Section A7 **Annex Point IIA 7.5**	**Ecotoxicological Profile Including Environmental Fate and Behaviour** A7.4.1.3 Growth inhibition test on algae

	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

A7.4.1.3 Growth inhibition test on algae (metabolite)

			Official use only
		1. REFERENCE	
1.1	**Reference**	Grade, R. and Wydra, V. (2007) Toxicity of NC 7312 (AE C507312) Metabolite of Bendiocarb to *Desmodesmus subspicatus* in an algal Growth Inhibition Test. [redacted] Document M-287279-01-1 7.4.1.3/02 30 April 2007 Unpublished	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Bayer CropScience AG	
1.2.2	Companies with letter of access	n.a.	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	OECD 201 (adopted March 2006) and Dir. 92/69/EEC C.3, 1992	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3. MATERIALS AND METHODS	
3.1	**Test material**	Bendiocarb metabolite NC 7312 (AE C507312)	
3.1.1	Lot/Batch number	30,40,41	
3.1.2	Specification	-	
3.1.3	Purity	99.2 %	
3.1.4	Composition of product	Not applicable	
3.1.5	Further relevant properties	-	
3.1.6	Method of analysis	HPLC/UV	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	See Table A7.4.1.3-7.	X
3.3	**Reference substance**	None	
3.3.1	Method of analysis for reference substance	Not applicable	
3.4	**Testing procedure**		
3.4.1	Culture medium	Algal medium	X
3.4.2	Test organisms	*Desmodesmus subspicatus* (see Table A7.4.1.3-8).	X

3.4.3	Test system	See Table A7.4.1.3-9	
3.4.4	Test conditions	See Table A7.4.1.3-10	X
3.4.5	Duration of the test	72 hours	
3.4.6	Test parameter	Cell density and growth rate	
3.4.7	Sampling	0, 24, 48 and 72 hours	
3.4.8	Monitoring of TS concentration	Yes	X
3.4.9	Statistics	E_BC_{50}, E_yC_{50} and E_rC_{50} and if possible the corresponding EC_{10} values and their 95 %-confidence limits were calculated by Probit analysis. The NOE_rC and the LOE_rC for algal growth were determined by Bonferroni t-test (ToxRat Version 2.09, 30.10.2005)	
		4. RESULTS	
4.1	**Limit test**	None	
4.1.1	Concentration	Not applicable	
4.1.2	Number/ percentage of animals showing adverse effects	Not applicable	
4.2	**Results test substance**		
4.2.1	Initial concentrations of test substance	0.30, 0.95, 3.1, 9.8, 31.3 and 100.0 mg NC 7312/L	X
4.2.2	Actual concentrations of test substance	At the start of the test 99 % of the nominal test concentration was found. After 72 hours test duration, 93 % of the nominal values were determined. Thus, during the test period of 72 hours the algae were exposed to a mean of 96 % of nominal. Therefore, all reported results are related to nominal concentration of the test item.	
4.2.3	Growth curves	See Figure A7.4.1.3-3.	
4.2.4	Concentration / response curve	-	
4.2.5	Cell concentration data	See Table A7.4.1.3-11.	
4.2.6	Effect data (cell multiplication inhibition)	See Table A7.4.1.3-11.	
4.2.7	Other observed effects	-	
4.3	**Results of controls**	See Table A7.4.1.3-11.	
4.4	**Test with reference substance**	Not applicable	
4.4.1	Concentrations	-	
4.4.2	Results	-	

		5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The purpose of this test was to determine the inhibitory effect of the test item NC 7312 (AE C507312) metabolite of bendiocarb on the growth of the freshwater green algal species *Desmodesmus subspicatus*. Exponentially growing cultures of this unicellular algal species were exposed to the test item under defined conditions, added to test water at various concentrations. The inhibition of growth in relation to control cultures was determined over a test period of 72 hours, and thus over several algal generations. The test method and the test species *Desmodesmus subspicatus* were recommended by the test guidelines. The purpose of the analytical part of this study was to verify the concentrations of NC 7312 in the test medium (0.30, 0.95, 3.1, 9.8, 31.3 and 100.0 mg NC 7312/L). NC 7312 was analysed after 0 h and 72 h test exposure.	
5.2	**Results and discussion**	A significant inhibition of the growth rate was observed at test concentrations of 3.1, 9.8, 31.3 and 100.0 mg NC 7312/L. The 72-hour NOE_rC was determined to be 0.95 mg NC 7312/L. The 72-hour LOE_rC was determined to be 3.1 mg NC 7312/L.	
5.2.1	NOE_rC (72-h)	0.95 mg NC 7312/L (based on nominal concentration)	
5.2.2	E_rC_{50} (72-h)	88.3 mg NC 7312/L (based on nominal concentration)	
5.2.3	E_bC_{50} (72-h)	-	X
5.3	**Conclusion**		
5.3.1	Reliability	1	
5.3.2	Deficiencies	No	

