiocarb (DT50 0.7–1.5 days). There was a further study reported in the EU DAR for a sandy loam soil from the US which had a methiocarb DT50 value of 14.3 days (DFOP model). This was ten-fold higher than the DT50 results obtained from the German soils as reported in the EU DAR report.

For the runoff modelling, the higher value of 14.3 days will be applied. The DotE 2006 assessment specified longer half-lives in soil (4–56 days), however, a final soil half-life value was not recommended or applied in exposure modelling. In their assessment, the EU applied a methiocarb soil half-life value of 0.8 days, but when considering the pellet product, used a value of 5.7 days based on a water/sediment study undertaken with the pellet product

(that study was not provided to the APVMA). Further, the EU applied a very high Koc =10000 L/kg for the pellet product. For this assessment, it is assumed the methiocarb present in the pellet is available for runoff and the methiocarb Kd value will be used. While it may be that when incorporated into the pellet the methiocarb is less available, the pellets themselves may be more susceptible to runoff in surface flow but there is no information available on this to refine any modelling. It is therefore considered appropriate to apply the higher methiocarb half-life value (14.3 days) and the active constituent Kd value (5.63 L/kg).

5.2.1 Methiocarb runoff assessment

A tiered approach to runoff was undertaken and applied as an evidence-based assessment at the highest tier whereby Australian data for slopes, rainfall, soils and hydrology were considered within a regional context (see Appendix 1).

In some cases, insufficient data exist at tier 3, for example, appropriate hydrology and soil data for the Ord in WA, or for the Northern Territory. These situations were considered in the uncertainty analysis.

Given the continued conservatism in the modelling, if 90% of catchments are protected (predicted in-stream concentrations below the RAC) by the measured in-stream flows, then the use of the chemical is acceptable.

In considering the outcomes of the modelling below, the following should also be noted:

- As stream flow data is not available for Tasmania, a conservative surrogate was used, namely dryland Victorian streamflow data; and
- Runoff curve numbers are not available for irrigated situations (horticulture or pastures). Consequently, the modelling applied the most conservative runoff curve number for each situation where soil infiltration capacity is considered poor, as may be expected if a rainfall event occurs following irrigation, when soils attaining field capacity have a maximum run-off.

There were little or no available data to undertake equivalent modelling for the ACT and NT. Hence the assessment that followed only presented outcomes for the six states. The range of soil types throughout the six states (very clay based soils in Queensland to sand dominated soils in the southern part of Western Australia) were considered a suitable spread and other regions were expected to fall within that range.

Step 1 calculations

The runoff assessment was considered for different agricultural use situations. In the first instance, the maximum application rate (22 kg product/ha; 440 g ac/ha) was modelled. For the Step 1 calculations a 90th percentile slope value was applied with the assumption that 50% of the treated area contributes to runoff. Application was assumed direct to soil with no interception.

a) Cereal and oilseed crops

The assessment was undertaken for the “Dryland Cropping; Grain, straight row” runoff curves.

Table 16: Step 1 calculations, cereal and oilseed crops

State	Rainfall (mm)	Slope (%)	Runoff (mm)	Runoff (% active)	Water concentration (µg/L)	Risk Quotient
New South Wales	27	1.89	2.92	0.03	0.793	1.0
Queensland	19	1.97	2.79	0.05	1.137	1.5
South Australia	28	2.49	2.92	0.04	1.043	1.4
Tasmania	21	2.59	2.74	0.06	1.378	1.8
Victoria	27	1.18	2.92	0.02	0.475	0.62
Western Australia	44	2.46	3.54	0.03	0.767	1.0

b) Horticulture - orchards, berry crops and vineyards

Table 17: Step 1 calculations, horticulture, orchards

State	Rainfall (mm)	Slope (%)	Runoff (mm)	Runoff (% active)	Water concentration (µg/L)	Risk Quotient
New South Wales	27	4.27	2.99	0.09	2.084	2.7
Queensland	17	4.27	2.39	0.11	2.740	3.6
South Australia	28	5.36	2.91	0.11	2.609	3.4
Tasmania	20	12.39	2.60	0.41	10.312	13.4
Victoria	27	2.85	2.96	0.05	1.280	1.7
Western Australia	46	3.78	3.29	0.05	1.142	1.5

c) Horticulture – vegetable crops

Table 18: Step 1 calculations, horticulture, non-orchards

State	Rainfall (mm)	Slope (%)	Runoff (mm)	Runoff (% active)	Water concentration (µg/L)	Risk Quotient
New South Wales	16	4.27	2.26	0.11	2.775	3.6
Queensland	8	4.27	1.34	0.13	3.478	4.5
South Australia	17	5.36	2.40	0.14	3.641	4.7
Tasmania	11	12.39	1.78	0.51	13.487	17.5
Victoria	16	2.85	2.25	0.07	1.705	2.2
Western Australia	27	3.78	2.15	0.05	1.358	1.8

d) Pastures

Table 19: Step 1 calculations, horticulture, non-orchards

State	Rainfall (mm)	Slope (%)	Runoff (mm)	Runoff (% active)	Water concentration (µg/L)	Risk Quotient
New South Wales	24	5.85	2.76	0.13	3.249	4.2
Queensland	17	1.10	2.48	0.02	0.608	0.79
South Australia	25	2.87	2.85	0.05	1.349	1.8
Tasmania	19	8.26	2.57	0.24	6.079	7.9
Victoria	24	3.87	2.78	0.08	1.960	2.6
Western Australia	39	3.17	3.35	0.05	1.109	1.4

The highest risk quotients for these Step 1 calculations resulted from modelling runoff in vegetable cropping situations with the highest risk from that use identified in Tasmania. The 90th percentile slope in horticultural growing regions in Tasmania is significantly steeper than other states, leading to the high risk quotients. Rainfall data was obtained from the main horticultural growing catchments in Tasmania (Coal River, Huon River, Piper-Ringarooma Rivers and Tamar River catchments – MCAS-S data) for development of in-stream modelling. The stream flow data for Tasmania are not readily obtainable and, in the first instance as a surrogate, the dryland Victorian streamflow data was applied. These are not likely to be reflective of stream flows in Tasmania, but were considered to be more conservative so an acceptable outcome by applying the Victorian stream flow data is likely to be protective of Tasmanian stream flows also.

In-stream analysis

The in-stream analysis assumes that 20% of a catchment is treated at any one time. Because the whole of the treated area is taken to contribute to the runoff, the mean slopes for any given area were applied rather than the 90th percentile slopes. The slope distributions tend to be exponential with a higher contribution of shallower slopes. The horticultural growing zones in Tasmania have the steepest slopes for both 90th percentile (12.4%) and mean (5.4%) slopes.

In addition, regional specific rainfall data are considered. The rainfall values in the Step 1 calculations above do not reflect real world. Rather, they represent the value required to give the highest concentration in the standard water body for the runoff curve that reflects the growing situation and the soil profile for the different states. These values are quite high. For example, with the vegetable cropping scenario, up to 34 mm rain is required in the sand dominated soil profile of Western Australia while 18 mm is required for the loamy soils applied in Tasmania.

Long term rainfall data have been obtained throughout the country. At this stage, horticulture specific rainfall datasets have not been developed for the mainland states with the exception of Queensland. For Tasmania, rainfall data have been obtained and the appropriate rainfall values developed for the main horticultural growing catchments in that State.

Stream flow

Streamflow data libraries for horticulture (with the exception of Queensland) and in pasture dominated regions are yet to be developed. The dryland streamflow data was applied as a surrogate. In many cases there is overlap between pasture (identified in MCAS-S as either “Grazing modified pastures” or “Irrigated pastures” and the dryland regions. Further, several major horticultural regions also overlap with dryland cropping zones. Where overlap does not exist, additional rainfall data have been obtained and developed for the higher rainfall coastal zones of the different states. These data resulted in higher rainfall values being applied for New South Wales and Western Australia with respect to pastures, orchards and vegetable crops.

In Queensland, no additional catchments were considered. A separate model has already been developed for Queensland production horticultural zones and was applied in the in-stream assessment for orchards and vegetable crops below. As a land use, pasture is limited in QLD (grazing natural vegetation is more prevalent) and where pasture use is identified, the catchments are covered already for either dryland or production horticulture. Therefore, an acceptable risk for those uses will result in an acceptable risk for use in pasture.

Grain (Dryland cropping)

Step 1 calculations identified a potential risk in Queensland, South Australia and Tasmania and these three states were considered further with in-stream assessments.

Table 20: Dryland cropping in-stream analysis for runoff risk, Queensland (mean slope = 0.86%)

Season	Percent Stream Flow	Rainfall (mm/d)	Runoff (% active)	Receiving waters protected (%)
Autumn	25	12.9	0.0031	>99
Autumn	75	30.6	0.0146	>99
Spring	25	12.6	0.0029	>99
Spring	75	30.1	0.0143	>99
Summer	25	13.1	0.0033	>99
Summer	75	30.7	0.0147	>99
Winter	25	12.7	0.0030	>99
Winter	75	29.0	0.0137	>99

Table 21: Dryland cropping in-stream analysis for runoff risk, South Australia (mean slope = 1.08%)

Season	Percent Stream Flow	Rainfall (mm/d)	Runoff (% active)	Receiving waters protected (%)
Autumn	25	18.8	0.0025	95.3
Autumn	75	29.8	0.0076	>99
Spring	25	18.8	0.0025	96.9
Spring	75	30.2	0.0078	>99
Summer	25	20.4	0.0032	91.9
Summer	75	36.5	0.0106	>99
Winter	25	18.0	0.0021	98.4
Winter	75	25.9	0.0058	>99

Table 22: Dryland cropping in-stream analysis for runoff risk, Tasmania (mean slope = 1.13%)

Season	Percent Stream Flow	Rainfall (mm/d)	Runoff (% active)	Receiving waters protected (%)
Autumn	25	11.8	0.0015	96.7
Autumn	75	24.4	0.0114	>99
Spring	25	11.7	0.0014	97.9
Spring	75	21.9	0.0096	>99
Summer	25	12.0	0.0017	>99
Summer	75	25.0	0.0118	>99
Winter	25	12.1	0.0018	98.5
Winter	75	22.9	0.0103	>99

For application in dryland cropping situations (cereals and oilseed crops), the use of methiocarb up to the maximum rate is acceptable.

Orchards (not including Queensland)

The following calculations were initially performed for orchards with a bare soil inter-row. Where an unacceptable risk is identified, the calculations were re-run with a pasture inter-row. In the event this demonstrates an acceptable risk due to the more favourable runoff curve, a use restriction was recommended accordingly. If an unacceptable risk continues to be demonstrated, a timing restriction was recommended.

Table 23: Horticulture orchards in-stream analysis for runoff risk, New South Wales (mean slope = 1.85%)

Season	Percent Stream Flow	Rainfall (mm/d)	Runoff (% active)	Receiving waters protected (%)
Autumn	25	17.1	0.0047	>99
Autumn	75	45.2	0.0272	>99
Spring	25	15.9	0.0037	>99
Spring	75	38.0	0.0217	>99
Summer	25	16.9	0.0045	98.9
Summer	75	42.3	0.0250	>99
Winter	25	17.4	0.0049	>99
Winter	75	45.9	0.0277	>99

Table 24: Horticulture orchards in-stream analysis for runoff risk, South Australia (mean slope = 2.33%)

Season	Percent Stream Flow	Rainfall (mm/d)	Runoff (% active)	Receiving waters protected (%)
Autumn	25	18.8	0.0058	91.3
Autumn	75	30.8	0.0187	98.3
Spring	25	19.2	0.0062	93.4
Spring	75	28.0	0.0157	99.0
Summer	25	19.1	0.0061	83.0
Summer	75	33.7	0.0217	98.6
Winter	25	17.9	0.0048	97.1
Winter	75	26.4	0.0140	>99

Table 25: Horticulture orchards in-stream analysis for runoff risk, South Australia (mean slope = 2.33%) – Summer application with a pasture inter-row:

Season	Percent Stream Flow	Rainfall (mm/d)	Runoff (% active)	Receiving waters protected (%)
Summer	25	19.1	0.0030	92.7
Summer	75	33.7	0.0154	>99

In South Australia, application should not occur to orchards with bare soil in the inter-row spaces.

Table 26: Horticulture orchards in-stream analysis for runoff risk, Tasmania (mean slope = 5.38%)

Season	Percent Stream Flow	Rainfall (mm/d)	Runoff (% active)	Receiving waters protected (%)
Autumn	25	11.7	0.0117	86.1
Autumn	75	22.7	0.0658	93.7
Spring	25	11.3	0.0096	91.9

Season	Percent Stream Flow	Rainfall (mm/d)	Runoff (% active)	Receiving waters protected (%)
Spring	75	20.3	0.0547	96.4
Summer	25	11.9	0.0128	73.1
Summer	75	23.2	0.0681	89.6
Winter	25	11.5	0.0107	95.1
Winter	75	21.0	0.0580	98.7

Table 27: Horticulture orchards in-stream analysis for runoff risk, South Australia (mean slope = 2.33%) – Summer and autumn application with a pasture inter-row:

Season	Percent Stream Flow	Rainfall (mm/d)	Runoff (% active)	Receiving waters protected (%)
Autumn	25	11.7	0.0010	97.6
Autumn	75	22.7	0.0445	95.8
Summer	25	11.9	0.0010	>99
Summer	75	23.2	0.0465	94.3

In Tasmania, application should not occur to orchards with bare soil in the inter-row spaces.

Table 28: Horticulture - orchards in-stream analysis for runoff risk, Victoria (mean slope = 1.24%)

Season	Percent Stream Flow	Rainfall (mm/d)	Runoff (% active)	Receiving waters protected (%)
Autumn	25	18.1	0.0035	94.0
Autumn	75	31.7	0.0107	>99
Spring	25	17.4	0.0031	96.3
Spring	75	27.7	0.0086	>99
Summer	25	19.5	0.0042	88.1
Summer	75	34.0	0.0119	>99
Winter	25	17.4	0.0031	97.9
Winter	75	29.6	0.0096	>99

Table 29: Horticulture orchards in-stream analysis for runoff risk, Victoria (mean slope = 1.24%) – Summer application with a pasture inter-row:

Season	Percent Stream Flow	Rainfall (mm/d)	Runoff (% active)	Receiving waters protected (%)
Summer	25	19.5	0.0024	96.0
Summer	75	34.0	0.0087	>99

In Victoria, application should not occur to orchards with bare soil in the inter-row spaces.

Table 30: Horticulture orchards in-stream analysis for runoff risk, Western Australia (mean slope = 1.64%)

Season	Percent Stream Flow	Rainfall (mm/d)	Runoff (% active)	Receiving waters protected (%)
Autumn	25	19.3	0.0007	>99
Autumn	75	27.3	0.0021	>99
Spring	25	17.8	0.0005	>99

Season	Percent Stream Flow	Rainfall (mm/d)	Runoff (% active)	Receiving waters protected (%)
Spring	75	21.8	0.0011	>99
Summer	25	18.8	0.0006	>99
Summer	75	27.6	0.0021	>99
Winter	25	19.2	0.0007	>99
Winter	75	26.6	0.0019	>99

Vegetable crops (not including Queensland)

Table 31: Horticulture non-orchards in-stream analysis for runoff risk, New South Wales (mean slope = 1.85%)

Season	Percent Stream Flow	Rainfall (mm/d)	Runoff (% active)	Receiving waters protected (%)
Autumn	25	17.1	0.0097	98.2
Autumn	75	45.2	0.0385	>99
Spring	25	15.9	0.0082	98.7
Spring	75	38.0	0.0321	>99
Summer	25	16.9	0.0094	97.8
Summer	75	42.3	0.0360	>99
Winter	25	17.4	0.0100	98.9
Winter	75	45.9	0.0391	>99

Table 32: Horticulture non-orchards in-stream analysis for runoff risk, South Australia (mean slope = 1.22%)

Season	Percent Stream Flow	Rainfall (mm/d)	Runoff (% active)	Receiving waters protected (%)
Autumn	25	18.8	0.0066	90.6
Autumn	75	30.8	0.0151	98.7
Spring	25	19.2	0.0069	93.5
Spring	75	28.0	0.0132	>99
Summer	25	19.1	0.0068	81.6
Summer	75	33.7	0.0170	>99
Winter	25	17.9	0.0059	96.7
Winter	75	26.4	0.0121	>99

The risk in summer at the 25th percentile flow rate is acceptable at 11 kg/ha (220 g ac/ha).

Table 33: Horticulture non-orchards in-stream analysis for runoff risk, Tasmania (mean slope = 5.38%)

Season	Percent Stream Flow	Rainfall (mm/d)	Runoff (% active)	Receiving waters protected (%)
Autumn	25	11.7	0.0319	75.3
Autumn	75	21.0	0.0869	91.7
Spring	25	11.3	0.0293	83.5
Spring	75	20.3	0.0830	94.7
Summer	25	11.9	0.0332	60.9
Summer	75	23.2	0.0985	83.5
Winter	25	11.5	0.0306	90.4
Winter	75	22.7	0.0959	97.8

At half the application rate (220 g ac/ha), the risk in spring is essentially mitigated, however, the risk in autumn and summer remains unacceptable at the 25th percentile stream flow (83.2% and 69.7% protection respectively). Even at the common rate of 110 g ac/ha, the risk in summer is unacceptable (78.9% protection).

Table 34: Horticulture non-orchards in-stream analysis for runoff risk, Victoria (mean slope = 1.2%)

Season	Percent Stream Flow	Rainfall (mm/d)	Runoff (% active)	Receiving waters protected (%)
Autumn	25	18.1	0.0070	90.1
Autumn	75	31.7	0.0169	98.5
Spring	25	17.4	0.0065	93.8
Spring	75	27.7	0.0142	>99
Summer	25	19.5	0.0081	79.2
Summer	75	34.0	0.0184	>99
Winter	25	17.4	0.0065	96.5
Winter	75	29.6	0.0155	>99

If the rate of application is restricted to 11 kg/ha (220 g ac/ha), the identified risk in summer is reduced with the protection level predicted to be 88.7%. Given the additional conservatism in the modelling and the acceptable risk at all other times and for both stream flow percentiles, the risk in Victoria from application of methiocarb to vegetable crops is considered acceptable with a rate restriction applicable in summer.

Table 35: Horticulture non-orchards in-stream analysis for runoff risk, Western Australia (mean slope = 1.64%)

Season	Percent Stream Flow	Rainfall (mm/d)	Runoff (% active)	Receiving waters protected (%)
Autumn	25	19.3	0.0021	93.4
Autumn	75	30.9	0.0079	>99
Spring	25	17.8	0.0014	95.5
Spring	75	25.8	0.0052	>99
Summer	25	18.8	0.0018	88.6
Summer	75	33.9	0.0095	96.5
Winter	25	19.2	0.0020	97.0
Winter	75	30.6	0.0077	>99

The risk in summer at the 25th percentile flow rate is acceptable at 11 kg/ha (220 g ac/ha).

Pastures (not including Queensland)

The Step 1 assessment identified a potential risk in all states except Queensland. These states were considered with an in-stream analysis.

Table 36: Pasture in-stream analysis for runoff risk, New South Wales (mean slope = 2.54%)

Season	Percent Stream Flow	Rainfall (mm/d)	Runoff (% active)	Receiving waters protected (%)
Autumn	25	17.1	0.0091	98.3
Autumn	75	45.2	0.0465	>99
Spring	25	15.9	0.0073	98.8
Spring	75	38.0	0.0378	>99

Season	Percent Stream Flow	Rainfall (mm/d)	Runoff (% active)	Receiving waters protected (%)
Summer	25	16.9	0.0088	97.9
Summer	75	42.3	0.0431	>99
Winter	25	17.4	0.0095	99.0
Winter	75	45.9	0.0473	>99

Table 37: Pasture in-stream analysis for runoff risk, South Australia (mean slope = 1.25%)

Season	Percent Stream Flow	Rainfall (mm/d)	Runoff (% active)	Receiving waters protected (%)
Autumn	25	18.8	0.0045	92.7
Autumn	75	30.8	0.0124	98.9
Spring	25	19.2	0.0048	95.0
Spring	75	28.0	0.0106	>99
Summer	25	19.1	0.0047	86.6
Summer	75	33.7	0.0141	>99
Winter	25	17.9	0.0039	97.5
Winter	75	26.4	0.0096	>99

A potential risk is identified in South Australia in summer for the low stream flow percentile. This value is approaching the acceptable 90%. Further the assessment was based on the highest application rate. The label notes that this rate should be applied to pasture where it is tall or dense, and in these situations, it is reasonable to expect a significant amount of interception will result. EFSA (2014) provides an interception value for “Grass” at the point of stem elongation (BBCH 20-39) of 60%. This rate of interception alone is sufficient to mitigate the risk such that the percent of protection of receiving waters in summer at the 25th percentile in South Australia is 91.5% and the risk from use in pasture is considered acceptable.

Table 38: Pasture in-stream analysis for runoff risk, Tasmania (mean slope = 1.25%)

Season	Percent Stream Flow	Rainfall (mm/d)	Runoff (% active)	Receiving waters protected (%)
Autumn	25	11.6	0.0094	88.0
Autumn	75	23.0	0.0458	95.6
Spring	25	11.2	0.0080	92.8
Spring	75	20.4	0.0380	97.5
Summer	25	11.9	0.0104	75.8
Summer	75	23.9	0.0484	93.9
Winter	25	11.2	0.0080	96.0
Winter	75	19.9	0.0365	>99

Applying the interception value of 60%, the risk in Autumn was mitigated (percent receiving waters protected =90.6%). However, the identified risk in summer at the low stream flow percentile remains with 80.6% of receiving waters predicted to remain protected. Reducing the rate to 11 kg/ha (220 g ac/ha) results in an acceptable risk in summer.

Table 39: Pasture in-stream analysis for runoff risk, Victoria (mean slope = 1.68%)

Season	Percent Stream Flow	Rainfall (mm/d)	Runoff (% active)	Receiving waters protected (%)
Autumn	25	18.1	0.0067	90.4
Autumn	75	31.7	0.0189	98.3
Spring	25	17.4	0.0060	94.1
Spring	75	27.7	0.0154	99.0
Summer	25	19.5	0.0080	79.3
Summer	75	34.0	0.0208	98.8
Winter	25	17.4	0.0060	96.6
Winter	75	29.6	0.0171	>99

Applying the interception value of 60%, the identified risk in summer at the low stream flow percentile remains with 84.2% of receiving waters predicted to remain protected. Reducing the rate to 11 kg/ha (220 g ac/ha) results in an acceptable risk in summer.

Table 40: Pasture in-stream analysis for runoff risk, Western Australia (mean slope = 1.38%)

Season	Percent Stream Flow	Rainfall (mm/d)	Runoff (% active)	Receiving waters protected (%)
Autumn	25	19.3	0.0010	>99
Autumn	75	27.3	0.0031	>99
Spring	25	17.8	0.0006	>99
Spring	75	21.8	0.0016	>99
Summer	25	18.8	0.0008	>99
Summer	75	27.6	0.0032	>99
Winter	25	19.2	0.0009	98.8
Winter	75	26.6	0.0029	>99

Queensland production horticulture

The Queensland assessment was performed on a monthly time scale.

Queensland's key production horticulture regions were identified (see Appendix 2, Growcom (2013)). In this assessment, the southern regions of Border Rivers and Condamine-Culgoa catchments fall within the dryland cropping zones, and were considered in the above analysis on a State basis. The other production horticulture regions were considered separately. There is no significant horticulture production in the Queensland NRM regions of the Southern Gulf, Desert Channels and South West QLD, and these were not considered in the following analysis.

Mean slope values within horticultural growing areas and the approximate area (ha) of horticultural activities in the different regions were obtained by the ABARE MCAS-S data.

In the following assessment, only 25th percentile stream flow data and the associated rainfall values were assessed. If there was a greater risk identified at the 75th percentile stream flow and rainfall data, it was noted separately. For the orchards assessment, a bare soil inter-row area was assessed in the first instance.

Cape York and Northern Gulf

Cape York stream flow data was used as a surrogate for the Northern Gulf region. The slopes in the growing areas differ and they were therefore assessed separately. The area identified for production horticulture is ~4800 ha for these two regions combined.

Table 41: Cape York in-stream analysis for runoff risk (mean slope, 0.75%)

Month	Rainfall (mm)	Vegetable crops		Orchards	
		Runoff (% active)	Receiving waters protected (%)	Runoff (% active)	Receiving waters protected (%)
January	16.0	0.008	>99	0.006	>99
February	14.6	0.007	>99	0.005	>99
March	16.4	0.009	>99	0.006	>99
April	14.0	0.007	>99	0.004	>99
May	11.5	0.005	>99	0.003	>99
June	10.1	0.004	>99	0.002	>99
July	10.4	0.004	>99	0.002	>99
August	10.0	0.004	>99	0.002	>99
September	10.6	0.004	97.4	0.002	98.5
October	13.0	0.006	92.7	0.004	95.7
November	13.6	0.007	87.9	0.004	90.2
December	16.0	0.008	98.7	0.006	>99

The identified risk in vegetable crops is mitigated at a rate of 11 kg/ha (220 g ac/ha).

Table 42: Northern Gulf in-stream analysis for runoff risk (mean slope, 1.80%)

Month	Rainfall (mm)	Vegetable crops		Orchards	
		Runoff (% active)	Receiving waters protected (%)	Runoff (% active)	Receiving waters protected (%)
January	16.0	0.021	>99	0.014	>99
February	14.6	0.019	>99	0.012	>99
March	16.4	0.022	>99	0.015	>99
April	14.0	0.018	>99	0.011	>99
May	11.5	0.013	>99	0.007	>99
June	10.1	0.010	>99	0.004	>99
July	10.4	0.011	>99	0.005	>99
August	10.0	0.010	98.5	0.004	>99
September	10.6	0.011	95.1	0.005	97.1
October	13.0	0.016	85.9	0.009	90.1
November	13.6	0.017	82.1	0.010	85.5
December	16.0	0.021	96.8	0.014	97.8

The identified risk in orchards and vegetable crops (October) is mitigated at a rate of 11 kg/ha (220 g ac/ha), and in vegetable crops (November) is mitigated at a rate of 5.5 kg/ha (110 g ac/ha).

Wet tropics

Table 43: Wet Tropics, in-stream analysis for runoff risk

Month	Rainfall (mm)	Vegetable crops		Orchards	
		Runoff (% active)	Receiving waters protected (%)	Runoff (% active)	Receiving waters protected (%)
January	17.5	0.043	>99	0.014	>99
February	16.7	0.040	>99	0.012	>99
March	16.5	0.040	>99	0.015	>99
April	14.7	0.034	>99	0.011	>99
May	13.5	0.030	98.9	0.007	>99
June	12.5	0.026	98.8	0.004	>99
July	12.2	0.025	98.2	0.005	99.0
August	12.2	0.025	97.4	0.004	98.5
September	12.2	0.025	96.7	0.005	98.1
October	12.2	0.025	95.7	0.009	97.5
November	13.7	0.030	94.8	0.010	96.8
December	14.5	0.033	95.6	0.014	97.2

Burdekin

Mean slope = 0.80%. Area to production horticulture ~18000 ha.

Table 44: Burdekin, in-stream analysis for runoff risk

Month	Rainfall (mm)	Vegetable crops		Orchards	
		Runoff (% active)	Receiving waters protected (%)	Runoff (% active)	Receiving waters protected (%)
January	16.5	0.009	>99	0.006	>99
February	15.8	0.009	>99	0.006	>99
March	14.8	0.008	>99	0.005	>99
April	13.8	0.007	>99	0.004	>99
May	12.4	0.006	>99	0.003	>99
June	12.7	0.006	>99	0.004	>99
July	12.6	0.006	>99	0.004	>99
August	12.7	0.006	>99	0.004	>99
September	14.6	0.008	97.1	0.005	98.62
October	12.7	0.006	90.8	0.004	95.35
November	13.5	0.007	>99	0.004	>99
December	14.4	0.008	>99	0.005	>99

Mackay/Whitsunday

Mean slope = 2.02%.

Table 45: Mackay/Whitsunday, in-stream analysis for runoff risk

Month	Rainfall (mm)	Vegetable crops		Orchards	
		Runoff (% active)	Receiving waters protected (%)	Runoff (% active)	Receiving waters protected (%)
January	14.2	0.021	98.6	0.013	>99
February	15.8	0.024	>99	0.016	>99
March	16.2	0.025	>99	0.017	>99
April	13.2	0.019	>99	0.011	>99
May	11.9	0.016	97.1	0.008	98.2
June	13.7	0.020	96.1	0.012	97.4
July	11.6	0.015	96.1	0.008	97.7
August	11.0	0.014	94.3	0.007	96.7
September	12.2	0.016	93.8	0.009	96.4
October	14.0	0.020	88.1	0.013	96.0
November	12.2	0.016	89.5	0.009	>99
December	14.0	0.020	93.8	0.013	96.9

The identified risk in vegetable crops is mitigated at a rate of 11 kg/ha (220 g ac/ha).

Fitzroy

Mean slope = 1.89%. Area to production horticulture ~7200 ha.

Table 46: Fitzroy, in-stream analysis for runoff risk

Month	Rainfall (mm)	Vegetable crops		Orchards	
		Runoff (% active)	Receiving waters protected (%)	Runoff (% active)	Receiving waters protected (%)
January	14.2	0.017	87.0	0.013	90.4
February	15.8	0.019	88.6	0.016	91.2
March	16.2	0.020	88.4	0.017	90.8
April	13.2	0.020	85.2	0.011	88.2
May	11.9	0.021	88.4	0.008	91.5
June	13.7	0.016	89.4	0.012	93.1
July	11.6	0.018	82.0	0.008	87.3
August	11.0	0.016	80.6	0.007	87.6
September	12.2	0.016	82.6	0.009	88.6
October	14.0	0.017	83.5	0.013	89.6
November	12.2	0.016	85.4	0.009	89.8
December	14.0	0.017	85.8	0.013	90.3

In the Fitzroy catchment, there was a significant amount of data available for stream flow and rainfall and the results for the 75% percentile stream flow provide adverse outcomes with <90% protection for all months of the year. For orchards, when a pasture inter-row was modelled, there were still a large number of months where protection did not exceed 90% of receiving waters. If, the application rate is reduced to 11 kg/ha (220 g ac/ha), there is only a slight risk identified at the higher stream flow in April, July and August.

At the label rate of 5.5 kg/ha (110 g ac/ha), the runoff risk from use in both orchards and vegetables is acceptable.

Mary/Burnett

Mean slope = 1.56%. Area to production horticulture ~16800 ha.

Table 47: Mary Burnett, in-stream analysis for runoff risk

Month	Rainfall (mm)	Vegetable crops		Orchards	
		Runoff (% active)	Receiving waters protected (%)	Runoff (% active)	Receiving waters protected (%)
January	13.6	0.015	>99	0.009	>99
February	13.8	0.015	>99	0.009	>99
March	13.4	0.014	98.7	0.009	>99
April	12.4	0.013	98.0	0.007	>99
May	13.2	0.014	97.7	0.008	99.0
June	12.5	0.013	>99	0.007	>99
July	17.0	0.020	97.0	0.014	98.3
August	11.8	0.012	98.1	0.006	>99
September	14.4	0.016	96.3	0.010	98.2
October	14.4	0.016	94.0	0.010	97.5
November	12.4	0.013	96.5	0.007	98.7
December	13.5	0.014	97.8	0.009	>99

South East Queensland

Mean slope = 1.68%. Area to production horticulture ~52000 ha (includes horticultural areas from the Border Rivers and Condamine catchments).

Table 48: Mary Burnett, in-stream analysis for runoff risk

Month	Rainfall (mm)	Vegetable crops		Orchards	
		Runoff (% active)	Receiving waters protected (%)	Runoff (% active)	Receiving waters protected (%)
January	14.4	0.017	85.8	0.011	89.9
February	13.5	0.016	93.9	0.009	96.2
March	13.3	0.015	91.8	0.009	94.4
April	12.4	0.014	91.1	0.008	94.1
May	13.1	0.015	91.9	0.009	94.6
June	12.6	0.014	94.4	0.008	96.6
July	13.7	0.016	94.3	0.010	96.6
August	12.0	0.013	92.8	0.007	96.1
September	11.4	0.012	88.9	0.006	93.6
October	13.0	0.015	85.1	0.009	91.4
November	13.4	0.015	87.4	0.009	92.2
December	14.0	0.016	86.7	0.010	91.5

South East Queensland is another region where the risk is higher at the higher 75th percentile stream flow. In orchard situations with a bare soil inter-row, an unacceptable risk is identified from October through to the end of January. This risk remains at the 75th percentile stream flow even when modelling orchards with a pasture inter-row. However, a lower rate of 11 kg/ha (220 g ac/ha) results in an acceptable risk in orchard situations irrespective of the inter-row characteristics.

With vegetable use, at a lower rate of 11 kg/ha (220 g ac/ha), the risk is essentially mitigated in all months for both stream flows with two exceptions at the 75th percentile stream flow, namely, January (88.8% protection) and October (88.8% protection). Given the closeness of these levels to 90% protection, and noting the acceptable risk at all other times of the year for both 25th and 75th percentile stream flow, the overall risk from the use of methiocarb in South East Queensland in vegetables can be considered acceptable with a rate restriction to 11 kg product/ha.

5.2.2 Uncertainties with methiocarb runoff assessment

The outcomes of the risk assessment need to be considered in context with the following uncertainties:

Uncertainty	Likely impact
Acceptable level of protection.	The conclusion of an acceptable risk is made if the percent of receiving waters where the in-stream concentration is above the RAC does not exceed 10%. This is not based on any national policy and an acceptable level of protection may vary between protection goals. For example, where runoff is expected to lead to exposure of highly sensitive areas, the appropriate level of protection may be higher than that where runoff will expose highly degraded areas.

Uncertainty	Likely impact
Lack of specific data for Northern Territory, Australian Capital Territory and the Ord region of Western Australia	There are insufficient data for these regions for soil characteristics and hydrology to allow an equivalent assessment for Step 1 or the in-stream analysis. For this assessment they have been assumed to fall within the range of results determined for other regions given the wide range of soil types, slopes, rainfall patterns and hydrology through these different regions.
No Tasmanian stream flow data available.	Used Victorian stream flow as a surrogate but with Tasmanian rainfall data. The Victorian stream flow data were from the dryland cropping districts and so are considered conservative and are expected to, if anything, overestimate the risk outcomes.
No horticulture specific stream flow data sets with the exception of Queensland.	There are overlaps in horticultural areas with dryland cropping in QLD, NSW and VIC. The dryland stream flow data sets from the different states were used in the horticultural in-stream assessments, but took account of possible higher rainfall values in some horticultural areas compared with dryland. These are therefore considered conservative and are expected to, if anything, overestimate the risk outcomes.
No pasture specific stream flow data sets with the exception of Queensland.	There are overlaps in pasture areas with dryland cropping in several states. The dryland stream flow data sets from the different states were used in the pasture in-stream assessments, but took account of possible higher rainfall values in some pasture areas compared with dryland. These are therefore considered conservative and are expected to, if anything, overestimate the risk outcomes.
The Regulatory Acceptable Concentration (RAC) was based on the acute toxicity *to Daphnia magna* and using the mean measured concentration.	The runoff assessments are undertaken based on peak predicted concentrations, and in this regard, the use of initial measured concentrations to calculate the EC50 in the critical study is probably more appropriate. However, the test was a semi-static test with renewal of test solutions at 24 h so such an assessment is not possible. The runoff assessment, while performed as an in-stream analysis, is taken as a surrogate for lentic water bodies where there is no flow. Therefore, residence time in the water was not considered further.
Composite curve numbers based on most conservative soil hydrological groups.	The highest risks were associated with the composite curve numbers generated for row crops where there was no crop residue and no additional management practices that may reduce runoff such as contouring or terracing. Runoff curves can be generated for these practices, but should only be applied on a case by case basis.

5.2.3 Metabolites runoff assessment

Highest slopes (90th percentile slopes for Step 1 calculations) were associated with horticultural activities, and the runoff curve for vegetable (row) crops gives a slightly higher predicted runoff than that for orchards. The metabolites were assessed only with the vegetable crop scenario for step 1.

Input parameters are provided in Table 15 above.

If no risk was identified at this step, then aquatic risk from the main soil metabolites is considered acceptable:

a) M01

Table 49: Step 1 calculations, M01 metabolite, vegetable crop uses

State	Rainfall (mm)	Slope (%)	Runoff (mm)	Runoff (% active)	Water concentration (µg/L)	Risk Quotient
New South Wales	21	4.27	2.58	0.44	6.960	1.1
Queensland	14	4.27	2.20	0.57	9.118	1.4
South Australia	22	2.81	2.66	0.26	4.157	0.64
Tasmania	17	12.39	2.67	2.33	34.49	5.6
Victoria	21	2.85	2.59	0.27	4.320	0.66
Western Australia	34	3.78	3.20	0.29	4.444	0.68

The steep 90th percentile slopes in Tasmania, and clay dominated soil profile in Queensland resulted in a predicted risk at Step 1 from exposure to M01 through runoff. A slight risk was also identified for NSW at Step 1. This assessment was unreliable as it assumed the full conversion (63% of parent for M01) occurs with runoff of the full amount happening at the same time. Clearly, such an approach will overestimate the runoff concentrations.

Nonetheless, an in-stream analysis for Tasmania was considered with application of the lower mean slope value. Additionally, the three Queensland production horticulture regions identified as potentially most at risk from parent compound runoff were considered in an in-stream analysis for M01.

Table 50: In-stream analysis for runoff risk of M01 metabolite for vegetable crops in Tasmania, 5.38% mean slope

Season	Streamflow (%)	Rainfall (mm)	Runoff (% formed metabolite)	Percent receiving waters protected
Autumn	25	11.7	0.1491	86.6
	75	21.0	0.4059	97.1
Spring	25	11.3	0.1371	91.5
	75	20.3	0.3881	98.1
Summer	25	11.9	0.1551	74.4
	75	23.2	0.4605	96.7
Winter	25	11.5	0.1432	95.1
	75	22.7	0.4483	>99

A potential risk in summer and autumn at low stream flow rates is identified. In terms of risk compared to the parent compound for this use pattern (see Table 33), that from the metabolite is lower than from the parent, so the risk management measures identified for the parent compound are also expected to mitigate the identified risk from this metabolite.

Table 51: In-stream analysis for runoff risk of M01 metabolite for vegetable crops in selected QLD production horticulture regions

Month	Northern Gulf		Mackay/Whitsunday		Fitzroy	
	Runoff (% applied)	Percent protected	Runoff (% applied)	Percent protected	Runoff (% applied)	Percent protected
January	0.100	>99	0.097	>99	0.081	93.3
February	0.088	>99	0.112	>99	0.089	94.0

Month	Northern Gulf		Mackay/Whitsunday		Fitzroy	
	Runoff (% applied)	Percent protected	Runoff (% applied)	Percent protected	Runoff (% applied)	Percent protected
March	0.104	>99	0.116	>99	0.092	93.3
April	0.083	>99	0.087	>99	0.092	91.3
May	0.061	>99	0.073	98.7	0.096	94.5
June	0.048	>99	0.092	98.4	0.073	95.2
July	0.050	>99	0.070	98.4	0.082	91.4
August	0.047	>99	0.064	97.5	0.073	91.7
September	0.052	97.6	0.076	97.6	0.076	92.5
October	0.074	93.4	0.095	>99	0.078	93.5
November	0.080	88.4	0.076	>99	0.077	92.8
December	0.100	98.8	0.095	>99	0.080	94.3

The risk is lower from the M01 metabolite than the parent compound. Periods identified in the Cape York and Northern Gulf regions for no application of methiocarb pellets will also be protective against aquatic exposure to M01.

b) M04, M05 and M10

These metabolites are significantly less toxic than the parent compound and M01. Their fractions of formation are also much lower. The risk to aquatic organisms from exposure to these soil metabolites formed in soil are considered acceptable.

5.3 Soil Organisms

In the DotE 2006 assessment it was observed that toxicity of methiocarb to earthworms is clearly dependent on soil organic matter content, with toxicity decreasing in line with increasing soil organic matter. The most sensitive acute earthworm toxicity value was an LC_{50} = 60.7 mg/kg soil with 2.5% peat. New data submitted to the review did not alter the risk conclusions of the previous assessment with respect to earthworms.

In their assessment, DotE concluded that while methiocarb should not persist in the environment, it has a very broad spectrum of biological activity consistent with its ability to inhibit acetylcholinesterase. Non-target organisms including earthworms and beetles are likely to be killed by the use of methiocarb, particularly snail bait formulations.

The concentration of methiocarb in the actual slug pellet is 20000 mg/kg, so direct consumption of the pellet will clearly have the potential for adverse effects and the previous conclusion therefore remains unaltered.

However, metabolite toxicity data are now provided and showed potentially higher toxicity from some metabolites than shown with methiocarb. Simple risk quotients were calculated below to consider potential risk of earthworms exposed to methiocarb and its metabolites through soil, assuming the available active constituent leaches out of the pellets and is distributed through the top soil. The metabolite LC_{50}s reported in Table 13 above were based on test systems with 10% peat. A corrected LC_{50} (10% peat LC_{50} X 0.5) was used in the risk calculations below. This correction was performed because the high level of organic matter in the test system may result in lower

bioavailability (increased sorption) than occurs in agricultural soils where organic matter is likely to be considerably less than 10%.

Table 52: Acute earthworm risk quotients for methiocarb and metabolites

Substance	Fraction	PECsoil mg/kg dw[1]	LC50corr mg/kg dw	Risk quotient
Methiocarb	1	0.59	60.7	0.01
M01	0.63	0.37	39	0.01
M04	0.293	0.17	>500	<0.01
M05	0.176	0.10	>500	<0.01
M10	0.126	0.074	281	<0.01

[1] *Top 5 cm soil, 1500 kg/m3 soil density.*

The risk from methiocarb metabolites is no greater than that from the parent compound.

Test data are also available for the four main soil metabolites to the predatory soil mite (*Hypoaspis aculeifer*) and the collembola (*Folsomia candida*). M01 was the most toxic to the soil mite with a NOEC = 10 mg/kg. Based on the predicted maximum soil concentration for this metabolite above, the risk quotient = 0.037, which indicates an acceptable risk to these soil organisms.

M10 was the most toxic metabolite to the collembola with a NOEC = 10 mg/kg. Based on the predicted maximum soil concentration for this metabolite above, the risk quotient = 0.007, which indicates an acceptable risk to these soil organisms.

6 CONCLUSIONS AND RECOMMENDATIONS

6.1 Mesurol Snail and Slug Bait

New data showed residues in earthworms following field use of representative formulations. A revised assessment of secondary poisoning potential to Australian birds indicated a high risk from this exposure route. However, evidence provided in additional field tests in a wide range of cropping situations treated with methiocarb pellets indicated that, in reality, birds are unlikely to be impacted either lethally or sub-lethally.

Additional toxicity data for methiocarb and metabolites to soil organisms have required an update to the soil organism risk assessment. It was demonstrated that the risk from methiocarb metabolites was no greater than that from the parent compound and the overall risk to soil organisms was considered acceptable.

The additional data provided have resulted in a lower aquatic end-point than previously applied and resulted in a re-assessment of runoff. Based on the maximum application rate (22 kg product/ha), risks from dryland cropping use were considered acceptable. Highest risks were associated with the use in vegetables.