Table A7.4.1.3-7 Preparation of TS Solution for Poorly Stable or Volatile Test Substances

Criteria	Details
Dispersion	A concentrated stock solution of the test medium of nominal 100 mg NC 7312/L was prepared by dissolving 70 mg NC 7312 into 700 mL test water by ultrasonification for 5 minutes and intense stirring for 15 minutes. After adjusting the pH-value from 7.5 to 7.9 with 0.1M NaOH, adequate volumes of this stock solution were diluted with test water to prepare all test concentrations.
Vehicle	Not applicable
Vehicle control performed	Not applicable
Other procedures	-

Table A7.4.1.3-8 Test organisms

Criteria	Details
Species	*Desmodesmus subspicatus*, formerly *Scenedesmus subspicatus*
Strain	No. 86.81 SAG
Source	Sammlung von Agenkulturen Pfanzenphysiologisches Institut der Unversität Göttingen - Germany
Laboratory culture	Yes
Method of cultivation	The algae were cultivated in the laboratories of IBACON under standardised conditions according to the test guidelines.
Pretreatment	None
Initial cell concentration	5 x 10^3 cells/mL

Table A7.4.1.3-9 Test system

Criteria	Details
Volume of culture flasks	50 ml
Culturing apparatus	Erlenmeyer flasks
Light quality	4440– 8880 Lux (continuous illumination)
Procedure for suspending algae	Continual stirring (with magnetic stirrers)
Number of vessels / concentration	Three (per concentration) and 6 (control)
Test performed in closed vessels	Yes (with a glass dish)

Table A7.4.1.3-10 Test conditions

Criteria	Details
Test temperature	23°C (± 2°C)
pH	7.9 (start) – 8.4 (end)
Aeration of dilution water	Not specified
Light intensity	4440 – 8880 Lux
Photoperiod	Continuous illumination

Table A7.4.1.3-11 Algal Cell Densities (Mean Values) During the test Period of 72 Hours

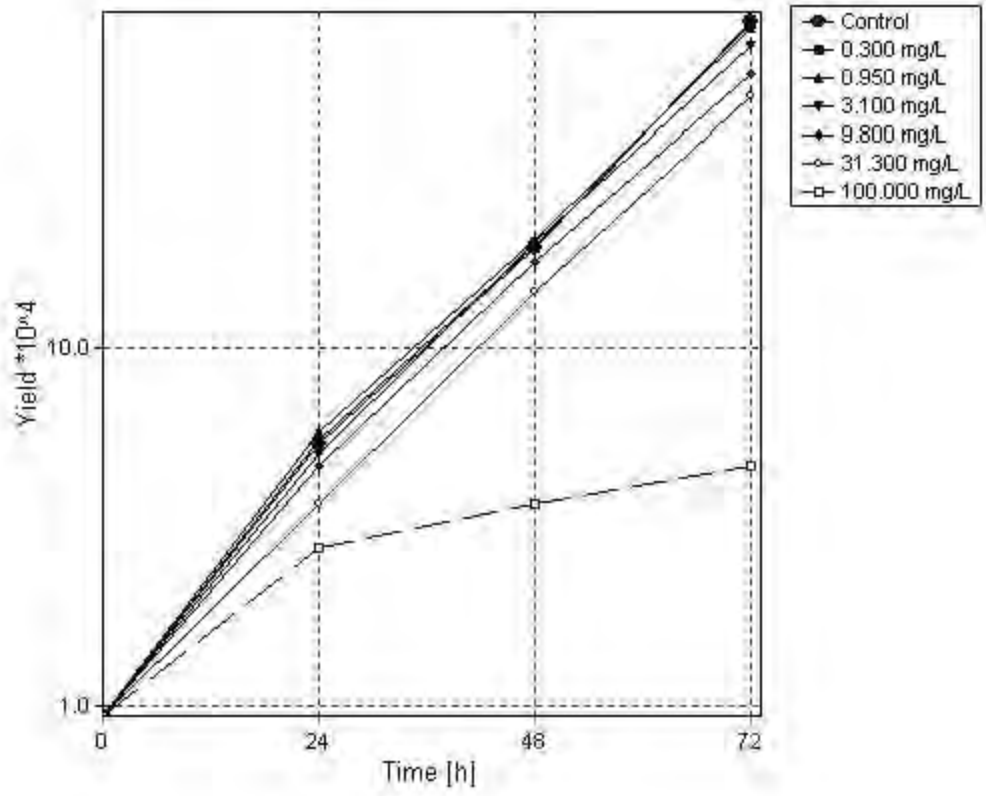
Test-Substance concentration (nominal) [mg NC 7312/L]	Density (x 10^4 cells/mL)	Growth Rate (day^{-1})	Inhibition Growth Rate (%)
Control	79.6	1.690	0.0
0.30	79.1	1.688	0.1
0.95	76.7	1.678	0.7
3.1	68.3	1.639	3.0*
9.8	56.9	1.577	6.7*
31.3	49.6	1.533	9.3*
100	4.7	0.740	56.2*
Temperature [°C]	23		
pH	7.9 – 8.4		
Oxygen [mg/l]	-		

*: mean value significantly different from the control (Bonferroni t-test ($\alpha = 0.05$, one side)

Table A7.4.1.3-12 Validity Criteria for Algal Growth Inhibition Test According to OECD Guideline 201

	Fulfilled	Not fulfilled
Cell concentration in control cultures increased at least by a factor of 16 within 3 days	X	
Concentration of test substance ≥ 80 % of initial concentration during test	X	

Figure A7.4.1.3-3 Growth Curves of *Desmodesmus subspicatus*, Incubated for 72 Hours at Different Concentrations of NC 7312



	EVALUATION BY COMPETENT AUTHORITIES
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	12th June 2007
Materials and methods	The Applicant's version is acceptable, noting the following: **3.2** The UK CA notes that the preparation of the test substance stock solution, described in Table A7.4.1.3-7, required ultrasonication and intense stirring to obtain a nominal concentration of 100 mg test substance litre^{-1} although its solubility in water was stated to be 20g l^{-1}. **3.4.1** The culture medium is stated to be 'Algal medium'. From the composition given in the study report, the medium appears to be equivalent to the OECD TG 201 medium given in OECD guideline 201, with the only difference being that it contains 0.08 mg l^{-1} $FeCl_3.6H_2O$, rather than 0.064 mg l^{-1} as stated in the OECD guideline. The UK CA accepts the use of this medium for this study. **3.4.2** There are a number of minor typographical errors in the source of the test organism given in Table A7.4.1.3-8 compared to the study report. According to the study report the source should be 'Sammlung von Algenkulturen Pflanzenphysiologisches Institut der Universität Göttingen – Germany' where the bold characters indicate those that were omitted in this study summary. **3.4.4.** The test conditions for the test temperature and light intensity that are given in Table A7.4.1.3-10 are not those actually observed. The test temperature observed was 23°C at 24, 48 and 72 hours, whilst the light intensity ranged from 6870-7440 lux with a mean of 7082 lux. **3.4.8** The analysis of the test substance was carried in duplicate for all test substance concentrations except 0.30 mg NC7312 l^{-1} at both 0 h and 72 h. For the control samples only one of the duplicate samples was analysed at each sampling time.
Results and discussion	The Applicant's version is acceptable. **4.2.1** The UK CA notes that the test substance concentrations chosen were based on a previous non-GLP range-finding test. However, in this definitive study these concentrations showed 72 h algal growth rate inhibitions varying from 0.1-56.2 % compared to the preferred range of 5-75 % given in the OECD guideline 201. The UK CA accepts that the concentration range was appropriate based on previous experience carried out and the test results are acceptable.
Conclusion	The Applicant's version is acceptable, noting the following: **5.2.3** The E_bC_{50} (72-h) has not been given in this study summary, although its value has been calculated in the study report to be 36.2 mg NC7312 l^{-1}.
Reliability	1
Acceptability	Acceptable
Remarks	