In many cases, rate restrictions in summer months are proposed in the southern states of Victoria, South Australia and Tasmania. It appears counter intuitive that, as these are the driest months, the risks from runoff are expected to be lower. However, the current methodology for estimating the runoff does not allow for a consideration of probability of a runoff event occurring. Nonetheless, given the lower probability of actual rainfall occurring in these southern states during the summer months, the APVMA proposes the following statement in lieu of the rate restrictions proposed for Victoria, Tasmania and South Australia:

> DO NOT apply in summer if rainfall of more than 10 mm per day is forecast for the next 48 hours.

The following table summarises the outcomes of the runoff assessment in terms of identified risks for different uses and the associated risk management options that would allow the risk to be considered acceptable.

Table 53: Risk management recommendations for runoff risk

Use situation	State/region	Risk management recommendations
Pasture	VIC	DO NOT apply more than 11 kg/ha in summer
Pasture	TAS	DO NOT apply more than 11 kg/ha in summer
Orchards	VIC	DO NOT apply to orchards with bare soil inter-rows
Orchards	TAS	DO NOT apply to orchards with bare soil inter-rows
Orchards	SA	DO NOT apply to orchards with bare soil inter-rows
Orchards	Northern Gulf	DO NOT apply more than 11 kg/ha in November.
Orchards	Fitzroy	DO NOT apply more than 11 kg/ha in the Fitzroy catchment.
Orchards	SE Queensland	DO NOT apply more than 11 kg/ha in the South East QLD catchments.
Vegetables	VIC	DO NOT apply more than 11 kg/ha in summer
Vegetables	TAS	DO NOT apply more than 11 kg/ha in spring
Vegetables	TAS	DO NOT apply more than 5.5 kg/ha in autumn
Vegetables	TAS	DO NOT apply in Tasmania in summer
Vegetables	SA	DO NOT apply more than 11 kg/ha in summer
Vegetables	WA	DO NOT apply more than 11 kg/ha in summer
Vegetables	Cape York	DO NOT apply more than 11 kg/ha in November
Vegetables	Northern Gulf	DO NOT apply more than 11 kg/ha in October
Vegetables	Northern Gulf	DO NOT apply more than 5.5 kg/ha in November
Vegetables	Mackay/Whitsunday	DO NOT apply more than 11 kg/ha in October and November
Vegetables	SE Queensland	DO NOT apply more than 11 kg/ha in the South East QLD catchments.

Additionally, the label for Mesurol Snail and Slug Bait contains the following instruction aimed at minimising harmful effects to birds and mammals: “After filling the applicator, clean up spilled pellets so that they are not eaten by animals and birds”. It is recommended that this instruction be varied by insertion of “immediately” after “pellets” as the longer the spilled pellets remain available, they present the possibility of being eaten by non-target species with concomitant poisoning. The revised statement would read:

> “After filling the applicator, clean up spilled pellets immediately so that they are not eaten by animals and birds.”

6.2 Baysol Snail and Slug Bait

The same instruction aimed at minimising harmful effects to birds and mammals appears on the label of the home garden product Baysol. Therefore, it is recommended that this instruction be varied by insertion of “immediately” after “pellets”. The revised statement would read:

> “Clean up spilled pellets immediately so that they are not eaten by animals and birds.”

Appendix

APPENDIX 1. DESCRIPTION OF RUNOFF METHODOLOGY DATA

The PERAMA model has been developed as an integrated model for undertaking environmental assessments of pesticides within the Australian regulatory framework. The software incorporates relevant real-world data with respect to slopes, soil types, rainfall and stream flow rates to allow a spatial and temporal assessment of runoff risk in Australia.

Runoff has been modelled following the methodology described in the APVMA's refinement of aquatic exposure estimates in environmental runoff assessments for pesticides in dryland cropping regions (http://apvma.gov.au/node/15696).

Following the consultation, some amendments have been made to the methodology (consultation paper still to be updated). These amendments relate to predicting edge of field concentrations, not the application of the stream flow data sets applied at the highest tier of assessment. The PERAMA software is based on the updated approach.

The edge of field concentration is still assessed by the following equation:

Equation 1

$$L\%_{runoff} = \left(\frac{Q}{P}\right) X\ f\ X \exp\left(-\frac{3XLn2}{DT_{50\ soil}}\right) X\ 100/(1+Kd)$$

The term "f" relates to several correction factors:

f1 is the slope factor, where f1 = 0.02153 X slope + 0.001423 X slope2 (for slopes < 20%);

f2 reflects the influence of plant interception, PI (%) where f2 = 1 – (PI/100);

f3 = reflects the influence of a densely-covered buffer zone where f3 = 0.083WBZ, and WBZ is the width of the buffer zone in metres. If the buffer zone is not densely covered with plants, the width is set to zero. Vegetative filter strips are not currently considered in Australian assessments, and thus this factor remains at zero.

The runoff assessment is performed in a tiered approach, namely, a screening level assessment, a first step of refinement where runoff curves from different cropping systems along with soil profiles for different regions are considered, and a final tier of assessment where receiving water characteristics in different use regions are considered along with regional specific rainfall values. The highest tier of assessment is undertaken in both spatial and temporal scales.

To perform these assessments, even at the screening level, a significant amount of Australian specific data have been analysed and applied. These data relate to slopes in different land use areas along with soil profiles, rainfall values and streamflow data in different regions. The following sections describe how these data were obtained and are applied in the different tiers of the runoff risk assessment.

Australian soil profiles and influence on runoff curves

Concern was raised that the Q/P runoff curves (Q = runoff in mm; P = rainfall in mm) in the current consultation paper will underestimate runoff from soils heavier than "loamy" soils, as the runoff curves were only available for "Sandy" and "Loamy" soils.

Consequently, a major change in the calculation of "Q" has been adopted, using the data and approach provided by the US Department of Agriculture (USDA). A combination of a hydrologic soil group (soil), a land use and treatment class (cover) is a hydrologic soil-cover complex. Tables and graphs of runoff curve numbers (CNs) assigned to such complexes are available from the USDA in their National Engineering Handbook Part 630. The chapters for this handbook are available electronically. The four hydrologic soil groups (HSGs) based on clay content are A (< 10% clay), B (10-30% clay), C (30-40% clay) and D (> 40% clay).

In addition, the Australian Grains research & Development Corporation (GRDC) and others have provided measured information on soil clay characteristics in Australian dryland cropping regions (www.soilquality.org), which allows classification of these regions within the hydrologic soil group (HSG) groupings. This information can be combined with the USDA curve-number information to develop composite runoff curves for the different regions.

The clay content information can be found at www.soilquality.org.au. The following table summarises the HSG contributions for dryland cropping for each State:

Table 54: Fraction of different hydrologic soil groups (HSGs) in dryland cropping zones for different States

State	Number of measurements	< 10% clay (A)	10-20% clay (B)	20-40% clay (C)	> 40% clay (D)
Queensland	97	0.0206	0.0103	0.1650	0.8041
New South Wales	575	0.1374	0.4383	0.3253	0.0991
Victoria	120	0.1333	0.4083	0.4000	0.0583
Tasmania	219	0.0091	0.1826	0.3607	0.4475
South Australia	167	0.1018	0.5030	0.3892	0.0060
Western Australia	2004	0.7425	0.1891	0.0619	0.0000

Composite curve numbers have been derived for the different states. Separate curve numbers are derived for the different scenarios based on the hydrologic soil group curve numbers identified in the USDA National Engineering Handbook, and the relative contribution for each soil group from the different state profiles. The formula applied is:

Equation 2

$$Composite\ Curve\ Number = \sum (CN_i\ X\ f_i)$$

Where: CN = USDA Curve number for soil hydrological group *i*; f = fraction of contribution for soil hydrological group *i*.

The above contributions are based on soil data in dryland cropping regions. In the absence of further analysis of soil clay contents for other cropping systems, at this stage PERAMA adopts them for different situations in the

same states. For example, when considering Queensland production horticulture, which is undertaken on Natural Resource Management areas, the same soil profile is applied in all areas.

To maintain a conservative approach, the composite curve numbers are based on the most conservative hydrologic condition assigned to the USDA curve numbers, that is, a "poor" hydrologic condition. This is based on combinations of factors that affect infiltration and a poor hydrologic condition adopting factors that impair infiltration and tend to increase runoff. PERAMA currently applies the following scenarios for a range of cropping categories and application types:

Table 55: Range of scenarios currently available in PERAMA and corresponding USDA cover types and curve numbers

PERAMA Scenario	USDA Cover type	Curve number for USDA soil hydrologic group			
		A	B	C	D
Turf, Turf farms	Pasture, fair (50-75% ground cover)	49	69	79	84
Turf, Golf courses	Pasture, fair (50-75% ground cover)	49	69	79	84
Turf, Playing surfaces	Pasture, good (>75% ground cover)	39	61	74	80
Stubble retention/no till	Pasture, poor (<50% ground cover)	68	79	86	89
Row crop, Straight row	Row crops, straight row	72	81	88	91
Row crop, contoured	Row crops, contoured	70	79	84	88
Rights-of-way	Specifically derived scenario. Non-region specific. Applies CN 86 for background catchment and CN93 for rights-of-way.				
Pasture	Pasture, poor (<50% ground cover)	68	79	86	89
Orchards, pasture inter row	Orchard or tree farm; 50% wooded, 50% pasture	57	73	82	86
Orchards, bare soil inter row	AEA derived; 50% wooded, 50% bare soil	61	76	84	89
Legume	Close seeded or broadcast legumes	66	77	85	89
Grain, Straight row	Small grain, straight row	65	76	84	88
Fallow, Crop residue	Fallow, crop residue	76	85	90	93
Fallow, Bare soil	Fallow, bare soil	77	86	91	94

Determination of slope for screening assessment

Slope values for the different cropping categories and regions have been determined based on a two-way analysis between slopes and land use from the Australian Government Department of Agriculture and Water Resources multi-criteria analysis shell (MCAS-S) tool, Version 3.1-2014. This tool is available at: http://www.agriculture.gov.au/abares/data/mcass. The MCAS data is based on the 2011 dataset with a 2 km^2 resolution.

In order to obtain more reliable results, 15 different slope class sizes were assessed from 0–2% in 0.25% increments, then from 2–2.5%, 2.5–3%, 3–4%, 4–6%, 6–8%, 8–10% and 10–20%. To remain conservative, returned results were assumed to be at the upper value of the mean slope range being assessed. For example, the area identified in the 3-4% slope class was assumed to all have a mean slope of 4%.

The following figure shows this analysis for the 0–2% slopes in the Mackay/Whitsunday region of QLD for sugarcane and horticultural areas:

Figure 1: Examples of slopes analysis

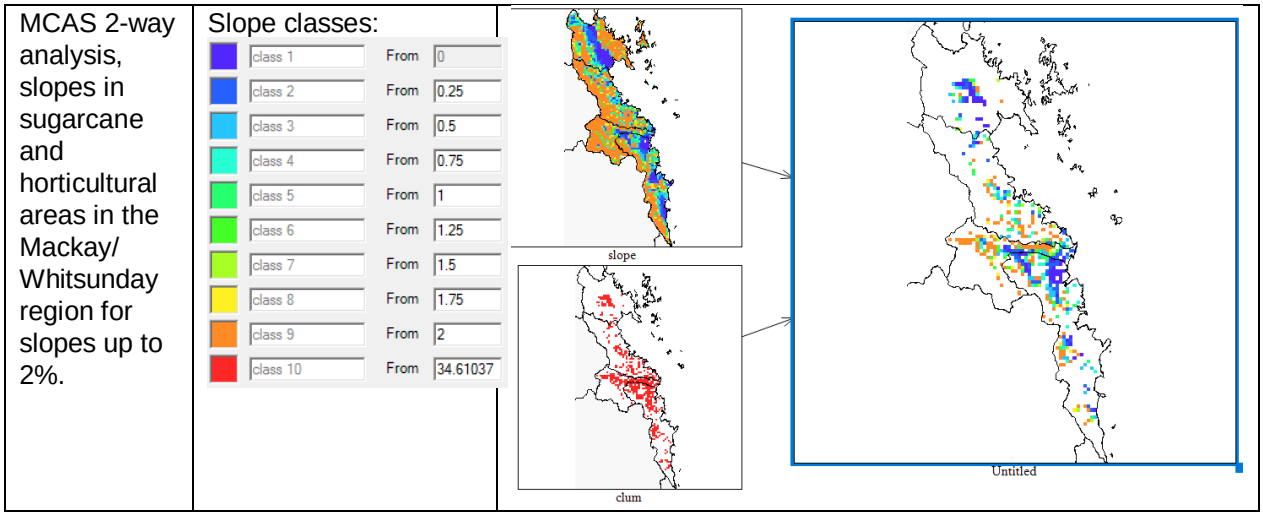


Table 56: Land use categories applied in calculating slope values

Cropping category	MCAS Land Use Category (Categories) applied		
Dryland	Dryland cropping		
Tropical/subtropical	Dryland horticulture	Irrigated horticulture	
Sugarcane	Dryland horticulture	Irrigated horticulture	Irrigated cropping
Horticulture, orchards	Dryland horticulture	Irrigated horticulture	
Horticulture, non-orchards	Dryland horticulture	Irrigated horticulture	
Pasture	Grazing modified pastures	Irrigated pastures	
Turf, golf courses	Urban	Rural residential	
Turf, turf farms	Dryland horticulture	Irrigated horticulture	
Turf, playing fields	Scenario specific. Set at 2%.		

There were insufficient data in the Mackay/Whitsunday region for tropical/subtropical horticulture so the data set was extended to include irrigated cropping.

While slopes for horticulture orchards and horticulture non-orchards were based on the same data set, orchard slopes in South Australia were restricted to an analysis of the Onkaparinga River Catchment, which resulted in steeper slopes than for total South Australian horticulture.

The slope data are not normally distributed. Low slope values are generally far more prevalent than high ones, and an exponential distribution is assumed.

Consequently, 90th percentile slope values have been calculated from the mean as follows:

$$90th\ percentile\ slope = -\mu\ X \log(0.1)$$

Where: μ = mean slope (%); Log = natural (base e) logarithm.

In the runoff modelling, the 90th percentile slope is applied in the first step of refinement while the mean slope is applied in the higher tier of assessment.

Screening runoff risk assessment – water column

This is a screening assessment only. It is taken as a worst case and substances that pass this level do not require further assessment.

Table 30 shows the soil profile with the highest runoff propensity is Queensland with > 80% of topsoils containing > 40% clay while Table 31 shows the worst case scenario based on runoff curve numbers to be a fallow, bare soil (highest curve numbers). Therefore, the worst case composite runoff curve is generated for Queensland under fallow, bare soil conditions. This composite curve number is applied in the screening assessment regardless of the situation being assessed. Substances that pass this level do not require any further assessment.

The screening level assessment is performed using the traditional standard water body scenario applied in Australian assessments whereby a 10 ha catchment feeds a 1 ha surface area water body with an initial depth of 10 cm. The rainfall value in this scenario that results in the maximum receiving water body concentration is 8 mm. At rainfall greater than this, additional runoff begins to dilute the concentration.

It is clear from the 90th percentile slope figures above that slopes can be significantly steeper in Tasmania than other horticultural/pasture/turf areas in the country. At the initial screening assessment, the slope is fixed at 8%. This is expected to cover > 90% of situations at the screening step. Horticultural, turf (golf courses) and pasture uses in Tasmania have 90th percentile slopes exceeding this value and in such cases, modelling runoff risk for Tasmania should proceed immediately to the Step 1 refinement.

Step 1 runoff refinement (some spatial considerations)

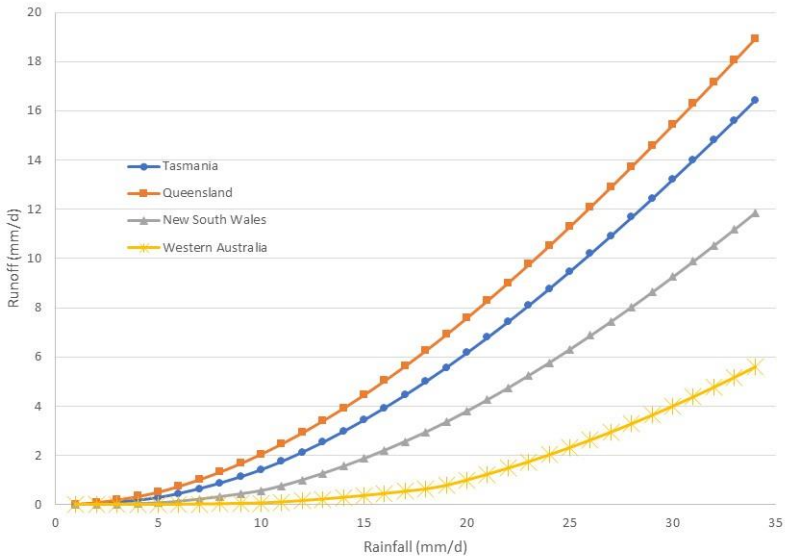
The first step of refinement from the screening assessment takes additional spatial influences into account in terms of soil profiles. These influence the runoff curve (Q/P) in Equation 3 above. This refinement step also moves from the worst case runoff curve for fallow, bare soil to more appropriate curves that consider influences of hydrologic soil-cover complexes. The range of scenarios currently available in PERAMA and corresponding USDA cover types and curve numbers are shown in Table 31 above. The following figures shows the influence of soil types and cover type on predicted runoff:

Influence of soil type on runoff curves

The profile most dominated by clay is found in Queensland and the profile most dominated by sand is found in Western Australia. For the same rainfall value, the predicted runoff rates are significantly different.

The following runoff curves for Queensland, Tasmania, New South Wales and Western Australia are shown for fallow, bare soil situation. For a given 30 mm rainfall, the predicted runoff from these different soil profiles is 15.4, 13.2, 9.3 and 4.0 mm/d. This demonstrates the importance of considering different regional characteristics in the runoff assessment rather than the “one size fits all” approach that has traditionally been applied in Australian risk assessments.

Figure 2: Influence of different soil profiles on runoff curves



Influence of cover type on runoff curves

The range of scenarios currently available in PERAMA and corresponding USDA cover types and curve numbers are shown in Table 55 above. Composite curve numbers are calculated for each soil type based on the contribution of the hydrologic soil group. In PERAMA, a rounded curve number value was not used. Rather, a composite curve number was calculated as follows.

Equation 3: Composite Curve Number Equation

$$CCN = \sum CN_i \; X \; F_i$$

Where:

CNN = Composite Curve Number;

CN*i* = Curve number for soil hydrologic group, *i*;

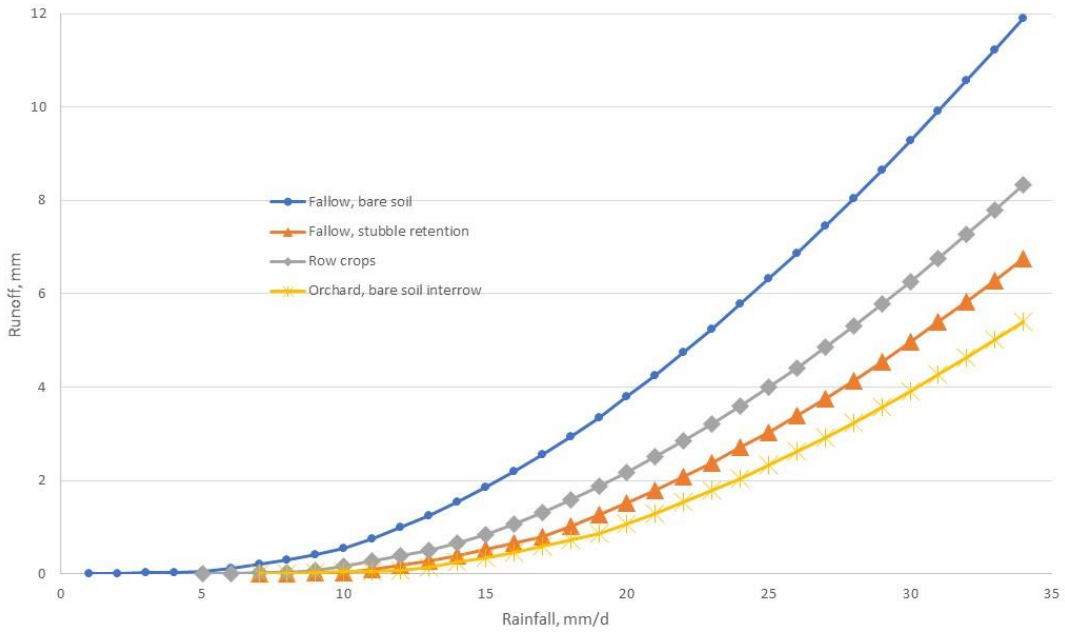
F*i* = Fraction of contribution of soil profile to soil hydrologic group, *i*.

An example is provided as follows:

Table 57: Development of composite curve number, Victoria, Row crop, straight row, poor hydrologic condition

Hydrologic soil group	< 10% clay (A)	10-20% clay (B)	20-40% clay (C)	> 40% clay (D)
Curve number (USDA)	72	81	88	91
Contribution from state soil profile	0.1333	0.4083	0.4000	0.0583
SUM contribution	83.18 – Final curve number applied for Victoria			

Figure 3: Influence of different soil cover on runoff curves



For a 30 mm rain event, the different curve numbers predict runoff of 9.3 mm, 5.0 mm, 6.3 mm and 3.9 mm in fallow (bare soil), fallow (stubble retention), row crops and orchards with a bare soil inter row, respectively. This demonstrates the importance of considering different ground cover situations in the runoff assessment rather than the "one size fits all" approach that has traditionally been applied in Australian risk assessments.

Limitation of step 1 refinement level

While this level of refinement allows for regional consideration in terms of slopes (90th percentile values), soil types and their influence on the runoff curve, and ground cover from different cropping systems, it still considers runoff in the standard Australian scenario of a 10 ha catchment feeding a 1 ha pond 15 cm deep. The associated rainfall values applied are those that result in the highest predicted water concentration, and do not reflect reality. As an example, when considering runoff from the composite curve number for each state in orchards (bare soil interrow), rainfall values of 18, 28, 28, 21, 29 and 47 mm are applied for Queensland, New South Wales, Victoria, Tasmania, South Australia and Western Australia, respectively. This is clearly unrelated to actual likely rainfall found in different regions. The likelihood of a 47 mm/d rainfall event in the orchard growing regions of Western Australia is low, but reflects the sandy soil profile. While the value in Queensland may be more realistic, at this level of refinement, it means not all regions are being considered equally.

In order for a more equal consideration, actual regional specific rainfall data require consideration. This is applied in the Step 2 level of refinement if needed. In addition, characteristics of receiving waters are also taken into account. The development of the stream flow data and rainfall data allow the assessments to be performed in both space and time.

Step 2 refinement (In-stream analysis; spatial and temporal consideration)

Background catchment and its influence

There are additional factors that require consideration in analysing the rainfall and stream flow data prior to undertaking the highest level of refinement in the assessment. Firstly, the stream flow data are “fixed” in that they are historic and represent the characteristics of their catchments. This means that for a given stream flow rate, any increase in rainfall will result in higher in-stream concentrations. Therefore, it is important that the rainfall value applied in the assessment is realistic.

In order to determine an appropriate rainfall value, a background catchment has been modelled, recognising that a certain amount of rain is required prior to runoff commencing. This background catchment is based on the USDA curve numbers for “Herbaceous – mixture of grass, weeds and low-growing brush, with brush the minor element”. The curve numbers are then applied for hydrologic soil groups A, B, C and D are 58, 71, 81 and 89, respectively, and composite curve numbers generated then for each state based on their respective soil profiles.

A "Fair" hydrologic condition has been applied to account for areas in the catchment that may be more susceptible to runoff. A trigger of 0.1 mm runoff was adopted such that where runoff was predicted to be <0.1 mm, it was not considered in determining the minimum rainfall value. The reason is that, for soils with any clay content, the commencement of runoff occurs at much the same rainfall (~7 mm). This does not allow for consideration of the contribution of the higher runoff component of the soils.

Based on the above composite curves, the background rainfall values for QLD, NSW, VIC, TAS, SA and WA are set at 9 mm, 12 mm, 15 mm, 9 mm, 16 mm and 16 mm respectively. The WA high sand component actually predicts 23 mm required prior to the commencement of runoff, but this is considered too high to obtain meaningful results and the SA value has been applied as a default for Western Australia.

Determination of final rainfall values

The background catchment rainfall values are applied to the cumulative frequency distributions of historic rainfall for different regions/states to obtain 25th percentile and 75th percentile rainfall levels for application in the in-stream analysis such that the cumulative frequency distributions commence from the background rainfall value.

Rainfall statistics were obtained from weather stations within cropping areas of interest, and the number of stations assessed was proportionate to the size of the area being considered. Where > 4 stations were assessed within a region, the 90th percentile values have been applied in PERAMA, which provides a conservative value of rainfall. For smaller growing regions, for example within the Queensland production horticulture NRMs, mean rainfall values were adopted as only a small number of weather stations were assessed and variability between these was (understandably) not high.

In order to perform the temporal assessment, rainfall data were separated by season for state based assessments (dryland, horticulture, pasture, turf) and by month for tropical/subtropical production horticulture assessments. The monthly time scale was considered important due to rainfall patterns being summer dominated and the wet season time period not necessarily corresponding with standard seasons.

PERAMA assesses for a current total of 528 different rainfall values depending on cropping category, state, region, percentile stream flow being assessed or the time period being considered. The following table provides a summary of some of these rainfall values to demonstrate the variability in time and space.

Table 58: 25th and 75th percentile rainfall values for dryland cropping in some States applied in PERAMA

	Queensland		Tasmania		Western Australia	
Percentile stream flow	25th	75th	25th	75th	25th	75th
Summer	13.1	30.7	12.0	25.0	20.3	39.2
Autumn	12.9	30.6	11.8	24.4	19.3	32.0
Winter	12.7	29.0	12.1	22.9	18.5	27.6
Spring	12.6	30.1	11.7	21.9	18.6	28.1

It may appear curious that rainfall values for Western Australia are significantly higher than those for Queensland or Tasmania. This, however, reflects the soil profile where a much greater amount of rain is required to generate runoff in the very sandy soils found in Western Australia. These figures still do not include a measure of probability of the rainfall values. Rainfall probability is not assessed further in PERAMA, but may be a further refinement option if required. For example, the 75th percentile rainfall value in Western Australia in summer is 39.2 mm/d. This is compared to the 75th percentile stream flow rate. Stream flows in Western Australia in the dryland areas are very small, but may still occur. However, the likelihood of a 39.2 mm rainfall event is low. Based on rainfall data from 45 weather stations throughout the Western Australian wheat belt and applying 90th percentile results, the probability of any day actually being wet (> 0.1 mm rain) is only 8.9%. The probability of any of these wet days resulting in rainfall exceeding 39.2 mm is only 2.6%. Therefore, the overall probability of a rainfall event in the summer months of 39.2 mm/d is 0.2%. Even with the lower 25th percentile value of 20.3 days, the overall probability of exceeding this in the summer months is < 1%.

Conversely, in the summer rainfall dominated Queensland dryland cropping regions, the probability of a wet day in summer is 22.1% and the probability of exceeding the 13.1 mm/d value on a wet day is relatively high at 21.1%.

Stream flow rates – consideration of base flow

In addition to runoff waters entering the stream/river, flow rates can already exist from other sources such as stream base flow and runoff waters originating from elsewhere in the catchment. In rainfall runoff models used to estimate design floods, rainfall runoff/filtration is classified as quick flow (surface runoff) and base flow is essentially the result of groundwater flow (Tularam and Ilahee, 2005). The methodology described for refined runoff assessments in this document focuses on in-stream concentrations that result from quick flow, which is a direct result of rainfall.

An important component of the stream analysis, both in the proposed methodology here and that described in the European FOCUS[2] stream scenarios, relates to base flow. If there was no consideration of a baseflow component, stream flow rate percentiles would be based on the cumulative frequency distribution curves using the total dataset and therefore overestimation of in-stream concentrations would be likely. This is because the increased flow resulting from rainfall induced runoff would not be considered as an additional flow rate. However, the rainfall value

[2] FOCUS – FOrum for Co-ordination of pesticide fate models and their USe. Approved versions of FOCUS simulation models and FOCUS scenarios are available at: http://esdac.jrc.ec.europa.eu/projects/focus-dg-sante

used to predict runoff concentrations would remain the same, so in-stream concentrations would be overestimated.

In the FOCUS scenarios, to derive a baseflow component to the hydrological flows feeding the surface water bodies, parameters quantifying the catchment “Base Flow Index” (BFI) were needed. The BFI quantifies the fraction of long-term total flow in a catchment that is represented by base flow. This parameter was derived from an estimated soil hydrological class at each representative field site for the scenarios available in Europe. Estimated soil hydrological classes have associated set of empirically-derived coefficients describing stream flow characteristics. Based on the soil hydrological characteristics for each of the FOCUS surface water scenarios, BFI values of between 0.17–0.79 were adopted.

Such a classification tool is not available in Australia. To remain conservative, a simple method for calculating an individual baseflow index for each monitoring station during each separate season has been applied. For the analysis, positive flow is considered to be > 0.001 m/s (~0.09 km/h). Using the standard stream of 3 m wide and 15 cm deep, this equates to a flow rate in terms of volume of around 1.15 L/s (0.1 ML/d) and this low rate has been used as the positive flow cut-off in an attempt to reduce possible inconsistencies in measuring low flow conditions between the different monitoring stations.

All the long term monitoring flow data have been separated by season and the time (%) of positive flow determined for each season.

The probability of rainfall within the season of interest for each region has been obtained by taking the lower 10th percentile of rainfall probability for each season. This is a likelihood of ‘any’ rainfall, not rainfall that could generate runoff as that value will be highly variable depending on other factors such as soil type and slopes within different catchments.

A unique BFI has then been calculated for each monitoring station for each season as the difference between the time (%) in positive low and the probability of any rainfall. In many cases, particularly in drier seasons, the stream flow data showed positive flow periods to be less than the frequency of rainfall. This is not surprising as the rainfall likelihood was for any rainfall, not the amount resulting in runoff (which would be a lower likelihood). In these instances, the BFI was set at 0 meaning that any flow in these systems is assumed to be the result of quick-flow only. It demonstrates the conservatism of the approach. If the probability of rainfall resulting in runoff was considered, the probability is significantly reduced, which would in turn lead to a higher BFI (less conservative for in-stream concentrations).

The stream flow data for South Australia were sparse. In this regard, there is essentially no surface water in South Australian dryland cropping regions west of the Spencer Gulf. Victorian dryland stream flows have been applied as a surrogate for South Australia at this stage.

Similarly, there appear to be stream flow data available within Tasmania, but the data are not easily obtained. Currently, Victorian dryland stream flows are used for in-stream analysis in Tasmania. Efforts are underway to compile an appropriate Tasmanian stream flow data base and PERAMA will be updated accordingly when possible.

Stream flow rates- cumulative frequency curves

To model the in-stream concentrations, the in-stream equation in Probst (2005) has been applied as an extension to Equation 1 as follows:

Equation 4: In Stream Calculation Module

$$Pc = L\%runoff \text{ X } Pa \text{ X } \left(\frac{1}{Qstream \text{ X } \Delta T}\right)$$

Where:

Pc = simulated mean pesticide in-stream concentration (µg/L);

L% runoff = % of application dose available in runoff water as dissolved substance (Equation 1);

Pa = amount of pesticides applied to the simulation area (µg);

Qstream = peak stream flow during rain events (L/s);

ΔT = duration of heavy rain event (seconds).

The standard assumption is that the intensity over short periods is much more than the intensity determined over a 24 h period, and in this method, the 24 h rainfall event is assumed to all occur over a duration of 1 hour for purposes of mixing with in-stream flow rates to predict the in-stream concentration (that is, ΔT = 3600 seconds). This is considered to be a conservative assumption but is thought to be applicable in predicting higher-risk "first flush" events and is applied in PERAMA for the 25th percentile flow rates. A 2 hour rainfall intensity is applied for the 75th percentile flow rates.

For a given state or region being assessed, all stream flow rates from individual monitoring stations are considered to derive a cumulative frequency curve of in-stream concentrations for any given time period or stream flow percentile. In its totality, this is a complex and time consuming process.

Example of total Step 2 refinement process

A worked example is provided here to demonstrate the total methodology that is applied for PERAMA to predict a percentage of receiving waters in a given region at a given time where the in-stream concentration remains below an aquatic toxicity threshold. The examples are based on a fictitious residual herbicide with a soil half-life of 26 days, Kd = 0.40 L/kg and an aquatic regulatory acceptable level of 6 µg/L. The application rate is 1500 g ac/ha. A fallow with stubble retention/no till scenario is modelled for Tasmania for the 25th percentile stream flow value in Autumn only:

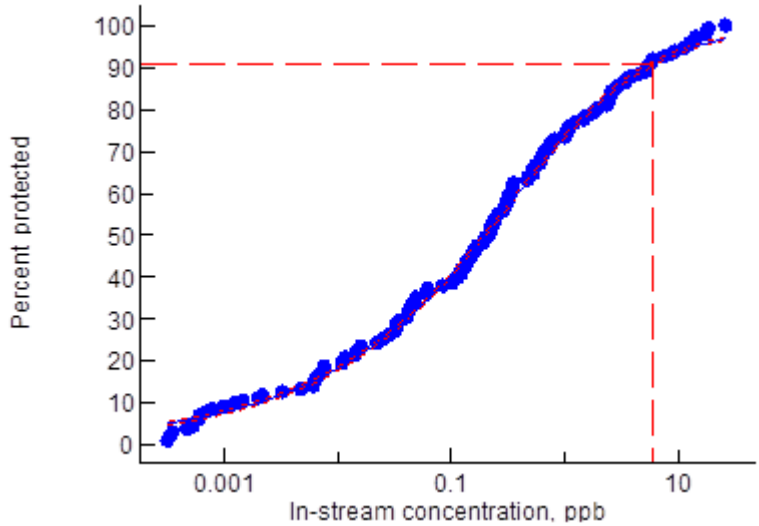
1. Calculate Q/P within Equation 1 – this is based on the composite runoff curve developed with the Tasmanian soil profile:
   Q = (((-0.000099 * ([Rainfall] ^ 3)) + (0.0162 * ([Rainfall] ^ 2))) + (-0.137 * [Rainfall]))
   The rainfall value was determined to be 11.8 mm/d, Q = 0.476; Q/P = 0.04,

2. Calculate % runoff: Filling out Equation 1, %runoff = 0.015 in the dissolved phase.

3. Calculate in-stream concentrations based on real world stream flow data:

This step is performed for every monitoring station within a region being assessed. Victorian streamflow data are being applied as a surrogate data set for Tasmania in PERAMA at this stage and that data library contains stream flow data from 145 individual monitoring stations. For all stations, the 25th percentile stream flow rate is calculated. A cumulative frequency curve for in-stream concentrations is then constructed:

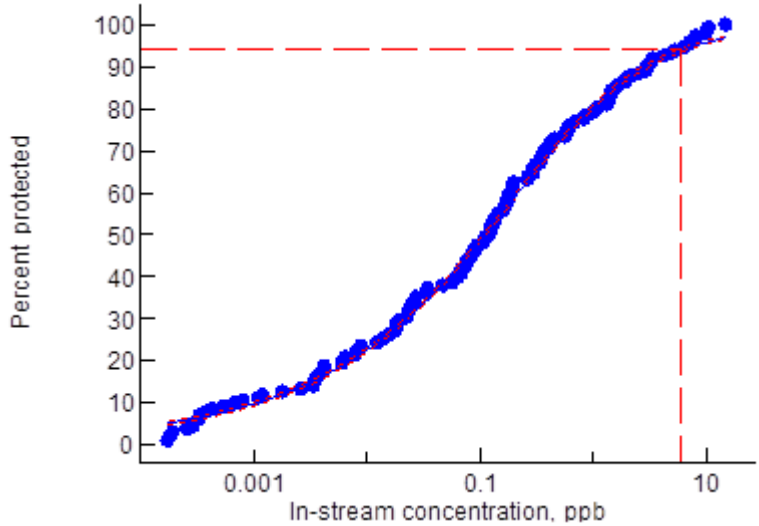
Figure 4: Tasmania, percent protected, Autumn application, Fallow, stubble retention



The percent protected is predicted to be 91% of receiving waters.

With the software, the same exercise can rapidly be performed to see the change in protection level if the pre-emergence application is made to formed fields rather than those still in fallow – the following distribution is based on the composite curve number for grains, straight row:

Figure 5: Tasmania, percent protected, Autumn application, grains, straight row



Runoff is predicted to decrease to 0.008% in the dissolved phase with the same rainfall amount.

The percent protected is predicted to be 94% of receiving waters.

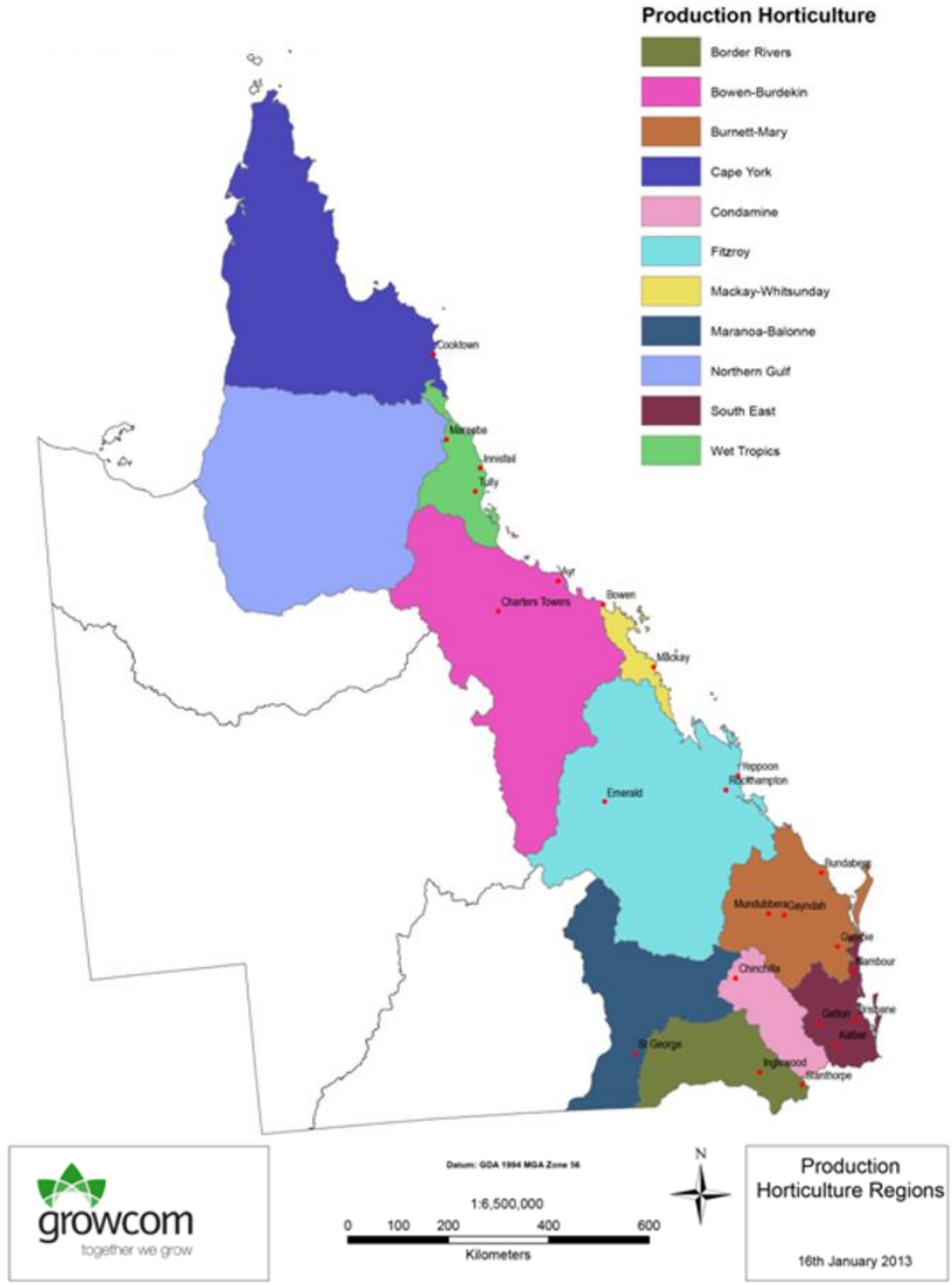
This short example has required calculations of 145 in-stream concentrations for two scenarios and the underlying non-linear regression modelling to determine the percent of protection. Done long hand, this in itself is a time

consuming exercise. To undertake a single scenario assessment for a state based assessment, a total of 871 in-stream concentrations are calculated for two stream flow percentiles across all states. Distributions are developed for six states, 4 seasons and 2 stream flow percentiles (48 distributions) for a single scenario.

When considering tropical and subtropical situations, distributions are developed for 9 different regions, 2 stream flow percentiles and 12 time periods because the assessment is undertaken monthly. This totals 216 different distributions for a single scenario.

The time required for such refined assessments is a limiting factor, and the scope for human error is large given the high number of different curve numbers, slope values, rainfall values and stream flow values that need to be included. However, all the distribution algorithms have been developed and incorporated into PERAMA. It is now possible to undertake single scenario assessments rapidly and compare scenarios rapidly and consistently.

APPENDIX 2: QUEENSLAND'S KEY PRODUCTION HORTICULURE GROWING REGIONS



SOURCE: http://www.growcom.com.au/_uploads/QldProdHortWDP.pdf

APPENDIX 3. ADDITIONAL ENVIRONMENTAL FATE DATA

Route and rate of degradation in soil

Photolysis

Title	[Methiocarb]: Photolysis of methiocarb on soil surface
Reference	Stupp, H.-P. 2002
Test Guideline	EC-Directive 91/414/EEC Annex II , the SETAC Procedures for Assessing the Environmental Fate and Ecotoxicity of Pesticides and the US EPA Pesticide Assessment Guidelines
Data Validity	1
Data Relied On	Yes - the data were considered to be critical and were relied on in this assessment.

The photodegradation of methiocarb was studied on thin layers (approx. 3 mm) of a sandy loam soil (Howe, Indiana, USA: pHCaCl2 = 6.7, org. C = 1.12%). The test system consisted of glass vessels sealed with a quartz glass cover plate, attached with traps for collection of CO2 and volatile organic compounds. The test was conducted as a mass balance study with radiolabelled parent compound [phenyl-1-14C]Methiocarb using 2.59 µg ac/3 g dry mass of soil, corresponding to about 120 g ac/ha (calculated for a soil density of 1.5 g/cm³ and 1 cm depth of soil).

The water content of soil was adjusted to 75% of the 1/3 bar soil moisture. Portions of treated soil (3 g dry mass) were continuously irradiated in a xenon chamber (Suntest®) simulating sunlight at a temperature maintained at 20°C. In the test duplicate samples as well as dark controls (single samples) were taken for processing and analysis 0, 0.25, 1, 2, 5, 7 and 9 days after treatment (DAT). The total amount of soil was extracted at room temperature and additionally under aggravated conditions. The crude extracts were analysed by means of radio-thin-layer chromatography (TLC). For identification of the transformation products co-chromatography methods with the reference standards were used.

The test conditions outlined in the study protocol were maintained throughout the study. The total recovery of radioactivity (means of duplicates) ranged from 97.2 (±0.3)% to 103.8 (±0.7)% for the irradiated samples, and from 98.0% to 106.0% for the dark controls. The complete material balance found at all sampling intervals of the tests demonstrated that no significant radioactivity dissipated from the vessels or was lost during processing. The degradation kinetics was calculated using linear regression tools of MS-EXCEL.