Section A7 Annex Point IIA 7.5	Ecotoxicological Profile Including Environmental Fate and Behaviour A7.4.1.3 Growth inhibition test on algae

	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

7.4.1.4 Inhibition to microbiological activity

		1. REFERENCE	Official use only
1.1	**Reference**	Bruns (2005) Bendiocarb – Toxicity to Bacteria [redacted] Document MO-05-010465 7.4.1.4/01 8 June 2005 Unpublished.	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Bayer CropScience AG	
1.2.2	Companies with letter of access	n.a.	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Directive 88/302 EEC.	X
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3. MATERIALS AND METHODS	
3.1	**Test material**	Bendiocarb	
3.1.1	Lot/Batch number	OP2455029	X
3.1.2	Specification	As given in Section 2.	
3.1.3	Purity	99.0 %	
3.1.4	Composition of product	Not applicable	
3.1.5	Further relevant properties	-	
3.1.6	Method of analysis	None	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Add to 130 ml deionised water and stirred overnight.	X
3.3	**Reference substance**	3,5-dichlorophenol (97 %)	
3.3.1	Method of analysis for reference substance	None	
3.4	**Testing procedure**		
3.4.1	Culture medium	Water	X
3.4.2	Inoculum / test organisms	Mixed populations of aquatic organisms (activated sludge) (see Table A7.4.1.4-1)	

3.4.3	Test system	See Table A7.4.1.4-2	
3.4.4	Test conditions	See Table A7.4.1.4-3	X
3.4.5	Duration of the test	3 hours	
3.4.6	Test parameter	Inhibition of respiration rate	
3.4.7	Analytical parameter	Based on nominal concentrations	
3.4.8	Sampling	None	
3.4.9	Monitoring of TS concentration	None	
3.4.10	Controls	-	
3.4.11	Statistics	Not reported	X
		4. RESULTS	
4.1	**Preliminary test**	Not reported	
4.2	**Results test substance**		
4.2.1	Initial concentrations of test substance	100, 320, 1024, 3277 and 10,000 mg a.s./L	
4.2.2	Actual concentrations of test substance	Not measured	
4.2.3	Concentration / response curve	See Figure A7.4.1.4-1	X
4.2.4	Effect data	See Table A7.4.1.4-4	
4.2.5	Other observed effects	-	
4.3	**Results of controls**	See Table A7.4.1.4-4	
4.4	**Test with reference substance**	See Table A7.4.1.4-5	
		5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	An activated sludge study was run according to Directive 88/302/EEC and under GLP conditions. Nominal concentrations of 100, 320, 1024, 3277 and 10,000 mg a.s./L were tested. The respiration rate of the activated sludge was measured after a contact time of 3 hours. The respiration rate of the same activated sludge in the presence of various concentrations of the test item, under otherwise identical conditions, was also measured. The inhibitory effect of the test item at a particular concentration was expressed as a percentage of the mean of the respiration rates of two controls. An EC_{50} value was calculated from the respiration rates at different test item concentrations.	

5.2	**Results and discussion**	Bendiocarb showed 57.1 % respiration inhibition of activated sludge at a test item concentration of 10,000 mg a.s./L. The EC_{50} is 845 mg a.s./L.	X
5.3	**Conclusion**		X
5.3.1	Reliability	1	X
5.3.2	Deficiencies	No	

Table A7.4.1.4-1 Inoculum / Test organisms

Criteria	Details
Nature	Activated sludge
Species	Mixed population of aquatic organisms
Strain	-
Source	Domestic sewage treatment plant
Sampling site	Aeration tank
Laboratory culture	-
Method of cultivation	-
Preparation of inoculum for exposure	In accordance with test method
Pretreatment	Aeration of the activated sludge; daily feed with synthetic medium
Feeding of animals during test	Yes

Table A7.4.1.4-2 Test system

Criteria	Details
Culturing apparatus	Incubators
Number of culture flasks/concentration	
Aeration device	Stirring
Measuring equipment	Oxygen, pH, temperature
Test performed in closed vessels due to significant volatility of TS	Not reported

Table A7.4.1.4-3 Test conditions

Criteria	Details
Test temperature	19.3 – 20.2°C
pH	8.1
Aeration of dilution water	16 hours before incubation
Suspended solids concentration	480 mg/L

Table A7.4.1.4-4 Results – Bendiocarb and Controls

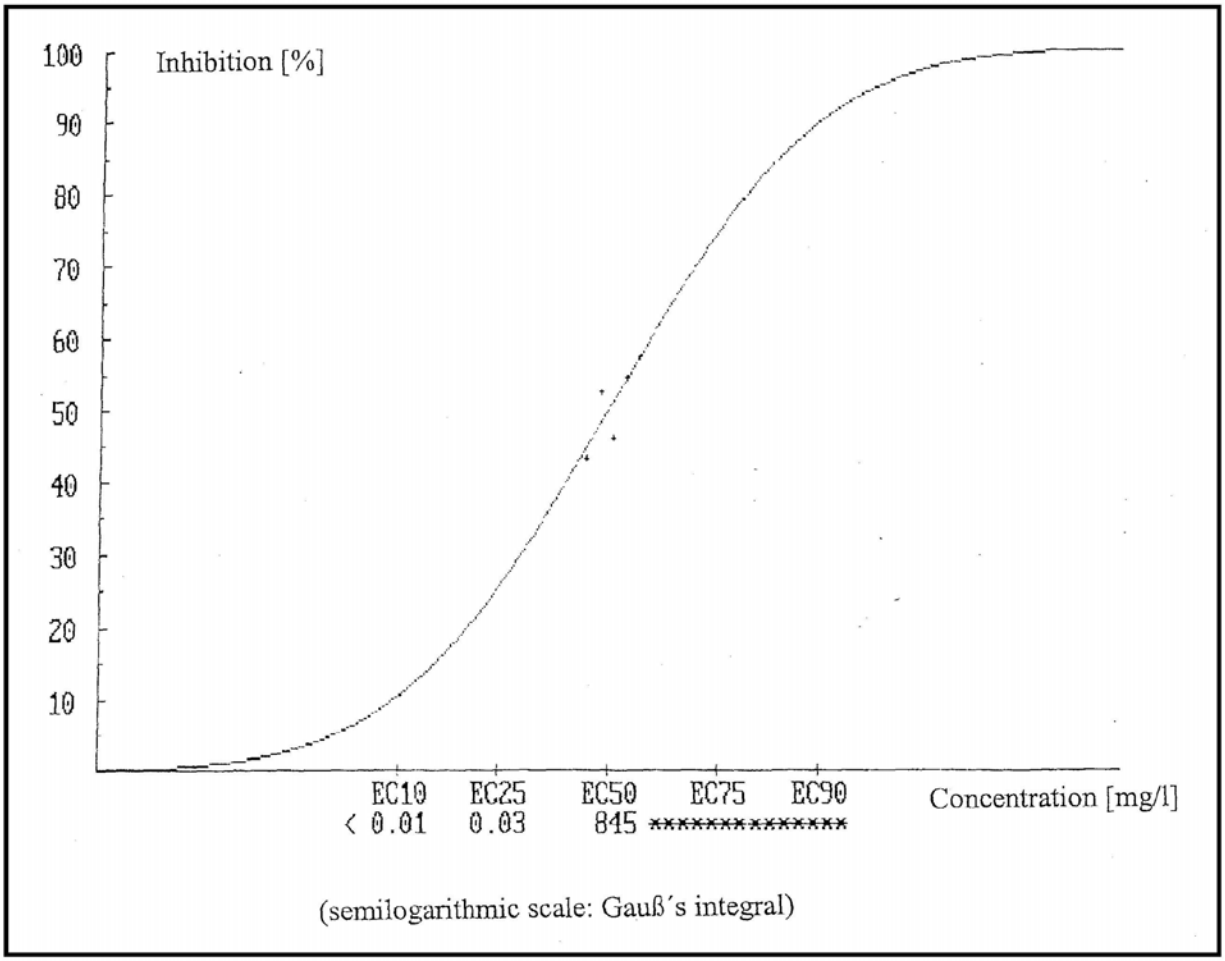
Test concentration (mg a.s./L)	Respiratory rate test item (mg/L h)	Phys.-chem. O_2 consumption (mg/L h)	Respiratory `rate - phys.-chem. O_2 consumption (mg/L h)	Inhibition (%)
100	24.0	*0.0	24.0	42.9
320	20.0	*0.0	20.0	52.4
1024	22.8	*0.0	22.8	45.7
3277	19.2	*0.0	19.2	54.3
10000	18.0	0.0	18.0	57.1
Control, mean	42.0			
Control 1	42.0			
Control 2	42.0			

* The physico-chemical oxygen consumption has been determined at 10,000 mg a.s./L test substance concentration. As no physico-chemical oxygen consumption was observed at that test item concentration this observation also holds true for the lower test item concentrations.