Distribution of Radioactivity in the Extracts (mean of irradiated samples, % AR)

Conditions	DAT	Extracted (%AR)	Methiocarb	M01	M03	M02	M04
Irradiated	0	102.08	98.5	3.1	ND[2]	ND	ND
	0.25[1]	89.41	37.21	15.51	ND	1.2	33.51
	1	100.16	34.2	57.2	0.6	1.4	6.3
	2	95.05	18.3	51.4	1.2	2.6	17.5
	5	83.8	15.8	31.7	1.5	3.1	26.1
	7	81.85	16.9	27.9	1.4	2.2	28.8
	9	76.97	12	28.9	1.5	2.4	26.4
Dark	0.25	105.41	97.8	5.5	ND	ND	0.6
	1	103.83	93.2	9.4	ND	ND	0.6

Conditions	DAT	Extracted (%AR)	Methiocarb	M01	M03	M02	M04
	2	100.97	84.1	13.9	ND	ND	1.2
	5	99.22	77.6	18.3	ND	ND	2.7
	7	99.06	74.2	21.3	ND	ND	2.8
	9	94.78	68.6	20.7	ND	ND	3.8

[1] The extract at 0.25 DAT were not considered valid. [2] ND = Not detected.

Minor (<1% AR) amounts of M10 were found in the irradiated samples. Three unknown metabolites (up to 2.2% AR) were found during the exposure period in the irradiated samples.

Degradation Kinetics of Methiocarb on the Surface of Soil Howe

	Irradiated soil samples	Dark soil samples (control)
Rate const. [/day]	0.918	0.040
DT50 [days]:	0.76	17.2
DT75 [days]:	1.51	34.4
DT90 [days]:	2.51	57.2
Square of R:	0.878	0.954
DT50 expressed as solar summer days in Phoenix*)	3.88	N/A

* Evaluated according to radiometry.

Photomineralization of the test substance to radiolabeled carbon dioxide accounted for max. 8.0% (mean) of the applied radioactivity (AR) on DAT-7 indicating the complete breakdown of the test substance. In dark samples no mineralization was observed. Non-extracted residues (bound residues after hot extraction) were max. 12.6% of AR (DAT-9) in irradiated samples (under dark conditions max. 3.2% of AR on DAT-9).

In irradiated samples the major metabolites observed were the oxidation product methiocarb sulfoxide (max. 57.2% of AR at DAT-1) and the consecutive metabolite methiocarb sulfoxide phenol (max. 28.8% of AR at DAT-7). Other metabolites (i.e. methiocarb sulfone, methiocarb phenol, methiocarb methoxy sulfone and three not identified metabolites) accounted for max. 3.1% of AR.

In dark samples the same metabolites were observed but to less extent. Methiocarb sulfoxide was max. 21.3% of AR on DAT-7 and the consecutive metabolite methiocarb sulfoxide phenol was max. 3.8% of AR on DAT-9. Other metabolites were not detected (< 0.1% of AR). Not extracted residues after hot extraction were max. 3.2% of AR at DAT-9. Volatiles were max. 0.2% of the AR.

Photodegradation of methiocarb on soil surface was very fast. The degradation product was methiocarb sulfoxide. Further hydrolysis of methiocarb sulfoxide generated methiocarb sulfoxide phenol, which was transformed to carbon dioxide. Additionally, a significant formation of bound residues occurred.

Aerobic biodegradation

Title	Aerobic degradation and metabolism of methiocarb in soil
Reference	Brumhard, B. 2002
Test Guideline	Commission Directive 95/36/EC amending Council Directive 91/414/EEC, and the SETAC Procedures for Assessing the Environmental Fate and Ecotoxicity of Pesticides
Data Validity	1

Data Relied On	Yes - the data were considered to be critical and were relied on in this assessment.

The aerobic degradation of methiocarb was studied in four different soils (sandy loam Laacher Hof AXXa, pHCaCl2 = 6.4, org. C = 1.8%; silt loam Frankenforst, pHCaCl2 = 7.6, org. C = 0.72%; silt Hoefchen am Hohenseh 4a, pHCaCl2 = 7.2, org. C = 2.62%; loamy sand Standard soil BBA 2.2, pHCaCl2 = 6.3, org. C = 2.48%) for a maximum of 120 days under aerobic conditions in the dark at 20°C. The soil moisture corresponded to 40% of maximum water holding capacity each. [Phenyl-1-14C]- labelled methiocarb was applied at the nominal rate of 0.13 mg ac/kg dry soil, equivalent to the proposed single maximum use rate of 100 g ac/ha calculated for 5 cm depth of soil.

The test system consisted of Erlenmeyer flasks attached with traps for collection of CO2 and volatile organics. Entire flasks were processed and investigated at days 0 (approx. 2 hrs), 0.25, 1, 3, 7, 17, 45, 80 and 120. The methiocarb residues were analysed by thin-layer chromatography. For identification of the transformation products co-chromatography and spectrocopic methods (LC/MS and LC/MS/MS, NMR) were used.

Results: During the study the total recovery of radioactivity (RA) in soil Laacher Hof AXXa ranged from 86.8% to 101.9% of applied radioactivity (AR; mean of duplicates), and the mean was 98.0% (SD) ±4.5). In soil Frankenforst the total recovery of RA ranged from 92.6% to 102.0%, and the mean was 97.8% (SD ±2.8). In soil Hoefchen the total recovery of RA ranged from 92.6% to 100.2%, and the mean was 97.0% (SD ±2.5). In soil BBA 2.2 the total recovery of RA ranged from 96.0% to 102.0%, and the mean was 99.2% (SD ±1.6). Recovery of vessel day 120 of soil Hoefchen was <<90% owing to losses of volatile radioactivity during quantitation procedure of the 14CO2. Therefore this sample was not included into the mean calculation. The complete material balance found at all sampling intervals demonstrated that no significant RA dissipated from the vessels or was lost during processing.

Degradation kinetics of Methiocarb in Four German Soils

	Laacher Hof AXXa	Frankenforst	Hoefchen	BBA 2.2
Parameter:	Simple 1st Order			
k (1/days)	0.502	0.547	0.937	0.475
DT50 (days)	1.4	1.3	0.7	1.5
DT75 (days)	2.8	2.5	1.5	2.9
DT90 (days)	4.6	4.2	2.5	4.9
R^2	0.999	1.00	0.993	0.999

Methiocarb is quickly degraded in soils. In the four soils Laacher Hof AXXa, Frankenforst, Hoefchen and Standard soil BBA 2.2 the mean DT50 was 1.2 days. Furthermore, the data indicated that the major metabolites were continuously degraded, that no metabolite accumulated towards the end of the study, and the formed bound residues were participating in the natural carbon cycle of soil.

Degradation of Methiocarb and Behaviour of Metabolites in Soil Laacher Hof AXXa. Mean Values in % of applied radioactivity

Incubation time [d]	Extracted radioact.	Methiocarb	M01	M04	M05	M02	M10
0	96.0	80.0	15.4	<0.1	n.d.	n.d.	n.d.
0.25	95.0	72.8	20.6	1.4	n.d.	n.d.	n.d.
1	91.9	48.6	35.8	6.6	n.d.	n.d.	n.d.
3	86.5	17.7	48.2	18.2	n.d.	n.d.	n.d.
7	71.1	2.9	34.9	30.5	1.5	n.d.	n.d.
17	51.0	1.6	14.1	25.1	8.0	0.3	n.d.
45	31.1	n.d.	9.5	2.7	1.9	0.2	8.6
80	22.8	n.d.	1.6	3.2	6.4	n.d.	9.4
120	6.1	n.d.	0.6	0.9	1.5	n.d.	2.7

Degradation of Methiocarb and Behaviour of Metabolites in Soil Frankenforst. Mean Values in % of applied radioactivity

Incubation time [d]	Extracted radioact.	Methiocarb	M01	M04	M05	M02	M10
0	96.3	77.4	17.1	0.9	n.d.	n.d.	n.d.
0.25	91.5	68.8	17.7	4.8	n.d.	n.d.	n.d.
1	93.2	45.9	33.7	13.1	n.d.	n.d.	n.d.
3	81.5	14.5	32.2	32.5	1.5	n.d.	n.d.
7	56.7	0.8	13.8	35.8	4.8	n.d.	n.d.
17	24.8	n.d.	0.9	9.0	6.1	n.d.	3.3
45	7.7	n.d.	0.5	0.7	0.4	n.d.	1.5
80	4.1	n.d.	0.2	0.2	0.2	n.d.	0.6
120	4.0	n.d.	0.1	0.5	0.2	n.d.	0.3

Degradation of Methiocarb and Behaviour of Metabolites in Soil Hoefchen am Hohenseh 4a. Mean Values in % of applied radioactivity

Incubation time [d]	Extracted radioact.	Methiocarb	M01	M04	M05	M02	M10
0	97.4	80.0	15.2	0.9	n.d.	n.d.	n.d.
0.25	92.6	59.5	28.3	4.7	n.d.	n.d.	n.d.
1	88.2	30.1	46.4	11.2	n.d.	n.d.	n.d.
3	83.0	8.2	50.4	20.5	1.9	n.d.	n.d.

7	66.9	4.3	27.2	21.9	10.8	1.9	n.d.
17	33.8	n.d.	3.9	7.5	11.1	n.d.	9.8
45	10.3	n.d.	1.2	0.8	0.3	n.d.	7.7
80	7.7	n.d.	0.7	0.8	1.1	n.d.	3.4
120	5.6	n.d.	0.3	0.4	0.5	n.d.	2.6

Degradation of Methiocarb and Behaviour of Metabolites in Standard Soil BBA 2.2. Mean Values in % of applied radioactivity

Incubation time [d]	Extracted radioact.	Methiocarb	M01	M04	M05	M02	M10
0	97.0	84.9	11.0	<0.1	n.d.	n.d.	n.d.
0.25	95.2	73.8	20.3	0.8	n.d.	n.d.	n.d.
1	97.2	51.6	40.0	4.7	n.d.	n.d.	n.d.
3	92.4	20.4	58.8	12.3	n.d.	n.d.	n.d.
7	87.5	4.7	56.1	23.3	0.2	1.7	n.d.
17	62.8	n.d.	12.8	17.8	19.8	2.0	7.0
45	28.5	n.d.	1.6	3.3	9.0	<0.1	13.2
80	16.5	n.d.	0.8	1.3	2.3	n.d.	9.4
120	9.8	n.d.	0.5	0.4	0.7	n.d.	5.3

In the course of the experiment several metabolites were detected and quantified together with unaltered methiocarb. Four major metabolites, exceeding 10% of AR at any time during the study, and two minor degradation products appeared. Metabolite methiocarb-sulfoxide (M01) reached its maximum between 1 and 3 days after application in all soils and ranked highest in soil BBA 2.2 (58.8% of AR). In all soils, sulfoxide declined towards the end of the study. Metabolite methiocarb-sulfoxide phenol (M04) is a degradate of sulfoxide and reached its maximum between on day 7 in all soils. The maximum amount of sulfoxide phenol was observed in soil Frankenforst with 35.8% of AR and again it declined towards the end of the study in all soils. Major metabolite methiocarb-sulfone phenol (M05) reached its maximum on day 17 uniformly in all soils. The maximum amount of sulfone phenol was observed in soil BBA 2.2 with 19.8% of AR. Also this metabolite rapidly declined until study termination. The fourth major metabolite was spectroscopically identified as methiocarb-methoxy-sulfone (M10). This metabolite accounted for max. 13.2% in soil BBA 2.2. Its detection started at sampling interval day 17 and was matched by the complete disappearance of methiocarb. Additionally, methiocarb-sulfone (M02) was detected in minor amounts but only in a small time window (7 to 45 days after application). One unknown minor metabolite appeared accounting for max. 6.6%.

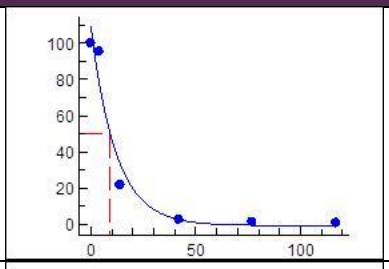
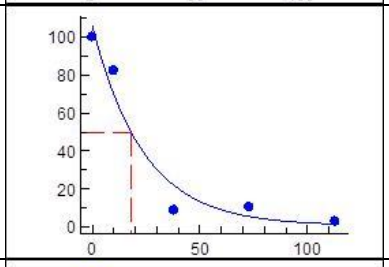
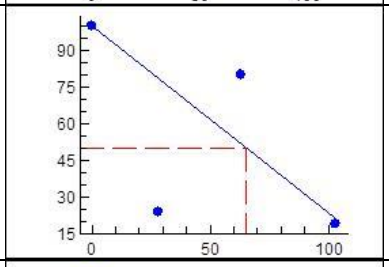
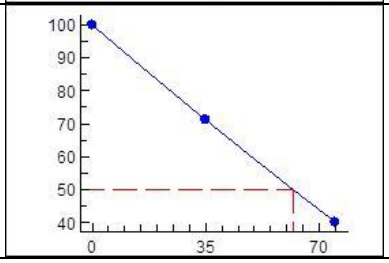
A significant formation of bound residues occurred along with the overall metabolism of the parent compound. The maximum level was reached after about 3 to 6 weeks already ranging from ca 32% to 58% of AR. In all four soils methiocarb underwent intense mineralization yielding amounts of $^{14}CO_2$ of up to 58% in 120 days of incubation.

The current laboratory study demonstrated that methiocarb is rapidly degraded and mineralized in aerobic soil. The DT50 in soil was found to be in the range of ca. 1 day (0.7–1.5 d), independent on the soil type. All of its metabolites are further degraded and, therefore, not expected to accumulate in soil.

Metabolite formation and decline: This study provides additional information on the formation and decline of the main metabolites. Half-life values have been modelled by the APVMA for use in the risk assessment. The data

from the time of peak formation to the end of the study have been fitted with a 2-phase exponential decay model (Double First Order in Parallel, DFOP model), a 1 phase exponential decay model or a linear decay model depending on the number of data points and which approach resulted in the best fit. The longest half-life for each metabolite will be used. The following summarises the results (longest half-life only shown here):

Metabolite soil half-life determinations – "X" axis = days; "Y" axis = % degradation from time of peak formation (day = 0)

Metabolite	Soil		Model/DT50 (days)
M01	BBA 2.2		1-phase exponential decay model. DT50 = 9.24 days
M04	Laacher Hof		1-phase exponential decay model. DT50 = 18.0 days
M05	Laacher Hof		Single First Order DT50 = 65.3 days
M10	BBA 2.2		Single First Order DT50 = 62.0 days

Adsorption, desorption and mobility in soil

Adsorption/desorption studies

Title	Estimation of the adsorption coefficient (Koc) of methiocarb-sulfoxide on soil using high performance liquid chromatography (HPLC)
Reference	Sommer, H. 2000
Test Guideline	OECD Guideline for the Testing of Chemicals, "Estimation of the Adsorption Coefficient (K_{OC}) on Soil using High Performance Liquid Chromatography (HPLC)
Data Validity	1
Data Relied On	Yes - the data were considered to be critical and were relied on in this assessment.

The objective of this study was to determine the mobility of methiocarb-sulfoxide in soil by estimation of the adsorption coefficient. In the present study the adsorption coefficient KOC of methiocarb-sulfoxide was investigated using High-Performance Liquid Chromatography (HPLC).

The retention time of the test substance measured in this study was used to calculate KOC -values for methiocarb-sulfoxide.

Using the HPLC estimation method the adsorption coefficient (K_{OC}) is deduced from the capacity factor (k') using a calibration plot of log k' versus log K_{OC} of the selected reference compounds.

Variability of the retention times from repetitive injections was low, confirming HPLC system stability throughout the analysis period.

The LogK_{OC} value estimated for methiocarb-sulfoxide was determined to be 1.495 (Koc = 31 L/kg). This compared to an estimated LogKoc for methiocarb in the same test system of 3.10 (Koc = 1,260 L/kg).

Title	Adsorption/desorption of [14C]-methiocarb methoxy sulfone on four different soils
Reference	Moendel, M. 2002
Test Guideline	EC, Commission Directive 95/36/EC Amending Council Directive 91/414/EEC, OECD Guideline 106 and Pesticide Assessment Guideline, Subdivision N, Environmental Fate, US EPA,§163-1
Data Validity	1
Data Relied On	Yes - the data were considered to be critical and were relied on in this assessment.

The adsorption of 14C-labelled methiocarb methoxy sulfone (M10) was investigated in four different soils (BBA 2.1 (I), Laacher Hof AXXa (II), Laacher Hof AIII (III), Hofchen am Hohenseh 4a (IV)). Based on results from preliminary tests a soil/solution ratio of 1:1 corresponding to 20 g soil and 20 mL solution was used for soils I and III, and a soil/solution ratio of 1:2.22 was used for soils II and IV. The shaking period in order to reach equilibrium was 24 hours for soils II, III, and IV, and 48 hours for soil I.

When applying the test item at concentrations corresponding to 1.003, 0.300, 0.100, 0.030, and 0.010 mg/L 0.01 M CaCl2 solution, the proportion of M10 adsorbed on soil BBA 2.1 ranged from 48.8% to 67.7%, on soil Laacher Hof AXXa from 55.9% to 70.5%, on soil Laacher Hof AIII from 57.3% to 72.5%, and on soil Hofchen am Hohenseh 4a from 52.5% to 66.5% (mean values).

The chromatographic analysis of the clear centrifuged supernatants, after establishment of the equilibrium, showed that more than 99% of the measured radioactivity could be assigned to be the unchanged test item.

The adsorption constants K_F, determined by means of the Freundlich adsorption isotherm, as well as the soil carbon-based sorption constant K_F,oc, were determined. The K_F and K_F,oc (expressed as L/kgL/kg) were determined as follows:

Freundlich Constants Describing the Adsorption of Methiocarb Methoxy Sulfone

Soil	Class	% org C	1/n	K_F-value	K_F oc-value	r^2
BBA 2.1	Sand	0.38	0.8405	0.9027	237.6	0.9992
Laacher Hof AXXa	Sandy loam	1.02	0.8586	2.5700	252.0	0.9998
Laacher Hof AIII	Silt loam	0.98	0.8414	1.2078	123.2	0.9997
Hofchen am Hohenseh 4a	Silt	1.55	0.8620	2.4881	145.0	0.9999

Freundlich Constants Describing the Desorption of Methiocarb Methoxy Sulfon

Soil	1/n	K_F	K_F,oc	r^2
BBA 2.1	0.8383	1.0762	283.2	0.9992
Laacher Hof AXXa	0.8477	3.0122	295.3	0.9998
Laacher Hof AIII	0.8319	1.4164	144.5	0.9996
Hofchen am Hohenseh 4a	0.8530	2.5988	167.7	0.9998

Desorption tests showed that between 16.5% and 28.3% of the adsorbed test item was desorbed again on soil I and soil II while on soil III the desorption rate ranged between 11.0% and 20.5%. The desorption rate on soil IV

ranged between 19.9% and 29.9%. K_F,oc for desorption (expressed as L/kg) were determined to be in the range from 145 to 295.

In case of the highest concentrations (1.003 mg/L) a second and third desorption step was conducted with all soils. The K_F,oc for desorption (expressed as L/kgL/kg) were determined to be in the range from 90 to 193.

Based on the soil sorption parameters measured in this study and classification of soil mobility potential according to BRIGGS, the Methiocarb Methoxy Sulfone is considered to be low mobile in BBA 2.1 (sand), Laacher Hof AXXa (sandy loam), and Hofchen am Hohenseh 4a (silt), and intermediate mobile in Laacher Hof AIII (silt loam).

Title	Adsorption/desorption of [14C] methiocarb sulfone phenol on four different soils
Reference	Moendel, M. 2002
Test Guideline	EC, Commission Directive 95/36/EC Amending Council Directive 91/414/EEC, OECD Guideline 106 and Pesticide Assessment Guideline, Subdivision N, Environmental Fate, US EPA,§163-1
Data Validity	1
Data Relied On	Yes - the data were considered to be critical and were relied on in this assessment.

The adsorption of [1-^{14}C] Methiocarb Sulfone Phenol (M05) was investigated in four different soils (BBA 2.1, Laacher Hof AXXa, Laacher Hof A III, Hofchen am Hohenseh 4a). Based on results from preliminary tests a soil/solution ratio of 1:1 corresponding to 20 g soil and 20 mL solution was used for all soils. The shaking period in order to reach equilibrium was 24 hours.

When applying the test item at concentrations corresponding to 1.006, 0.303, 0.101, 0.030, and 0.010 mg/L 0.01 M $CaCl_2$ solution, the proportion of M05 adsorbed on soil BBA 2.1 ranged from 41% to 55%, on soil Laacher Hof AXXa from 63% to 73%, on soil Laacher Hof AIII from 50% to 66%, and on soil Hofchen am Hohenseh 4a from 60% to 71%.

The chromatographic analysis of the clear centrifuged supernatants, after establishment of the equilibrium, showed that more than 99% of the measured radioactivity could be assigned to be the unchanged test item.

The adsorption constants KF, determined by means of the Freundlich adsorption isotherm, as well as the soil carbon-based sorption constant K_F,oc, were determined. The K_F and K_F,oc (expressed as L/kg) were determined as follows:

Freundlich Constants Describing the Adsorption of Methiocarb Sulfone Phenol

Soil	% org C	1/n	K_F-value	K_F oc-value	r^2
BBA 2.1	0.38	0.8704	0.6195	163.0	0.9996
Laacher Hof AXXa	1.02	0.9023	1.5386	150.8	1.0000
Laacher Hof AIII	0.98	0.8431	0.9057	92.4	0.9999
Hofchen am Hohenseh 4a	1.55	0.8886	1.3377	86.3	1.0000

Freundlich Constants Describing the Desorption of Methiocarb Sulfone Phenol

Soil no.	Soil	1/n	K_F	$K_{F,oc}$	r^2
I	BBA 2.1	0.8688	0.7615	200.4	0.9993
II	Laacher Hof AXXa	0.8958	I.7420	170.8	1.0000
III	Laacher Hof AIII	0.8383	1.0555	107.7	0.9999
IV	Hofchen am Hohenseh 4a	0.8744	1.4998	96.8	1.0000

Desorption tests showed that between 14% and 19% of the adsorbed test item was desorbed again on soil II and soil IV while on soil I the desorption rate ranged between 24% and 31%. The desorption rate on soil III ranged between 16% and 25%. The K_F,oc for desorption (expressed as L/kg) were determined to be in the range from 97 to 200.

For all soils a second and third desorption step was conducted with the highest concentrations (1.006 mg/L). The KF,oc for desorption (expressed as L/kg) were determined to be in the range from 62 to 145.

Leaching studies

Title	Leaching behaviour of Mesurol® (methiocarb) DRAZA Slug Pellets in soil columns during aging
Reference	Babczinski, P. & Schramel, O.2001
Test Guideline	BBA Guidelines for Testing of Plant Protectants in the Registration Procedure, Part IV, 4-2, December 1986, Commission Directive 95/36/EC Amending Council Directive 91/414/EEC, July 1995, SETAC Europe Procedures for Assessing the Environmental Fate and Ecotoxicity of Pesticides, March 1995
Data Validity	1
Data Relied On	Yes - the data were considered to be critical and were relied on in this assessment.

The present study describes the leaching potential of methiocarb (active substance in "Schneckenkorn" Mesurol) and its relevant metabolite methiocarb-sulfoxide (M01) after application of slug pellets to soil columns. The standardized soil column leaching set-up chosen was suitable to investigate in detail the leaching potential of the relevant methiocarb residues from this slow-release formulation under controlled worst case irrigation conditions. To meet the specific release characteristics of the pellet formulation, the study design did not comply with an official guideline study. However, its design ("non- saturated hydraulic conditions") was close to SBA-Guidelines Part IV, 4-2, to the Commission Directive 95/36/EC and to SETAC Procedures for Assessing the Environmental Fate and Ecotoxicity of Pesticides.

The study was conducted with a sandy loam soil (laacher Hof AXXa) and with a sand soil (BBA 2.1).

Soil	Textural class	Organic carbon (%)	pH ($CaCl_2$)	Sand (%)	Silt (%)	Clay (%)
Laacher Hof AXXa	Sandy loam	1.02	6.32	72.4	22.6	5
BBA 2.1	Sand	0.38	5.6	89.6	8.1	2.3

5 kg formulated product per hectare (equivalent to 150 g ac/ha) was taken as use pattern application rate of DRAZA RB3 slug pellets. Each column was applied with one pellet (11.1 mg) which corresponded to an overdose

rate of approximately 11-fold. The test substance (DRAZA RB3 pellets) was applied on top of packed 30 cm soil columns and covered with a soil layer of approximately 5 mm. The pellets were aged in soil (on the top of the column) for a period of 12 weeks at room temperature. This procedure is considered as worst case aging conditions since the soils were constantly held at maximum water holding capacity. Once a week the soil columns were irrigated using two different irrigation intensities corresponding to 2000 and 6000 mm per year. These regimes have to be considered as worst to extreme case irrigation conditions (worst case middle Europe: "Hamburg wet": 800 mm/year). Considering the sectional area of the columns of 19.6 cm^2, the resulting amount of irrigation water (0.01 M $CaCl_2$ solution) was 75 and 225 mL per week. These volumes were applied as single irrigation events using a peristaltic pump at a flow rate of ca. 8 mL per hour resulting in irrigation durations of approximately 10 and 30 hours at the beginning of each week. The mobility of ac and M01 during aging of the pellets in the soil was investigated by analysing the leachate and the soil segments by means of HPLC-MS/MS.

The study period of 12 weeks combined with intermittent weekly irrigation was appropriate to evaluate the leaching potential of methiocarb and MSO from the DRAZA RB3 slow-release pellets.

Content of methiocarb and methiocarb-sulfoxide found in the leachates and soil columns from the columns filled with soils Laacher Hof AXXa and BBA 2.1 (Mean values from two columns)

Substance	Laacher Hof AXXa		BBA 2.1	
	Leachate (% applied)	Soil (% applied)	Leachate (% applied)	Soil (% applied)
2000 mm/year				
Methiocarb	0.00	32.41	0.00	48.5[1]
M01	0.00	1.62	0.00	0.5[2]
6000 mm/year				
Methiocarb	0.00	51.71	0.03	22.3[1]
M01	0.01	2.22	0.20	<0.1[2]

[1] Located exclusively in the top soil segment (0-5 cm). [2] Located mainly in the top soil segment.

It is concluded from this study using pellets that methiocarb and its metabolite methiocarb-sulfoxide are unlikely to leach into deeper subsoil or ground water reservoirs.

Residues in earthworms

Since July 2015 there are only two registered methiocarb products, namely Baysol Snail & Slug Bait (product # 51851: a 20 g/kg pellet home garden product); and Mesurol Snail and Slug Bait (product # 33274: a 20 g/kg pellet commercial product). New data have been provided to the APVMA providing residue levels in earthworms and slugs following application of methiocarb snail pellets in different cropping situations. These data are important in considering potential secondary poisoning to birds when consuming contaminated earthworms and the results are reported below.

Title	Field Monitoring of Small Mammals on Oilseed Rape Fields Treated with Mesurol Slug Bait RB4 ("Schneckenkorn Mesurol").
Reference	Wolf et al, 2003
Test Guideline	Pesticides and Wildlife – Field Testings: Recommendations of an international workshop on terrestrial field testing of pesticides, attached to Pesticide Effects on Terrestrial Wildlife, Somerville and Walker (ed.), Taylor & Francis, London, 1990.
Data Validity	1
Data Relied On	Yes - the data were considered to be critical and were relied on in this assessment.

Field monitoring was conducted on two study fields and their surroundings in Germany. Both sites were commercial cultivated oilseed rape fields. The test material used was Mesurol RB4. Fields were treated twice with approximately 2 weeks between applications. Monitoring activities focussed on exposure of slug pellets at the soil surface after application, on the occurrence of poisoned slugs and of impacted earthworms as potential food for vertebrates, on mammalian activities and their abundance on the fields and in the surroundings and on the occurrence of vertebrate mortality. The following information describes the results of residue analysis in earthworms and slugs.

On the day after each application and at least every third day during the post-treatment period (for two weeks) earthworms and slugs (dead or alive) were collected on different parts of the field. Sampling times consisted of three searchers each searching for 20 minutes (1 man hour per sampling). Individuals were counted and samples analysed for methiocarb, M01 and methiocarb-sulfone.

From all 80 plots (both fields, all transects) the mean number of exposed pellets immediately after the first application was 27.9 pellets/m^2. It decreased to 14.7 pellets/m^2 at 9 days after application.

1 and 3.0 kg product/ha (~120 g ac/ha) on field 2. The second application for both fields was 3.0 kg product/ha (~120 g ac/ha). The following table summarises the residues levels in earthworms over the monitoring period of the study:

Field 1			Field 2		
Days after application (1st /2nd app)	Worms/surface	Residues (mg/kg fw)	Days after application (1st /2nd app)	Worms/surface	Residues (mg/kg fw)
1	4	<0.003	1	4	<0.003
4	31	0.05	4	40	19.75
7	293	49.02	7	150	39.07
10	230	30.33	10	208	27.06

Field 1			Field 2		
13	195	32.95	13/1	27	29.46
14/1	67	24.15	16/4	96	23.93
17/4	211	20.67	19/7	38	24.45
20/7	128	32.74	22/10	87	39.73
23/10	129	18.38	26/14	93	15.46
26/13	69	27.34			

The number of earthworms found on the soil surface tended to increase with increasing rainfall. The analysis of one sample of slugs (field 2, 1 day after the second application) produced a content of 334 mg/kg fw. On average, M01 was present in earthworms at concentrations of 25–30% that of the parent compound. The average methiocarb residues in earthworms over the course of the study were 26.2 mg/kg (field 1) and 27.4 mg/kg (field 2). When including M01, "total" methiocarb averaged ~32.5 mg/kg fw in both fields.

Title	Field Monitoring of Birds on Oilseed Rape Fields Treated with Mesurol Slug Bait RB4 ("Schneckenkorn Mesurol") and RB2 ("Mesurol Schneckenkorn").
Reference	Wolf and Wilkens, 2003
Test Guideline	Pesticides and Wildlife – Field Testings: Recommendations of an international workshop on terrestrial field testing of pesticides, attached to Pesticide Effects on Terrestrial Wildlife, Somerville and Walker (ed.), Taylor & Francis, London, 1990.
Data Validity	1
Data Relied On	Yes - the data were considered to be critical and were relied on in this assessment.

Field monitoring was conducted on two study fields and their surroundings in Germany. Both sites were commercial cultivated oilseed rape fields. The test material used was Mesurol RB4 (first application) and RB2 (second application) pellets. Monitoring activities focussed on exposure of slug pellets at the soil surface after application, on the occurrence of poisoned slugs and of impacted earthworms as potential food for birds, on bird activities and abundance on the field before and after application and on the occurrence of vertebrate mortality. The following information describes the results of residue analysis in earthworms and slugs.

On the day after each application and at least every third day during the post-treatment period (for two weeks) earthworms and slugs (dead or alive) were collected on different parts of the field. Sampling times consisted of three searchers each searching for 20 minutes (1 man hour per sampling). Individuals were counted and samples analysed for methiocarb, M01 and methiocarb-sulfone.

The application rate was around 3.8 kg product/ha for the first application (~150 g ac/ha) and 5.1 kg product/ha for the second application (2% ac; ~100 g ac/ha). The following table summarises the residues levels in earthworms over the monitoring period of the study.

Field 1			Field 2		
Days after application (1st /2nd app)	Worms/surface	Residues (mg/kg fw)	Days after application (1st /2nd app)	Worms/surface	Residues (mg/kg fw)
1	0	-	1	16	44.95
4	10	<0.003	4	133	31.97

Field 1			Field 2		
7	132	39.64	7	125	29.22
10	237	39.47	10	115	44.3
13	77	41.54	13	132	20.82
16./1	36	25.31	16./1	213	16.77
19./4	74	20.07	19./4	170	18.75
22./7	102	13.79	22./7	127	12.06
25./10	41	9.84	25./10	82	17.6
28/13	58	17.7	28/13	92	10.84

From all 80 plots (both fields, all transects) the mean number of exposed pellets immediately after the first application was 32.8 pellets/m^2. It decreased to 20.0 pellets/m^2 at 12 days after application.

The average content of total residues of methiocarb in worms found on the study fields decreased continuously during the observation period. The second application had no significant effect on the residue content of methiocarb in earthworms. The residue level after the application of RB2 was approximately half that after application of RB4 corresponding to the 50% lower active constituent content of the RB2 formulation. The average "total residues" (methiocarb + M01) following the first application but before the second application was 49.5 mg/kg (field 1) and 42.5 mg/kg (field 2). The average residues following the second application until the end of the monitoring period was 23.8 mg/kg (field 1) and 19.9 mg/kg (field 2).

The analysis of one sample of slugs (field 2, 1 day after the second application) produced a content of 136 mg/kg fw. On average, M01 was present in earthworms at concentrations of 24–38% that of the parent compound. The average methiocarb residues in earthworms over the course of the study were 25.9 mg/kg (field 1) and 24.7 mg/kg (field 2). When including M01, "total" methiocarb averaged ~28.5 to 35.9 mg/kg fw.

Fate and behaviour in water

Hydrolysis

Title	Hydrolysis of [phenyl-1-^{14}C]methiocarb-sulfoxide in sterile buffer solutions
Reference	Sneikus, J. 2001
Test Guideline	SETAC Europe Procedures for Assessing the Environmental Fate and Ecotoxicity of Pesticides, March 1995 and European Communities No L 172, 95/36/EC, Placing of the Plant Protection Products on the Market, July 14, 1995
Data Validity	1
Data Relied On	Yes - the data were considered to be critical and were relied on in this assessment.

The hydrolysis of methiocarb sulfoxide (M01) was studied in 0.01 M buffer solutions, which were adjusted to pH 5, 6 and 7. The experiment was carried out according to the EC Commission Directive 95/36/EC, 1995 as well as the SETAC-Europe guidelines.

The test solutions were prepared with radiolabelled parent compound ([phenyl-1- ^{14}C]Methiocarb sulfoxide) at a concentration of about 10 mg/L. The solutions in the pre-test were incubated for a maximum period of 7 days under sterile conditions in the dark at 50°C, and the sampling intervals were 0, 2.5 and 6 hours and 1, 2, 5 and 7 days.

The solutions in the main-test (pH 5 and pH 6) were incubated for a maximum period of 30 days under sterile conditions in the dark at 25°C, and the revised sampling intervals were 0, 2 and 6 hours and 1, 2, 4, 7, 14, 21 and 30 days as deduced from the results of the pre-test. The maximum incubation period for the pH 7 samples was 4 days with sampling intervals after 0, 2, 6, 24 and 30 hours as well as 2, 3 and 4 days.

The complete material balances found in all solutions demonstrated that no radioactivity dissipated from the solutions by means of volatilization.

Hydrolysis of Methiocarb sulfoxide at 50 °C (pre-test) in pH 5, 6 and 7 Buffers: TLC Analysis, Components Expressed as % of Applied Radioactivity (= 100%)

Incubation time	pH 5	pH6	pH7
0 hrs	98.4	97.3	91.8
2 hrs	90.8	46.4	0.5
6 hrs	83.3	18.1	0.3
1 d	53.6	0.3	0.2
2 d	29.6	0.2	0.2
5 d	7.7	n.d.	n.d.
7 d	3.0	n.d.	0.4

Hydrolysis of Methiocarb sulfoxide at 25 °C Expressed as % of Applied Radioactivity (= 100%)

Incubation time	pH 5	pH6	Incubation time	pH7
0 hrs	97.4	96.5	0 hrs	90.4

Incubation time	pH 5	pH6	Incubation time	pH7
2 hrs	96.0	95.3	2 hrs	76.9
6 hrs	97.0	94.3	6 hrs	63.0
1 d	97.5	80.5	24 hrs	22.3
2 d	95.2	74.3	30 hr	16.5
4 d	93.1	63.8	2 d	6.1
7 d	89.2	43.9	3d	1.9
14d	81.8	17.7	4d	0.8
21d	76.1	8.4		
30 d	65.6	2.4		

The degradation rate of M01 in the buffer solutions was calculated by pseudo first order reaction kinetics using the calculation program ®ModelManager with the Simple First Order Model (SFO):

Kinetic results of Methiocarb sulfoxide hydrolysis at 50 °C

	50°C		25°C		20°C	
Test Solution	DT50 (d)	r^2	DT50	r^2	DT50	r^2
pH 5 (0.01 M citrate buffer)	1.19	0.999	54.8	0.994	122	-
pH 6 (0.01 M citrate buffer)	0.09	0.995	6.1	0.996	15	-
pH 7 (0.01 M phosphate buffer)	0.01	1.000	0.5	0.999	1	-

Considering the hydrolytic behaviour determined under environmental pH conditions it is expected that hydrolytic processes will contribute to the degradation of M01 in the environment.

Photolysis

Title	Photolysis of [phenyl-1-^{14}C]methiocarb in sterile aqueous buffer pH5
Reference	Hellpointer, E. 2002
Test Guideline	Official Journal of the European Communities, No. L 172 (EN), July 14, 95. Commission Directive 95/36/EC amending Council Directive 91/414/EEC, Annex II, Fate and Behavior in the Environment SETAC-Europe: Procedures for Assessing the Environmental Fate and Ecotoxicity of Pesticides, March 1995, Section 10 (Aqueous Photolysis) EPA Pesticide Assessment Guidelines, Subdivision N, § 161-2, 1982, Photodegradation Studies in Water
Data Validity	1
Data Relied On	Yes - the data were considered to be critical and were relied on in this assessment.

In the present study the phototransformation of Methiocarb was investigated in aqueous solution (sterile, 10 mM acetate buffer, pH 5) under continuous artificial light exposure at a constant temperature of about 25°C.

The test was conducted as a mass balance study with [phenyl-1-^{14}C] Methiocarb using an initial concentration of 0.989 mg methiocarb/L buffer. Individual aliquots of 10 mL of sterile test solution maintained at 25°C were

continuously irradiated in a xenon chamber (®Suntest) simulating sunlight. Duplicate test vessels were taken after 1, 2, 3, 6, 8 and 10 days of irradiation, and dark controls were investigated after 0, 6 and 10 days. The crude test solutions were investigated by liquid scintillation counting and analysed by radio-HPLC to determine the content of Methiocarb and its degradation products. For identification of the transformation products co-chromatography methods with the reference standards were used. In addition, some product zones were isolated and characterised by LC-MS and LC-MS/MS methods.

The degradation curve and regression analysis of methiocarb was calculated with the evaluation program ®ModelManager (Environmental Kinetics), Version 1.1, developed and published by Cherwell Scientific Ltd. Oxford, UK.

The total recovery of radioactivity from individual test vessels ranged from 98.9% to 101.6% for the irradiated samples, and from 99.4% to 103.4% for the dark controls. The complete material balance found at all sampling intervals of the test demonstrated that no significant radioactivity dissipated from the vessels or was lost during processing.

Degradation of Methiocarb and Formation of Metabolites (data expressed as % of AR)

	DAT [d]	Parent'	M04	M01	4 isolated product peaks	2 isolated product peaks	Minor metab.	Volatile compounds	
								CO2	Org.
Test	0	99.4	n.d.	n.d.	n.d.	n.d.	n.d.	n.m.	n.m.
Irradiated	1	98.2	n.d.	2.4	n.d.	n.d.	n.d.	0.1	0.1
Samples	2	93.8	n.d.	4.5	n.d.	n.d.	n.d.	0.6	<0.1
	3	89.9	n.d.	6.2	n.d.	1.7	n.d.	1.3	<0.1
	6	62.1	2.1	20.2	3.7	2.8	n.d.	4.3	0.1
	8	56.1	2.7	18.0	5.3	5.5	1.1	6.3	0.1
	10	39.3	3.4	25.1	5.9	5.7	<LOQ	9.0	0.1
Dark	6	103.0	n.d.	n.d.	n.d.	n.d.	n.d.	0.1	0.1
Samples	10 *	103.3	n.d.	n.d.	n.d.	n.d.	n.d.	<0.1	0.1

** No duplicate in the case LOQ: 1.0% of AR.*

The study demonstrated that methiocarb was moderately photodegraded in aqueous buffer solution under experimental conditions. In contrast to the dark controls, where no degradation was measured, methiocarb was photodegraded to 39% of AR within 10 days. The experimental DT50 of methiocarb calculated assuming simple first order model was 8.17 days of continuous irradiation in the ®Suntest unit (rate constant k = 0.0848 d-1, R^2 = 0.952).

In the course of the experiment several ^{14}C peak zones were detected in the irradiated test solutions. However, only M01 was considered major and reached 25% after 10 days of continuous irradiation. The maximum portion of radiolabelled CO_2 was 9% AR after 10 days of light exposure. All other product peak zones corresponded to max. 5.7% AR at any time during the study.

Based on the experimental half-life of methiocarb of 8.17 days the half-life under environmental conditions was calculated to be 31 solar summer days at Phoenix (AZ, USA) or 48 solar summer days at Athens (Greece).

It is concluded from this study that under environmental conditions solar radiation does not significantly contribute to the degradation of methiocarb in aqueous solution.

Aerobic degradation

Title	[Phenyl-1-^{14}C]methiocarb: Aerobic aquatic metabolism in two water-sediment systems
Reference	Heinemann, O. 2005
Test Guideline	EU Commission Directive 95/36/EC amending Council Directive 91/414/EEC (Annexes I and II, Fate and Behaviour in the Environment), 1995-07-14
Data Validity	1
Data Relied On	Yes - the data were considered to be critical and were relied on in this assessment.

The aerobic biotransformation of methiocarb was studied in two water-sediment systems (Angler Weiher: water pH 7.6, total organic carbon <2 mg/L, sediment texture sand, pH 7.1, organic carbon 0.37 % from Leverkusen, Germany and Hoenniger Weiher: water pH 8.0, total organic carbon <2 mg/L, sediment texture silt loam, pH 6.6, organic carbon 3.0 % from a location near Wipperfuerth, Germany) for a maximum of 90 days in the dark at 20°C.[Phenyl-1-^{14}C] methiocarb was applied to the water body at a rate of 12.5 µg test item per batch, corresponding to 46.6 kBq per batch and 24 µg/L water.

The water-sediment ratio used was 3/1 (v/v). The test system consisted of laboratory microcosm flasks attached to traps for the collection of CO2 and organic volatiles. Samples were analysed at 0, 1, 3, 7, 14, 30, 62 and 90 days of incubation. In addition, a day 2 sample was taken for the Angler Weiher water-sediment system.

The water samples were concentrated for analysis, and the sediment samples were extracted twice with acetonitrile/water/glacial acetic acid (50/50/0.1, v/v/v), followed by a single extraction with pure acetonitrile at ambient temperature and finally by an aggravated microwave extraction with acetonitrile/water/glacial acetic acid (8/2/1, v/v/v) at 60°C. For chromatography, the combined sediment extracts were concentrated. Methiocarb residues were analysed by reversed phase HPLC equipped with a diode array detector and a radioactivity detector. The results were confirmed by normal phase radio AMD-TLC for representative samples. Identification of the transformation products was achieved by co-chromatography with reference compounds.

Nonlinear regression analysis was used to define the degradation rate constants, and linear regression analysis was used to determine the radioactive detector response Arithmetic means were used for all LS measurements, and for the mentioned replicates.

The material balances of the water-sediment systems ranged from 97.7 % to 103.7 % and from 92.0 % to 101.9 % of the applied radioactivity (AR) for Angler Weiher and Hoenniger Weiher, respectively, with overall mean ± standard deviations of 100.7± 2.0 % and 97.1 ± 2.8 %, respectively.

Biotransformation of Methiocarb, HPLC Components Expressed as % of AR (Mean) in Water-Sediment System under Aerobic Conditions: Angler Weiher System

Compound	Source	DAT [days]								
		0	1	2	3	7	14	30	62	90
Methiocarb	Water	91.5	54.2	31.2	20.0	2.7				
	Sediment	2.6	14.9	14.5	14.6	6.9	3.0	0.7		
M03	Water	5.7	11.7	13.9	15.2	3.9				
	Sediment		1.1	2.5	4.0	8.4	12.4	13.7	7.8	7.5
M05	Water				0.5	2.4	1.1	2.3	3.1	4.2
	Sediment							1.1	1.5	2.4
M04	Water		10.0	20.9	25.5	34.1	33.3	20.4	11.3	9.1
	Sediment		1.1	3.0	3.0	4.3	6.9	6.3	7.0	3.8
Total extractable residues	Water	97.2	75.9	67.2	63.3	79.3	39.2	26.7	18.4	13.8
	Sediment	2.6	18.5	21.3	21.6	19.6	22.3	21.8	17.3	13.7
Total 14CO2			0.7	1.7	1.4	1.9	8.5	14.9	20.8	25.3
Non-extractable residues	Sediment	0.3	4.3	11.3	15.1	27.2	32.6	37.9	46.3	45.2
Total recovery (%)	TOTAL	100.2	99.5	101.5	101.3	98.0	102.6	101.4	102.7	97.9

Blank boxes: values < LOD, DAT: days after treatment.

Biotransformation of Methiocarb, HPLC Components Expressed as % of AR (Mean) in Water-Sediment System under Aerobic Conditions: Hoenniger Weiher Systems

Compound	Source	DAT [days]							
		0	1	3	7	14	30	62	90
Methiocarb	Water	93.7	60.5	38.8	16.7	4.0	0.4		
	Sediment	2.7	27.8	36.7	33.9	25.0	15.3	8.7	7.7
M03	Water	3.8	3.6	6.1	6.8	1.9			
	Sediment			2.9	9.6	16.5	15.5	14.1	11.8
M05	Water					0.4	0.4		
	Sediment						0.9	0.6	
M04	Water		2.2	5.5	12.6	9.5	5.7	3.7	1.1
	Sediment			1.1	2.1	2.9	4.0	5.9	1.3
Total extractable residues	Water	97.5	67.6	52.1	37.1	16.7	6.5	3.7	2.0
	Sediment	2.7	29.4	41.8	46.1	44.4	37.5	29.3	20.7
Total $^{14}CO2$			0.1	0.3	1.1	3.6	7.0	9.4	12.3
Non-extractable residues	Sediment	0.3	1.5	5.1	12.3	30.7	47.9	52.5	58.6
Total recovery (%)		100.6	98.6	99.3	96.5	95.3	97.0	94.8	93.6

The following DT_{50} / DT_{90} values of methiocarb were calculated:

System		DT^{50} [d] Methiocarb	DT^{90} [d] Methiocarb	Best Fit Kinetic Model; X^2 criterion
Water	Angler Weiher	1.3	4.4	SFO; 1.4 %
	Hoenniger Weiher	2.5	8.2	SFO; 9.4 %

Sediment	Angler Weiher	4.3	14	SFO; 8.3 %
	Hoenniger Weiher	27	90	SFO; 8.2 %
Entire System	Angler Weiher	2.1	6.8	SFO; 3.5 %
	Hoenniger Weiher	8.7	29	SFO; 8.5 %

Overall, three transformation products were detected, methiocarb phenol (M03), methiocarb sulfoxide phenol (M04) and methiocarb sulfone phenol (M05). The major transformation products methiocarb phenol and methiocarb sulfoxide phenol declined until study termination not exceeding 11.8 % of the AR in all compartments, the minor transformation product methiocarb sulfone phenol remained in Angler Weiher at a very low level (below 5 % of the AR until study termination). There were significant non extractable residues in the sediment and these residues were not characterised further.

Bioconcentration

Title	Bioconcentration, depuration & determination of residues of methiocarb-phenol in fish (Lepomis macrochirus)
Reference	Dorgerloh, M., Weber, E. & Eberhardt, R. 2002
Test Guideline	OECD Test Guideline 210 (1992); OPPTS 850.1400 (1996); ASTM E 1241-92 (1992)
Data Validity	1
Data Relied On	Yes - the data were considered to be critical and were relied on in this assessment.

The objective of this study was to quantify the methiocarb-phenol (M03) residues in tissues of fish (edible and non-edible) under flow-through conditions in order to calculate the bioconcentration factor (BCF). A total of 64 young bluegill sunfish (Lepomis macrochirus, with a mean body weight of 4.3 g and a mean body length of 6.6 cm) were exposed to a flow-through treatment and an untreated control. A dosing system was used to maintain a mean water concentration (nominal) of 30 µg [^{14}C]- Methiocarb-phenol/L for a 28-day exposure period. After exposure the test fish were placed in clean water for 14 days in order to determine the depuration of [^{14}C]- Methiocarb-phenol.

The test substance was analysed for M03 and metabolites in the aquarium water during the exposure phase (day 0, day 1 and day 28) to show the concentration of test compound to which the fish were exposed. The parent compound accounted for 94–97% of the determined radioactivity and was stable in test water during the exposure phase of the study.

The uptake rate constant (Ku), depuration rate constant (Kd), time for half clearance and the kinetic bioconcentration factors (based on total radioactive residues) for edible parts and for whole fish were determined using the OriginTM non-linear kinetic modelling computer program for this bioconcentration study. The total radiolabelled residues (sum of radiolabelled compounds, parent, metabolites and mineralization products) concentrated in bluegill sunfish with a bioconcentration factor of about 132 time for whole fish. When exposure ceased, the residues were depurated with a half-life of 0.77 days. After 14 days in uncontaminated water, 95% of the mean plateau radioactivity were depurated from whole fish. Accumulation of total residues in edible parts was less (49.6 time) than in whole fish (132 time).

In edible parts and viscera, 77% and 71% of the total radioactive residue (TRR) could be identified, respectively. 12% and 18% of the TRR were characterised by their behaviour in the extraction and RP-HPLC analysis, respectively. The metabolites characterised accounted for 6 and 9 peaks in HPLC profiles, respectively. No single peak not identified was > 5% of the TRR.

Residues of M03 determined in fish samples accounted for 15% of the TRR in edibles and 9% of the TRR in viscera. Metabolites and degradation products identified were: Methiocarb-phenol-GA, Methiocarb-phenol-SA, Methiocarb-sulfoxide- phenol-SA, Methiocarb-sulfoxide-phenol-GA and Methiocarb-sulfoxide-phenol.

The steady- state-BCF for M03 (based on whole fish, wet weight) was 14.5, the steady-state-BCF for M03 (normalised to 6% lipid content) was 10.9.

Fate and behaviour in air

Title	Calculation of the chemical lifetime of methiocarb in the troposphere
Reference	Hellpointer, E. 2000
Test Guideline	Guideline for the testing of plant protectants in the registration procedure, Federal Biological Institute for Agriculture and Forestry, Braunschweig, FRG, part IV, 6-1, July 1990
Data Validity	1
Data Relied On	Yes - the data were considered to be critical and were relied on in this assessment.

Based on the calculation according to Atkinson the chemical lifetime of the parent compound Methiocarb in the air is assessed by AOPWIN (version 1.87). A value of at the most 20 hours results, with respect to the OH radical reaction, only.

The calculated overall OH reaction rate of 13.5 x 10^{-12} [cm^3/molecules sec] is mainly obtained by addition reactions to the aromatic ring (kar = 8.42 x 10^{-12} [cm^3/molecules sec]), by reactions at the nitrogen (kN-C(=O)-o- = 2.70 x 10^{-12} [cm^3/molecules sec]), and by hydrogen abstractions at several sites (kH-abs total = 2.35 x 10^{-12} [cm3/molecules sec]).

Based on the before-mentioned calculated overall OH rate constant and using a 12-hrs- day with 1.5 x 10^6 OH radicals/cm^3 (for details see Appendix 4) values as follows were assessed:

=> half-life of methiocarb in air = 9.5 hours

corresponding to a

=> chemical lifetime of methiocarb in air = 13.8 hours.

A more conservative assessment of the overall OH radical rate constant could be made by using, for example, onlyhalf of the estimated rate in case of all assumed figures (i.e. taking only kar = 4.21 x 10^{-12} [cm^3/molecules sec],). This would result in an overall OH reaction rate of 9.25 x 10^{-12} [cm3/molecules sec], and a chemical lifetime of methiocarb in the air of (‘t) = 20 hrs.

The before mentioned estimations do not consider any contribution of an attack by other radicals (i.e. by nitrate radicals). Whenever the active ingredient is applied during early afternoon (in opposite to early morning or late afternoon), it is to be expected that the chemical lifetime is shorter at that moment, as during the day the OH radical concentration in the troposphere may increase unto 5 x 106 radicals/cm^3. On the other hand, the OH radical concentration in the night decreases to zero.

APPENDIX 4. ADDITIONAL ENVIRONMENTAL EFFECTS DATA

Birds and mammals

Acceptance: bait palatability

Title	Acceptance of Mesurol slug pellets RB 4 (3.9% purity) by Japanese Quail (*Coturnix coturnix japonica*)
Reference	Barfknecht, R 2002
Test Guideline	Guidelines for testing plant protection products in the registration-process", Part VI, No.25-1: "Testing of baits, granules and treated seeds for hazards to birds - acceptance tests" (Variant A, aggravated conditions) of the Federal Biological Research Centre for Agriculture and Forestry (BBA), Braunschweig, from March 1993.
Data Validity	1
Data Relied On	Yes - the data were considered to be critical and were relied on in this assessment.

This study assessed the acceptance of Mesurol slug pellets RB 4 (3.9% purity) by the Japanese quail *(Coturnix coturnix japonica*) as a representative for granivorous birds. The study revealed whether the slug pellets are palatable and taken up in quantities, which are toxic to birds.

Birds were housed indoors in aviaries with a ground area of 2 x 2 m and a height of 2 m. Four aviaries were arranged in a line on a concrete floor surface, covered with a uniform layer of quartz sand. Drinking water was served by a green-coloured reservoir dispenser inside each aviary. A photoperiod of 12 hours light was maintained by Neon tubes which were arranged in line above the aviaries. Additionally, natural daylight reached the hall through windows in the roof and in one wall. Seven days before the day of treatment, body weights were recorded.

On the evening before the exposure day all birds were weighed again and the group size was reduced to 4 males and 4 females with special consideration of less acclimatized or injured individuals.16 hours prior to exposure, birds were deprived from food. Immediately before start of exposure, all birds were removed from the aviaries. Then the quartz sand layer on the ground of each aviariy was renewed and 32 g of the standard seed diet was offered on plastic plate. This amount of untreated seeds corresponds to 25% of the average daily feed demand for a group of 8 Japanese Quails. Subsequently, 96 g of Mesurol slug pellets RB 4 (75% of the average daily feed demand for the group) was presented on the same plastic plate 40x60 cm) to ease the reweight of the pellets that remain after the exposure time. The birds were then returned to their respective aviaries.

Observations on mortality, signs of intoxication and feeding activity were made continuously during the first hour of exposure, followed by an hourly observation until the end of the 8 hours exposure period. At termination of exposure, birds were removed from the aviaries. The food/sample mixture was removed for reweighting. The birds were returned into their test cages and were observed for 14 days, with free access to untreated standard seed diet. Further body weight determinations were performed 6 days post-treatment and at study termination.

At the end of the study all surviving birds were sacrificed by CO2 asphyxiation. Gross pathological necropsies were carried out on all quails that died before study termination.

Summary of sign of intoxication

Replicate No.	#Toxic Signs / #Dead / # Dosed			
	Males	Females	Combined	Observed Effects
1	1/L/4	1/0/4	2/L/8	AP, DM
2	2/L/4	1/0/4	3/L/8	AP, DM, SV
3	1/L/4	2/0/4	3/L/8	AP, DM
4	1/0/4	1/L/4	2/L/8	AP, DM, SV

AP= apathy, OM= discoordination movement, SV= salivation.

Average Body Weight Data of Treated Males and Females

		Body Weights (g)							
		d -7		d -1		d 6		d 14	
Replicate No.1	mean	196	(n=8)	200.4	(n=8)	201.7	(n=7)	201.1	(n=7)
Replicate No.2	mean	197.5	(n=8)	199.5	(n=8)	196.7	(n=7)	188	(n=7)
Replicate No.3	mean	202.8	(n=8)	197.3	(n=8)	197.0	(n=7)	196.0	(n=7)
Replicate No.4	mean	192.4	(n=8)	196.3	(n=8)	205.3	(n=6)	207.1	(n=7)

Standard Diet and Mesurol Slug Pellets Intake (g)

	Standard diet (inweight 32g/aviary)	Slug pellets (inweight 96g/aviary)
Aviary No.1	26.4	5.6
Aviary No.2	26.7	7.1
Aviary No.3	21.6	6.2
Aviary No.4	25.3	8.2
mean	25.0	6.8
SD	2.3	1.1
% intake	78.1%	7.1%

Behaviour: One hour after the start of exposure, signs of intoxication were observed on seven quails in different aviaries such as reduced vigilance, disturbance of coordination, trembling, apathy and salivation. The symptoms were only observed on the exposure day. The surviving birds recovered completely.

Mortality: Mortalities were observed at the day of exposure. From two to five hours after the start of exposure, four quails died (three male and one female), one from each aviary. The following gross pathology showed slug pellets in the crop, the gizzard and in one case in the intestines. One quail had an increased gall bladder while another one showed an increased spleen.

Body Weight Development: The evaluation of body weights showed ignorable variations from d-1 to d+6 as well as from d+6 and d+14.

Food Consumption: Most of the standard diet was consumed (78%), while slug pellets were mostly avoided. Some birds took up some pellets and showed signs of intoxication. Since the birds were housed in groups, it was impossible to determine the individual uptake.

The results of the study are in compliance with those of an acceptance test (aggravated conditions = unrealistic exposure), which was performed in 1983 (Hermann) according to BSA 25-1, (Variant A, aggravated conditions) with a slightly different formulation (dry extruded in 1983; wet extruded in 2001). The ratio of premature dead quails was identical (4 of 32). Since no differences were observed between the two formulations, the further results of the previous test were considered valid also for the new formulation. Therefore an exposure scenario according Variant B (more realistic conditions) was not tested any more.

Title	Acceptance of Mesurol Slug Pellets RB4 (ac. methiocarb), by canary birds (*Serinus canaria*) under realistic exposure conditions
Reference	Barfknecht, R 2002
Test Guideline	Commission Directive 96/46EC of 16 July 1996 amending Council Directive 91/414EEC Concerning the Placing of Plant Protection Compounds on the Market
Data Validity	1
Data Relied On	Yes - the data were considered to be critical and were relied on in this assessment.

The study objective was to determine the acceptance of slug pellets under realistic exposure conditions by canaries (*Serinus canaria*), as a representative for granivorous birds. The study investigated whether slug pellets are palatable and taken up in quantities, which are toxic to birds.

Sixteen well-developed canaries were selected, individually weighed and housed in 4 groups of 4 birds in aviaries (2m x 2m x 2m). Natural soil was dispersed on the surface of the aviaries to generate realistic exposure conditions. Since slug pellets are not natural food, a special acclimation on that food item was not performed. A total of 32 g standard food was served per aviary (= 8 g per bird) per day, which corresponded to the daily food demand of a canary. The food was evenly scattered on the surface.

On the first exposure day 1.2 g slug pellets (=28 pellets/m2), corresponding to an application rate of 3 kg formulation/ha and the food demand for 3 days (96 g) were dispersed on the ground of each aviary. The exposure period lasted from 10 am on the first day until 4pm on the third day. Afterwards the soil was removed.

Observations on mortality, signs of intoxication and feeding activity were made hourly (from 8 am to 5pm) on the exposure days in a way that disturbance were avoided as much as possible. During the following post-exposure period of 4 days standard food was offered ad libitum. Body weight was measured on day -7, 0, +2 and at study termination.

No mortalities were reported. No influence on body weight development was observed. The canary birds as representatives of granivorous birds were able to peck selectively the seeds from the surface. There was no indication of any accidental ingestion of slug pellets. The birds showed neither signs of intoxication nor any signs of behavioural changes.

Average Body Weight Data of Treated Canaries

		Body Weights (g)							
		d -7		d 0		d +2		d +7	
Aviary No.1	mean	18.4	(n=4)	19.2	(n=4)	20.3	(n=4)	19.8	(n=4)
Aviary No.2	mean	22.9	(n=4)	23.0	(n=4)	24.7	(n=4)	23.9	(n=4)
Aviary No.3	mean	16.8	(n=4)	17.7	(n=4)	18.7	(n=4)	17.3	(n=4)
Aviary No.4	mean	19.8	(n=4)	20.3	(n=4)	21.4	(n=4)	20.1	(n=4)

Title	Acceptance of Mesurol Slug Pellets RB4 (ac Methiocarb) by domestic pigeons (*Columbia livia f. domestica*) under aggravated conditions
Reference	Barfknecht, R 2001
Test Guideline	BBA 25-1 , Variant A, aggravated test condition modified (prolongated)
Data Validity	1
Data Relied On	Yes - the data were considered to be critical and were relied on in this assessment.

The study objective was to assess the acceptance of slug pellets by the domestic pigeon (*Columba livia f. domestica*), as a representative for large granivorous birds. The study should reveal whether the slug pellets are palatable and taken up in quantities, which are toxic to birds.

After one week of acclimatisation to the test conditions, slug pellets were offered to 10 singly housed domestic pigeons for 8 hours following a 16-hours starvation period: 30 g slug pellets and 10 g of standard food were spread out on plastic trays in each aviary. After the exposure the remaining food was removed and weighed.

The birds were observed for signs of intoxication as well as for effects on feed consumption and body weight. The same exposure scenario was repeated on the next two days. On the fourth exposure day a mixture of 10 g slug pellets and 10 g grit was offered at the same time as the standard food in order to test whether pigeons would take up slug pellets instead of natural grit for grinding the food. In contrast to the first three exposure days there was no starvation period before.

Body weight was measured at the beginning of the acclimatisation, the day before exposure, after the third exposure day (when the rigid feeding regime was finished) and at the end of the study. Intoxication pattern and feeding activity over three 8 hour exposure periods and observations over a 7 day post-exposure period (including eventually time and amount of mortalities as well as type, severity and duration of changes in behaviour). Statistical Procedures: A statistical evaluation of body weight data appeared unnecessary due to the clear data-pattern.

Neither behavioural changes nor any signs of an intoxication were observed. During the exposure day no pigeon was observed to consume slug pellets. If at all, only very small amounts of slug pellets were taken up. The small differences between inweight and reweight of the slug pellets are probably caused by the difficulties in recollecting all particles. The standard food was almost completely consumed.

All birds showed an apparent reduction of body weights during the exposure period. This reduction is not an effect of the substance, since minimal amounts of slug pellets seed were consumed, but an indication of the harsh feeding regime during the exposure. The reduction of body weight was almost compensated during the next 7

days. At study termination the body weights of all birds (with the exception of the bird of cage 8) were in the same range as at the beginning of the test (-1.6%). Since the bird of cage 8 showed no signs of intoxication during the exposure period and the following 7 days, the observed body weight reduction in the last post-treatment week is not attributed to the test substance.

Short term (dietary)

Title	Technical Mesurol: A subacute dietary test with bobwhite quails
Reference	Barfknecht, R. & Hancock, G/2002
Test Guideline	U.S.EPA Ecological Effects Test Guidelines; OPPTS 850.2200 (1996); OECD Test Guideline 205 (1984)
Data Validity	1
Data Relied On	Yes - the data were considered to be critical and were relied on in this assessment.

Singly housed sub-adult birds of 9 to 12 weeks of age were used in the experiments. A total of 10 birds per group across 5 treatment groups (213 mg ac/kg food, 470 mg ac/kg food, 1033 mg ac/kg food, 2273 mg ac/kg food, 5000 mg ac/kg food) and a control were housed in stainless steel cages with sloping floors, approximately 56 L x 28 W x 27 H cm, with a 1 week acclimation period. Bedding (deotized cage board) was changed at least weekly. The light was set at 8 h light / 16 h dark with a 30 minute dawn/dusk cycle. Room temperature was maintained at 70 ± 4°F. Releative humidity within the room was 25 to 80%.

Diet homogeneity was determined by analysing samples of the feed from the 213 mg ac /kg food and the 5000 mg ac /kg food concentrations after mixing.

The exposure period was individually dependent on body weight development, avoidance and mortality. If severe food avoidance was seen, the test diets were removed from the affected birds and replaced with normal diet. The avoidance was considered serious if over 3 days a bird consumed less than 30% of its mean per day food consumption during the pre-exposure period; and/or a body weight reduction of more than 30% was determined.

Health observations were made daily during the re-exposure period. Observations for clinical signs of toxicity, including appearance of excreta, was made three times after diet administration on day 0. Birds were observed twice daily thereafter, except on weekends or holidays when birds were observed once per day. All birds were weighed weekly from the start of the 7-day pre-treatment period, including days 0, +7, +14, +21 and +28 (the end of the treatment period, the end of the post-treatment period, and the end of any extension period.) Any birds that were dead were weighed prior to postmortem examination.

Feed consumption was measured weekly during the pre-treatment and post-treatment periods, twice on day 0 and daily during the remainder of treatment.

A gross examination was performed on all birds found dead during the course of the study. When the treatment period ended, half of the surviving birds were necropsied for gross changes. All other birds were fed untreated diet for further 7 days. They were sacrificed at study termination and necropsied for gross changes.

Summary (mean values per group) of findings

Treatment group (mg/kg)	Day 0-4 Food Intake Rate (%, g/bw)	Average daily dose (mg ac/kg bw)	% premature deaths
0	9.2	0.0	0

Treatment group (mg/kg)	Day 0-4 Food Intake Rate (%, g/bw)	Average daily dose (mg ac/kg bw)	% premature deaths
213	8.5	18.3	0
470	7.5	34.0	0
1033	4.4	46.6	30
2273	2.5	56.1	60
500	1.3	64.6	30

All birds in the top two treatment groups were dead by day 14. Methiocarb was shown to be a strong repellent, since even at the lowest concentration a slight (not significant) avoidance of the treated food could be observed. This became very obvious at all higher treatment groups. The drastically reduced food intake remained during the whole exposure period at the two highest test concentration (2273 mg ac/kg food; 5000 mg ac/kg food), while the birds at the medium concentrations adapted to the food source: Birds at 470 mg ac/kg food consumed similar amount of food as the control from day +2 on, birds at 1033 mg ac/kg food from day 7 onwards.

Reduced food consumption was a criterion to switch birds from treated food to untreated. From the 15 birds in the top three exposure groups which were switched based on this criterion to normal diet, 10 recovered completely and consumed comparable amounts of food as the control bird, whilst five birds died one day after the switching. Reduced body weight was another criterion for switching birds to standard diet. It was not surprising that most of the birds switched because of reduced food consumption hit also this criterion, when birds were weighed again on day +7. Four of the switched birds had already recovered so that their body weight was higher than the trigger value of 70%. From the five birds which were switched because of reduced body weight, two died shortly after switching, while the others recovered completely.

Mean Body weight development (g)

Concentration ac. mg /kg food	day -7	day 0	day +7	day +14	day +21	day +28
Control	224.8	233.3	248	253	257.9	248.8
213	236.5	246.3	255.2	266	270.7	272.6
470	223.4	238.5	235.5	247	230.6	245
1033	238.7	248.1	194	199.1	239	257
2273	228	239.6	151.8	197		
5000	238.5	248.4	170.4	218.6		

During the exposure period the birds of the higher treatment groups lost body weight to a higher degree. After switching to normal diet, the body weights increase rapidly.

Observation: Birds were observed daily for symptoms of intoxication. In all treatment groups some cases of diarrhea and soft excrements were observed. Since this occurred in the control as well, it cannot be attributed to the test substance.

A typical symptom of acetylcholinesterase inhibition is ataxia, which was found in two of the 470 mg ac/kg group, in one bird of the 1033 mg ac/kg group, in five birds of the 2273 mg ac/kg group and in four birds of the 5000 mg ac/kg group. Of the 12 birds that showed ataxia, six died prematurely and the other recovered completely during the study. Birds from the higher test levels (from 1033 mg ac/kg food on) showed hyporeactivity as an additional symptom. It occurred as well in birds which died prematurely as well as in those which stayed on treated food until the end of the designed exposure period or recovered completely after switching to the standard diet. It should be

mentioned that hyporeactivity is not a good indicator of intoxication, but may be also caused by general weakness (e.g. starvation).

Gross Necropsy: No relevant pathological changes were found in the survivors. The only finding noted in prematurely dead birds was emaciation, which was found in all of them.

Due to the great variability in the clinical history of the birds (no dose response) an LD50 could not be evaluated. That is not surprising for a substance which is well known for its repellency. It was observed that birds refused the food to an extent that severe signs of starvation occurred. Since all prematurely dead birds were emaciated, the starvation has to be considered the main reason for death.

Title	Acceptance of earthworms, treated with Mesurol Slug Pellets (4% methiocarb ac), by Japanese quail (*Coturnix coturnix japonica*)
Reference	Barfknecht, R. 2002
Test Guideline	Commission Directive 96/46EC of 16 July 1996 amending Council Directive 91/414EEC Concerning the Placing of Plant Protection Compounds on the Market
Data Validity	1
Data Relied On	Yes - the data were considered to be critical and were relied on in this assessment.

The study tested the acceptance of earthworms treated with 4% methiocarb by the Japanese quails (*Coturnix cotumix japonica*), as a representative for earthworm consuming birds. The study should reveal whether the treated earthworms are palatable and taken up in quantities, which are toxic to birds.

Japanese quails are known to consume earthworms rapidly. As proven earthworm eaters, quails were housed in three groups of six birds. During acclimation the birds were exposed to dead untreated earthworms two days before the exposure (30 g per group). The earthworms, cut into appropriate pieces, were exposed on wet paper. Additionally standard diet (150 g per group) was offered in dispensers during the acclimation period. The consumption of standard diet and earthworm was measured.

After a starvation time of 16 hours, the birds were exposed to one tray with approximately 30 g dead untreated earthworms and one tray with ca 30 g methiocarb treated dead earthworms for four hours while one control group was exposed only to dead untreated earthworms. After the exposure period birds were switched to standard diet ad libitum. The birds were observed during the whole exposure period and for three days post exposure. The consumption of earthworms was determined. The concentration of Methiocarb RB4 in the earthworms was analysed.

Methiocarb was determined in earthworm samples ranging from 74.5–97.4 mg/kg. Methiocarb sulfoxide was determined at 4.8 to 7.16 mg/kg. Methiocarb-sulfone was not found above the LOQ of 0.01 mg/kg.

Observation and Behaviour Control: All birds consumed earthworms. After 90 minutes all earthworms were eaten. Treatment group 1: five of six birds consumed earthworms but only three of them untreated ones. At the end of exposure 62 % of the untreated and 48% of the treated earthworms were eaten. Treatment group 2: All birds fed on earthworms, treated as well as untreated. At the end of the exposure all untreated and 59% of the treated earthworms were consumed. No signs of intoxication were observed.

Body weight development: The birds of the two treatment groups lost body weight compared to the control group. This observation has to be considered to be within a natural variation since the two treatment groups consumed apparently less standard food on the acclimation days. The birds of these groups consumed more food on the exposure day than the control and showed no indications of intoxication. Therefore the body weight loss cannot be attributed to the test substance.

Some birds preferred the untreated worms, while others ate both treated and untreated worms to a similar extent. The concentration of methiocarb in the earthworms was too low to evoke apparent avoidance or any signs of intoxication.

Field

Title	Field monitoring of birds on oilseed rape fields treated with Mesurol Slug Bait RB4 (“Schneckenkorn Mesurol”) and RB2 (“Mesurol Schneckenkorn”)
Reference	Wolf, C. & Wilkens, S. 2003
Test Guideline	EU Council Directive 91/414/EEC amended by the Commission Directive 96/68/EC. Pesticides and Wildlife - Field Testings: Recommendations of an international workshop on terrestrial field testing of pesticides, attached to Pesticide Effects on Terrestrial Wildlife, Somerville & Walker (ed.), Taylor & Francis, London 1990
Data Validity	2
Data Relied On	Yes - the data were considered to be critical and were relied on in this assessment.

This study was conducted to investigate the exposure and possible impact of a MESUROL slug pellet application on rape fields on individual birds and the natural bird community. The fields were treated under conditions of commercial agricultural practice with MESUROL used as slug pellet to reduce crop damage. The occurring birds were investigated by field observations using the 'scan sampling' technique. Furthermore, during carcass searches at regular intervals after a MESUROL slug pellet treatment, the study fields were surveyed for dead individuals.

In addition, the exposure scenario was investigated in detail by evaluating the spatial and temporal exposure pattern of slug pellets in the field and the occurrence of contaminated earthworms and slugs on the soil surface, including analysis of their methiocarb residue levels. The field monitoring was conducted on two study fields and their surroundings near Soest, North-Rhine-Westphalia, Germany. Both sites were commercial cultivated oilseed rape (OSR) fields. The test material used was MESUROL RB4 and RB2 slug pellets (first application RB4, second application RB2, active ingredient methiocarb). Monitoring activities focused on exposure of slug pellets at the soil surface after the application, on the occurrence of poisoned slugs and of impacted earthworms as potential food for birds, on bird activities and abundance on the field before and after the application and finally on the occurrence of vertebrate mortality.

Exposure was measured by transect counts of pellets every third day (from day 1 after the first application until day one after the second application) at four transects (10 plots each) on every field.

To quantify the occurrence of earthworms and slugs, the fields were searched (one man hour per search) every 3rd day. Worms and slugs found were collected, counted and analysed for residues of methiocarb.

Behavioural observations were performed to estimate bird activities prior to and after application and to record abnormal behaviour (i.e. signs of intoxication) after the application. The observation technique was the scan sampling technique with one observation interval every 5 min. Observations were mainly performed in the early

morning and in the evening before sunset for a duration of 3 h per session. On the day of the first application and during the three subsequent days, observations were performed within the whole daylight period.

To quantify wildlife mortality, carcass searches were performed on the study fields and their surroundings on transect routes every 3rd day after the application. Causes of death of carcasses were ascertained by gross pathology, residue analyses and measurements of Acetyl-Cholin-Esterase (AChE) activity in dissected brain samples.

Results:

Test substance	Methiocarb (Mesurol slug bait RB4 (4% ac.) and RB 2 (2% ac.))		
Test object	natural bird communities on two study fields		
Exposure			
Mean number of pellets on the soil surface [pellets/m2]	on day of 1st application	32.8	
	12 days after 1st application	20.0	
	on day of 2nd application (RB2)	49.9	
Number of sampled earthworms on the soil surface after the application	0 to 237 (mean 99) per man-hour of search, depending on weather conditions and soil humidity		
Number of sampled slugs on the soil surface after the application	0 to 66 (mean 10) per man-hour of search, depending on weather conditions and soil humidity		
Ac. content of sampled earthworms	31 mg ac/kg fresh weight (average, range: 0 to 59)		
Ac. content of sampled slugs	156 mg ac/kg fresh weight (1 sample only)		
Bird Monitoring			
Number of bird species, that visited the fields before and after the application	period	field 1	field 2
	before appl.	22	21
	after appl.	24	16
Dominant nutritional guild, which was observed on the fields after the application	large (>80g) potentially earthworm eating birds		
Feeding activity of large (>80 g) earthworm eaters on the fields [Individuals/observation-interval/ha]	period	field 1	field 2
	before appl.	0.26	0.11
	after 1st appl.	0.10	0.07
	after 2nd appl.	0.02	0.21
Species observed ingesting potentially contaminated earthworms	*Turdus* spp.*, Columba palumbus, Milvus milvus, Falco tinnunculus , Buteo buteo, Circus pygarus*		
Signs of intoxication	none		
Carcass Search			
Mortality of birds caused by Methiocarb poisoning	none		
Mortality of mice and voles [mean no. of dead individuals, corrected for search efficiency and standardised on 1000 m transect length (10 m width) during the whole study period after the treatment	cause of death	treatment related	other and unknown reasons
	midfield area	13	5
	field border area	13	28

Application of Mesurol slug bait (RB4 or RB2) on winter rape fields in Germany had no impact on birds feeding on those fields. Earthworms were ingested in low amounts without affecting bird individuals sublethally (i.e. behaviour impacts) or lethally. In general, feeding activity of birds decreased after application of slug pellets on the study fields due to a generally reduced food availability on the fields after the drilling process of the crop (except second application on field 2: foraging activity increased slightly, birds were mainly attracted by

ploughing activities on theneighbouring field and were foraging on earthworms on the study field occasionally). Large (>80g b.w.) earthworm eating birds were the only relevant nutritional guild regularly observed foraging on the fields. Some carcasses of small mammals were found on and around the study fields after Mesurol slug pellet application. In general no differences were observed between the effects of RB4 and RB2 slug bait formulations.

Title	Exposure of birds in different crops to Mesurol RB4 slug pellets in France in spring – attractiveness of those fields, species of concern and impacts
Reference	Wilkens, S. 2007
Test Guideline	None
Data Validity	2
Data Relied On	Yes - the data were considered to be critical and were relied on in this assessment.

The aim of this study was to investigate the potential impact of Mesurol RB4 application in freshly treated maize, sugar beet and sunflower fields on the natural bird community. The study was conducted in different sugar beet fields in the department Aube (Champagne- Ardenne region) in north-eastern France, and in sunflower and maize fields in the department Tarn (Midi-Pyrénées region) in south-western France. Both regions are typical cultivation areas for the crops concerned.

In order to gain information on the occurrence of birds, bird activity was observed by scan sampling once in each field over an entire daylight period. Every ten minutes a defined section of the study field was scanned, records being taken of species, behaviour and any incidents. Each field was completely searched for dead birds. Predator removal tests and carcass search efficiency tests were conducted to evaluate the actual carcass detection rate. In order to quantify the exposure of Mesurol RB4 slug pellets and potential food items (seeds, earthworms, slugs), sample counts were carried out in each field.

Results: In order to estimate the proportion of carcasses removed by predators a defined number of carcasses (adult and scallywag quails) were placed randomly within a study field. The presence of the carcasses was checked after approximately 12 and 24 hours.

Carcasses removed by predators

field	crop	maize	sugar beet	sunflower
	no.	7	10	3
carcass distribution	date	26.04.2006	12.04.2006	17.05.2006
	time	08:10 - 08:50	06:40 - 07:30	07:50 - 08:30
	carcasses inside field	9	8	9
	carcasses outside field	3	4	6
	total no. of carcasses	12	12	15
first check after ~ 12h	date	26.04.2006	12.04.2006	17.05.2006
	time	20:00 - 20:15	18:10 - 18:20	19:55 - 20:15
	carcasses lost inside field	5	1	8
	carcasses lost outside field	1	0	1
	carcasses lost total	6	1	9
	removal inside field	55.6 %	12.5 %	88.9 %
	removal outside field	33.3 %	0.0 %	16.7 %
	removal total	50.0 %	8.3 %	60.0 %
	date	27.04.2006	13.04.2006	18.05.2006
	time	07:45 - 08:00	09:45 - 10:00	07:10 - 07:25
	carcasses lost inside field	6	7	8
	carcasses lost outside field	1	3	1

second check after ~24h	carcasses lost total	7	10	9
	removal inside field	66.7 %	87.5 %	88.9 %
	removal outside field	33.3 %	75.0 %	16.7 %
	removal total	58.3 %	83.3 %	60.0 %

	MAIZE	SUGAR BEET	SUNFLOWER
BIRD OBSERVATION			
Worked fields	15	10	16
Mean observed area [ha]	1.49	1.53	2.30
Mean no. of scans	88.5	84.2	90.1
Observed species per field	2 to 15	1 to 10	1 to 17
Total number of species	35	25	28
Frequency of occurrence of foraging birds [%] (top five species; given is the mean of the results for each field)	Magpie (4.18) Carrion Crow (2.93) Red-legged Partridge (2.88) Turtle Dove (2.33) Feral Pigeon (2.13)	Pied Wagtail (2.73) Blackbird (2.50) Rook (2.13) Grey Partridge (1.55) Skylark (1.54)	Wood Pigeon (4.61) Red-legged Partridge (2.35) Feral Pigeon (1.96) Domestic Fowl (1.51) Carrion Crow (1.39)
Abundance of foraging birds [ind./ha/scan] (top five species; given is the mean of the results for each field)	Feral Pigeon (0.056) Red-legged Partridge (0.045) Carrion Crow (0.045) Magpie (0.034) Turtle Dove (0.026)	Pied Wagtail (0.038) Blackbird (0.026) Rook (0.025) Skylark (0.022) Grey Partridge (0.017)	Wood Pigeon (0.074) Domestic Fowl (0.029) Feral Pigeon (0.027) Red-legged Partridge (0.018) Stock Pigeon (0.012)
Relative Risk Index(top five species; given is the mean of the results for each field; the index varies from 0 (no risk) to1 (high risk))	Magpie (0.026) Red-legged Partridge (0.023) Carrion Crow (0.022) Wood Pigeon (0.014) Turtle Dove (0.014)	Pied Wagtail (0.018) Grey Partridge (0.014) Blackbird (0.013) Rook (0.013) Skylark (0.013)	Wood Pigeon (0.027) Red-legged Partridge (0.022) Feral Pigeon (0.014) Carrion Crow (0.011) Black Kite (0.010)
Bird incidents	none	none	none
EXPOSURE ASSESSMENT (headlands / midfield)			
Worked fields	15	10	16
Area per field [m2]	5 / 5	5 / 5	5 / 5
Mesurol RB4 [pellets/m2] (mean of single results)	15.8 / 13.5	31.6 / 20.9	16.3 / 13.3
Slugs [no./m2] (mean of single results)	<0.1 / 0.1	0 / 0	0.1 / 0.1
Earthworms [no./m2] (mean of single results)	0.1 / <0.1	0 / 0	<0.1 / 0.1
Seeds [no./m2] (mean of single results)	0.2 / 0.1	0.1 / 0	<0.1 / 0
CARCASS SEARCH			
Worked fields	15	10	16
Mean area searched [ha]	6.11	9.36	5.80
Total area searched [ha]	91.6	93.6	92.8
Carcasses found	one mallard chick (residues below LOQ1)	none	one pied wagtail (residues below LOQ[1])

Search efficiency [% of placed carcasses found]	100	58.3	85.7
Removal [% of placed]	50.0 / 58.3	8.3 / 83.3	60.0 / 60.0

[1] LOQ = 0.04 mg/kg for methiocarb, methicarb-sulfone and methiocarb-sulfoxide.

Maize: It can be concluded that the exposure of birds to Mesurol RB4 slug pellets applied on maize fields is very low. Freshly treated fields were not especially attractive to birds, the abundance of foraging birds was very low and no impacts of Mesurol RB4 on birds could be detected. Five species can be named as species of concern: magpie, red-legged partridge, carrion crow, turtle dove and feral pigeon.

Sugar beet: It can be concluded that the exposure of birds to Mesurol RB4 slug pellets applied on sugar beet fields is very low. Freshly treated fields were not attractive to birds, the abundance of foraging birds was very low and no impacts of Mesurol RB4 on birds could be detected. Five species can be named as species of concern: pied wagtail, blackbird, rook, grey partridge and Skylark.

Sunflower: It can be concluded that the exposure of birds to Mesurol RB4 slug pellets applied on sunflower fields is very low. Freshly treated fields were not especially attractive to birds, the abundance of foraging birds was very low and no impacts of Mesurol RB4 on birds could be detected. Six species can be named as species of concern: wood pigeon, red-legged partridge, feral pigeon, domestic fowl, carrion crow and stock pigeon

The exposure of birds to Mesurol RB4 slug pellets applied on maize, sugar beet and sunflower fields is very low. Freshly treated fields were not especially attractive to birds, the abundance of foraging birds was very low and no impacts of Mesurol RB4 on birds could be detected.

Title	Mesurol RB4: Field monitoring of birds and mammals in artichoke fields in Spain (Campo de Cartagena and Huerta de Lorca 2007)
Reference	Barfknecht, R 2008/
Test Guideline	None
Data Validity	2
Data Relied On	Yes - the data were considered to be critical and were relied on in this assessment.

The present study aims to examine possible side-effects of Mesurol slug pellets RB4 on bird and mammal communities after an application on artichoke fields in Spain. The study was conducted in the Autonomic Region of Murcia, in particular on the Campo de Cartagena and in the Huerta de Lorca, located in the South Eastern part of Spain. The monitoring was performed in March and April 2007.

On each site a bird census was performed on the day -1. After the application, on each site at least 3 searches for dead or impacted birds and mammals were performed (day +1, +2 or +3). On the application day, no carcass search was carried out in order not to chase the birds away. Instead of it, a bird observation was performed in the afternoon to scan for impacted birds. During the carcass search, a team of 2–6 people paced the test area. The team walked along the artichoke field in parallel rows.

When carcasses were found, further carcass searches were performed. This activity stopped after a maximum of five post application searches or when two following searches were negative.

All detected carcasses were collected and determined to species level. The place of finding, the circumstances of the finding and the conditions of the carcasses including signs of intoxication were recorded. A gross necropsy was performed to look for slug pellets in the digestive tract. When they were found, no further analysis was necessary and the carcass could be disposed.

Results:

Observations of birds: In summary 54 bird species were detected, but most of them in the surrounding (e.g. wading birds) or only flying over the field like swifts and swallows. The artichoke fields were visited by 19 species. The most regularly observed birds were crested larks, corn buntings, linnets, and yellow wagtails. They were found on half of the fields and are therefore good candidates for focal species in artichoke fields. In regard of quantity, the same species were also the most abundant ones. In one field (field 3) the house sparrow was similarly abundant. During carcass searches on field 4, one linnet nest with 4 eggs and one corn bunting nest with 5 eggs were detected.

Effects on exposed bird species: During all bird observation periods as well as during the carcass search activities, neither mortalities nor behavioural impacts on these birds were found.

Observation of mice: On fields 2 and 3 in the Campos de Cartagena, 40 life traps each were installed to evaluate a list of existing small mammal species. Since on these sites the trapping did not show any indications of mouse activity (no mouse in traps, no indication of oat flake consumes), an extreme low abundance was supposed for these fields and the activity was stopped. On the other sites no mouse trapping was performed.

Findings at carcass searches after day 0

		Field No.					
Found Object	Findings	1	2	3	4	5	6
Feather Spot	Black Bird		x				
Skin	Rabbit			x			
Two dead beetle	Carbide beetle			x			
Wood Mouse	Slug pellets found in				x		
Wood Mouse	Slug pellets found in					x	
Wood Mouse	Slug pellets found in					x	
Part of magpie	Wing					x	
Wood Mouse	Slug pellets found in					x	
Wood Mouse	Slug pellets found in					x	
Wood Mouse	Slug pellets found in					x	
Wood Mouse	Slug pellets found in						x

Efficiency of the carcass search

Date	Area	Carcass Laid out	Found	Disappeared	Finding Rate [%]
2007-03-29	Site No. 5	20	17	3	85
2007-04-04	Site No. 2	19	17	2	89
Total		39	34	5	87

Efficiency of carcass search: The efficiency of the search team recovery rate (of the positioned dummies) was tested on site 2 and on site 5. The finding rates amounted to 89 % and 85%.