Table A7.4.1.4-5 Results – Test with Reference Substance (3.5-Dichlorophenol)

Test concentration (mg/L)	Respiratory rate (mg/L h)	Inhibition (%)
2.5	38.0	9.5
5	36.0	14.3
10	20.0	52.4
20	6.0	85.7
40	3.6	91.4

Figure 7.4.1.4-1 Concentration Effect Relation (Semi-Logarithmic Scale)



EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	4/03/2008
	2.1 The Applicant has noted in the study report that this study was carried out according to Directive 88/302/EEC and is in most parts identical with OECD guideline 209.
Materials and methods	The Applicant's version is acceptable, noting the following: **3.1.1** The batch number given in the study report is OP24550029 and not OP2455029 as indicated in this study summary. **3.2** The test substance was prepared by adding it to deionised water and then stirring the mixture overnight. No information was initially provided on the resulting concentration of test substance after the stirring (i.e. at the beginning of the 3 h test) and bendiocarb is known to rapidly hydrolyse (half-life of 48.1 h at pH 7 and 43.8 minutes at pH 9). Hence, the UK CA asked the Applicant to recalculate the actual test substance concentrations used in the test, based on the initial concentrations prior to stirring and hydrolytic degradation. The Applicant's recalculation has been included below the UK CA comments on this study. **3.4.1** The Applicant has provided additional infomrtaion regarding the culture medium. These solutions were freshly prepared at the start of the study using a stock solution at a concentration of 0.5 g l^{-1}. A synthetic sewage feed was prepared by dissolving the following amounts of substances in one litre of water: 16 g peptone, 11 g meat extract, 3 g urea, 0.7 g NaCl, 0.4 g $CaCl_2.2H_2O$, 0.2 g $MgSO_4.7H_2O$ and 2.8 g K_2PO_4. **3.4.4** Table A7.4.1.4-3 gives the test conditions for the test substance concentrations and duplicate controls. The UK CA notes that the physico-chemical oxygen consumption control falls outside these values, having a pH of 7.2 and a temperature of 20.9 °C during the exposure phase of this study. This is acceptable since these vessels contain no activated sludge. **3.4.11** No statistical method has been reported for the EC_{50} determination for the test substance, although probit analysis was used to determine the EC_{50} of the reference substance (3,5-dichlorophenol).
Results and discussion	The Applicant's version is considered acceptable, when data are based on the recalculated test substance concentrations which allow for the hydrolytic degradation of bendiocarb during the pre-test stirring overnight. **4.2.3** The concentration response curve plotted (Figure 7.4.1.4-1) is based on five % inhibition values ranging over a very small range i.e. from 42.9 % to 57.1 %. In addition, no details have been given of the statistical method used to determine the EC_{50} and no 95 % confidence intervals have been generated. The UK CA considers this reduces the accuracy of the result obtained, but does not consider it invalidates the study.
Conclusion	The Applicant's version is acceptable, when data are based on the recalculated