Results of carcass search: Prior to day 0 only two feather spots (feral pigeon), one nest including eggs (linnet), one cast, and some old bones from a sheep or goat were found in the test sites On test sites 1, 2 and 3, all situated in the Campo de Cartagena, no carcass was found after application. On the other sites single mice were found dead, all of them containing slug pellets. Therefore it has to be assumed that they were lethally intoxicated. On field 4 (also in the Campo de Cartagena), only on day +1 a dead wood mouse was detected, but no further on the following days. The same happened on site 6 in the Huerta de Lorca. On site 5 (Huerta de Lorca) 3 wood mice were detected on day +1, none on day +2, one each on day +3 and +4, none on day +5. Altogether 7 dead mice were detected.

Overall the application of Mesurol RB4 had no impact on birds while some wood mice individuals were at risk, appearing to be lethally intoxicated.

Title	Methiocarb – Exposure of birds and mammals in cabbage and potato fields in France to slug pellets – species of concern and impacts
Reference	Von Blanckenhagen, F., Muenderle, M. & Lueckmann, J.2008/
Test Guideline	None
Data Validity	1
Data Relied On	Yes - the data were considered to be critical and were relied on in this assessment.

The aim of the study was to investigate the potential impact of soil-applied ‘methiocarb RB4 slug pellets (Mesurol Pro)’ at a rate of 3.0 kg/ha on the natural bird and mammal community present on freshly planted cabbage and pre-harvest potato fields in France. Therefore, bird species and number of individuals making use of treated fields were recorded and observed throughout the first week following the application. Within this period, the fields were systematically searched for bird and small mammal carcasses.

Eight freshly planted cabbage fields (comprising in total 15.7 ha) and eight pre- harvest potato fields (comprising in total 27.0 ha) in Brittany (France) were selected as typical for the cultivation of these crops. The surrounding of the potato fields was more structured and no soil cultivation took place for the past months. The cabbage fields were surrounded by less structured surroundings and soil cultivation took place before application.

In order to provide an insight into the composition of the bird community found in freshly planted cabbage and pre-harvest potato fields and to gain further information on the impact of ‘methiocarb RB4 slug pellets (Mesurol Pro)’ on individual birds, scan sampling was performed after application. The number of bird individuals and any signs of intoxication or otherwise abnormal behaviour of any bird was recorded in a pre-selected area of the study fields. In total, 304 scans (every 10 minutes one scan) within each crop were conducted. Each individual bird and its behaviour was recorded. Abundance and frequency of occurrence were calculated to describe the resident bird community in freshly planted cabbage and pre-harvest potato fields. Additionally, the percentage of foraging birds and the Relative Risk Index (RRI) were calculated for each individual bird species.

Each of the 16 study fields was completely searched twice (111 man hours) to find dead birds and mammals. Carcass search efficiency tests and carcass removal tests were also conducted to evaluate the actual carcass

detection rate. Carcasses were analysed for methiocarb residues (LOQ = 0.04 mg/kg), including the parent and the metabolites methiocarb-sulfoxide and methiocarb-sulfone.

Data recording and analysis: All data were collated and analysed using standard spreadsheet applications. A complete list of species observed was then compiled and the species were ranked in a list according to the order of importance: mean abundance > FOscan > RRI.

Results:

Bird monitoring after the application: In freshly planted cabbage fields, a total of 1,711 individual bird contacts were allocated to 12 species and in pre-harvest potato fields, 114 individual bird contacts were allocated to 14 species. No symptoms of intoxication or any kinds of abnormal behaviour of the applied insecticide 'methiocarb RB4 slug pellets (Mesurol Pro)' on the avian community of freshly planted cabbage and pre-harvest potato fields could be detected during the bird scans, including the bird species with the highest Relative Risk Indices (RRI).

Carcass search: Carcass search efficiency tests in freshly planted cabbage and pre-harvest potato fields resulted in a mean recovery rate of 75% and 72%, respectively, indicating that carcass search was effective. Carcass removal test in freshly planted cabbage and pre-harvest potato fields resulted in 5% and 3% of carcasses removed by predators after 24 hours, respectively, indicating low scavenger pressure.

Seven small mammal carcasses (4 wood mice, 2 red-toothed shrews, 1 unidentifiable mouse) were found on the freshly planted cabbage fields (mean 0.6 small mammal carcasses/ha/plot). A total of 35 small mammal carcasses (32 wood mice, 2 white-toothed shrews, 1 house mouse) and one bird carcass (robin) were found on the pre-harvest potato fields (4.6 small mammal carcasses/ha/plot and 0.1 bird carcasses/ha/plot, respectively).

Higher carcass numbers of small mammals and birds in potato fields can be attributed to the more structured surrounding area, which provides densely populated source habitats for birds and small mammals, which frequently move into the study fields. In contrast, the cabbage fields were surrounded by less structured surroundings. Additionally, the cabbage fields were drilled shortly before application resulting in reduced mammal populations due to the disturbance by agricultural work whereas the soil surface of the potato fields have been left undisturbed for the past months.

All residue levels of methiocarb (including the parent and the metabolites methiocarb-sulfoxide and methiocarb-sulfone) measured in all carcasses were above the LOQ, except in one white-toothed shrew carcass. Residues in the different taxa varied: the shrews (*Crocidura* spec., *Sorex* spec.) contained methiocarb residues between <LOQ and 8.3 mg methiocarb/kg. Except two specimens of wood mice (*Apodemus sylvaticus*), which contained residue traces of 0.13 and 0.14 mg methiocarb/kg only, all other wood mice showed residue levels >16 mg methiocarb/kg. Based on the toxicity profile of methiocarb to small mammals it can be concluded that 'methiocarb RB4 slug pellets (Mesurol Pro)' were related to the death of 92% of the wood mice (n = 36) and 50% shrews (n = 4). As for the other species of mammal carcasses found, it can suspected that their deaths were also linked to 'methiocarb RB4 slug pellets (Mesurol Pro)'.

The residues of methiocarb (including metabolites) in the bird carcass was above the LD50 of 5 mg methiocarb/kg for birds known from the literature. It cannot be excluded that this particular case was related to methiocarb contaminated food, since robins typically feed on soil-dwelling arthropods, and mean residue levels in the moribund carabid beetles collected from the field were 149 mg methiocarb/kg. However, given the high abundance

and high exposure of this bird species in the potato fields, concomitant with high carcass search efficiency and low carcass removal it appears to be conclusive that this incident can be considered as exceptional.

The five most abundant bird species in freshly planted cabbage fields were black-headed gull, magpie, herring gull, wood pigeon and lesser black-backed gull, and in pre-harvest potato fields robin, blackbird, dunnock, wood pigeon and great tit. Residue analysis of recorded carcasses indicate that 'methiocarb RB4 slug pellets (Mesurol Pro)' was involved in the death of wood mice and shrews in most cases (92% and 50%, respectively). Additionally methiocarb-contaminated insects cannot be excluded as a reason for the exceptional death of one robin. More structured surroundings and less disturbances through agricultural management led to higher densities of small mammals in the pre-harvest potato fields compared to freshly planted cabbage fields.

Effects on aquatic organisms

Fish Acute

Title	Acute toxicity of methiocarb to rainbow trout (*Oncorhynchus mykiss*) in a 96-hour semi-static test
Reference	Peither, A. 2000
Test Guideline	OECD No. 203 and Directive 92/69/EEC, C.1
Data Validity	1
Data Relied On	Yes - the data were considered to be critical and were relied on in this assessment.

The objective of this 96-hour study was to evaluate the acute toxicity of methiocarb to fish. Seven fish per test concentration were exposed for 96 h in a semi-static test (daily test medium renewal) to nominal test item concentrations of 0.16, 0.35, 0.80, 0.16, 3.6 and 8.0 mg ac/L, to a control and a solvent control. The recorded effects were the mortality and symptoms of intoxication of the test fish.

According to pre-experiments the test item was not sufficiently stable in water. Therefore a semi-static test procedure was chosen with a daily test medium renewal to keep exposure concentrations as constant as possible during the test period of 96 hours. To confirm the maintenance of the test item concentrations during the test medium renewal periods of one day, duplicate samples were taken out of all test media, the control and the solvent control at the end of the first test medium renewal period (from Day 0 to Day 1). In the analysed test medium samples from the start of the test medium renewal periods the measured test item concentrations ranged between 82 and 97% of the nominal values. The mean measured test concentrations in the freshly prepared test media (calculated as the average over all measurements per test concentration) varied in the range of 82 to 91% of the nominal values. This shows the correct dosage of the test item.

The test fish were observed after approximately 4, 24, 48, 72 and 96 hours test duration for symptoms of intoxication and mortality. Dead fish were removed once daily and discarded. The LC50 and the 95% confidence interval at the observation dates were calculated by Probit Analysis. The NOEC, LOEC, LCO, LLC and LC100 were determined directly from the raw data.

Results:

Nominal concentration of the test item (mg/L)	Observation time Number of affected fish / number of dead fish, and observed symptoms of intoxication:				
	4 hours	24 hours	48 hours	72 hours	96 hours
Control 0.16 0.35 0.80 1.6 3.6 8.0	0 / 0 0 / 0 0 / 0 1 / 0 SR 2 / 0 TS 7 / 3 TS, SR 7 / 7	0 / 0 0 / 0 1 / 1 7/0 SR, TS 7/5 7 / 7	0 / 0 0 / 0 1 / 1 7 / 1 SR, TS 7 / 5 SR,TS	0 / 0 0 / 0 2 / 1 TS 7 / 1 SR, TS 7 / 5 TS	0 / 0 0 / 0 2 / 1 TS 7 / 1 SR, TS 7 / 5 TS
LC50 95% CI	- -	1.2 0.82-1.8	1.1 0.73-1.6	1.1 0.73-1.6	1.1 0.73-1.6

TS: Tumbling during swimming, BO: Fish mainly at the bottom of the aquarium, SR: Fish lying on side or back on the bottom, GA: Exopthalmus.

Title	Acute toxicity of methiocarb to bluegill (*Lepomis macrochirus*) in a 96-hr flow-through test
Reference	Peither, A. 2000
Test Guideline	OECD No. 203 and Directive 92/69/EEC, C.1
Data Validity	1
Data Relied On	Yes - the data were considered to be critical and were relied on in this assessment.

The objective of this 96-hour toxicity test was to evaluate the acute toxicity of methiocarb to fish. For this purpose, young bluegill (*Lepomis macrochirus*) were exposed in a flow-through test to aqueous test media. Seven fish per test concentration were exposed for 96 h in a flow-through test to nominal test item concentrations of 0.08, 0.16, 0.36, 0.80, 1.6, 3.6 and 8.0 mg ac/L. Additionally, a control (test water without test item) and a solvent control (100 µL/L N,N-Dimethylformamide) were tested in parallel. The test concentrations were based on the results of two range-finding tests and the results of pre-experiments to the solubility of the test item in test water.

The analytically determined test item concentrations in the freshly prepared application solutions varied in the range from 88 to 100 % of the nominal values. This shows the right preparation of the application solutions. In the aged application solutions, values from 87 to 99% of nominal were found. Thus, methiocarb was sufficiently stable in the application solutions during the renewal period of two days. the concentrations of the test item in the analysed test medium samples from the concentrations of nominal 0.16 to 1.6 mg/L were a range from 86 to 110% of the nominal values during the 96 hour test period. The mean measured test concentrations (calculated as the average over all measurements per test concentration) were in the range of 88 to 98% of nominal. Therefore, the reported biological results are related to the nominal concentrations. The analytical results from the test concentrations of nominal 3.6 and 8.0 mg/L were not taken into account, since these concentrations were not a relevant part of the concentration effect curve. The concentration of nominal 8.0 mg/L was obviously above the water solubility limit of the test item in the test water.

The test fish were observed after approximately 3, 24, 48, 72 and 96 hours test duration for symptoms of intoxication and mortality. Dead fish were removed once daily and discarded.

The LC50 and the 95% confidence interval at the observation dates were calculated by probit analysis. The two highest concentrations of nominal 3.6 and 8.0 mg/L were not taken into account at the probit analysis, since they were not a relevant part of the concentration effect curve (the same biological results were obtained in the next lower concentration of 1.6 mg/L). The NOEC, LOEC, LLC, LCO and LC100 were determined directly from the raw data.

Results:

Nominal concentration of the test item (mg/L)	Observation time Number of affected fish1 / number of dead fish, and observed symptoms of intoxication:				
	4 hours	24 hours	48 hours	72 hours	96 hours
Control	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0

0.08 0.16 0.36 0.80 1.6 3.6 8.0	0 / 0 0 / 0 0/0 1/1 3/0 TS,SR 7/0 TS 7/0 TS, SR, BO	0 / 0 0 / 0 3/0 SR,TS 7/3 SR,TS 7/5 SR** 7/3 SR** 7/5 SR**	0 / 0 0 / 0 3/2 TS 7/3 TS BO 7/7 7/3 SR** 7/7	0 / 0 0 / 0 7/2 BO,TS 7/3TS, BO 7/4 SR**	0 / 0 0 / 0 7/2BO,TS 7/3 SR, TS, 7/4 SR**
LC50 95% CI	>8.0 -	1.0 0.71-1.5	0.65 0.44-0.96	0.65 0.44-0.96	0.65 0.44-0.96

*TS: Tumbling during swimming, BO: Fish mainly at the bottom of the aquarium, SR: Fish lying on side or back on the bottom, ** All fish showed weak gill movement only.*

Aquatic invertebrates - acute

Title	Acute toxicity of methiocarb to *Daphnia magna* in a 48-hour semi-static immobilization test
Reference	Peither, A. 2000
Test Guideline	OECD No. 202, Part I and Directive 92/69/EEC, C.2
Data Validity	1
Data Relied On	Yes - the data were considered to be critical and were relied on in this assessment.

The objective of the 48-hour toxicity test was to evaluate the influence of methiocarb on the mobility respectively survival of *Daphnia magna*. For this purpose a semi-static test was performed, exposing juvenile daphnids for 48 hours nominal concentrations of 2.2, 4.6, 10, 22 and 46 µg ac/L. Additionally, a control (test water without test item) and a solvent control (100 µL/L N,N-Dimethylformamide) were tested in parallel.

In the analysed test media of nominal 4.6, 10, 22 and 46 µg/L, the measured concentrations at the start of the test media renewal periods were in the range of 76 to 104% of the nominal values. At the end of the test medium renewal periods of 24 hours the measured concentrations were 59 to 72% of nominal. The mean measured concentrations (calculated as the average over all measurements) were 4.0, 7.4, 17 and 37 µg/L respectively. All reported biological results are based on the mean measured test item concentrations.

The immobility or mortality of the daphnids was determined by visual controls after 24 and 48 hours of exposure. Those organisms not able to swim within 15 seconds after gentle agitation of the test beaker were considered to be immobile.

The 24-hour and 48-hour EC50 and their 95% confidence limits were calculated by probit analysis. The NOEC and LOEC were determined directly from the raw data. The TEC was calculated as the geometric mean of NOEC and LOEC.

The highest test concentration of nominal 46 µg/L was not taken into account for the calculation of the 48-hour EC50, by the study authors because in the next lower concentration the same biological result was obtained and thus the concentration of nominal 46 µg/L was not a relevant part of the concentration-effect curve. The lowest test concentration of nominal 2.2 µg/L was not taken into account by the study authors for probit analysis, because this concentration was below the NOEC determined in this test and thus not analysed.

Results

Influence of methiocarb on the mobility of *Daphnia magna*

Concentration [µg/L] Nominal (Mean measured)	No. of beaker	No. of daphnids tested	No. of immobilized daphnids after		Mean % of immobilized daphnids after	
			24 h	48 h	24 h	48 h
Control	1 2	10 10	0 0	0 0	0	0
Solvent control	1 2	10 10	0 0	0 0	0	0
2.2 (n.a.)	1 2	10 10	0 0	0 0	0 0	0 0
4.6 (4.0)	1 2	10 10	0 0	1* 0	0	5*
10 (7.4)	1 2	10 10	0 0	5 4	0	45
22 (17)	1 2	10 10	3 5	10 10	40	100
46 (37)	1 2	10 10	10 10	10 10	100	100

n.a.: not analysed, not estimated as significant toxic effect.*

The following results were calculated based on mean measured concentrations:

Exposure	24 h semi-static	48 h semi-static
EC50 (µg/L)	18	7.7
(95% confidence limits)	16–21	6.7–8.8
Lowest observed effect concentration (LOEC)	17	7.4
No observed effect concentration (NOEC)	7.4	4.0

Title	Acute toxicity of methiocarb SC 500 to the waterflea *Daphnia magna* in a water-sediment system
Reference	Dorgerloh, M 2007
Test Guideline	Higher tier study, conducted under principal consideration of OECD-Guideline No. 202 (2004)
Data Validity	1
Data Relied On	Yes - the data were considered to be critical and were relied on in this assessment.

The 48 hour (h) acute exposure is to evaluate possible effects on viability of *Daphnia magna* caused by the test item during static exposure in a water-sediment system. *Daphnia magna* (1st instars < 24 h old, 6 x 5 animals per treatment group and control), were exposed in a static test system for 48 hours (without feeding) to the nominal initial concentrations of 11.2, 22.3, 44.7, 89.4 and 179 µg form./L (corresp. to 5, 10, 20, 40 and 80 µg ac/L).

The chemical analysis of methiocarb spiked in the overlying water of the basins at test initiation ranged between 103.6 % and 108.5 % (mean: 105.4 %) of the corresponding nominal concentrations, thus all results are based on nominal initial concentrations.

Exposure concentrations of methiocarb were measured only at start of the 48 hours exposure period in the overlying water phase of the whole water-sediment test system. After 48 hours, behaviour of the water fleas was visually evaluated by counting mobile daphnids, defined as animals with swimming movements (slight movements of antennae were not interpreted as swimming movement) within approximately 15 seconds after gentle agitation of the test vessel. Additionally all possible signs of sublethal effects had to be recorded. Statistical evaluation was performed on a 5% level of significance (p=0.05).

Results:

Toxicity to *Daphnia magna* (based on nominal initial concentrations):

Test Concentration		Exposed daphnids (=100%)	Immobilised daphnids after 48 h of exposure	
µg ac/L	µg form./L		n	%
Control (0)	0	30	2	6.7
5	11.2	30	2	6.7
10	22.3	30	12	40.0
20	44.7	30	25	83.3
40	89.4	30	28	93.3
80	179	30	30	100

Validity criteria were met for the study. The sediment parameters measured directly after preparation, at start of equilibration time (day -18) fulfilled the guideline requirements with a water content of 30.9%, pH of 7.0 and an organic carbon content of 2.3 %.

Observations on sub-lethal effects revealed abnormal behaviour of the exposed daphnids from test concentrations of 22.3 to 89.4 µg form./L.

Statistical significant differences compared to control findings (α = 0.05) were established for test concentrations from 22.3 to 179 µg form./L, resulting in a NOEC of 11.2 µg form./L, corresponding to 5 µg ac/L.

The EC50 for immobility after 48 hours of static exposure in a water sediment test system is, 29.2 µg form./L, corresponding to 13.1 µg ac/L.

Aquatic invertebrates - chronic

Title	Influence of methiocarb SC 500 on development and reproductive output of the waterflea *Daphnia magna* in a static water-sediment test system after multiple spiking
Reference	Dorgerloh, M 2007
Test Guideline	EU Council Directive 91/414/EEC (1991)
Data Validity	1
Data Relied On	Yes - the data were considered to be critical and were relied on in this assessment.

The primary objective of this 21 day chronic-toxicity test is to evaluate possible effects on reproductive capacity and mortality (immobilisation) of *Daphnia magna* caused by the test item during static exposure in a water-sediment system after multiple spiking (three times). *Daphnia magna* (1st instars < 24 h old, 10 x 1 animal per test level), exposed in a static test system for 21 days to a total of three water spikes of three nominal initial concentrations of 1.12, 4.47 and 17.9 µg form./L (corresponding to 0.5, 2.0 and 8.0 µg ac/L), freshly prepared and repeatedly admixed to the overlying water at 7 days intervals (on study days 0, 7 and 14).

During the study, the measured concentrations of the test item in the overlying water were analysed four times on days 0, 7, 14 and 21 at all test concentrations and the control. The results of chemical analysis of methiocarb in the freshly prepared test solutions directly after application on day 0, 7 and 14 ranged between 97% and 104% (mean: 100%) of nominal for day 0, 102% to 112% (mean: 108%) of nominal for day 7 and 110% to 111% (mean: 110%) of nominal for day 14. Additional measurements of the freshly prepared application stocks on day 0, 7, 14 were 107, 108 and 109 % of nominal (on average). Due to the analytical findings, all results are based on nominal concentrations of the test item in the overlying water for each spike.

The corresponding concentrations of the aged test solutions on day 7, 14 and 21 ranged between 8% and 9% (mean: 9%), 4% and 6% (mean: 5%) and 6% of nominal. The partitioning of the active ingredient between water and sediment is known from water/sediment studies done under comparable conditions. The analysed concentrations of the aged exposure solutions reflect the expected fast dissipation of the active ingredient from the water body.

All freshly emerged neonates were counted and removed at least three times per week from the appearance of the first brood on until study-termination. Possible adverse effects on the juveniles had to be recorded. Counted offspring were discarded afterwards.

Behaviour of the parental animals was repeatedly assessed during the whole exposure period, at least at the timepoints of neonate counting. Therefore, the number of immobile water fleas (=animals showing no swimming movements within 15 seconds after slight agitation of the vessel) was recorded for each test vessel separately. Slight movements of antenna was not interpreted as swimming movement. Additional observations for sub-lethal effects (like clinging of animals to the water surface) were also performed. The individual onset of maturity was recorded separately for each parental female.

At study termination, the body length of all surviving parent animals was determined by measurement under a scaled binocular

Results:

Toxicity of methiocarb SC 500 to Daphnia magna, based on nominal concentrations of the formulation after each spike.

Nominal initial treatment (µg form./L))	Parental endpoints		Reproductive endpoints		
	Body length (mm)	Survival (%)	Cumulative offspring per parent animal (n)	Parent age at first offspring emergence (days)	Neonates behaviour (% unaffected neonates)
Control	5.0	80	298	9.3	100 %
3 x 1.12	4.8	90	253	10.1	100 %

Nominal initial treatment (µg form./L))	Parental endpoints		Reproductive endpoints		
	Body length (mm)	Survival (%)	Cumulative offspring per parent animal (n)	Parent age at first offspring emergence (days)	Neonates behaviour (% unaffected neonates)
3 x 4.47	5.0	80	292	9.4	100 %
3 x 17.9	5.0	90	283	9.6	100 %

Validity criteria were met. No sub-lethal effects had been observed of the survived daphnia over the whole exposure period

The overall chronic NOEC for 21 days of static exposure after multiple spiking (3 times) of methiocarb SC 500 in a water- sediment - system to Daphnia magna expressed as nominal test concentration is > 3 x 17.9 µg form./L (corresponding to > 3 x 8 µg ac/L).

Algae and aquatic plants

Title	Toxicity of methiocarb to *Scenedesmus subspicatus* in a 72-hour algal growth inhibition test
Reference	Peither, A. 2000
Test Guideline	OECD Test Guideline 201 (2006); Directive 92/69/EEC, C.3
Data Validity	1
Data Relied On	Yes - the data were considered to be critical and were relied on in this assessment.

The objective of this test was to determine the inhibitory effect of methiocarb on the growth of the freshwater green algal species *Scenedesmus subspicatus*. A static, non-renewal exposure system was used. The inhibition of growth in relation to control cultures was determined over a test period of 72 hours, and thus over several algal generations. *Scenedesmus subspicatus* was exposed under static conditions (stirring cultures) for 72 h. The following nominal concentrations were tested: 0.10, 0.32, 1.0, 3.2 and 10 mg ac/L.

The quantities of the test item found at the beginning of the test ranged from 72 to 90% of nominal concentrations. The quantities of the test item found at Day 3 were 13 to 33% of nominal. The losses could be due to hydrolysis of the test item in water. The mean measured test concentrations (calculated as the average over all measurements per test concentration) varied in the range of 46 to 60% of the nominal values. Therefore, the reported biological results are related to the mean measured test item concentrations.

The algal cell densities in the samples were determined by counting with an electronic particle counter (Coulter Counter®, Model ZM), with at least two measurements per sample. In addition, after the test period of 72 hours, a sample was taken from the control and from a test concentration with reduced algal growth (nominal 3.2 mg/L). The shape of the algal cells was microscopically examined.

Inhibition of algal growth was determined from: the area under the growth curves AUC (= biomass) and the specific growth rates for exponentially growing cultures. The EbC50 and ErC50 (the concentrations of the test item corresponding to 50% inhibition of algal biomass b respectively growth rate r compared to the solvent control), and the corresponding EC10 and EC90 and their 95%-confidence limits were calculated by probit analysis.

Validity criteria were met. No remarkable observations were made concerning the appearance of the test media with the nominal concentrations up to and including 3.2 mg/L. These test media were clear solutions throughout the test period. In the highest test concentration of nominal 10 mg/L a small part of the test item was lying on the bottom of the test vessel at all observation times (although the test media were continuously stirred).

Areas under the growth curves (AUC) and percentage inhibition of AUC (Iauc) during the test period

Mean measured concentration (mg/L)	Areas under the Growth Curves (AUC) and % Inhibition of AUC					
	0-24 h		0-48 h		0-72 h	
	AUC	Iauc	AUC	Iauc	AUC	Iauc
solvent control	74	0.0	429	0.0	1820	0.0
0.052	65	11.4	408	4.8	1786	1.8
0.18	64	13.6	392	8.6	1725	5.2
0.60	54	26.8	286	33.3	1309	28.1
1.5	30	59.3	122	71.5	335	81.6
4.6	9	88.1	35	91.8	79	95.6

Growth rates r and percentage inhibition of r (Ir) during the test period

Mean measured concentration (mg/L)	Growth Rate r (1/day) and % Inhibition of r					
	0–24 h		0–48 h		0–72 h	
	r	Ir	r	Ir	r	Ir
solvent control	1.96	0.0	1.60	0.0	1.51	0.0
0.052	1.86	4.9	1.59	0.4	1.51	0.2
0.18	1.84	6.0	1.57	2.0	1.50	0.8
0.60	1.70	13.1	1.38	13.6	1.42	6.0
1.5	1.25	36.1	0.91	43.1	0.87	42.5
4.6	0.55	72.0	0.45	71.8	0.39	74.3

The following end-points were calculated:

Parameter (0-72 h)	Growth rate r (mg/L mean measured)	Biomass b (mg/L mean measured)
EC50 95%-conf. limits	2.2 1.5–3.7	0.82 0.38–1.7
EC10 95%-conf. limits	0.60 0.23–0.95	0.23 0.027–0.47
NOEC	0.18	0.052

At the microscopic examination of the shape of the algal cells after 72 hours test period no difference was observed between the algae growing in the test concentration of 1.5 mg/L (nominal 3.2 mg/L) and the algal cells in the control. Thus, the shape of the algal cells growing at least up to this test concentration was obviously not affected.

Sediment organisms

Title	*Chironomus riparius* 28-day chronic toxicity test with methiocarb (tech.) in a water-sediment system using spiked water
Reference	Bruns, E. 2006
Test Guideline	OECD Guideline 219: "Sediment-Water Chironomid Toxicity Test Using Spiked Water" (adopted 13 April 2004)
Data Validity	1
Data Relied On	Yes - the data were considered to be critical and were relied on in this assessment.

The aim of the study was to determine the influence of the test item on emergence and development of *Chironomus riparius* for 28-days in a static water-sediment- system (spiked water exposure). First instar of *Chironomus riparius* larvae, 4 beakers per test concentration and control with 20 animals each were exposed for 28 days to initial nominal concentrations in the overlying medium (spiked water application) of 0.01, 0.02, 0.04, 0.08, 0.16 and 0.32 mg ac/L of a water-sediment system.

Dissolved oxygen concentrations ranged in the water phase from 7.2 to 8.7 mg O_2/L (7.2 mg O_2/L= 80 % O_2 - saturation), the water pH values ranged from 8.1 to 8.6 and the water temperature ranged from 20.0°C to 20.6°C measured from parallel beakers of each test concentration over the whole period of testing. Recoveries of methiocarb were measured three times during the study: 1 hour, 7 days and 28 days after application in one additional test container of each nominal initial test concentrations of 0.01, 0.04 and 0.32 mg ac/L and control (only on day 0) of the overlying water and the pore water of the sediment. Results are based on nominal initial concentrations in mg ac/L of the test item in the overlying water:

Analysis in the overlying water and pore water

Initial nominal conc.	analytical results of Methiocarb, means of two analyses each (mg ac/L)					
mg ac/L	1 hour / day 0		day 7		day 28	
	analysed conc.	% of initial nominal	analysed conc.	% of initial nominal	analysed conc.	% of initial nominal
control	overlying water					
	<0.0011	-	n.a.	-	n.a.	-
solvent control	<0.0011	-	n.a.	-	n.a.	-
0.01	0.0091	91.1	<0.0011	0	<0.0011	0
0.04	0.0341	85.3	0.00192	4.8	<0.0011	0
0.32	0.298	93.1	0.0121	3.8	<0.0011	0
average %	-	89.8	-	2.9	-	0
	pore water[1]					
control	<0.0011	-	n.a.	-	n.a.	-
solvent control	<0.0011	-	n.a.	-	n.a.	-
0.01	<0.0011	0	<0.0011	0	<0.0011	0
0.04	0.00357	0.6	0.00139	0.2	<0.0011	0
0.32	0.02780	0.6	0.0110	0.2	0.00170	0.04
average %	-	0.4	-	0.2	-	0.01

n.a.: not analysed, [1]calculated to the real volume of pore water and the applied amount of ac.

The test vessels were also observed at least three times per week to make a visual assessment of any behavioural differences compared to the control. The sex, time point of emergence and number of emerged midges was recorded daily during the period of emergence. As only fully emerged adults are relevant for the endpoints of this study, larvae which did not yet mature were not taken into account for emergence ratios and development time. To determine number and sex of emerged adults, the covering plates of each test container were carefully moved and the midges, which mostly stayed at the sides of the vessels, were enumerated; after identification of the sex (male midges have feathered antennae) midges were removed.

Because it is not possible to introduce the same number of female and male organisms as larvae into the individual test beakers, the evaluation of the emergence ratio cannot be performed for individual sexes. Thus, for the statistical analysis of the emergence ratio, male and female results are pooled. Emergence ratio, which is related to mortality. Influence on emergence and development rate after 28 days (based on nominal initial concentrations of the test item in the overlying water):

Validity: The emergence in the control(s) had to reach at least 70% of introduced larvae at the end of the test. The emergence should occur between 12 and 23 days after their insertion into the control vessels. The oxygen content in the water body had to be > 60 % of saturation at the end of the test in all test vessels. The pH of the overlying water had to be between 6 and 9 in all test vessels. The water temperature did not differ by more than ± 1°C over the whole exposure period. A total of 92.5 % of the inserted (n= 160) larvae matured into adults in the pooled controls after 28 days, fulfilling the guideline requirements.

Start of emergence was on day 13 and 14 for the controls and test concentrations from 0.01 to 0.16 mg ac/L. The start of emergence was reduced for one day at the highest test concentration of 0.32 mg ac/L.

Concentration	Number of emerged midges	Emergence of inserted larvae			Development pooled sex Rate (/day)
initial nominal mg ac/L		total (%)	male (%)	female (%)	
Controls*	148	92.5	46.9	45.6	0.062
0.01	76	95.0	41.3	53.7	0.062
0.02	78	97.5	41.3	56.2	0.060
0.04	77	96.2	56.2	40.0	0.064
0.08	75	93.8	46.3	47.5	0.063
0.16	67	83.7	36.2	47.5	0.063
0.32	19	23.7	11.2	12.5	0.062

** control and solvent control were pooled.*

The Chi^2-Test indicates no statistically different sensitivities of sexes. Therefore male and female results were pooled for further statistical analyses to increase the statistical power. Statistical significance (α= 0.05) on emergence ratio and development rate of females was only evaluated for 0.32 mg ac/L (= LOEC), resulting in an NOEC of 0.16 mg ac/L. For the development rate of male and pooled sex, no statistical significance could be established up to the highest test concentration, resulting in an NOEC of > 0.32 mg ac/L.

Endpoints	NOEC	LOEC	EC50
emergence ratio (pooled sex) (95 % confidence limits)*	0.160	0.320	0.275
development rate (pooled sex)	≥ 0.32	> 0.32	> 0.32

** due to mathematical reasons, the calculation of the confidence limits was not possible.*

Title	Acute toxicity of methiocarb (tech.) to larvae of *Chironomus riparius* in a 48 h static laboratory test system
Reference	Silke, G. 2014
Test Guideline	EU Directive 91/414/EEC Regulation (EC) No 1107/2009 US EPA OCSPP 850.SUPP.
Data Validity	1
Data Relied On	Yes - the data were considered to be critical and were relied on in this assessment.

The objective of this 48 hour (h) toxicity test was to evaluate the acute immobilisation to larvae of *Chironomus riparius* (1st instar) caused by the test item. As the primary endpoint, a concentration causing 50 % immobility to larvae of *Chironomus riparius* (24 h and 48 h -EC50) was determined. Larvae of *Chironomus riparius* (1st instars < 2–3 days old, 6 beakers per test concentration and control(s), with 5 animals each) were exposed for 48 hours in a static test system (water only) to concentrations of 0.05, 0.11, 0.23, 0.50 and 1.08 mg ac/L.

Quantitative amounts of analysed ac. were measured in all freshly prepared test levels on day 0, and control(s). On day 2, at the end of exposure, additionally all aged test levels including control(s) were measured. The analysed pure metabolite found in all freshly prepared test levels on day 0 in reference to nominal concentrations ranged between 95 and 100 % (average 98%). In aged test levels on day 2 there were analytical findings between 56 and 76% (average 64%) of nominal. Due to the recoveries of < 80% of nominal after 2 days of exposure, all results are based on mean measured concentrations.

Measurements of the water temperature were done continuously in one negative control vessel and recorded hourly by a data logger. Additionally water parameters (temperature, pH and oxygen) were measured in the freshly prepared test solutions of each test concentration on day 0 and on day 2 in the combined test solutions of each test concentration. Beside immobility, a possible occurrence of symptoms was recorded and evaluated after 24 and 48 hours of exposure

All validity criteria were met: Control mortality was below 15% within 48 hours. Dissolved oxygen was > 3 mg oxygen/L in the control and in all test concentrations. The water pH values ranged from 7.8 to 8.0 and the water temperature ranged from 20.4°C to 20.7°C over the whole period of testing, fulfilling the guideline requirements.

Acute toxicity of test item to first instar-larvae of *Chironomus riparius* after 48 hours (based on mean measured concentrations) are shown in the table below:

Nominal concentrations [mg ac/L]	Mean measured concentrations [mg ac/L]	Exposed chironomids (=100%)	Immobility			
			24 h		48 h	
			n	%	n	%
control	-	30	0	0	1	3.3
solvent-control	-	30	0	0	1	3.3
0.05	0.040	30	1	3.3	1	3.3
0.11	0.086	30	4	13.3*	10	33.3*
0.23	0.198	30	12	40.0*	29	96.7*
0.50	0.390	30	25	83.3*	30	100.0*

Nominal concentrations [mg ac/L]	Mean measured concentrations [mg ac/L]	Exposed chironomids (=100%)	Immobility			
			24 h		48 h	
			n	%	n	%
1.08	0.868	30	30	100.0*	30	100.0*

** statistically significant (α = 0.05).*

Statistical results of probit analysis conducted for determination of EC50 values (based on mean measured concentrations)

Probit analysis for data obtained after	NOEC [mg ac/L] (mean measured)	EC 50 [mg ac/L] (mean measured)	lower 95% CI [mg ac/L] (mean measured)	upper 95% CI [mg ac/L] (mean measured)
24 hours	0.040	0.200	0.163	0.246
48 hours	0.040	0.103	0.089	0.121

Metabolites – effects on aquatic organisms

Fish - Acute

Title	Acute toxicity of methiocarb-phenol to rainbow trout (*Oncorhynchus mykiss*) in a 96-hour static test
Reference	Peither, A. 1999
Test Guideline	OECD No. 203, Directive 92/69/EEC, C.1
Data Validity	1
Data Relied On	Yes - the data were considered to be critical and were relied on in this assessment.

The objective of this 96-hour study was to evaluate the acute toxicity of the test item methiocarb-phenol (M03) to fish. For this purpose, young rainbow trout were exposed in a static test to aqueous test media containing the test item at nominal test item concentrations of 0.22, 0.46, 1.0, 2.2, 4.6 and 10 mg/L. Additionally a control was tested in parallel.

In the duplicate test medium samples, taken at the start of the test, the concentrations of the test item methiocarb-phenol were analysed from the nominal test item concentrations of 1.0 to 4.6 mg/L. In the duplicate samples from the aged test media the concentrations of the test item were analysed from the nominal test item concentrations of 1.0 and 2.2 mg/L (96 hour samples) and from the nominal test item concentration of 4.6 mg/L (24 hour sample). The analytically determined mean concentrations of the test item methiocarb-phenol in the analysed test media from the start of the test varied in the range from 93 to 95% of the nominal values. The slight losses especially in the lowest test concentration could be due to adsorption of the test item during the performance of the test. The total mean measured test item concentrations (calculated as the average over all measurements per test concentration) ranged from 83 to 92% of the nominal values. Since the test item concentrations were sufficiently stable during the test period of 96 hours, the samples taken after 48 hours were not analysed. All reported biological results are related to the nominal concentrations of the test item.

One glass aquarium with 30 litre test medium was used for each test concentration and the control. The test vessels were labelled with the RCC project number and all necessary additional information to assure unmistakable identification. At the start of the test seve fish were introduced into each aquarium in a random order. The test media were slightly aerated during the test period. The fish were not fed during the test.

The test fish were observed after approximately 2.5, 24, 48, 72 and 96 hours test duration for symptoms of intoxication and mortality. Dead fish were removed daily and discarded.

The LC50 was calculated for all observation dates. However, calculation by probit analysis or Moving Average Interpolation was not possible due to the steep concentration-effect relationship. Instead the LC50-values were determined as the geometric mean value of the two consecutive test concentrations with 0% and 100% mortality, and the 95% confidence intervals for the LC50 as the test concentrations with 0% and 100% mortality. The NOEC, LOEC, LCO and LC100 were determined directly from the raw data. The samples from the nominal test item concentrations of 0.22 and 0.46 mg/L were not analysed, because at these test concentrations no biological effects were observed, and thus they were clearly below the 96-hour NOEC. The test item concentration of nominal 10 mg/L was not analysed, since the same high toxic effect was determined in the lower analysed test item concentration of nominal 4.6 mg/L. The test item concentrations of nominal 10 mg/L was therefore not considered as being a relevant part of the concentration- effect curve.

Nominal concentration of the test item (mg/L)	Observation time Number of affected fish / number of dead fish, and observed symptoms of intoxication:				
	2.5 hours	24 hours	48 hours	72 hours	96 hours
Control 0.22 0.46 1.0 2.2 4.6 10	0 / 0 0 / 0 0 / 0 0 / 0 7 / 0 TS, BO, SR 7 / 0 SR, KR, 7 / 7	0 / 0 0 / 0 0 / 0 0 / 0 7 / 0 TS, BO, SR 7 / 7	0 / 0 0 / 0 0 / 0 0 / 0 7 / 0 TS, SR, GA	0 / 0 0 / 0 0 / 0 0 / 0 7 / 0 TS, BO, SR, GA	0 / 0 0 / 0 0 / 0 0 / 0 7 / 0 TS, BO, SR, GA
LC50 95% CI	6.8 4.6-10	3.2 2.2-4.6	3.2 2.2-4.6	3.2 2.2-4.6	3.2 2.2-4.6

TS: Tumbling during swimming, BO: Fish mainly at the bottom of the aquarium, SR: Fish lying on side or back on the bottom, GA: Exopthalmus.

Title	Methiocarb-sulfone-phenol - Acute toxicity (96 hours) to rainbow trout (*Oncorhynchus mykiss*) in a static test
Reference	Dorgerloh, M. & Sommer, H. 2001
Test Guideline	OECD Test Guideline 203 (1992); OPPTS 850.1075 (1996); EPA-FIFRA § 72-1, Directive 92/69/EEC, C.1 (1992)
Data Validity	1
Data Relied On	Yes - the data were considered to be critical and were relied on in this assessment.

An acute 96 h toxicity test was conducted in order to determine the toxicity of methiocarb-sulfone-phenol (M05) to rainbow trout (*Oncorynchus mykiss*). The primary measure for acute toxicity was mortality. Sublethal and behavioural observations were made during the course of the study. Ten fish per test concentration were exposed for 96 h under static test conditions to nominal (mean measured) concentrations of 6.25 (6.02), 12.5 (12.1), 25.0 (24.2), 50.0 (48.4) and 100 (97.5) mg test item (pure metabolite)/L.

The test item was homogeneously mixed into the aquaria. Under the test conditions the test item was stable. Based on analytical determination of methiocarb-sulfone-phenol (in water by HPLC) mean measured values between 98% and 99% of nominal were found. Nominal (mean measured) concentrations of 6.25 (6.02), 12.5 (12.1), 25.0 (24.2), 50.0 (48.4) and 100 (97.5) mg test item (pure metabolite)/L. All reported results refer to mean measured concentrations of the test item.

Dissolved oxygen, water temperature and pH values were determined daily in each aquarium, water temperature was additionally measured in the control aquarium and recorded hourly with a data logger. Analytical determinations of the active ingredient concentrations were made in the test medium at the beginning of the test, after 48h and at test termination. Whenever possible, the LC50 values and the 95%-confidence intervals were calculated every 24-hours using a computer program, which estimated the LC50 using one of three statistical techniques: moving average, binomial probability or probit analysis. The appropriate method was determined according to the data characteristics

There was no mortality or sub-lethal effects in any test concentration except the highest. At 24 h in the highest (97.5 mg/L) group, fish showed the following symptoms: were inactive or displayed abnormally low activity; showed loss of equilibrium with lateral deviation from their normal orientation; displayed mucous evacuation of the intestines. At this concentration all fish died by 72 h.

The no-observed-effect-concentration (NOEC) was 48.4 mg/L. The LC50 (96 h) of M03 to rainbow trout (*Oncorhynchus mykiss*) was determined to be 68.7 mg/L with a 95% confidence interval from 48.4–97.5 mg/L.

Title	Methiocarb-sulfoxide-phenol - Acute toxicity (96 hours) to rainbow trout (*Oncorhynchus mykiss*) in a static test (limit test)
Reference	Dorgerloh, M. & Sommer, H. 2001
Test Guideline	OECD Test Guideline 203 (1992); OPPTS 850.1075 (1996); EPA-FIFRA § 72-1, Directive 92/69/EEC, C.1 (1992)
Data Validity	1
Data Relied On	Yes - the data were considered to be critical and were relied on in this assessment.

The effect of methiocarb-sulfoxide-phenol (M04) to rainbow trout (*Oncorhynchus mykiss*) was studied in a static test (limit test). For every test concentration one aquarium was used and was labelled with a study number, a consecutive number and the nominal concentration of the test item.

Immediately prior to the test, water samples were taken from the centre of the aquaria for analytical determination of the active ingredient concentration. Under test conditions the test item was stable. Based on analytical determination of M04 (in water by HPLC) a mean measured value of 101 % of nominal was found. All reported results refer to mean measured concentrations of of 106 mg/L.

At the start of the test, thirty fish were randomly introduced into each aquarium using a randomization table. During the test, fish were examined after four hours and then daily for mortalities and signs of poisoning.

Dissolved oxygen, water temperature and pH values were determined daily in each aquarium, water temperature was additionally measured in the control aquarium and recorded hourly with a data logger. Analytical determinations of the active ingredient concentrations were made in the test medium on day 0, on day 2 and at test termination.

In this test 30 fish were exposed to a nominal (mean measured) concentration of 104 (106) mg/L. A control group of 30 fish was exposed to untreated test water. There were no mortality or sub lethal effects in the control or treatment group. The 96 h LC50 was >106 mg/L.

Concern was raised that the Q/P runoff curves (Q = runoff in mm; P = rainfall in mm) in the current consultation paper will underestimate runoff from soils heavier than “loamy” soils, as the runoff curves were only available for “Sandy” and “Loamy” soils.

Title	Methiocarb-methoxy-sulfone - Acute toxicity (96 hours) to rainbow trout (*Oncorhynchus mykiss*) in a static test
Reference	Dorgerloh, M. & Sommer, H. 2001
Test Guideline	OECD Test Guideline 203 (1992); OPPTS 850.1075 (1996); EPA-FIFRA § 72-1, Directive 92/69/EEC, C.1 (1992)

Data Validity	1
Data Relied On	Yes - the data were considered to be critical and were relied on in this assessment.

An acute 96 h toxicity test was conducted in order to determine the toxicity of methiocarb-methoxy-sulfone (M10) to rainbow trout (*Oncorynchus mykiss*). The primary measure for acute toxicity was mortality. Sublethal and behavioural observations were made during the course of the study rainbow trout (*Oncorhynchus mykiss*, mean body length 5.4 cm, mean body weight 1.7 g), 10 fish per test concentration were exposed for 96 h under static test conditions to nominal (mean measured) concentrations of 3.125 (3.09), 6.25 (6.00), 12.5 (12.2), 25.0 (24.8) and 50.0 (45.9) mg test item (pure metabolite)/L.

Under the test conditions the test item was stable. Based on analytical determination of Methiocarb-methoxy-sulfone (in water by HPLC) mean measured values between 92% and 100% of nominal were found. All reported results refer to mean measured concentrations of the test item.

Dissolved oxygen, water temperature and pH values were determined daily in each aquarium, water temperature was additionally measured in the control aquarium and recorded hourly with a data logger. Whenever possible, the LC50 values and the 95%-confidence intervals were calculated every 24-hour using a computer program which estimated the LC50 using one of three statistical techniques: moving average, binomial probability or probit analysis. The appropriate method was determined according to the data characteristics.

Results:

Cumulative Mortality and Behavioural Observations: Total number of fish tested at each concentration: 10

Mean measured concentration (mg/L)	4 h		24 h		48 h		72 h		96 h	
	Dead	Obs.	Dead	Obs.	Dead	Obs.	Dead	Obs.	Dead	Obs.
control	0	0	0	0	0	0	0	0	0	0
3.09	0	0	0	0	0	0	0	0	0	0
6.00	0	0	0	0	0	0	0	0	0	0
12.2	0	0	0	0	0	10 AP, DF	0	10 AP, DF	0	10 AP, DF
24.8	0	IO BO, DF, OB, AP	0	10 BO, DF, OB, SD, BA	3	10 TS, DF, OB, SD, BA	4	10 TS, DF	4	10 TS, DF
45.9	0	10 DF, BO, SR	0	10 BO, DF, SD, SR, BA	10	10	+	+	+	+

Dead fish are added to the sum of fish with symptoms.

Abbreviations of behavioural observations:

AP: were inactive or displayed abnormally low activity. BO: laid inactive on the bottom of the aquarium

DF: turned dark in coloration SR : laid on their sides or backs

TS: showed loss of equilibrium wiht lateral deviation from their normal orientation. OB: remained for unusually long periods at the water surface

BA: had swollen bellies

SD: displayed mucous evacuation of the intestines

+ ... no observations, all fish dead

The NOEC was 6.00 mg/L. The 96 h LC50 was 26.8 mg/L (95% CI 12.2-45.9 mg/L) calculated by binomial probability.

Title	Methiocarb-sulfoxide – Acute toxicity (96 hours) to rainbow trout (*Oncorhynchus mykiss*) in a semi-static test
Reference	Dorgerloh, M. 2000
Test Guideline	OECD Test Guideline 203 (1992); OPPTS 850.1075 (1996); EPA-FIFRA § 72-1, Directive 92/69/EEC, C.1 (1992)
Data Validity	1
Data Relied On	Yes - the data were considered to be critical and were relied on in this assessment.

An acute 96h toxicity test was conducted to estimate the toxicity of methiocarb-sulfoxide (M01) to rainbow trout (*Oncorhynchus mykiss*). Ten fish per test concentration were exposed for 96 h under semi-static test conditions to nominal (mean measured) concentrations of 0.625 (0.591), 1.25 (1.17), 2.50 (2.38), 5.00 (4.77) and 10.0 (9.13) mg/L.

Analytical determinations of M01 (in water by HPLC) were made in all freshly prepared test media in the aquaria at each beginning as well as after the longest period (24 hours) of use. Mean measured values between 93% and 97% of nominal were detected. All reported results refer to mean measured concentrations of the test item.

Dissolved oxygen, water temperature and pH values were determined daily in each aquarium, water temperature was additionally measured in the control aquarium and recorded hourly with a data logger. Whenever possible, the LC50 values and the 95%-confidence intervals were calculated every 24-hour using a computer program which estimated the LC50 using one of three statistical techniques: moving average, binomial probability or probit analysis. The appropriate method was determined according to the data characteristics.

Dissolved oxygen (DO) concentrations ranged from 96 to 104 percent oxygen saturation in all aquaria. The pH values ranged from 6.9 to 7.2 in all aquaria. The test temperature ranged from 11.4°C to 12.7°C (daily means).

Cumulative Mortality and Behavioural Observations: Total number of fish tested at each concentration: 10

Mean measured concentration (mg/L)	4 h		24 h		48 h		72 h		96 h	
	Dead	Obs.	Dead	Obs.	Dead	Obs.	Dead	Obs.	Dead	Obs.
control	0	0	0	0	0	0	0	0	0	0
0.591	0	0	0	0	0	0	0	0	0	0
1.17	0	0	0	0	0	10 H	0	10 H	0	10 H
2.38	0	0	0	10 H	0	10 H	0	10 H, AT	0	10 H, AT
4.77	0	0	0	10 H	0	10 H, AT	0	10 OB, AT	0	10 OB, AT
9.13	0	10 AT	0	10 OB, AT, BO	10	10	+	+	+	+

Dead fish are added to the sum of fish with symptoms.

+ ... no observations, all fish dead

Abbreviations of behavioural observations: BO: on bottom of aquarium lying

AT: labored respiration

H: hyperactive-exaggerated response to stimulus or disturbance

OB: at water surface - rising and remaining unusually long at the surface

There were neither mortalities nor signs of poisoning in the control group.

The LC50 (96 h) of M01 to rainbow trout was 6.60 mg/L with a 95% confidence interval from 4.77–9.13 mg/L.

Aquatic invertebrates - Acute

Title	Acute toxicity of methiocarb-sulfone-phenol (tech.) to water fleas (*Daphnia magna*)
Reference	Hendel, B. 2001
Test Guideline	OECD Test Guideline 202, adopted version 4 April, 1984 and Draft document, October 2000 Deviations: none (test duration 48 hours)
Data Validity	1
Data Relied On	Yes - the data were considered to be critical and were relied on in this assessment.

This study assessed the acute toxicity of methiocarb-sulfone-phenol (M05) (tech.) to water fleas (*Daphnia magna*). Young Daphnia magna (1st instars < 24 h old, 3 x 10 animals per concentration) in a static test system were exposed for 48 h to nominal concentrations of 0, 10, 18, 32, 56 and 100 mg/L.

Measured concentrations of methiocarb sulfone-phenol were 99 to 104% (for an average of 101.6 %) of the nominal concentrations at the beginning of the test. These results indicate that the test concentrations prepared in this test correspond to nominal concentrations.

If possible, the EC50-values and the 95 percent confidence limits are calculated by an EC50 computer program developed by Dr. H.T. Ratte (Technical University Aachen) using the Probit-Analysis after the "Maximum-Likelihood" Method (according to Finney (1952)).

Validity criteria were met. After 48 h, there was 3%, 3%, 0%, 63% and 97% immobilisation in the 0, 10, 18, 32, 56 and 100 mg/L groups respectively. Sublethal effects were observed in the top two highest test concentrations.

The 48 h EC50 was 54 mg/L (95 %-confidence limits not calculable) and the NOEC was 32 mg/L (nominal).

Title	Acute toxicity of methiocarb-methoxy-sulfone (tech.) to water fleas (*Daphnia magna*)
Reference	Hendel, B. 2000
Test Guideline	OECD-Guideline No. 202 (April 1984)
Data Validity	1
Data Relied On	Yes - the data were considered to be critical and were relied on in this assessment.

The Acute Toxicity of methiocarb-methoxy-sulfone (M10) (tech.) to water fleas (*Daphnia magna*) was assessed. Young Daphnia magna (1st instars < 24 h old, 3 x 10 animals per concentration) in a static test system were exposed for 48 h to nominal concentrations of 0, 10, 18, 32, 56, 100 and 180 mg/L.

Measured concentrations of M10 were 85 to 98 % (for an average of 89.2 %) of the nominal concentrations at the beginning of the test. These results indicate that the test concentrations prepared in this test correspond to nominal concentrations. The measured concentrations at the end of the 48 hours exposure period indicate that the test substance was stable for the duration of this study.

The test vessels consisted of 100 mL glass beakers. Each test vessel contained 50 mL of the test solution with ten animals per vessel, three replicates per concentration. The beakers were covered with a plexiglass plate and placed in an environmental chamber at 20 ± 1 °C and a 16:8 light- dark cycle.

If possible, the EC50-values and the 95 percent confidence limits are calculated by an EC50 computer program developed by Dr. H.T. Ratte (Technical University Aachen) using the Probit-Analysis after the "Maximum-Likelihood" Method (according to Finney (1952)).

Validity criteria were met. There was no immobilisation or sublethal effects at test concentrations up to 56 mg/L. 7% and 23% immobilisation were observed in the 100 mg/L and 180 mg/L groups respectively.

The 48 h EC50 was >180 mg/L (95 % confidence limits not calculable) and the NOEC was 56 mg/L (nominal).

Title	Acute toxicity of methiocarb-sulfoxide (tech.) to water fleas (*Daphnia magna*) under flow through test conditions
Reference	Hendel, B. & Sommer, H. 2001
Test Guideline	OECD-Guideline No. 202 (April 1984)
Data Validity	1
Data Relied On	Yes - the data were considered to be critical and were relied on in this assessment.

This study assessed the acute toxicity of methiocarb-sulfoxide (M01) (tech.) to water fleas (*Daphnia magna*) under flow through test conditions. Young Daphnia magna (1st instars < 24 h old, 4 x 10 animals per concentration) in a flow through test system (renewal of test solutions every hour) were exposed for 48 h to concentrations (nominal initial) of control, solvent control, 0.018, 0.032, 0.056, 0.10, 0.18, 0.32 and 0.56 mg/L.

Averages of three analytical measurements of initial concentrations were 89% to 100% (mean: 95%). Concentrations of the metabolite were on average nearly 22% lower at the end of the one exposure period. The mean measured initial concentrations on days 0, 1 and 2 of the test were 89 to 100% of the nominal concentrations (for an average: 95%). These results indicate that the test concentrations prepared in this test correspond to nominal concentrations and therefore nominal initial concentrations were used for calculation.

Analysed concentrations of M01 in stock solutions

Nominal Concentration (mg/L)	Day 0 initial measured concentration (mg/L)	Day 2 initial measured concentration (mg/L)	Day 0 (% of nominal)	Day 2 (% of nominal)
180	165	161	92	89
320	290	286	91	89
560	540	532	96	95
1000	958	945	96	95
1800	1720	1740	96	97
3200	3100	3090	97	97
5600	5420	5380	97	96

The test vessels consisted of 400 mL glass beakers, labelled with a study number, concentration and replicate. Each test vessel contained 250 mL of the test solution with ten animals per vessel, four replicates per concentration. The test was placed in an environmental chamber at 20 ± 1 °C and a 16:8 light-dark cycle.

If possible, the EC50-values and the 95 percent confidence limits are calculated by an EC50 computer program developed by Dr. H.T. Ratte (Technical University Aachen) using the Probit-Analysis after the "Maximum-Likelihood" Method (according to Finney (1952)).

Validity criteria were met. The following observations were recorded:

Water flea toxicity of M01.

		Number of living animals		Immobilized Water Fleas (%)	
Nominal concentration (mg ac/L)	Replicate number	24 h	48h	24 h	48h
	1	10	10		
Control	2	10	10	0	5 ± 6
	3	10	10		
	4				
	1	9	9		
Solvent Control	2	10	10	2.5 ± 5	2.5 ± 5
	3	10	10		
	4	10	10		
	1	9	8		
0.018	2	10	10	2.5 ± 5	7.5 ± 10
	3	10	10		
	4	10	9		
	1	10	10		
0.032	2	10	10	2.5 ± 5	5.0 ± 10
	3	10	10		
	4	9	8		
	1	10	6 (5)		
0.56	2	9	4 (4) [6,4]	5.0 ± 10	52.5 ± 10
	3	9	5 (4) [6,4]		
	4	10	4 (4) [6,4]		
	1	10 (2)			
0.10	2	8 (3) [6,4]	2 (2) [6,4]	20.0 ± 14	90.0 ± 8
	3	7 (2) [6,4]	0		
	4	7 (3) [6,4]	1 (1) [6,4]		
	1	4 (1) ·	0		
0.18	2	4 (2) [6,4]	0	65.0 ± 6	97.5 ± 5
	3	3 (1) [5]	1 (1) [6,4]		
	4	3 (1) [6,4] (1) [7]	0		
	1	1 (1) ·	1 (1) [6,4]		
0.32	2	1 (1) [6,4]	0	87.5 ± 5	97.5 ± 5
	3	1 (1) [6,4,1]	0		
	4	2 (2) [6,4,1]	0		
	1	1 (1) "	0		
0.56	2	1 (1) [6,4, 1]	0	82.5 ± 10	100
	3	2 (2) [6,4,1]	0		
	4	3 (3) [6,4,1]	0		

( ) number of living animals with symptoms, if observed.
Symptoms:
1) Quick, trembling antennae movements.

3) Frequency of antennae movements clearly decreased.
5) Swimming movements showed coordination disturbances.
7) Animals cling to the water surface.
2) Frequency of antennae movements clearly increased.
4) Hardly any movements perceivable.
6) Animals lie at the bottom.
8) Animals cling together in clusters.

Mortality above 10% has been observed at 0.10 mg/L after 24 hours and at 0.056 mg/L after 48 hours (LOEC). No mortality above 10% and no occurrence of symptoms were observed at concentrations up to 0.056 mg/L after 24 hours and up to 0.032 mg/L after 48 hours (NOEC).

The 48 h EC50 was 0.056 mg/L (95 %-confidence limits 0.034 to 0.092 mg/L) and the NOEC was 0.032 mg/L (nominal).

Title	Acute toxicity of methiocarb-phenol to *Daphnia magna* in a 48-hour immobilization test
Reference	Peither, A. 1999
Test Guideline	OECD No. 202, Part I and Directive 92/69/EEC, C.2
Data Validity	1
Data Relied On	Yes - the data were considered to be critical and were relied on in this assessment.

The objective of the 48-hour toxicity test was to evaluate the influence of the test item methiocarb-phenol (M03) on the mobility of Daphnia magna. For this purpose a static test was performed, exposing juvenile daphnids for 48 hours to the test item, added to water at a range of concentrations. Young *Daphnia magna* (151 instars <24 h old, 2 x 10 animals per concentration) were exposed to test item concentrations (nominal) of 0.46, 1.0, 2.2, 4.6 and 10 mg/L.

The analytically determined mean concentrations of the test item methiocarb-phenol in the analyzed test media from the start of the test varied in the range from 91 to 94% (mean: 93%) of the nominal values. This shows the correct dosage of the test item. At the end of the test the mean test item concentrations ranged from 92% to 94% (mean: 93%). In the test media methiocarb-phenol was sufficiently stable during the test period of 48 hours. Therefore, all reported biological results are related to the nominal concentrations of the test item.

In each test concentration and the control, 20 daphnids were tested, divided into two groups of ten organisms, each group in 50 mL test medium in a 100-mL glass-beaker. The test was performed in a temperature controlled room. Water temperature: 20–22 °C during the test period.

The immobility or mortality of the daphnids was determined by visual controls after 24 and 48 hours of exposure. Those organisms not able to swim within 15 seconds after gentle agitation of the test beaker were considered to be immobile.

The 24-hour and 48-hour EC50 could not be calculated by Probit Analysis or Moving Average Interpolation due to the steep concentration-effect relationship. Instead, the 48-hour EC50 was determined as the geometric mean value of the two consecutive test concentrations with 0% and 100% immobility. The 24-hour EC50 was calculated by linear regression. The NOEC and LOEC were determined directly from the raw data

At the start and at the end of the test, the pH-values, the oxygen concentrations and the water temperature were determined in each test concentration and the control. The appearance of the test media was recorded at the start of the test and after 24 and 48 hours.

Validity criteria were met. No remarkable observations were made concerning the appearance of the test media. All test media were clear solutions throughout the entire test duration.

At the beginning and the end of the test period, the oxygen concentrations in the test media and the control were at least 8.4 mg/L, the pH-values ranged from pH 7.8 to 8.0

No immobility was observed in the control or any treatment group except the highest during the study. In the 10 mg/L group all daphnids were immobilie after 48 h.

The 48-hour EC50 was calculated to be 6.8 mg/L. The 48-hour NOEC was 4.6 mg/L nominal.

Title	Acute toxicity of methiocarb-sulfoxide to the waterflea *Daphnia magna* in a water-sediment test system
Reference	Dorgerloh, M. 2008
Test Guideline	Higher tier study, conducted under principal consideration of OECD-Guideline No. 202 (2004)
Data Validity	1
Data Relied On	Yes - the data were considered to be critical and were relied on in this assessment.

The objective of the 48-hour toxicity test was to evaluate the influence of the test item methiocarb-sulfoxide (M01) on the mobility of *Daphnia magna* in a water/sediment system. *Daphnia magna* (1st instars < 24 h old, 6 x 5 animals per treatment group and control), exposed in a static test system for 48 hours (without feeding) to the nominal initial concentrations of 0.10, 0.32, 1.00, 3.20 and 10.0 mg/L, freshly prepared and and mixed to the overlying water at start of exposure.

The chemical analysis of M01 spiked in the overlying water of the basins at test initiation ranged between 104 % and 118 % (mean: 111%) of the corresponding nominal concentrations, thus all results are based on nominal initial concentrations.

After 48 hours, behaviour of the water fleas was visually evaluated by counting mobile daphnids, defined as animals with swimming movements (slight movements of antennae were not interpreted as swimming movement) within approximately 15 seconds after gentle agitation of the test vessel. Additionally all possible signs of sublethal effects had to be recorded. Statistical evaluation was performed on a 5% level of significance (p=0.05).

Validity criteria were met. After 48 hours, immobility of 0%, 0%, 20%, 33.3%, 40% and 60% was recorded in the 0.10, 0.32, 1.00, 3.20 and 10.0 mg/L test groups respectively. Sublethal effects were recorded from 1 mg/L and higher.

The 48 h EC50 in the water sediment test system was calculated to be 4.64 mg/L and the 48 hours NOEC is 0.10 mg/L.

Aquatic invertebrates - Chronic

Title	Chronic toxicity of methiocarb-sulfoxide to *Daphnia magna* under flow-through conditions
Reference	Matlock, D. & Lam, C.V.2008
Test Guideline	FIFRA Guideline 72-4 (b) (1982), OPPTS Guideline 850.1300 (1996 draft), OECD Guideline 211 (1998)
Data Validity	1
Data Relied On	Yes - the data were considered to be critical and were relied on in this assessment.

A 21-day flow-through toxicity test was conducted to determine the chronic toxicity of methiocarb-sulfoxide (M01) to Daphnia magna. In a 21-day chronic test first instars of *Daphnia magna* (< 24 h old) were exposed to nominal (mean measured) concentrations of control (<0.40), solvent control (<0.40), 3.75 (1.66), 7.50 (3.28), 15.0 (6.52), 30.0 (13.4) and 60.0 (22.8) µg ac/L under flow-through conditions. Mean measured recoveries were within the range of 38 to 45% of the nominal concentrations. The discrepancy of measured and nominal values can be explained by the instability of M01 in water even under flow-through conditions. However analytical recoveries were consistent during the study in all test levels and reflect actual test concentrations. The toxicity values were calculated based on mean measured concentrations.

Parameters measured included sublethal effects, survival (immobilization), time to first brood release, reproduction (neonates per adult reproductive day) and growth (length and dry weight at study termination). Observations for sublethal effects and survival made daily, reproductive output (neonate counts) occurred at the time of first brood release and on Monday, Wednesday and Friday thereafter (to include the day of termination), growth determinations were made at the end of the exposure. To determine if the treatment groups were significantly different from the control, the reproduction data, survival data and parent daphnid growth data (length and dry weight) were analyzed by a one-way analysis of variance (ANOVA) followed by the Bonferroni t-test and William's test. A 21-Day EC50 based on mortality was calculated, using a computer program developed by Stephan et al. (1977) using the binomial probability method. The method selected was determined by the characteristics of the data, i.e., the number of concentrations in which immobility between 0 and 100 percent occurred and the 95% confidence intervals.

Results:

All validity criteria for this study were met. The following table summarises the findings.

Survival, Reproduction and Growth of Daphnia magna (reported as mean replicate values)

Mean Measured Concentrations (µg ac/L)	Survival (%) (not immobilized)	Time to First Brood (days)	Neonates/ Adult Repro. Day	Adult Body Length (mm)	Adult Dry Weight (mg)
Control	88	8.0	9.3	4.23	0.614
Solvent Control	98	8.0	9.4	4.21	0.490
1.66	98	8.0	9.3	4.04	0.554
3.28	100	8.0	11.3	4.36	0.728
6.52	88	8.0	10.7	4.31	0.737
13.4	63*	8.0	9.9	4.27	0.542
22.8	18*	8.0	10.4	4.23	0.702

**Statistically significant effect (P < 0.05)*

Immobilization of parent animals was analysed at test termination on study day 21. Percent immobilization ranged from 0 to 83%with a statistically significant difference at 13.4 and 22.8 µg ac/L compared to the pooled controls. There was no statistically significant differences with reproduction end-points or adult lengths and weights.

The NOEC was determined based on mean measured concentrations. The 21-day exposure to methiocarb-sulfoxide resulted in an NOEC of 6.52 µg ac/L.

Algae/aquatic plants

Title	Toxicity of methiocarb-phenol to *Scenedesmus subspicatus* in a 72-hour algal growth inhibition test
Reference	Peither, A. 1999
Test Guideline	OECD Test Guideline 201 (2006); Directive 92/69/EEC, C.3
Data Validity	1
Data Relied On	Yes - the data were considered to be critical and were relied on in this assessment.

The objective of this test was to determine the inhibitory effect of the test item methiocarb-phenol (M03) on the growth of the freshwater green algal species *Scenedesmus subspicatus*. Exponentially growing cultures of *Scenedesmus subspicatus* was exposed under static conditions (stirring cultures) for 72 h. The following concentrations were tested: 0.46, 1.0, 2.2, 4.6 and 10 mg test item/L (nominal). The quantities of the test item found at the beginning of the test were 95 to 96% of nominal concentrations. The quantities of the test item found at Day 3 were 93 to 94% of nominal. Therefore, all reported biological results are related to the nominal concentrations of the test item.

The test was started (0 hours) by inoculation of a biomass of 10,000 algal cells per mL test medium. These cells were taken from an exponentially growing pre-culture, which was set up 4 days prior to the test at the same conditions as in the test and was diluted with test water one day prior to the test. The test design included three replicates per test concentration and six replicates in the control. Volumes of 15 mL algal suspension per replicate were continuously stirred by magnetic stirrers in 50 mL Erlenmeyer flasks.

The algal cell densities in the samples were determined by counting with an electronic particle counter (Coulter Counter®, Model ZM), with two measurements per sample.In addition, after the test period of 72 hours, a sample was taken from the control and from a test concentration with reduced algal growth (nominal 4.6 mg/L). The shape of the algal cells was microscopically examined. Inhibition of algal growth was determined from: the area under the growth curves AUC (= biomass) and the specific growth rates µ for exponentially growing cultures.

Biomass (area under the curve) and growth rates were calculated for each test flask. Then the mean area and mean growth rate were determined as arithmetic mean value over all test flasks per treatment. The EbC50 and ErC50 and the corresponding EC10 and EC90 were calculated as far as possible by Probit Analysis for the determination of the LOEC and NOEC. The calculated mean biomass and the mean growth rate µ at the test concentrations were tested on significant differences to the control values by a Dunnett-test.

Results:

0-72 h biomass, growth rates and inhibition (%) compared to the control

Nominal concentration of the test item (mg/L)	0-72 h, Biomass		0-72 h Growth Rate	
	AUC	% Inhibition	Rate (/day)	% Inhibition
control	2314	0.0	1.62	0.0
0.46	2228	3.7	1.62	0.0
1.0	2191	5.3	1.62	0.3
2.2	2124	8.2	1.61	1.0
4.6	1833	20.8*	1.54	4.9*
10	319	86.2*	0.84	48.4*

** mean value significantly different from the control, Dunnett's Test, one sided, p = 0.05.*

At the microscopic examination of the shape of the algal cells after 72 hours test period no difference was observed between the algae growing in the test item concentration of nominal 4.6 mg/L and the algal cells in the control. Thus, the shape of the algal cells growing at least up to this test concentration was obviously not affected.

The 72 h ErC50 was 11 mg/L and the 72 h EbC50 was 6.0 mg/L. The corresponding EC10 values were 4.9 and 1.8 mg/L respectively. Confidence intervals could not be calculated. The study NOEC was 2.2 mg/L for both growth rate and biomass.

Title	Methiocarb-methoxy-sulfone – Influence on the growth of the green alga, *Scenedesmus subspicatus*
Reference	Dorgerloh, M. & Sommer, H. 2001
Test Guideline	STM E 1218 (1990), OECD 201 (1984), ISO 8692 (1989) and EEC C.3 (1992)
Data Validity	1
Data Relied On	Yes - the data were considered to be critical and were relied on in this assessment.

A static 72 h algal growth test was conducted to determine the effects of methiocarb-methoxy-sulfone (M10) on the growth of the green alga, *Scenedesmus subspicatus. Scenedesmus subspicatus* was exposed under static conditions (shake cultures) for 72 h. Algal growth in the controls was exponential over the entire test period. The following concentrations of the test item were tested: 5.00, 10.0, 20.0, 40.0, 80.0 and 160 mg/L. The quantities of the test item found at the beginning of the test in reference to the nominal concentrations, were 86 to 95 % (average 92 %). The quantities found at the end (day 3) were 87 to 96 % (average 92 %). Therefore the calculations are based on nominal concentrations of the test item.

The variable used to calculate the various response parameters was cell density as determined by direct cell counts or indirect calculation of cell numbers after measuring of cell density. Cell numbers were estimated photometrically.

The 0–72 h EC50 for biomass (EbC50) and for algal growth rate (ErC50) were calculated using probit analyses after Finney (1952), and the slopes of the regression lines were calculated following Litchfield and Wilcoxon (1949) The NOEC' s and LOEC's were calculated by an analysis of variance ("Dunnett's-Test"). The effect threshold was calculated as geometric mean between NOEC and LOEC.

Results:

Test validity criteria were met.

The following table summarises the 0–72 h results:

0-72 h biomass, growth rates and inhibition (%) compared to the control

Nominal concentration of the test item (mg/L)	0–72 h, Biomass		0–72 h Growth Rate	
	AUC	% Inhibition	Rate (/day)	% Inhibition
Control	981	0.0	1.27	0.0
5.00	1069	-9.0	1.33	-4.6
10.0	945	3.7	1.29	-1.4
20.0	1088	-1 1.0	1.33	-4.2
40.0	1082	-10.3	1.32	-3.9
80.0	635	35.2*	1.12	12.0*
160	171	82.6*	0.46	63.7*

** mean value significantly different from the control, Dunnett's Test, one sided, p = 0.05.*

The 72 h ErC50 was 137 mg/L and the 72 h EbC50 was 97.7 mg/L. The study NOEC was 40 mg/L for both growth rate and biomass.

Title	Methiocarb-sulfoxide-phenol – Influence on the growth of the green alga, *Scenedesmus subspicatus*
Reference	Dorgerloh, M. & Sommer, H. 2001
Test Guideline	Directive 92/69/EEC, C.3 (1992), OECD 201, ISO 8692, ASTM E 1218
Data Validity	1
Data Relied On	Yes - the data were considered to be critical and were relied on in this assessment.

A static 72 h algal growth test was conducted to determine the effects of methiocarb-sulfoxide-phenol (M04) on the growth of the green alga, *Scenedesmus subspicatus* was exposed under static conditions (shake cultures) for 72 h. Algal growth in the controls was exponential over the entire test period. The following concentration of nominal: 100 (99.6) mg/L was tested. Calculations are based on nominal values of the test item. The quantities of pure metabolite found at the beginning (day 0) and at the end (day 3) of the test in reference to the expected concentrations, were 96 and 95 %. The test was done as a limit test.

The variable used to calculate the various response parameters was cell density as determined by direct cell counts or indirect calculation of cell numbers after measuring of cell density.

Validity criteria were met. At the single application rate of 100 mg/L there was a 20.9% inhibition in biomass after 72 h compared with 27.5% and 27.1% after 24 h and 48 h respectively. This was considered statistically significant resulting in a NOEC for biomass <100 mg/L.

After 72 h the inhibition in growth rate was 3.9%. After 24 and 48 h the inhibition was 14.5% and 9.9% respectively. The difference at all time intervals was considered statistically significantly different to the control resulting in a NOEC for growth rate <100 mg/L.

Title	Methiocarb-sulfone-phenol – Influence on the growth of the green alga, *Scenedesmus subspicatus*
Reference	Dorgerloh, M. & Sommer, H. 2001
Test Guideline	Directive 92/69/EEC, C.3 (1992), OECD 201, ISO 8692, ASTM E 1218
Data Validity	1
Data Relied On	Yes - the data were considered to be critical and were relied on in this assessment.

A static 72 h algal growth test was conducted to determine the effects of methiocarb-sulfone-phenol (M05) on the growth of the green alga, *Scenedesmus subspicatus* was exposed under static conditions (shake cultures) for 72 h. Algal growth in the controls was exponential over the entire test period. The following concentration of nominal: 6.25, 12.5, 25.0 50.0 and 100 mg test item/L were tested. The quantities of pure metabolite found at the beginning of the test (day 0) in reference to the nominal concentrations, were 97 to 98 % (average 98 %). The quantities found at the end (day 3) were 96 and 97 % (average 97 %). Therefore the calculations are based on nominal concentrations of the test item.

The variable used to calculate the various response parameters was cell density as determined by direct cell counts or indirect calculation of cell numbers after measuring of cell density.

Results:

Validity criteria were met. The following table summarises the 0–72 h results:

0-72 h biomass, growth rates and inhibition (%) compared to the control

Nominal concentration of the test item (mg/L)	0–72 h, Biomass		0–72 h Growth Rate	
	AUC	% Inhibition	Rate (/day)	% Inhibition
Control	1685	0.0	1.51	0.0
6.25	1904	-13.0	1.55	-2.2
12.5	1795	-6.6	1.54	-1.8
25.0	1782	-5.8	1.51	0.2
50.0	1476	12.4	1.45	3.9*
100	573	66.0*	0.97	35.7*

** mean value significantly different from the control, Dunnett's Test, one sided, p = 0.05*

The study authors report a 72 h ErC50 was 120 mg/L and a 72 h EbC50 105 mg/L. These both exceed the maximum tested concentration and should be treated with some caution. The ErC50 is >100 mg/L. The NOEC for biomass (based on statistical significance) is 50 mg/L while that for growth rate is 25 mg/L.

Title	Methiocarb-sulfoxid -Influence on the Growth of the Green Alga, *Scenedesmus subspicatus*
Reference	Dorgerloh, M. & Sommer, H. 2001
Test Guideline	ASTM E 1218 (1990), OECD 201 (1984), ISO 8692 (1989) and EEC C.3 (1992)
Data Validity	1
Data Relied On	Yes - the data were considered to be critical and were relied on in this assessment.

A static 72 h algal growth test was conducted to determine the effects of the methiocarb-sulfoxide (M01) on the growth of the green alga, *Scenedesmus subspicatus*. Nominal test concentrations were 1, 2, 4, 8 and 16 mg/L.

Initial measured concentrations were approximately 40% of nominal and mean measured concentrations were approximately 20% of nominal. M01 was unstable under alkaline test conditions. Therefore the effect concentrations are reported as mean measured (day 0-3). These corresponded to 0.2, 0.41, 0.802, 1.58 and 3.2 mg/L.

The variable used to calculate the various response parameters was cell density as determined by direct cell counts or indirect calculation of cell numbers after measuring of cell density.

Validity criteria were met. The following table summarises the 0–72 h results:

0-72 h biomass, growth rates and inhibition (%) compared to the control

Mean measured concentration (mg/L)	0–72 h, Biomass		0–72 h Growth Rate	
	AUC	% Inhibition	Rate (/day)	% Inhibition
Control	1826	-	1.52	-
0.20	1664	8.8	1.49	2.0
0.41	1766	3.3	1.51	0.7
0.80	1299	28.8*	1.41	7.2*
1.58	804	56.0*	1.24	18.0*
3.20	163	91.0*	0.61	59.7*

** mean value significantly different from the control, Dunnett's Test, one sided, p = 0.05*

The 72 h ErC50 was 1.31 mg/L and the 72 h EbC50 was 2.75 mg/L. The study NOEC was 0.80 mg/L for both growth rate and biomass.

Effects on bees

Acute

Title	Effects of methiocarb technical (acute contact and oral) on honey bees (*Apis mellifera* L.) in the laboratory
Reference	Schmitzer, S. 2008
Test Guideline	OECD 213 and 214 (1998)
Data Validity	1
Data Relied On	Yes - the data were considered to be critical and were relied on in this assessment.

This study assessed the effects of methiocarb technical (acute contact and oral) on honey bees (*Apis mellifera*) under laboratory conditions. Thirty worker bees per treatment were exposed for 48 hours to doses of 0.30, 0.21, 0.12, 0.07 and 0.03 µg ac per bee for feeding (oral, value based on the actual intake of the test item) and to doses of 1.0, 0.50, 0.25, 0.13 and 0.06 µg ac per bee for topical application (contact).

Application in the Contact Test: A single 5 µL droplet of methiocarb technical in an appropriate carrier (acetone) was placed on the dorsal bee thorax using a Burkard Applicator. For the controls, one 5 µL droplet of a) tap water containing 0.5 % Adhasit* and b) pure acetone was used. The reference item was also applied in a 5 µL droplet (dimethoate made up inacetone). A 5 µL droplet was chosen in deviation to the guideline recommendation of a 1 µL droplet, since a higher volume ensured a more reliable dispersion of the test item.

Application in the Oral Test: Appropriate amounts of methiocarb technical or referenceitem dilutions in acetone were mixed with syrup (ready-to-use syrup; Apiinvert, Siidzucker, D-97195 Ochsenfurt; composition of the sugar component: 30 % Saccharose, 31 % Glucose, 39 % Fructose) in order to achieve the required test concentrations in a final dilution of 50 % syrup solution (45 % water, 50 % syrup and 5 % acetone (w/w)). For the controls, the same proportion of syrup, water and acetone was used (solvent control) and similarly, 50 % aqueous syrup solution was used for the negative control. The treated food was offered in syringes, which were weighed before and after introduction into the cages (duration of uptake was 3–6 hours for the test item treatments). After a maximum of 6 hours, the syringes containing the treated food were removed, weighed and replaced by ones containing fresh, untreated food.

Results:

Based on OECD test guideline validity criteria for control mortality were met.

The following table summarises the mortality results at 24 h and 48 h:

Mortality (%) of adult honey bees in standard adult contact and oral toxicity tests.

Contact toxicity test			Oral toxicity test		
µg/bee	24 h	48 h	µg/bee (measured)	24 h	48 h
Control	0	0	0	0	0
Solvent control	0	0	0	0	0
0.06	3.3	3.3	0.03	0	0
0.13	6.7	6.7	0.07	10.0	10.0
0.25	3.3	3.3	0.12	76.7	76.7
0.50	63.3	73.3	0.21	96.7	100
1.0	93.3	100	0.30	100	100

During the first 4 hours in the contact test, treatment related behavioural abnormalities (e.g. movement coordination problems and/or apathy) were observed in all dose groups except in the 0.06 µg ac/bee dose level. 24 hours following the application, these behavioural abnormalities still occurred in the two highest dose levels (1.0 and 0.50 µg ac/bee). During the 48 hours assessment no behavioural abnormalities were found any more in all dose levels.

For the oral test, the maximum nominal dose levels of the test item (0.50, 0.25 and 0.13 µg ac/bee) could not be achieved, because the bees did not ingest the full volume of treated sugar solution even when offered over a period of 6 hours. During the 4 hours assessment, behavioural abnormalities (e.g. movement coordination problems and/or apathy) were observed in the four highest dose groups. No behavioural abnormalities were observed in the 0.03 µg ac/bee dose group. After 24 hours all behavioural abnormalities had gone until test end (48 hours after application).

The 48 h contact LD50 was calculated to be 0.43 µg/bee (95% CI 0.36–0.52 µg/bee) while the 48 h oral LD50 was calculated to be 0.08 µg/bee (95% CI 0.07–0.09 µg/bee).

Title	Honey bee (*Apis mellifera L.*) larval toxicity test on methiocarb, technical, single exposure
Reference	Ehmke, A. & Muenz, J. 2015
Test Guideline	OECD TG 237 (2013)
Data Validity	1
Data Relied On	Yes - the data were considered to be critical and were relied on in this assessment.

The purpose of this study was to determine the acute toxicity of methiocarb, technical after a single exposure to honey bee larvae (*A. mellifera L.)* for a period of 72 hours. 36 synchronised first instar larvae of *A. mellifera*, obtained from three different honey bee colonies. Each of the three colonies represented one replicate with 12 larvae. The experimental unit was the individual grafting cell, containing a single larva. For the definite test, the 36 larvae of each treatment were pooled on one plate.

Each replicate was exposed for 72 hours to doses of 1.0, 0.4, 0.16, 0.064, 0.026 and 0.010 µg ac per larva via treated artificial diets (single exposure). An untreated, respectively a solvent control and a reference item (dimethoate, 98.5 % w/w) were included in the study. The mortality of the larvae was determined 24, 48 and 72 hours after application. The endpoint of the study was 72 hours after application. The presence of uneaten food was assessed qualitatively at the last assessment date, 72 hours after application.

Samples of the stock solution were taken to conduct an analytical determination of the content of the active ingredient on the day of application. The analytical determination of the samples was conducted via HPLC-UV and resulted in 1.004 g/L, corresponding to 103% of the nominal concentration of the stock solution.

Results obtained from the larvae treated with the test item and the reference item were compared to those obtained from the respective control. LD50 values of the test item were estimated with Probit Analysis (according to Finney 1971). The NOED of the test item was estimated using Fisher's Exact Test (pairwise comparison, one-sided greater, α = 0.05), which is a distribution-free test and does not require testing for normality or homogeneity prior to analysis.

Results:

Validity criteria were met.

Mortality of the larvae in the toxicity test

µg/larva	24 h	48 h	72 h
Control	0	0	0
Solvent control	0	0	0
0.010	0	0	2.8
0.026	0	2.8	2.8
0.064	0	8.3	13.9
0.16	11.1	30.6	33.3*
0.40	11.1	41.7	44.4*
1.0	22.2	52.8	58.3*

** = statistically significant different to the control.*

The 72 h LD50 was calculated to be 0.547 µg ac/larva with a no observed effect dose (NOED) of 0.064 µg ac/larva.

Title	Methiocarb (tech.) – Acute contact toxicity to the bumble bee, *Bombus terrestris* L. under laboratory conditions
Reference	Schmitt, H. 2014
Test Guideline	OECD 213 and 214 (1998)
Data Validity	1
Data Relied On	Yes - the data were considered to be critical and were relied on in this assessment.

This study, while noted on the data list, was not available for review. Bumble bees are not used for commercial pollination in Australia and it is not considered necessary to review the study.

Metabolite effects on soil arthropod species

Title	Methiocarb-methoxy-sulfone: Effects on reproduction of the predatory mite *Hypoaspis aculeifer* in artificial soil
Reference	Witte, B. 2013
Test Guideline	OECD 226, 2008
Data Validity	1
Data Relied On	Yes - the data were considered to be critical and were relied on in this assessment.

This study assessed effects of methiocarb-methoxy-sulfone (M10) on reproduction of the predatory mite *Hypoaspis aculeifer* in artificial soil. Adult females, approximately 9 days after reaching the adult stage, were tested in a 14-d exposure in treated artificial soil. One concentration of the test item was mixed homogeneously into the soil which was filled in glass vessels before the predatory mites were introduced on top of the soil; one concentration and one control were tested; 8 replicates/concentration and 8 replicates for the control, with 10 female predatory mites each. Feeding of the mites with cheese mites (*Tyrophagus putrescentiae*) ad libitum at test start and on day 2, 5, 7, 9 and 12.

Number of surviving adult female mites 14 days after test initiation was recorded (counted after extraction). Missing adult predatory mites were recorded as dead as it was assumed they would have died and degraded during the test period. The living predatory mites were observed for differences in morphology or any abnormalities at experimental end. Number of juvenile mites at day 14 after application, counted after extraction. Mortality data were statistically analysed using Fisher's Exact test (α = 0.05, one-sided greater). Reproduction data were tested for normal distribution and homogeneity of variance using Shapiro-Wilk's test and Levene's test (α = 0.05. As data were normally distributed and homogeneous, the further statistical evaluation was performed using Student t-test (pairwise comparison, α = 0.05, one-sided smaller). The determination of the NOEC and LOEC values was based on the results of the statistical evaluation.

All validity criteria for the study were met.

No statistically significant mortality was observed in the single test item treated group (3% mortality) compared to the control, where 11% of the adult mites died (Fisher’s Exact Test, α = 0.05, one-sided greater).

In the treatment group a total of 248 juveniles were counted at day 14 compared to 254 in the control group. Reproduction of the predatory mites exposed to methiocarb-methoxy-sulfone was not statistically significantly different compared to the control at the single test concentration of 100 mg test item/kg soil (Student t-test, α = 0.05, one-sided smaller).

Title	Methiocarb-sulfone-phenol: Effects on reproduction of the predatory mite *Hypoaspis aculeifer* in artificial soil
Reference	Witte, B. 2013
Test Guideline	OECD 226, 2008
Data Validity	1
Data Relied On	Yes - the data were considered to be critical and were relied on in this assessment.

The purpose of the study was to determine the effects of methiocarb-sulfone-phenol (M05) on mortality and reproduction of the predatory mite *Hypoaspis aculeifer*. Adult females, approximately 9 days after reaching the adult stage were exposed for 14-d in treated artificial soil. One concentration of the test item was mixed homogeneously into the soil which was filled in glass vessels before the predatory mites were introduced on top of the soil; one concentration and one control were tested; eight replicates/concentration and eight replicates for the control, with 10 female predatory mites each. Feeding of the mites with cheese mites (*Tyrophagus putrescentiae*) ad libitum at test start and on day 2, 5, 7, 9 and 12. Assessment of adult mortality and reproduction performed after 14 d.

Number of surviving adult female predatory mites 14 days after test initiation was recorded (counted after extraction). Missing adult predatory mites were recorded as dead as it was assumed they would have died and degraded during the test period. The living predatory mites were observed for differences in morphology or any abnormalities at experimental end. Number of juvenile mites at day 14 after application, counted after extraction.

Mortality data were statistically analysed using Fisher's Exact test (α = 0.05, one-sided greater). Reproduction data were tested for normal distribution and homogeneity of variance using Shapiro-Wilk's test and Levene's test (α = 0.05). As data were normally distributed and homogeneous, the further statistical evaluation was performed using Student t-test (pairwise comparison, α = 0.05, one-sided smaller).The determination of the NOEC and LOEC values was based on the results of the statistical evaluation.

Validity criteria were met.

No statistically significant mortality was observed in the single test item treated group (6% mortality) compared to the control, where 11% of the adult mites died (Fisher’s Exact Test, α = 0.05, one-sided greater).

In the treatment group a total of 268 juveniles were counted at day 14 compared to 254 in the control group. Reproduction of the predatory mites exposed to methiocarb-sulfone-phenol was not statistically significantly different compared to the control at the single test concentration of 100 mg test item/kg soil (Student t-test, α = 0.05, one-sided smaller).

Title	Methiocarb-sulfoxide phenol: Effects on reproduction of the predatory mite *Hypoaspis aculeifer* in artificial soil
Reference	Witte, B. 2013
Test Guideline	OECD 226, 2008
Data Validity	1
Data Relied On	Yes - the data were considered to be critical and were relied on in this assessment.

The purpose of the study was to determine the effects of methiocarb-sulfoxide phenol (M04) on mortality and reproduction of the predatory mite *Hypoaspis aculeifer*. Adults, approximately 9 days after reaching the adult stage were placed in glass containers (volume: 100 mL; diameter: 5 cm), tight screw top closure to avoid water evaporation, filled with approximately 20 g ± 1.0 g artificial soil dry weight.

The artificial soil was moistened to approximately half of the final water content 2 days before the application. The additional water required to achieve the final water content was added when applying the test item.

Number of surviving adult female predatory mites 14 days after test initiation was recorded (counted after extraction). Missing adult predatory mites were recorded as dead as it was assumed they would have died and degraded during the test period. The living predatory mites were observed for differences in morphology or any abnormalities at experimental end. Number of juvenile mites at day 14 after application, counted after extraction.

Mortality data were statistically analysed using Fisher's Exact test (α = 0.05, one-sided greater). Reproduction data were tested for normal distribution and homogeneity of variance using Shapiro-Wilk's test and Levene's test (α = 0.05). As data were normally distributed and homogeneous, the further statistical evaluation was performed using Student t-test (pairwise comparison, α = 0.05, one-sided smaller).The determination of the NOEC and LOEC values was based on the results of the statistical evaluation.

Validity criteria were met.

No statistically significant mortality was observed in the single test item treated group (1% mortality) compared to the control, where 11% of the adult mites died (Fisher's Exact Test, α = 0.05, one-sided greater).

In the treatment group a total of 256 juveniles were counted at day 14 compared to 254 in the control group. Reproduction of the predatory mites exposed to methiocarb-sulfone-phenol was not statistically significantly different compared to the control at the single test concentration of 100 mg test item/kg soil (Student t-test, α = 0.05, one-sided smaller).

Title	Methiocarb-sulfoxide: Effects on reproduction of the predatory mite *Hypoaspis aculeifer* in artificial soil
Reference	Witte, B. 2013
Test Guideline	OECD 226, 2008
Data Validity	1
Data Relied On	Yes - the data were considered to be critical and were relied on in this assessment.

The purpose of the study was to determine the effects of methiocarb-sulfoxide (M01) on mortality and reproduction of the predatory mite *Hypoaspis aculeifer*. Two experiments were conducted. An initial experiment did not provide a final result so a second experiment was performed studying lower test concentrations. First experiment: Control, 18, 32, 56, 100 and 178 mg/kg artificial soil. Second experiment: Control, 1.0, 1.8, 3.2, 5.6 and 10.0 mg/kg artificial soil.

Both experiments were performed according to the following test design: 14-d exposure in treated artificial soil. Different concentrations of the test item were mixed homogeneously into the soil, which was filled into glass vessels before the predatory mites were introduced on top of the soil. Five concentrations and one control were tested, four replicates/concentration and eight replicates for the control, with 10 female predatory mites each. Feeding of the mites with cheese mites (*Tyrophagus putrescentiae*) ad libitum at test start on day 2, 5, 7, 9, 12 (1st experiment) and on day 2, 4, 7, 9, 11 (2nd experiment).

Results were analysed using standard statistical procedures, Fisher's Exact Test (mortality), Williams t-test (reproduction), Probit Analysis (determination of ECx values).

Validity criteria were met. The following results were obtained:

Methiocarb-sulfoxide: Effect on the Predatory Mite Hypoaspis aculeifer in a 14-day reproduction study

	Treatment					
Methiocarb-sulfoxide [mg/kg soil dry weight]	**Control**	**18**	**32**	**56**	**100**	**178**
Mortality (day 14) [%]	4	18	23	18	65	63
* = significantly different	-	*	*	*	*	*
No. of juveniles (day 28)	230	123	168	140	54	20
* = significantly different	-	*	*	*	*	*
Reproduction in [%] of control (day 14)	-	53	73	61	23	9
2nd Experiment						
Methiocarb-sulfoxide [mg/kg soil dry weight]	**Control**	**1.0**	**1.8**	**3.2**	**5.6**	**10.0**
Mortality (day 28) [%]	6	8	3	8	8	8
Significance (experiment 2)	-	n.s.	n.s.	n.s.	n.s.	n.s.
No. of juveniles (day 28)	275	300	303	288	302	282
Significance (experiment 2)	-	n.s.	n.s.	n.s.	n.s.	n.s.
Reproduction in [%] of control (day 28)	-	109	110	105	110	102
Endpoints [mg test item/kg soil dry weight]						
NOEC (mortality)	10.0					
NOEC (reproduction)	10.0					
EC Values (reproduction)	EC10			EC20		
	10.34			17.82		
95% Confidence Limits	1.33 – 19.95			4.35 – 29.84		

Effects on earthworms

Acute

Title	Acute toxicity of methiocarb (tech.) to earthworms (*Eisenia fetida*)
Reference	Meisner, P. 2000
Test Guideline	OECD Test Guideline 207, (1984)
Data Validity	1
Data Relied On	Yes - the data were considered to be critical and were relied on in this assessment.

Adult earthworms (*Eisenia fetida*; 4 x 10 animals per concentration) were exposed in an artificial soil for 14 days to the concentrations of 0.1, 0.32, 1.0, 3.2, 10, 32, 100, 178, 316, 562 and 1000 mg ac/kg dry weight soil (nominal concentrations). The values reported in the study are nominal concentrations.

The test soil consisted of 69% fine quartz sand (84 % of the sand has a particle size of 0.06–0.2 mm), 10% dried, finely ground peat (sphagnum peat; pH 2–4), 20% kaolin (kaolinite content of about 36 %, pH value ca 7, "Kaolin W", from Erbsloh / Geisenheim) and around 1% calcium carbonate (pure) to adjust the pH value to 6 + 0.5 (figures refer to dry weight).

Seven days after the start of the study, the number of surviving earthworms was determined by emptying the soil out onto an inert surface and removing the earthworms by hand. The animals were then returned to the test container with the test soil. After 14 days, the weight, abnormal behaviour, observed symptoms as well as the number of surviving earthworms were determined. Earthworms which showed no reaction when prodded with a blunt probe were considered dead.

Results:

Methiocarb (tech.) acute toxicity to earthworms. Unless stated to the contrary, the figures in the table are means of n = 4 test containers, each containing ten earthworms.

Concentration (mg test substance / kg dry weight soil)	Mortality (%)	Weight alteration of (%)	the survivors U-Test **)
second study			
control	3 ± 5	+ 2 ± 4	
0.1	3 ± 5	+ 1 ± 4	
0.32	0	- 0 ± 3	
1.0	0	- 5 ± 5	+
first study			
control	0	+ 15 ± 4	
1.0	0	- 2 ± 4	+
3.2	0	- 14 ± 5	+
10 *)	0	- 22 ± 3	+
32 *)	3 ± 5	- 33 ± 4	+
100 *)	3 ± 5	- 40 ± 1	+
178 *)	3 ± 5	- 40 ± 2	+
316 *)	13 ± 5	- 41 ± 6	+
562 *)	25 ± 21	- 43 ± 4	+
1000 *)	48 ± 25	- 42 ± 5	+

***) Results of the U-test + = weights of control and the test concentration do differ significantly; - = weights of control and the test concentration do not differ significantly; *) earthworms became cramped.*

The LC50 (14 d) was 1322 mg test substance/kg dry weight substrate (95 % confidence limits 795 - 2197 mg/kg). Related to weight alterations and symptoms, the no-observed- effect-concentration (NOEC) was 0.32 mg test substance/kg dry weight soil.

Reproduction-Field

Title	Methiocarb RB 2.0 W: Sublethal toxicity to the earthworm *Eisenia fetida* in artificial soil
Reference	Friedrich, S. 2014
Test Guideline	OECD 222 (2004), ISO 11268-2 (1998)
Data Validity	1
Data Relied On	Yes - the data were considered to be critical and were relied on in this assessment.

The purpose of this study was to determine the sublethal effects of the Methiocarb RB 2.0 W (2.02 % w/w methiocarb, formulation type: RB (pellets)) on reproduction, mortality and growth of the earthworm *Eisenia fetida* by dermal and alimentary uptake using an artificial soil in a laboratory test.

Adult earthworms (*Eisenia fetida*, about 3 months old) were exposed to 66, 99, 148, 222, 333 mg test item/kg dry weight (d.w.) of soil. This corresponded to 3.3, 4.5, 6.5, 9.8 and 14.5 pellets per test vessel respectively. The test item pellets were evenly distributed by hand on the soil surface and then slightly pressed into the soil after the worms had been introduced. The test soil contained 68.5 % quartz sand, 20 % kaolin clay, 10 % sphagnum peat, 1 % food and 0.5 % $CaCO_3$, at 18.0–21.8 °C and a photoperiod: light : dark = 16 h : 8 h (570 lx) and were fed with horse manure. Mortality and biomass change were determined after 4 weeks and reproduction was determined after 8 weeks.

Shapiro-Wilk´s test and Levene´s test, respectively, were used to test the data for normality and homogeneity of variance. Fisher`s Exact Binomial Test with Bonferroni Correction and the Williams-t-test were used to compare the control with the independent test item groups. For statistical evaluation of the biomass change, the changed mean fresh weight of surviving worms per replicate was used.

All validity criteria were met. The physico- chemical parameters measured at the start and at the end of the tests met the guideline requirements.

Results:

Observations:

Methiocarb RB 2.0 W mg test item/kg d.w.						
	Control	66	99	148	222	333
	Mean number of pellets/test vessel					
	-	3.3	4.5	6.5	9.8	14.5
Mortality of adult worms after 4 weeks						
Mortality (%)	0.0	5.0	0.0	2.5	2.5	0.0

Methiocarb RB 2.0 W mg test item/kg d.w.						
Biomass change (change in fresh weight after 4 weeks relative to initial fresh weight)						
Mean (mg)	119.7	123.5	118.0	111.9	93.9	96.8
Mean (%)	33.6	34.9	33.2	31.6	26.5	27.1
Number of juveniles per surviving adult worm after 8 weeks						
Mean	11.9	12.0	11.4	12.0	10.1	10.0
Number of juveniles per replicate after 8 weeks						
Mean	119.3	115.0	112.3	119.5	98.8	100.3
Reproduction compared to control (%)						
% to control	100	96.4	94.1	100.2	82.8	84.1

No statistically significant differences between the control and test item were calculated for biomass and reproduction (Williams t-test; α = 0.05, one-sided smaller) and mortality (Fisher's Exact Binomial Test with Bonferroni Correction, α = 0.05, one-sided greater).

No effects on behaviour (including feeding activity) of the worms were observed during the test. Methiocarb RB 2.0 W caused no statistically significant change in biomass (change in fresh weight after 4 weeks relative to initial fresh weight) compared to the control group (Williams t-test, α = 0.05, one-sided smaller), although there did appear to be a general reduction in biomass as the exposure rates increased.

Based on the statistical evaluation of these results, the overall No-Observed-Effect-Concentration (NOEC) was determined to be ≥ 333 mg test item/kg soil d.w.

Title	Methiocarb RB4 and methiocarb RB2 slug pellets: Effect on the earthworm fauna within 1 year under field conditions
Reference	Kunze, C.L. 2003
Test Guideline	BBA (Federal Biological Research Centre for Agriculture and Forestry, Germany):Guidelines for the Testing of Plant Protection Products within Registration, Part VI, 2- 3 (January 1994): Effects of Plant Protection Products on Earthworms in the Field. ISO (International Standard Organisation): Draft Guideline CD 11268-3 (E):Soil Quality - Effects of Pollutants on Earthworms, Part 3: Guidance on the determination of effects in field situations (1999)
Data Validity	1
Data Relied On	Yes - the data were considered to be critical and were relied on in this assessment.

The effects of methiocarb RB4 slug pellets (4.16 % methiocarb,) and methiocarb RB2 slug pellets (1.96 % methiocarb) on earthworm populations under field conditions were studied. To ensure an abundant earthworm population, an area which was used as grassland for several years, was selected. It was ploughed and sown with winter wheat according to agricultural practice before the start of the study.

Four plots (= 4 replicates) within this area were treated once with 3 kg Methiocarb RB4/ha (1 x 3 kg/ha = nominal) on October 15, 2001 and four plots were treated twice with 3 kg Methiocarb RB4/ha (2 x 3 kg/ha = nominal) on October 15, 2001 and October 29, 2001. In addition, four plots were treated twice with 5 kg Methiocarb RB2/ha (2

x 5 kg/ha = nominal) on October 15, 2001 and October 29, 2001. Four untreated plots served as controls. All plots were screened for alive and dead earthworms on the soil surface within 32 days after the first application. The earthworm numbers and biomass was determined 5 weeks, 6 months and 12 months after the first application by sampling with formalin (formalin method). At each sampling time 16 samples per treatment (4 plots, 4 samples per plot) were collected. Data were analysed separately for the three treatment groups and the control group respectively for adult and juvenile worms, for alive and dead animals, and for tanylobous and epilobous species.

Results:

The mean number of earthworms in the control plots, was determined to be 75 earthworms/m^2 five weeks after the first application, 181 earthworms/m^2 six months after the first application and 176 earthworms/m^2 twelve months after the first application. Six different earthworm species were identified in the test area at different abundances: the tanylobous species *Lumbricus terrestris, Lumbricus rubellus* and *Lumbricus castaneus*, and the epilobous species *Aporrectodea caliginosa, Allolobophora chlorotica* and *Aporrectodea terrestris longa.*

Of the three epilobous species *Aporrectodea caliginosa* was the most abundant species for all categories (adult, juvenile and adult & juvenile), treatments and sampling times tested. *Aporrectodea caliginosa* comprised about 82% + 89% of the total epilobous species and about 20% + 48% of the total population sampled in control plots 5 weeks + 12 months after application. At 6 months sampling, *Aporrectodea caliginosa* made up 95% of the total epilobous species and 51% of the total population sampled in control plots.

For the tanylobous species the picture was more diverse. *Lumbricus terrestris* comprised about 21% of the total tanylobous species and about 16% + 10% of the total population sampled in control plots 5 weeks + 12 months after application. At 6 months sampling, *Lumbricus terrestris* comprised 28% of the total tanylobous species and 13% of the total population sampled in control plots and category "adult & juvenile".

Number of earthworms found on the soil surface: The earthworm numbers (dead and alive, all categories) per species class based on an area of 0.25 m^2 and summed up for 20 search days is presented for each treatment in the following table. The number of "total" earthworms counted on the soil surface during searching amounted to 6–8% of the earthworm numbers extracted from the soil by formaline, corrected by sampling efficiency 6 months after first application. The vast majority of worms found on the soil surface were tanylobous species. The number of earthworms collected on the soil surface varied with actual climatic conditions such as soil temperature and rainfall and were higher at 6 months than at 5 weeks after treatment.

Comparison to the sampled numbers 6 months after treatment is shown.

Treatment	Control plots	1x3 kg/ha Methiocarb RB4 plots	2x3 kg/ha Methiocarb RB4 plots	2x5 kg/ha Methiocarb RB2 plots
"total" earthworms on soil	0.35	4.28	3.41	3.61
abundance relative to earthworms in soil	0.56%	5.95%	6.50%	7.77%
"total tanylobous" earthworms on soil	0.29	4.03	3.23	3.24
abundance relative to earthworms in soil	1.00%	11.92%	12.83%	14.79%

Treatment	Control plots	1x3 kg/ha Methiocarb RB4 plots	2x3 kg/ha Methiocarb RB4 plots	2x5 kg/ha Methiocarb RB2 plots
"total epilobous" earthworms on soil	0.05	0.25	0.19	0.37
abundance relative to earthworms in soil	0.16%	0.65%	0.69%	1.51%

The absolute numbers of earthworms on the soil surface varied between the searching days and treatments. However, in all treatment groups there was a strong increase in the total number of earthworms on the soil surface compared to the control around 11–14 days after the first application of slug pellets. The numbers increased a second time for treatment group 2 x 3 kg Methiocarb RB4/ha and for treatment group 2 x 5 kg Methiocarb RB2/ha. In contrast numbers of earthworms on the soil in the control group remained comparatively low for the whole screening period.

The strong increase in worms on the soil surface compared to the control, which was biphasic in areas repeatedly treated with slug pellets was mainly due to the juvenile tanylobous species. Most of the sampled earthworms belonged to the species *Lumbricus terrestris*. Two other tanylobous species, *Lumbricus rubellus* and *Lumbricus castaneus* (comprised in the group "other tanylobous species) which mainly live on the soil surface in the grass layer were also identified.

Worms of the epilobous species *Aporrectodea caliginosa, Allolobophora chlorotica* and *Aporrectodea terrestris* longa which are considered to be more relevant for arable soils were found at comparatively low numbers on the surface with *Aporrectodea caliginosa* being the most abundant.

The number of total earthworms (pooling: adult and juvenile, tanylobous and epilobous, dead and alive worms) between treated and untreated plots were statistically analysed using Wilcoxon, Man and Whitney U-test (one sided smaller, probability of error 5%) for each of the 20 search days.

In plots treated with 1 x 3 kg Methiocarb RB4/ha, significantly higher numbers of earthworms were found on the surface between days 7–17 after treatment. In plots treated with 2 x 3 kg Methiocarb RB4/ha, significantly higher numbers of earthworms were found on the surface between days 8–14 and at day 17, 22 and 29 after the first application. In plots treated with 2 x 5 kg Methiocarb RB2/ha, significantly higher numbers of earthworms were found on the surface between days 7–11, days 17–23 and at days 29 and 32 after first application.

Between days 6–14 and days 23–25 after the first treatment heavy rainfalls were measured. Heavy rainfall attracts earthworms to the soil surface. Thus not only slugs, but also earthworms that moved to the surface or moved on the surface were exposed to methiocarb pellets. Earthworms which came into contact with methiocarb on the soil surface might not have been able to bury themselves into the ground again anymore. The probability for a contact between an individual worm and a slug pellet was higher in plots treated repeatedly with methiocarb. Probability for a contact was also higher in plots treated with 2 x 5 kg Methiocarb RB2/ha than in plots treated with 2 x 3 kg Methiocarb RB4/ha due to higher numbers of pellets per m^2.

Adult earthworms; Relative Changes of Numbers & Biomass in soil

An application of 1 x 3 kg/ha RB4 had no statistically significant effect on the parameters "numbers" and "biomass" of all species at any sampling date.

An application of 2 x 3 kg/ha RB4 had no statistically significant effect on the parameter "numbers" at all sampling dates and for all species classes.

Application of 2 x 3 kg/ha RB4 caused statistically significant reductions in the parameter "biomass" in the class "total" and "total tanylobous" earthworms five weeks and six months after the first application. Recovery was observed at 12 months after first treatment.

For "total epilobous" species, a 60% reduction in biomass after five weeks and a statistically significant reduction in biomass six months after the first application were observed. As for the tanylobous species, these effects are considered transient, since biomass recovered to control level 12 months after the first application.

Application of 2 x 5 kg/ha RB2 had no statistically significant effect on the parameter "numbers" at all sampling times and for all species classes except for the class "total epilobous" earthworms, where numbers were statistically significantly reduced five weeks after the first application. This effect was transient and six and 12 months after the first application no statistically significant effects were observed in this treatment group. The treatment also had no effects on biomass of "total epilobous" species at any sampling date.

Biomass of surface active "total tanylobous" earthworms was statistically significantly reduced at all three sampling times. However biomass re-approached control levels within 12 months in the "total tanylobous" class.

In the class "total" earthworms, statistically significant reductions in biomass were observed five weeks and six months after first application. However, no significant effect on "total" earthworm biomass was detected after 12 months, and biomass had also recovered.

Juvenile earthworms; Relative Changes of Numbers & Biomass in soil

An application of 1 x 3 kg/ha RB4 had no statistically significant effects on the parameters "numbers" and "biomass" at all sampling times and for all species classes, except for a statistically significant reduction found for parameter "biomass" in the class "total" earthworms five weeks after the first application. This was a transient effect and biomass values of "total" earthworms returned to control levels six and 12 months after the first application.

An application of 2 x 3 kg/ha RB4 had no statistically significant effects on the parameter "numbers" at all sampling times and for all species classes.

Parameter "biomass" was statistically significantly reduced in all species classes five weeks after the first application. This was a transient effect and no statistically significant effects were determined six and 12 months after the first application and recovery of biomass can be concluded.

An application of 2 x 5 kg/ha RB2 had no statistically significant effects on the parameter "numbers" at all sampling times and for all species classes. The same was true for the parameter "biomass" and "total epilobous" and "total tanylobous" earthworms. Although there was no statistically significant reduction (at significance level $\alpha = 0.05$),

biomass of "total tanylobous" earthworms was reduced by 68% and biomass of "total epilobous" earthworms was reduced by 82% at five weeks sampling time with p (U) = 0.086 being slightly higher than 0.05. This resulted in a highly significant reduction of biomass for "total" earthworms five weeks and six months after first application. In contrast 12 months after first application, there was no statistically significant effect on biomass for any species class: "total", "total tanylobous" and "total epilobous" earthworms, and recovery of biomass can be concluded.

An application of 1 x 3 kg/ha RB4 did not affect the prevalent epilobous species *A.caliginosa* and the tanylobous species *L. terrestris*.

Application of 2 x 3 kg/ha RB4 and 2 x 5 kg/ha RB2 had transient effects on both species, but recovery was found 12 months after first application for all parameters measured.

An application of 1 x 3 kg Methiocarb RB4/ha had only negligible effects on the indigenous earthworm population.

An application of 2 x 3 kg Methiocarb RB4/ha caused significant reductions in the earthworm abundance during the first six months following treatment, but allowed full recovery of the indigenous earthworm population within 12 months.

Application of 2 x 5 kg Methiocarb RB2 also had significant effects which were slightly more pronounced as for 2 x 3 kg R84 on the tanylobous species. However, also in this treatment group, a clear recovery from effects was observed within 12 months after first treatment.

Title	Effects of slug pellets methiocarb RB 3 on the earthworm fauna of a winter wheat field
Reference	Heimbach, F., 2000
Test Guideline	BBA (Federal Biological Research Centre for Agriculture and Forestry, Germany): Guidelines for the Testing of Plant Protection Products within Registration, Part VI, 2 - 3 (January 1994): Effects of Plant Protection Products on Earthworms in the Field. ISO (International Standard Organisation): Draft Guideline CD 11268-3: Soil Quality - Effects of Pollutants on Earthworms, Part 3: Field (1996)
Data Validity	1
Data Relied On	Yes - the data were considered to be critical and were relied on in this assessment.

This test was designed to determine the effects of methiocarb slug pellets (ac content: 2.9 %) on earthworm populations under field conditions. For this purpose, the effect on earthworm abundance was determined in arable land, which had been used in the years before as a grassland area, because the populations here are high (up to 199 animals beneath 1 m2 of surface). In this pasture, an area measuring 40 x 50 m was used for the study. Methiocarb slug pellets were applied on fourteen designated plots in autumn 1998 in four application variants relevant to actual use: on four plots incorporated into the seed furrow during sowing of winter wheat; on four plots broadcasted 18 days after sowing, on four plots 35 days after sowing and on two plots twice, 18 and 35 days after sowing. Each time the application rate was 5 kg/ha.

Earthworm abundances were determined 11 weeks after sowing (= six weeks after the last application of the slug pellets), in spring 1999 and again at the end of the season in autumn 1999 about one year after sowing. The earthworm abundance was determined six weeks after the late broadcast application, during spring and autumn 1999 (16 samples per treatment at each sampling and eight samples for the double treatment). The mean density

of earthworms in the control was 40 individuals/m^2 six weeks after the late broadcast application, 111 individuals/m^2 in spring 1999 and 119 individuals/m^2 in autumn 1999. Differences in abundance were in the range of methodological and natural variations, which stem primarily from weather and soil conditions during sampling. Six different species of earthworms were found in the test field: the tanylobous species *Lumbricus terrestris, L. rubellus* and *L. castaneus*, and the epilobous species *Aporrectodea caliginosa, Allolobophora chlorotica* and *Aporrectodea terrestris longa*. During the first 3 months after sowing the surface of the plots was regularly searched—particularly during humid weather conditions—for dead worms and worms with behavioural changes or injuries.

The results were statistically evaluated by the U-test (after Wilcoxon, Mann & Whitney, SACHS 1978). The hypothesis tested was that the elements of the two random samples are derived from the same distribution (significance level P = 0.05, two-sided).

Results:

Some dead earthworms and worms with injuries were found on the soil surface after application. Tanylobous earthworms showed for plots with furrow incorporated seeds in only 2 out of 21 evaluation days significantly higher numbers of dead or injured worms as compared to the control (U-test, p= 0.05), which is not considered relevant. On plots with early broadcast, 7 out of 16 and on the plots with late and two broadcast applications, about half of the evaluations provided statistically higher numbers of dead or injured worms as compared to the control. These findings had been amplified by the high precipitation during the evaluation period causing many tanylobous earthworms to crawl onto the soil surface and to get into contact with the methiocarb. No difference between treatments and control was found for the epilobous species.

However, due to the concomitant transformation from grassland to arable field, the proportion of epilobous and tanylobous species was not representative in this experiment. In typical arable field, tanylobous species represent less than about one fourth of the total populations of earthworms, compared to about two thirds in this study. Species most frequently found at surface in this study were *L. rubellus* and *L. castaneus*, species which are not usually present in noticeable numbers in arable fields. The total effects on earthworm populations in arable fields can therefore be estimated to less than 5%, transitorily during the first weeks after application, which corroborates well with previous findings from similar field studies.

The earthworm abundance and biomass in soil (sum of adults and juveniles per 0.25 m^2):

Treatment	6 weeks	spring 1999	autumn 1999	6 weeks	spring 1999	autumn 1999
	Total earthworms					
	numbers			biomass (g)		
control	9.9 ± 6.2	27.8 ± 10.0	29.7 ± 13.0	4.3 ± 4.6	10.8 ± 6.6	17.2 ± 8.6
furrow	8.1 ± 8.0	22.8 ± 8.8	32.9 ± 12.8	2.6 ± 3.0	7.7 ± 4.6	19.7 ± 7.2
br. early	6.9 ± 6.8 *)	22.1 ± 12.6	25.3 ± 10.0	2.5 ± 2.1	8.1 ± 6.5	13.4 ± 5.1
br. late	9.1 ± 4.5	23.2 ± 7.6	24.6 ± 6.8	3.1 ± 2.0	6.9 ± 2.3	14.0 ± 6.2
br. twice	6.6 ± 5.4	23.8 ± 11.2	19.0 ± 12.7	2.2 ± 2.4	7.4 ± 6.0	8.6 ± 6.9 *)
	sum of tanylobous species					
	numbers			biomass (g)		
control	9.2 ± 6.0	17.4 ± 8.4	13.7 ± 11.2	3.7 ± 4.4	6.6 ± 4.6	7.2 ± 5.6
furrow	7.3 ± 7.4	15.7 ± 5.7	17.8 ± 9.0	1.8 ± 2.0	5.2 ± 3.4	10.4 ± 4.2

br. early	6.1 ± 5.8 *)	15.6 ± 10.6	11.4 ± 6.7	2.0 ± 1.7	5.2 ± 5.0	5.3 ± 3.5
br. late	8.3 ± 4.6	14.9 ± 6.0	1 1.2 ± 3.7	2.5 ± 2.0	4.0 ± 2.5	5.9 ± 4.2
br. twice	6.1 ± 4.8	15.1 ± 5.6	10.8 ± 6.1	2.1 ± 2.2	4.4 ± 4.0	3.9 ± 3.3
	sum of epilobous species					
	numbers			biomass (g)		
control	0.75 ± 0.86	10.4 ± 4.3	16.0 ± 6.1	0.62 ± 1.06	4.2 ± 2.8	10.0 ± 5.0
furrow	0.75 ± 1.00	7.1 ± 4.5 *)	15.2 ± 7.7	0.76 ± 1.20	2.5 ± 2.1	9.3 ± 6.0
br. early	0.81 ± 1.17	6.7 ±3.5 *)	13.8 ± 5.7	0.54 ± 0.69	3.0 ± 2.7	8.1 ± 3.3
br. late	0.75 ± 1.13	8.3 ± 3.6	13.4 ± 5.3	0.53 ± 1.00	2.8 ± 1.2	8.1 ± 3.6
br. twice	0.50 ± 0.76	8.6 ± 7.2	8.3 ± 7.4 *)	0.16 ± 0.26	3.0 ± 2.4	4.7 ± 4.0 *)

br.: broadcasted

**) = statistically significant difference to control (U-test, p= 0.05)*

Except of two cases the results do not indicate a significant reduction in earthworm abundance or biomass for any of the four treatment regimes, neither for tanylobous species nor for epilobous species or total earthworms. Only on two occasions the U-test (p = 0.05) provided a statistically significant difference to the control for the total of earthworms: early broadcast application (6 weeks after broadcast), and two broadcast applications (autumn 1999). Nevertheless, these findings are unidirectional with regard to the number of earthworms and their biomass, indicating that differences are caused by differences in species composition or fluctuations between age classes only.

In autumn 1999, significantly lower abundance and biomass of earthworms was found in plots with two applications; nevertheless, the former samplings six weeks after application and in spring 1999 did not show any effects of methiocarb slug pellet applications. Thus, a treatment related effect in autumn 1999 cannot be admitted. The numbers fluctuated between test plots, as usual for earthworm populations, without indication of effects of methiocarb slug pellets on any species.

Concerning the earthworm population in the soil, no lasting treatment related effects of the application of 5 kg/ha methiocarb slug pellets were observed in this study, although some transient effects, particularly with epilobous species, appear to have occurred.

Title	Methiocarb RB 2C W: Effects on earthworms under field conditions
Reference	Schulz, L. 2011
Test Guideline	ISO 11268-3 (1999) Technical Recommendations to ISO 11268-3 (1999), Kula et al. (2006)
Data Validity	1
Data Relied On	Yes - the data were considered to be critical and were relied on in this assessment.

The objective of this field study was to investigate possible effects and potential recovery of field populations of earthworms after application of Methiocarb RB 2C W. A field experiment lasting about one year was performed and the effects of the test item with regard to species composition, biomass, and abundance were compared to an untreated control and to a reference item (toxic standard; Nutdazim 50 FLOW®).

The trial was placed near Posthausen in Saxony/Germany. The test item was applied once at a rate of 450000 granules/ha (corresponding to approx. 5 kg test item/ha) during the spring activity period of earthworms (May

2010). Nutdazim 50 FLOW® (Carbendazim 500 g/L, nominal) was applied to the plots as toxic reference item at a rate of 10 kg nominal ac/ha (20 L product/ha).

Twelve plots, each 10 m x 10 m, were arranged in a 3 x 4 formation, each plot surrounded by a 2 m wide path between the plots. The set-up was a randomised block design. The assignment of the treatment groups to the plots was based on the results of a pre-sampling. The pre-sampling was conducted to determine the density, diversity and homogeneity of earthworm populations at the site. Defined areas were sampled to assess earthworm populations before application (about two weeks before application = pre-sampling), and three times after application, i.e. 1, 5 and 12 months after application. Earthworms were sampled from four 0.125 m² sampling areas per plot per sampling occasion by combining hand sorting with formalin extraction in the excavated hole. Adult earthworms were identified to the species level and juveniles were identified to species level if possible, otherwise to the genus level.

Total number, total biomass, total adult and total juvenile number, total adult and total juvenile number and biomass of single species, total adult and total juvenile number and biomass of endogeic and anecic were reported. With the Shapiro-Wilk's-test, data were analysed for normal distribution and with the Levene's test, data were analysed for homogeneity in variance. Data were reasonably normal in distribution and variances were reasonably equal. Therefore data were analysed as follows: Pre-treatment sampling: Data were analysed with a two-factorial analysis of variance (ANOVA) with treatment as fixed factor and block as random factor. Post-treatment sampling: Data were analysed by a one-sided Dunnett-test with treatment group < control at the 5 % significance level (equivalent to one-sided t-test for paired samples). Test item and reference item were analysed in separate analyses.

The current study meets all criteria required for a valid earthworm field study.

Mean abundance (individuals/m2) of the total earthworm population, total juveniles, total adults and of the dominant species L. terrestris and A. caliginosa – values reported as mean % of control.

	Sampling Time			
Observation group	Pre-sampling	1 month	5 months	12 months
Total worms	103.9	109.5	112.2	122.5
Total adults	85.5	102.7	115.3	76.4
Total juveniles	112.1	113.7	110.9	139.6
L. terrestris (anecic)	112.6	98.7	112.6	130.9
A. caliginosa (endogeic)	103.3	112.8	106.6	120.7

Mean biomass (g/m2) of the total earthworm population, total juveniles, total adults and of the dominant species L. terrestris and A. caliginosa – values reported as mean % of control.

	Sampling No.			
Observation group	Pre-sampling	1 month	5 months	12 months
Total worms	101.3	90.7	109.1	121.6
Total adults	95.9	78.3	101.6	79.2
Total juveniles	114.8	110.6	116.9	163.1
L. terrestris (anecic)	118.7	76.0	107.3	132.5
A. caliginosa (endogeic)	88.9	106.8	107.1	111.2

The mean earthworm abundance in the control plots was 256.0 /m² at trial start and 227.0 /m^2 at the end of the trial and the presence of the dominant species *Aporrectodea caliginosa* and *Lumbricus terrestris* representing different ecological groups indicated the suitability of the field site.

No statistically significant reductions in total earthworm abundance and biomass could be observed in the test item treatment neither one, five nor 12 months after application. Dominant earthworm species found in the field site were the endogeic species Aporrectodea caliginosa (70.3% of total earthworms at pre-sampling) and the anecic species Lumbricus terrestris (20.1% of total earthworms at pre-sampling). No statistically significant reductions in the abundance and biomass of these earthworm species and ecological groups (anecic and endogeic) could be observed in the test item treatment group compared to the control throughout the whole test period.

During surface monitoring from day 1 until day 29 after application, 43 dead earthworms were found on the soil surface of the test item treatment group. Mainly earthworms of the species Lumbricus terrestris were affected. The number of dead earthworms increased until day 10 after application, especially after rainfall and decreased gradually till the end of the surface monitoring period. The absence of statistical significant effects on the earthworm species L. terrestris over the course of the study underlines that the number of dead earthworms found during the monitoring period is of no biological relevance for the earthworm community.

The toxic reference item reduced total earthworm abundance by 73.9% at 1st sampling, 51.0% at 2nd sampling and 45.4 % at 3rd sampling. The statistically significant reduction of 73.9 % at 1st sampling (1 month after application) confirmed the validity of the test system.

It can be concluded from this study that after application of Methiocarb RB 2C W at a rate of 450 000 granules/ha corresponding to approx. 5 kg/ha, no adverse effects on earthworm field populations occurred.

Title	Residues of methiocarb in earthworms on the soil surface of an arable field after broadcast application of methiocarb RB 4 slug pellets
Reference	Heimbach, F. 2000
Test Guideline	OECD Test Guideline 222, (1984) and ISO 11268, part 2, (1998)
Data Validity	1
Data Relied On	Yes - the data were considered to be critical and were relied on in this assessment.

After the broadcast application of methiocarb slug pellets, sometimes immobile or intoxicated earthworms are observed on the soil surface of arable fields following rainfall periods. This study was designed to determine the residues of methiocarb in earthworms which can be found on the soils surface after an application of such pellets.

The test substance for this study was Methiocarb RB 4 Slug Pellets. Methiocarb slug pellets were applied on a designated area of 40 x 40 m at a broadcast application rate of 3.75 kg/ha (= 150 g ac/ha). The test area had been used for winter wheat until autumn the year before; after harvest it was left without further treatments until the start of this study in April 2000.

The earthworms were sampled at several days during the first three weeks after the application depending on actual weather conditions since earthworms crawl on the soil surface and are exposed to slug pellets only if there is enough rainfall. At these days, the soil surface of the test plots was searched for intoxicated earthworms. The abundance of such earthworms was low (<1 earthworm/m^2) Sampled earthworms were collected, identified,

weighed and deep frozen for analysis. Five different species of earthworms were found in the test field: the tanylobous species *Lumbricus terrestris*, *L. rubellus* and *L. castaneus*, and the epilobous species *Aporrectodea caliginosa* and *Aporrectodea terrestris longa*.

In total, 116 individuals with a total weight of 153.25 g were collected during the study, which were divided into nine analytical samples.

All earthworms showed clear symptoms of intoxication with delayed movements. They seemed to be hardened and some of them looked like a telephone cord. Nevertheless, all sampled individuals were still alive with a reaction to sensory stimuli.

Methiocarb concentrations [mg ac/kg wet weight] in earthworm samples

Sample No.	Day after application	Weight [g]	Methiocarb concentration [mg ac/kg]*)	
			individual values	mean value
1 2	1	22 20	27.8 40.8	34.3
1 2	8	19 11	51.1 56.2	53.7
1 2	9	8 21	34.9 25.8	30.4
1	10	15	44.7	
1 2	20	17 21	47.4 19.4	33.4
Overall mean			38.7	
SD			12.5	

**) based on the wet weight of earthworms (as sampled).*

Metabolites - Acute

Title	Acute toxicity of methiocarb-methoxy-sulfone to the earthworm *Eisenia fetida* in a 14-day test
Reference	Batscher, R. 2000
Test Guideline	OECD Test Guideline 207, (1984) and Directive 87/302/EEC, L 133
Data Validity	1
Data Relied On	Yes - the data were considered to be critical and were relied on in this assessment.

The purpose of this 14-day toxicity study was to assess the acute toxic effect of methiocarb-methoxy-sulfone (M10) to earthworms. For this purpose, the test item was mixed into an artificial soil (10% peat). Adult earthworm *Eisenia fetida* (at least two months old, 4 x 10 animals per concentration) were exposed for 14 days in an artificial soil to test item concentrations (nominal) of 10, 32, 100, 316, and 1000 mg per kilogram dry soil. The mortality and the symptoms of toxicity were assessed after 7 and 14 days, and the body wet weight was assessed after 14 days.

At the beginning (prior to exposure) and at the end of the test (Day 14), the test organisms of each test vessel were weighed (at the start each individually, at the end all together of each test vessel). Before weighing, the worms were quickly washed with water, surplus water was adsorbed on filter paper.For each test vessel, the difference of the mean body wet weight of the surviving test organisms between the start and the end of the test

was calculated. The changes in mean body weight of the surviving worms were compared to the control, and were statistically evaluated by means of a multiple Dunnett-test after a one-way analysis of variance (ANOVA).

After 7 and 14 days of exposure, the content of each single test vessel was emptied onto a plate. Then, the surviving worms were counted and any symptoms of toxicity or abnormal behaviour of the test organisms were recorded. Those worms which did not move after gentle mechanical stimulus to the front end were considered to be dead. After the 7-day assessment, the soil of each test vessel was refilled into the test vessel and the worms were replaced on the same test substrate surface.

Mortality data are based on the initial number of worms placed on top of the test medium minus the number of living worms found on the day of assessment of mortality (Day 7 and 14), since dead worms decompose in the soil during the exposure and hence cannot always be found.

Validity criteria were met.

The mean body wet weight of the worms in the control vessels was 388 mg at the beginning of the test, and 375 mg at the end of the study. Thus, the mean body wet weight of the surviving worms in the control had, on average, decreased slightly by 3% due to starvation during the exposure period of 14 days. The mean losses in body wet weight were at all test concentrations up to and including 316 mg/kg dry soil in the same range (mean decrease of 3 to 7%). According to the results of a Dunnett-test (one-sided smaller, $\alpha = 0.05$), the losses in mean body wet weight of the living worms were not significantly higher compared to the control value up to and including the highest test concentration without mortality of 316 mg/kg dry soil.

After 7 days of exposure, no mortality was observed in the control and at the test concentrations up to and including 316 mg/kg dry soil. At the highest test concentration of 1000 mg/kg dry soil, all worms were dead. The LC50 after seven days of exposure was determined to be 562 mg/kg dry soil with 95% confidence limits from 316 to 1000 mg/kg dry soil.

After 14 days of exposure, again in the control and up to and including the test concentration of 316 mg/kg dry soil, no significant mortality and no symptoms of intoxication or abnormal behaviour of the test organisms were observed. At the test concentration of 100 mg/kg dry soil, one worm was dead. However, this low mortality rate of 2.5% was not estimated as a toxic effect, because according to the test guidelines, a mortality rate up to 10% is considered as natural, and additionally, no signs of intoxication and no abnormal behaviour was observed at the next higher test concentration of 316 mg/kg dry soil. At the highest test concentration of 1000 mg/kg dry soil, all earthworms were dead already after seven days. Thus, the concentration-effect relationship was rather steep.

The LC50 at the observation intervals after 7 and 14 days could not be calculated by Probit analysis or Moving average interpolation due to the steep concentration-effect relationship. Instead, the LC50-values were determined as the geometric mean value of the two consecutive test concentrations with 0% and 100% mortality, and the 95% confidence interval for the LC50 as the test concentrations with 0% and 100% mortality. The 14-day LC50 was identical to the 7-day LC50: 562 mg/kg dry soil with 95% confidence limits from 316 to 1000 mg/kg dry soil. Correcting this value due to the high %OC in the test soil gives a 14 d LC50 ~280 mg/kg dry soil.

Title	Acute toxicity of methiocarb-sulfoxide-phenol to the earthworm *Eisenia fetida* in a 14-day test
Reference	Batscher, R. 2001

Test Guideline	OECD Test Guideline 207, (1984) and Directive 87/302/EEC, L 133
Data Validity	1
Data Relied On	Yes - the data were considered to be critical and were relied on in this assessment.

The purpose of this 14-day toxicity study was to assess the acute toxic effect of the test item methiocarb-sulfoxide-phenol (M04) to earthworms. For this purpose, the test item was mixed into an artificial soil (10% peat). Adult earthworm *Eisenia fetida* (about 5-7 months old, 4 x 10 animals per concentration) were exposed for 14 days in an artificial soil to test item concentrations (nominal) of 10, 32, 100, 316, and 1000 mg per kilogram dry soil. The mortality and the symptoms of toxicity were assessed after seven and 14 days, and the body wet weight was assessed after 14 days.

After seven and 14 days of exposure, the content of each single test vessel was emptied onto a plate. The surviving worms were counted and any symptoms of toxicity or abnormal behaviour of the test organisms were recorded. Those worms, which did not move after gentle mechanical stimulus to their front end were considered to be dead. After the 7-day assessment, the soil of each test vessel was refilled into the test vessel and the worms were replaced into the same test substrate.

Mortality data are based on the initial number of worms placed on top of the test medium minus the number of living worms found on the day of assessment of mortality (Day 7 and 14), since dead worms can decompose in the soil during the exposure and hence cannot always be found.

At the beginning (prior to exposure) and at the end of the test (Day 14), the test organisms of each test vessel were weighed (at the start each individually, at the end all together of each test vessel). The changes in mean body weight of the surviving worms were compared to the control, and were statistically evaluated by means of a multiple Dunnett-test after a one-way analysis of variance (ANOVA).

Compared to the control, the losses in mean body wet weight of the worms were not statistically significantly higher at any concentration up to and including the highest test concentration of nominal 1000 mg/kg dry soil (results of a Dunnett-test, one-sided, a = 0.05).

Mortality and symptoms of toxicity: After seven and 14 days of exposure, all test organisms survived in the control and at all test item concentrations up to and including the highest test concentration of nominal 1000 mg/kg dry soil with the exception of one earthworm in three of the four replicates of the nominal test item concentration of 32 mg/kg dry soil and one earthworm in a replicate of the test item concentration of 1000 mg/kg dry soil. However, these mortality rates of 7.5% and 2.5%, respectively, were not estimated as a toxic effect, because according to the test guidelines, a mortality rate up to 10% is considered as natural. Additionally, no concentration-response relationship was determined. Moreover, no abnormal behaviour or symptoms of toxicity were recorded on worms in any of the test treatments.

The test item proved to be non-toxic to the earthworm *Eisenia fetida* up to and including the test item concentration of 1000 mg/kg dry soil in this 14 day test.

Title	Acute toxicity of methiocarb-sulfoxide to the earthworm *Eisenia fetida* in a 14-day test
Reference	Batscher, R. 2000
Test Guideline	OECD Test Guideline 207, (1984) and Directive 87/302/EEC, L 133

Data Validity	1
Data Relied On	Yes - the data were considered to be critical and were relied on in this assessment.

The purpose of this 14-day toxicity study was to assess the acute toxic effect of the test item methiocarb-sulfoxide (M01) to earthworms. For this purpose, the test item was mixed into an artificial soil (10% peat). Adult earthworm *Eisenia fetida* (about 8 months old, 4 x 10 animals per concentration) were exposed for 14 days in an artificial soil to test item concentrations (nominal) of 10, 32, 100, 316, and 1000 mg per kilogram dry soil. The mortality and the symptoms of toxicity were assessed after seven and 14 days, and the body wet weight was assessed after 14 days.

Mortality and Symptoms of Toxicity: After seven and 14 days of exposure, the content of each single test vessel was emptied onto a plate. Then, the surviving worms were counted and any symptoms of toxicity or abnormal behaviour of the test organisms were recorded. Those worms which did not move after gentle mechanical stimulus to the front end were considered to be dead. After the 7-day assessment, the soil of each test vessel was refilled into the test vessel and the worms were replaced on the same test substrate surface.

Mortality data are based on the initial number of worms placed on top of the test medium minus the number of living worms found on the day of assessment of mortality (Day 7 and 14), since dead worms completely decompose in the soil during the exposure and hence cannot always be found.

The LC50 and the 95% confidence interval at the observation dates were calculated by Probit Analysis the NOEC and LOEC were determined directly from the raw data.

Body Weight: At the beginning (prior to exposure) and at the end of the test (Day 14), the test organisms of each test vessel were weighed (at the start each individually, at the end all together of each test vessel). The changes in mean body weight of the surviving worms were compared to the control, and were statistically evaluated by means of a multiple Dunnett-test after a one-way analysis of variance (ANOVA).

Validity criteria were met.

Body weight: The mean body wet weight of the worms in the control vessels was 481 mg at the beginning of the test, and 439 mg at the end of the study. Thus, the mean body wet weight of the surviving worms in the control had, on average, decreased slightly by 9% due to starvation during the exposure period of 14 days. According to the results of a Dunnett-test (one-sided smaller, $\alpha = 0.05$), the difference of the mean body wet weight of the living worms was not significantly different to the control value at the lowest test concentration of 10 mg/kg dry soil. First at the test concentration of 32 mg/kg dry soil, the mean loss in body wet weight of the surviving worms significantly increased compared to the control. From 32 to 316 mg/kg dry soil, a concentration-dependent increase of the mean body weight loss was determined.

Mortality and symptoms of toxicity: After seven days of exposure, no mortality was observed in the control and at the lowest test concentrations of 10 mg/kg dry soil. At the test concentration of 32 mg/kg dry soil, a mortality rate of 17.5% was determined. Some of the surviving worms were remarkably smaller than in the control. At the higher test concentrations of nominal 100, 316, and 1000 mg/kg dry soil, the mortality rates were 55, 87.5, and 100%, respectively. The surviving worms at the test concentration of nominal 316 mg/kg dry soil were less active than the worms in the control. The LC50 after 7 days of exposure was determined to be 90 mg/kg dry soil with 95% confidence limits from 70 to 115 mg/kg.

After 14 days of exposure, again in the control and at the test concentration of 10 mg/kg dry soil, no significant mortality and no symptoms of intoxication or abnormal behaviour of the test organisms were observed. In the control, two earthworms were dead. However, this low mortality rate of 5% is considered as natural and accepted by the guidelines. At the test concentration of 32 mg/kg dry soil, an unchanged mortality rate of 17.5% was determined. At the higher test concentrations of 100 and 316 mg/kg dry soil, the mortality rates were 60 and 95%, respectively. At the highest test concentration of 1000 mg/kg dry soil, all worms were dead already after seven days. The same symptoms of intoxication and abnormal behaviour as described above at the observation after seven days were observed at the end of the test.

The 14-day LC50 was calculated to be 78 mg/kg dry soil with 95% confidence limits from 62 to 97 mg/kg dry soil. Correcting for the high organic carbon content of the test soil, the 14 d LC50corr is approximately 39 mg/kg dry soil.

Title	Acute toxicity of methiocarb-sulfone-phenol to earthworms (*Eisenia fetida*)
Reference	Meisner, P. 2001
Test Guideline	OECD Test Guideline 207, (1984)
Data Validity	1
Data Relied On	Yes - the data were considered to be critical and were relied on in this assessment.

The purpose of this 14-day toxicity study was to assess the acute toxic effect of the test item methiocarb-sulfone-phenol (M05) to earthworms. Adult *Eisenia fetida* (4 x 10 animals per concentration) were exposed in an artificial soil for 14 days to the concentrations of 0.1, 1.0, 10, 32, 100, 316 and 1000 mg/kg dry weight soil (nominal concentrations). The values given are nominal concentrations.

The test soil consisted of 69 % fine quartz sand (84 % of the sand has a particle size of 0.06–0.2 mm), 10 % dried, finely ground peat (sphagnum peat; pH 2–4), 20 % kaolin (kaolinite content of about 36 %, pH value ca 7, "Kaolin W", from Erbsloh I Geisenheim) and around 1% calcium carbonate (pure) to adjust the pH value to 6 ± 0.5. The test soil was first mixed from these dry components in a plough-share mixer, and then moistened with a part of the final volume of water required.

Mortality was determined at 7 and 14 days. Changes in worm weights were determined at the end of the study.

With the exception of 5% mortality in the control group and 3% mortaility in the 32 mg/kg treatment group, no other mortality was recorded. There were no effects on worm body weights through the test. The 14 d LC50 was >1000 mg/kg soil dw.

Metabolites - Chronic

Title	Methiocarb-sulfoxide: Effects on reproduction and growth of earthworms *Eisenia fetida* in artificial soil
Reference	Witte, B. 2013
Test Guideline	OECD Test Guideline 222, (1984) and ISO 11268, part 2, (1998)
Data Validity	1
Data Relied On	Yes - the data were considered to be critical and were relied on in this assessment.

The purpose of this study was to investigate the effects of methiocarb-sulfoxide (M01) on the mortality, body weight, feeding activity and reproduction of the adult earthworm *Eisenia fetida*. Concentrations used were Control,

0.20, 0.36, 0.63, 1.12 and 2.00 mg/kg soil. There were 4 replicates per treatment and 8 replicates for the control with 10 worms each.

Assessment of adult worm mortality, behavioural effects and biomass development was carried out after 28 days exposure of adult worms in treated artificial soil. Reproduction rate (number of offspring) was assessed after additional 28 days (assessed 56 days after application).The numbers of dead adult earthworms, sublethally affected worms, body weights and the amount of food added to each test container (which approximately reflects the amount of food eaten) were listed for each test container (replicate).The number of offspring was listed for each test container (replicate) and treatment.Mortality data were analysed for significance by using the Fisher's Exact Test (one-sided greater, α = 0.05).The body weight change and reproduction data were tested for normal distribution and homogeneity of variance (α = 0.05) using the Shapiro-Wilk´s test and the Levene´s test, respectively.

As the data for body weight changes were normally distributed, homogeneous and not monotonous decreased, Dunnett´s t-test was used to compare treatment and control values (multiple comparison, two-sided, α = 0.05). As the data for reproduction were normally distributed and homogeneous Williams t-test was used to compare treatment and control values (multiple comparison, one-sided smaller, α = 0.05). The EC values and their 95% confidence limits were calculated by applying Probit-Analysis (Finney, 1971).

All study validity criteria were met.

No statistically significant increased mortality was observed in any treatment group. The NOEC for mortality was determined to be ≥ 2.00 mg test item/kg soil.

The body weight changes were significantly reduced compared to the control at the test concentrations of 0.36 and 2.00 mg test item/kg soil. Since at the test concentrations of 0.63 and 1.12 mg test item/kg soil no significant effects were observed, the effect at 0.36 mg test item/kg soil was not considered to be test item related (Dunnett´s t-test, α = 0.05, two-sided). The NOEC for body weight changes was determined to be 1.12 mg test item/kg soil.

The reproduction rates were not significantly different compared to the control in any of the test item treated groups (Williams t-test, α = 0.05, one-sided smaller). The NOEC for reproduction was determined to be 2.00 mg/kg soil. The EC10 was determined to be 2.3 mg/kg soil; the EC20 was determined to be 9.6 mg/kg soil (Probit Analysis, 95% confidence limits not determinable).

Title	Methiocarb-methoxy-sulfone: Effects on reproduction and growth of earthworms *Eisenia fetida* in artificial soil
Reference	Witte, B. 2013
Test Guideline	OECD Test Guideline 222, (1984) and ISO 11268, part 2, (1998)
Data Validity	1
Data Relied On	Yes - the data were considered to be critical and were relied on in this assessment.

The purpose of this study was to investigate the effects of methiocarb-methoxy-sulfone (M10) on the mortality, body weight, feeding activity and reproduction of the adult earthworm *Eisenia fetida*. A single treatment group of 100 mg/kg soil and a control were tested with eight replicates for the test item treatments and eight replicates for the control with 10 worms each.

Assessment of adult worm mortality, behavioural effects and biomass development was carried out after 28 days exposure of adult worms in treated artificial soil. Reproduction rate (number of offspring) was assessed after additional 28 days (assessed 56 days after application).

After four weeks, the artificial soil was transferred to a tray and adult worms were counted, removed and weighed per replicate after being washed under tap water and dried on paper towels. Missing earthworms and earthworms that failed to respond to gentle stimulation were considered to be dead. The remaining soil (without the adult worms) was then returned to the respective test containers.

The body weight change and reproduction data were tested for normal distribution and homogeneity of variance ($\alpha = 0.05$) using the Shapiro-Wilk´s test and the Levene´s test, respectively. As the data for body weight changes and reproduction were normally distributed and homogeneous in both cases, Student t- test was used to compare treatment and control values (pairwise comparison, two-sided for weight and one-sided smaller for reproduction, $\alpha = 0.05$).

All study validity criteria were met.

There were no treatment related effects on earthworm body weight.

No mortality was observed in any treatment group.

The reproduction rate was not significantly different compared to the control at the single test item concentration of 100 mg test item/kg soil (Student t-test, $\alpha = 0.05$, one-sided smaller). No behavioural abnormalities were observed in any of the treatment groups. The feeding activity in all the treated groups was comparable to the control.

The NOEC for mortality, growth, reproduction and feeding activity of the earthworm *Eisenia fetida* was determined to be 100 mg test item/kg soil.

Title	Methiocarb-sulfone-phenol: Effects on reproduction and growth of earthworms *Eisenia fetida* in artificial soil
Reference	Witte, B. 2013
Test Guideline	OECD Test Guideline 222, (1984) and ISO 11268, part 2, (1998)
Data Validity	1
Data Relied On	Yes - the data were considered to be critical and were relied on in this assessment.

The purpose of this study was to investigate the effects of methiocarb-sulfone-phenol (M05) on the mortality, body weight, feeding activity and reproduction of the adult earthworm *Eisenia fetida*. One concentration of the test item (100 mg/kg soil dw) was incorporated into the soil; two treatment groups (one test item concentration, one control); eight replicates for the test item treatments and eight replicates for the control with 10 worms each.

Assessment of adult worm mortality, behavioural effects and biomass development was carried out after 28 days exposure of adult worms in treated artificial soil. Reproduction rate (number of offspring) was assessed after additional 28 days (assessed 56 days after application).

The numbers of dead adult earthworms, sublethally affected worms, body weights and the amount of food added to each test container (which approximately reflects the amount of food eaten) were listed for each test container (replicate).The number of offspring was listed for each test container (replicate) and treatment. The body weight

change and reproduction data were tested for normal distribution and homogeneity of variance (α = 0.05) using the Shapiro-Wilk´s test and the Levene´s test, respectively. As the data for body weight changes and reproduction were normally distributed and homogeneous in both cases, Student t- test was used to compare treatment and control values (pairwise comparison, two-sided for weight and one-sided smaller for reproduction, α = 0.05).

All study validity criteria were met.

There were no treatment related effects on earthworm body weight.

No mortality was observed in any treatment group.

The reproduction rate was not significantly different compared to the control at the single test item concentration of 100 mg test item/kg soil (Student t-test, α = 0.05, one-sided smaller). No behavioural abnormalities were observed in any of the treatment groups. The feeding activity in all the treated groups was comparable to the control

The NOEC for mortality, growth, reproduction and feeding activity of the earthworm *Eisenia fetida* was determined to be 100 mg test item/kg soil.

Title	Methiocarb-sulfoxide phenol: Effects on reproduction and growth of earthworms *Eisenia fetida* in artificial soil
Reference	Witte, B. 2013
Test Guideline	OECD Test Guideline 222, (1984) and ISO 11268, part 2, (1998)
Data Validity	1
Data Relied On	Yes - the data were considered to be critical and were relied on in this assessment.

The purpose of this study was to investigate the effects of methiocarb-sulfoxide phenol (M04) on the mortality, body weight, feeding activity and reproduction of the adult earthworm *Eisenia fetida*. One concentration of the test item 100 mg/kg soil was incorporated into the soil; two treatment groups (one test item concentration, one control); eight replicates for the test item treatments and eight replicates for the control with 10 worms each.

The numbers of dead adult earthworms, sublethally affected worms, body weights and the amount of food added to each test container (which approximately reflects the amount of food eaten) were listed for each test container (replicate).The number of offspring was listed for each test container (replicate) and treatment. The body weight change and reproduction data were tested for normal distribution and homogeneity of variance (α = 0.05) using the Shapiro-Wilk´s test and the Levene´s test, respectively. As the data for body weight changes and reproduction were normally distributed and homogeneous in both cases, Student t-test was used to compare treatment and control values (pairwise comparison, two-sided for weight and one-sided smaller for reproduction, α = 0.05).

All study validity criteria were met.

There were no treatment related effects on earthworm body weight.

No mortality was observed in any treatment group.

The reproduction rate was not significantly different compared to the control at the single test item concentration of 100 mg test item/kg soil (Student t-test, α = 0.05, one-sided smaller). No behavioural abnormalities were observed in any of the treatment groups. The feeding activity in all the treated groups was comparable to the control.

The NOEC for mortality, growth, reproduction and feeding activity of the earthworm *Eisenia fetida* was determined to be 100 mg test item/kg soil.

Effects on other soil non-target micro-organisms

Title	Methiocarb RB 2.0 W: Effects on the activity of soil microflora (nitrogen transformation test)
Reference	Schulz, L. 2013
Test Guideline	OECD 216 (2000)
Data Validity	1
Data Relied On	Yes - the data were considered to be critical and were relied on in this assessment.

The purpose of this study was to determine the effects of Methiocarb RB 2.0 W on the activity of soil microflora with regard to nitrogen transformation in a laboratory test. A loamy sand soil was exposed for 28 days to 6.67 and 66.67 mg test item/kg soil dry weight, which were considered equivalent to 5 and 50 kg test item/ha. The test pellets were finely ground and homogenized prior to application. The nitrogen transformation was determined in soil enriched with lucerne meal (concentration in soil 0.5 %). NH_4-nitrogen, NO_3- and NO_2-nitrogen were determined by an Autoanalyzer at different sampling intervals (0, 7, 14 and 28 days after treatment).

The microbial biomass of the test soil was 30.56 mg C/100 g soil dw.

The coefficients of variation in the control (NO3-N) were maximum 3.0 % and thus fulfilled the demanded range (<15 %).

The following results were recorded:

Effects on nitrogen transformation in soil after treatment with Methiocarb RB 2.0 W

Time Interval (days)	Control			6.67 mg test item/kg soil dry weight equivalent to 5 kg test item/ha				66.67 mg test item/kg soil dry weight equivalent to 50 kg test item/ha			
	Nitrate-N1			Nitrate-N1			% difference to control	Nitrate-N1			% difference to control
0-7	4.32	±	0.21	4.39	±	0.49	+1.7 n.s.	4.64	±	0.14	+7.5 n.s.
7-14	1.28	±	0.43	1.70	±	0.40	+32.7 n.s.	0.91	±	0.28	-28.6 n.s.
14-28	0.90	±	0.10	0.72	±	0.10	-20.5 n.s.	0.99	±	0.03	+9.2 n.s.

The calculations were performed with unrounded values

1) Rate: Nitrate-N in mg/kg soil dry weight/time interval/day, mean of 3 replicates and standard deviation

n.s. = No statistically significant difference to the control (Student-t-test for homogeneous variances, 2-sided, p α 0.05.

The test item methiocarb RB 2.0 W caused a temporary stimulation and a temporary inhibition of the daily nitrate rate at the tested concentrations of 6.67 mg/kg and 66.67 mg/kg dry soil, respectively, at time interval 7–14 days after application.

However, no adverse effects of methiocarb RB 2.0 W on nitrogen transformation in soil could be observed at both tested concentrations at the end of the test, 28 days after application (time interval 14–28). Differences from the control of -20.5 % (test concentration 6.67 mg/kg dry soil) and +9.2 % (test concentration 66.67 mg/kg dry soil) were measured at the end of the 28-day incubation period (time interval 14–28).

Methiocarb RB 2.0 W caused no adverse effects (difference to control < 25 %) on the soil nitrogen transformation (measured as NO_3-N-production) at the end of the 28-day incubation period. The study was performed in a field soil at concentrations up to 66.67 mg test item/kg dry soil, which are equivalent to application rates up to 50 kg test item/ha.

Metabolites

Title	Influence of the metabolite methiocarb-methoxy-sulfone on the microbial mineralization of nitrogen in soils
Reference	Anderson, J.P.E 2000
Test Guideline	Guidelines for the Official Testing of Plant Protectants, Part VI, 1-1, Influence on the Activityof the Soil Microflora, BBA Braunschweig, Germany, March 1990 (2nd ed.).ISO/DIS 1036-6:1992, OECD/OCDE Guideline No. 216, Adopted: 21st January 2000,OECD/OCDE Guideline No. 216 (2000)
Data Validity	1
Data Relied On	Yes - the data were considered to be critical and were relied on in this assessment.

The objective of the experiment was to determine the influence of methiocarb-methoxy-sulfone (M10) on nitrogen mineralization in an agricultural soil. The purity of the metabolite was 98.5 %. A loamy sand soil was exposed for 28 d to 1.33 mg/kg soil dry weight. Lucerne-grass-green meal was added to soil (5 g/kg dry weight soil) to stimulate nitrogen transformation.

After mixing, soil samples equivalent to 250 g dry weight were poured into 500 mL brown glass bottles and these were closed with parafilm. Three replicates were prepared per treatment. Each bottle was marked with an identification number and the quantity of product added to the soil. The soil was held in the dark at 20 ± 2°C and about 40% water capacity.

The quantities of soluble nitrogen in the soil were determined immediately before the start of the experiment. For this purpose, triplicate untreated and unamended samples of the soil (corresponding to 10 g dry wt) were extracted with 1 N KCl by agitating for 60 min on a horizontal shaker at ca. 150 rpm.

The carbon content of the metabolically active microbial biomass in the soil was determined at the start of the experiment.

During the 28-day experiments, M10 had no influence on the turnover of nitrogen in a loamy sand soil amended with lucerne-grass-green meal.

Title	Influence of the metabolite methiocarb-sulfoxide on the microbial mineralization of nitrogen in soils
Reference	Anderson, J.P.E 2000
Test Guideline	Guidelines for the Official Testing of Plant Protectants, Part VI, 1-1, Influence on the Activityof the Soil Microflora, BBA Braunschweig, Germany, March 1990 (2nd ed.).ISO/DIS 1036-6:1992, OECD/OCDE Guideline No. 216, Adopted: 21st January 2000,OECD/OCDE Guideline No. 216 (2000)
Data Validity	1
Data Relied On	Yes - the data were considered to be critical and were relied on in this assessment.

The objective of the experiment was to determine the influence of methiocarb-sulfoxide (M01) on nitrogen mineralization in an agricultural soil. The purity of the metabolite was 98.2 %. A loamy sand soil was exposed for 28 d to 1.47 mg/kg soil dry weight. Lucerne-grass-green meal was added to soil (5 g/kg dry weight soil) to stimulate nitrogen transformation.

After mixing, soil samples equivalent to 250 g dry weight were poured into 500 mL brown glass bottles and these were closed with parafilm. Three replicates were prepared per treatment. Each bottle was marked with an identification number and the quantity of product added to the soil. The soil was held in the dark at 20 ± 2°C and about 40% water capacity.

During the 28-day experiments, M01 had no influence on the turnover of nitrogen in a loamy sand soil amended with lucerne-grass-green meal.

Title	Influence of the metabolite methiocarb-sulfone-phenol on the microbial mineralization of nitrogen in soils
Reference	Anderson, J.P.E, 2001
Test Guideline	Guidelines for the Official Testing of Plant Protectants, Part VI, 1-1, Influence on the Activityof the Soil Microflora, BBA Braunschweig, Germany, March 1990 (2nd ed.).ISO/DIS 1036-6:1992, OECD/OCDE Guideline No. 216, Adopted: 21st January 2000,OECD/OCDE Guideline No. 216 (2000)
Data Validity	1
Data Relied On	Yes - the data were considered to be critical and were relied on in this assessment.

The objective of the experiment was to determine the influence of methiocarb-sulfone-phenol (M05) on nitrogen mineralization in an agricultural soil. The purity of the metabolite was 98.3%. A loamy sand soil was exposed for 28 d to 1.20 mg/kg soil dry weight. Lucerne-grass-green meal was added to soil (5 g/kg dry weight soil) to stimulate nitrogen transformation.

During the 28-day experiments, M05 had no influence on the turnover of nitrogen in a loamy sand soil amended with lucerne-grass-green meal.

Title	Influence of the metabolite methiocarb-sulfoxide-phenol on the microbial mineralization of nitrogen in soils
Reference	Anderson, J.P.E, 2000
Test Guideline	Guidelines for the Official Testing of Plant Protectants, Part VI, 1-1, Influence on the Activityof the Soil Microflora, BBA Braunschweig, Germany, March 1990 (2nd ed.).ISO/DIS 1036-6:1992, OECD/OCDE Guideline No. 216, Adopted: 21st January 2000,OECD/OCDE Guideline No. 216 (2000)
Data Validity	1
Data Relied On	Yes - the data were considered to be critical and were relied on in this assessment.

The objective of the experiment was to determine the influence methiocarb-sulfoxide-phenol (M04) on nitrogen mineralization in an agricultural soil. The purity of the metabolite was 99%. A loamy sand soil was exposed for 28 d to 1.09 mg/kg soil dry weight. Lucerne-grass-green meal was added to soil (5 g/kg dry weight soil) to stimulate nitrogen transformation.

During the 28-day experiments, M04 had no influence on the turnover of nitrogen in a loamy sand soil amended with lucerne-grass-green meal.

Effects on other non-target organisms (flora and fauna) believed to be at risk

Title	Methiocarb RB 4: Extended laboratory study to evaluate the effects on the spider, *Pardosa* spp. (Araneae, Lycosidae)
Reference	Hermann, P. 2001
Test Guideline	BBA Guideline VI 23-2.1.9 (WEHLING & HEIMBACH 1994) including recentimprovements by the ring test group (HEIMBACH et al. 2000) and Guidance Document for Regulatory Testing Procedures for Pesticides with Non-Target Arthropods (BARRETT et al. 1994)
Data Validity	1
Data Relied On	Yes - the data were considered to be critical and were relied on in this assessment.

The effect of Methiocarb RB 4 (nominal content of ac: 4%) at a rate of 6000 g, corresponding to 240 g ac, per hectare on lycosid spiders of the genus *Pardosa* was determined in the laboratory. Spiders (34 per treatment group) were individually exposed in test units filled with moist natural standard soil (LUFA 2.1). One pellet (mean weight: 0.0107 g product) was placed in the centre of each exposure unit (soil surface: approximately 178 cm2) which is equivalent to a rate of 6011.2 g product/ha (approximately 1.002 times of the recommended rate). Deionized water was used for the control treatment. Perfekthion (ac dimethoate) was applied at 900 g ac/ha (nominal) in the toxic reference treatment. Test duration was 14 days. During the exposure time the spiders were fed with *Drosophila* flies (strain unable to fly). Mortality and feeding rate were assessed. Mortality in the toxic reference treatment was 88.24%.

Mortality of each treatment group was recorded for each date and calculated as percentage of the number of lycosid spiders put in at the start of the test. This calculation was also done for each sex separately. The mortality caused by the test substance was corrected by the mortality in the control using the formula of SCHNEIDER-ORELLI (1947).

The mean number of consumed flies per lycosid spider per assessment date was calculated by summing up the number of consumed flies per spider for all replicates and dividing this sum by the number of spiders. The mean food consumption per lycosid spider in the first week was calculated as the mean value from the assessment values of day 1, day 2, day 3 and day 4.

The mean food consumption per lycosid spider in the second week was calculated as the mean value from the assessment values on day 8 and day 11.

In order to receive the food consumption per lycosid spider for the entire observation period, the mean value of all single assessment dates was calculated. This calculation was also done for females and males separately Mortality data were analysed for significance using Fisher's Exact test, which is a distribution-free test and does not require testing for normality or homoscedasticity prior to analysis (ZAR, 1984).

The number of consumed *Drosophila* flies per lycosid spider per assessment date was tested for normality and homoscedasticity using Shapiro-Wilk's test and residual analysis (ZAR, 1984). Statistical evaluation is based on mean values for single spiders. Each value was calculated for the period of time during which the spider was alive. Since the assumptions were met for the data of the first or the second week, Dunnett's Test was used to analyse for significant differences between control and treatment effects for each week separately. Considering all assessments together during the whole observation period, assumptions were not met, and therefore the Kruskal-Wallis-Test was used.

Validity criteria were met.

The spiders in the control group and in the group with Methiocarb RB 4 showed normal activity during the entire exposure period. In the toxic standard treatment 19 spiders showed symptoms of paralysis during the first 4 hours after treatment. A total of 22 spiders were found dead on day +1, approximately 24 hours after application.

The mean number of flies consumed per spider per day during the period of exposure to Methiocarb RB 4 was 2.81 compared to 2.69 in the control. The mean feeding rate in the toxic standard treatment group was 2.51 for the total observation period. The feeding rate during exposure to Methiocarb RB 4 compared to the control group was calculated as 104.46%. The feeding rate of the toxic standard treatment relative to the control was calculated as 93.31%. No statistically significant effects compared to the control were observed in the test substance treatment group.

No abnormal behavioural effects were observed m the control and m the Methiocarb RB 4 treatment group.

Title	Methiocarb RB 4: An extended laboratory study conducted on natural soil to evaluate the effects on the staphylinid beetle, *Aleochara bilineata* Gyll. (Coleoptera, Staphylinidae)
Reference	Hermann, P. 2001
Test Guideline	MORETH & NATON (1992) and Guidance Document for Regulatory Testing Procedures for Pesticides with Non-Target Arthropods (BARRETT et al. 1994
Data Validity	1
Data Relied On	Yes - the data were considered to be critical and were relied on in this assessment.

This study assessed the effect of Methiocarb RB 4 at a rate of 6000 g product/ha, corresponding to 240 g ac per hectare on the staphylinid beetle *Aleochara bilineata* in the laboratory. Pairs (male + female) of rove beetles per replicate (4 replicates per treatment group) were exposed to the test substance in exposure units filled with moist natural standard soil (LUFA 2.1). One pellet (mean weight: 0.0115 g product) was placed in the centre of each exposure unit (soil surface: approximately 190 cm^2) which is equivalent to a rate of 6052.6 g product/ha (approximately 1.009 times of the recommended rate). Deionized water was used for the control treatment. Dursban 480 (ac chlorpyrifos) was applied at 1 L product/ha in the toxic reference treatment.

The complete life cycle of beetles - parental generation, mating and oviposition of parental generation, hatching of F 1 larvae, parasitisation period until emergence of F_1 adults - took place during the test. During the four week exposure time the beetles were fed with thawed *Chironomus* larvae. Pupae of the onion fly *Delia antiqua* were offered as hosts for the *Aleochara* larvae. The quantity of evaporated water was determined by weighing the exposure units and the water was replenished with deionized water. Thereafter this procedure was repeated at intervals of 2–4 days.

The emergence period of Fl adults lasted 56 days. The complete duration of the experimental phase was 89 days. The reproduction in the test substance and reference substance group compared to the control group was calculated from the mean numbers of emerged offspring.

Validity of the Test: The results are considered to be valid because the mean number (of four replicates) of offspring in the control group was 350 beetles and the reduction in parasitisation compared to the control in the toxic standard group was 50%. During the exposure and emergence period the temperature was above the target range of 20 ± 2°C one day and below the target range on three days. The results of the control group illustrate that these deviations had no impact on the validity and integrity of the study.

The following results were obtained:

Treatment group	Mean no. of offspring	Reproduction [%] relative to the control
Control	747.0	--
240 g ac/ha (nominal)	678.8	90.9
Toxic standard	0.80*	0.11

** statistically significantly different compared to the control*

Methiocarb RB 4 at a rate of 6000 g product per hectare did not significantly affect reproduction of the rove beetle *Aleochara bilineata* compared to the control. The product is therefore assessed as harmless for *A. bilineata* up to this rate.

Title	Methiocarb RB 2.0 W: Effects on the reproduction of the collembolan *Folsomia candida*
Reference	Friedrich, S. 2014
Test Guideline	OECD 232 (2009), ISO 11267 (1999)
Data Validity	1
Data Relied On	Yes - the data were considered to be critical and were relied on in this assessment.

The purpose of this study is to determine potential effects of different concentrations of Methiocarb RB 2.0 W on the reproductive output of the collembolan *Folsomia candida* as a representative of soil micro-arthropods during a test period of 28 days. Ten Collembola (9–12 days old) were exposed to 229, 458, 917, 1833 and 3667 mg/kg dry weight of soil containing 74.7 % quartz sand, 20 % kaolin clay, 5 % sphagnum peat and 0.3 % $CaCO_3$, at 19.9–22.0 °C and a photoperiod: light : dark = 16 h : 8 h (540 lx) and were fed weekly with granulated dry yeast. Mortality and reproduction were determined after 28 days.

The reference item boric acid (100% analysed) was tested in a separate study at concentrations of 44, 67, 100, 150 and 225 mg ac/kg soil dry weight.

Results:

Test item Test object Exposure	MTC RB 2.0 W *Folsomia candida* Artificial soil					
mg/kg soil dry weight (nominal)	Adult mortality (%)	Mean number of juveniles per test vessel± standard deviation			Reproduction (% of control)	Significance
Control	3.8	701	±	106	-	
229	2.5	690	±	76	98	-
458	5.0	687	±	95	98	-
917	5.0	665	±	72	95	-
1833	2.5	593	±	59	85	+
3667	40.0	435	±	63	62	+

No effects on behaviour of the collembolans were observed during the test. The NOEC for the mortality of parental collembolans was determined to be 1833 mg test item/kg soil dry weight.

For reproduction, statistically significant effects (Wiiliams-t-test, α = 0.05, one-sided smaller) on the number of juveniles compared to the control group were recorded at concentrations of 1833 and 3667 mg test item/kg soil dw. The NOEC was determined to be 917 mg test item/kg soil dry weight.

The EC10 and EC20 values for reproduction were calculated to be 1358 and 2133 mg test item/kg soil dry weight, respectively.

Metabolites

Title	Acute and reproduction toxicity of methiocarb-sulfoxide-phenol to the collembolan species *Folsomia candida* according to the ISO Guideline 11267 “Soil quality – inhibition of reproduction of Collembola (*Folsomia candida*) by soil pollutants” (1999)
Reference	Moser, Th. 2001
Test Guideline	ISO Guideline 11267 “Soil quality – inhibition of reproduction of Collembola (Folsomia candida) by soil pollutants” (1999)
Data Validity	1
Data Relied On	Yes - the data were considered to be critical and were relied on in this assessment.

The purpose of this study was to asses the effects of methiocarb-sulfoxide-phenol (M04) on the lethal and sublethal effects on the collembolan species *Folsomia candida* (Isotomidae) as a representative of the soil fauna. Ten Collembola (10–12 days old) were exposed to 6.25, 12.5, 25, 50 and 100 mg test item/kg artificial soil at 19–22°C and 400–800 Lux and were fed weekly with granulated dry yeast. Mortality and reproduction was determined after 28 days. Mortality: Springtails were classified as dead when they did not move. Reproduction: The number of juveniles was determined by directly counting using a digital photography system.

Validity criteria were met. The following results were reported:

Mortality and reproduction results

Concentration (mg test item/kg)	Percent mortality	Number of Juveniles (% of control)
Control	4.0	-
6.25	8.0	114.1
12.5	18.0	85.4
25	18.0	103.5
50	12.0	98.8
100	26.0	94.0

After 28 days, the Dunnett's test (1-sided, p = 0.05) showed no significant difference concerning the number of juveniles between the control and all concentrations tested. Therefore, the NOEC was determined as 100 mg test item/kg (the highest concentration tested).

With the highest concentration tested (100 mg test item/kg) 26% mortality was determined. The LC50 was >100 mg/kg soil dw.

Title	Acute and reproduction toxicity of methiocarb-sulfoxide to the collembolan species *Folsomia candida* according to the ISO Guideline 11267 “Soil quality – inhibition of reproduction of Collembola (*Folsomia candida*) by soil pollutants” (1999)
Reference	Moser, Th. 2001
Test Guideline	OECD Test Guideline 208
Data Validity	1
Data Relied On	Yes - the data were considered to be critical and were relied on in this assessment.

The purpose of this study asses the effects of methiocarb-sulfoxide (M01) on the lethal and sublethal effects on the collembolan species *Folsomia candida* (Isotomidae) as a representative of the soil fauna. Collembola (10–12 days old) were exposed to 6.25, 12.5, 25, 50 and 100 mg test item/kg artifical soil at 18–22°C and 510 Lux and were fed weekly with granulated dry yeast. Mortality and reproduction was determined after 28 days.

The test substrate (including collembolans) of each test vessel was poured individually into a water-filled Bellaplast vessel. After gentle stirring, the animals drifted to the surface (if aggregations of collembolans were observed, they were broken up by means of a gentle mechanical stirring). The water was coloured by black ink in order to increase the contrast between water and white collembolans. Than the adult collembolans were counted directly. Afterwards each test vessel was photographed by a digital camera and the number of juveniles was counted using the pictures.

Cochran's test was used to confirm homogeneity of the data. The NOEC was determined by a one-way Analysis of Variance (ANOVA), followed by Dunnet's test (1-sided; p = 0.05).

Validity criteria were met. The following results were reported:

Mortality and reproduction results

Concentration (mg test item/kg)	Percent mortality	Number of Juveniles (% of control)
Control	14	-
6.25	23	94.3
12.5	8	80.6*
25	10	94.8
50	20	85.4
100	32	58.0*

Fourteen percent of adult springtails died in the control. In the concentrations of 12.5 and 50 mg test item/kg, mortality rates of 8–20% were observed.

With the concentration of 6.25 and 100 mg test item/kg, mortality rates of 2% and 32% respectively were determined, which are higher than the required control mortality of 20%. However, no LC50 value was calculated.

Cochran's test confirmed the homogeneity of the data. After 28 days, the ANOVA and the Dunnett's test (1-sided, p 0.05) showed a significant difference concerning the number of juveniles between the control and the concentrations of 12.5 mg test item/kg and 100 mg test item/kg.

Since the treatment of 25 and 50 mg test item/kg did not differ significantly from the control and the concentration of 100 mg test item/kg showed a clearly decreased number of juveniles compared to all treatments including the control, the NOEC was regarded to be 50 mg test item/kg.

Title	Acute and reproduction toxicity of methiocarb-sulfone-phenol to the collembolan species *Folsomia candida* according to the ISO Guideline 11267 “Soil quality – inhibition of reproduction of Collembola (*Folsomia candida*) by soil pollutants” (1999)
Reference	Moser, Th. 2001
Test Guideline	ISO Guideline 11267 “Soil quality – inhibition of reproduction of Collembola (Folsomia candida) by soil pollutants” (1999)
Data Validity	1
Data Relied On	Yes - the data were considered to be critical and were relied on in this assessment.

The purpose of this study is to assess the effects of methiocarb-sulfone-phenol (M05) on the lethal and sublethal effects of the test item on the collembolan species *Folsomia candida* (Isotomidae) as a representative of the soil fauna. Ten Collembola (10–12 days old) per replicate (5) were exposed to 10, 32, 100, 316 and 1000 mg test item/kg artificial soil at 18–21°C and 440 Lux and were fed at the beginning and after 14 days with granulated dry yeast. Mortality and reproduction were determined after 28 days. The test item is not soluble in water; therefore, a volatile organic solvent was used. The stock solution was prepared in acetone. All concentrations to be tested were based on the content of Methiocarb-sulfone-phenol (98.6%).

Note: A first test run had been carried out but since the adult mortality was 26% in the water control and 36% in the solvent control (required :<20%) the study was repeated.

Validity criteria were met. The following results were reported:

Mortality and reproduction results

Concentration (mg test item/kg)	Percent mortality	Number of Juveniles (% of control)
Control	10	-
Solvent Control	8	111.6
10.00	18	126.3
32	20	94.8
100	10	98.8
316	18	94.8
1000	20	85.1

Ten percent of adult springtails died in the control and 8% in the solvent control. In all concentrations of the test item tested 18–20% mortality was observed. Therefore, no LC50 was calculated.

After 28 days, the Dunnett's test (1-sided, p: 0.05) showed no significant difference concerning the number of juveniles between the control and all concentrations of the test item tested. Therefore, the NOEC was determined as 1000 mg test item/kg (the highest concentration tested.

Title	Acute and reproduction toxicity of methiocarb-methoxy-sulfone to the collembolan species *Folsomia candida* according to the ISO Guideline 11267 “Soil quality – inhibition of reproduction of Collembola (*Folsomia candida*) by soil pollutants” (1999)
Reference	Moser, Th. & Scheffczyk, A. 2001
Test Guideline	ISO Guideline 11267 “Soil quality – inhibition of reproduction of Collembola (Folsomia candida) by soil pollutants” (1999)
Data Validity	1
Data Relied On	Yes - the data were considered to be critical and were relied on in this assessment.

The purpose of this study is to asses the effects of methiocarb-methoxy-sulfone (M10) on the lethal and sublethal effects of the test item on the collembolan species *Folsomia candida* (Isotomidae) as a representative of the soil fauna. Ten collembola (10–12 days old) per replicate (5) were exposed to 3.16, 10, 31.6, 100, and 316 mg test item/kg artifical soil at 18–21°C and 440 Lux and were fed at the beginning and after 14 days with granulated dry yeast. Mortality and reproduction were determined after 28 days. Mortality: Springtails were classified as dead when they did not move. Reproduction: The number of juveniles was determined by directly counting using a digital photography system.

The test item is not soluble in water; therefore, a volatile organic solvent was used. The stock solution was prepared in acetone. All concentrations to be tested were based on the content of methiocarb-methoxy-sulfone (99.1%).

Validity criteria were met. The following results were reported:

Mortality and reproduction results

Concentration (mg test item/kg)	Percent mortality	Number of Juveniles (% of control)
Control	8	-
Solvent Control	8	95.4

Concentration (mg test item/kg)	Percent mortality	Number of Juveniles (% of control)
3.16	16	95.9
10	14	92.2
31.6	20	67.5*
100	16	82.5
316	76	0.2*

Cochran's test confirmed the homogeneity of the data. After 28 days, the ANOVA and the Dunnett's test (1-sided, p s 0.05) showed a significant difference concerning the number of juveniles between the control and the concentrations of 31.6 and 316 mg test item/kg. Therefore, the NOEC was regarded to be 10 mg test item/kg. However, the reduction in juveniles at 100 mg/kg was not considered statistically significantly different suggesting the impact seen at 31.6 mg/kg soil was not treatment related and 100 mg/kg could be considered a more appropriate NOEC.

Eight percent of adult springtails died in the control as well as in the solvent control. In the concentrations of 3.16, 10, 31.6, and 100 mg test item/kg, mortality rates of 14–20% were observed. With the concentration of 316 mg test item/kg, 76% mortality was observed.

The LC50 value was calculated as 272.1 mg test item/kg.

ABBREVIATIONS

ac	Active constituent
APVMA	Australian Pesticides and Veterinary Medicines Authority
CAS	Chemical Abstracts Service
DotE	Department of the Environmen
EC	European Commission
EFSA	European Food Safety Authority
EU	European Union
ha	hectare
Kd	Partition (or distribution) coefficient
KOC	Soil adsorption coefficients
LC	Lethal concentration
LD	Lethal dose
NOEC	No Observed Effect Concentration
PEC	Probable Effects Concentration
PNEC	Predicted No Effect Concentration
QS	Quality Standard
RQ	Risk Quotient
US	United States
US EPA	US Environmental Protection Agency

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