	test substance concentrations given below this robust study summary. **5.2** The conclusion of a 3 h EC_{50} of 845 mg a.s. l^{-1} was considered, by the UK CA to be inaccurate as the test substance, which is known to rapidly hydrolyse, appears to have been stirred in water overnight. In addition, no analysis was done to determine the actual concentrations present in the test flasks. However, upon request the Applicant was able to provide recalculated test substance concentrations which allow for the hydrolytic degradation of bendiocarb during the pre-test stirring overnight. Taking a worst-case of pH 8.1 the test substance concentration remaining after 16 h of stirring (i.e. at the start of the 3 h test) was 8.61 % of the original. This correction was applied to the original EC_{50} value to determine a revised 3 h EC_{50} of 70.135 mg a.s. l^{-1}. **5.3** The validity criteria have not been mentioned here in this study summary, although it is mentioned within the study report that the validity criteria given in OECD 209 guideline i.e. - the respiration rates of the two controls are within 15 % of one another - the 3 h EC_{50} for 3,5-dichlorophenol is within the range 5 - 30 mg l^{-1} (value determined to be 9.8 mg l^{-1}) have been met. **5.3.1** The reliability has been reduced from 1 to 2 as the concentration range tested was insufficient to provide a wide-ranging biological response, and the actual concentrations used were estimated from hydrolytic degradation data because measured concentrations were not available.
Reliability	2
Acceptability	Acceptable
Remarks	All endpoints and data presented in the summary have been checked against the original study and are correct.
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Recalculation of Test Substance Concentration in 'Bendiocarb – Toxicity to Bacteria Study (provided by Applicant on 01/02/2007)

The Applicant confirmed that in this study (MO-05-010465), as indicated in page 11 of the study report, the test item was stirred for 16 hours before start of incubation time. In this study, the highest concentration tested was 10 g bendiocarb/L. As indicated below, the solubility of bendiocarb at 20°C is according to Stalker and Ward (A90138, 1992) as follows:

pH 3-5: 0.31 g/L
pH 7: 0.28 g/L

pH 9-11: 0.03 g/L

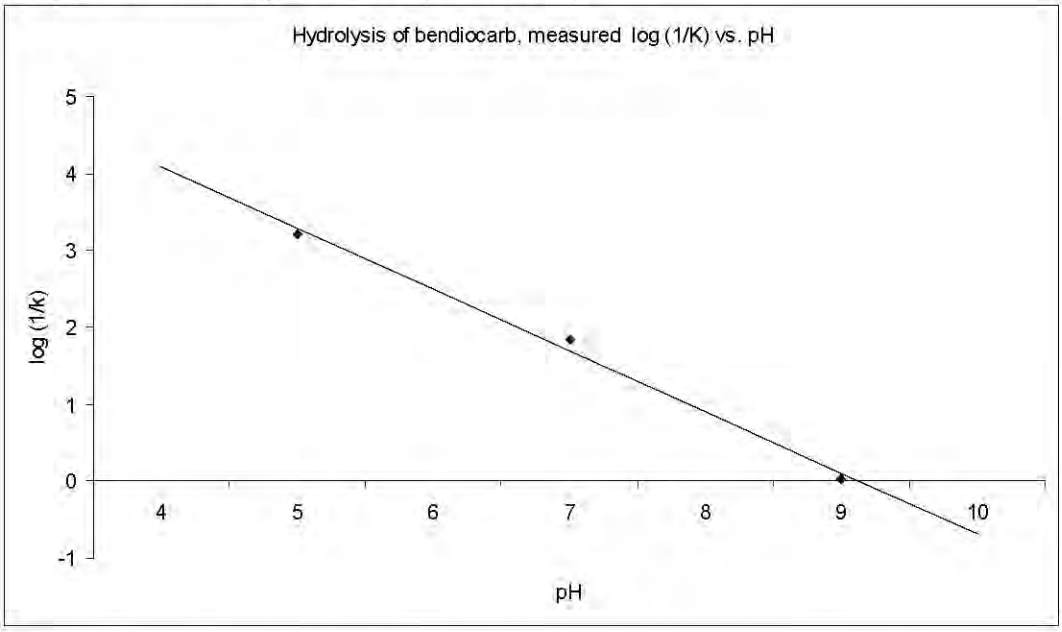
Therefore, the highest concentration tested far exceeds the solubility of bendiocarb in water, presenting a risk of non homogeneity. Consequently, for comparability purposes, all tested concentrations (in the range of 0.1 to 10 g/L) were treated in the same way and stirred for a certain period of time before the start of the incubation time; in this case 16 hours. This procedure is laid down in the Standard Operating Procedure of the study to avoid effect due to non homogeneous distribution in the activated sludge. This can be provided on request.

Considering the rate of hydrolysis of bendiocarb (study A90220), the concentration of bendiocarb likely to have been present at the start of the 3-hour test (after 16-hours stirring) has been calculated.

The table below presents the hydrolysis rate constants calculated ($K = \ln 2 / DT_{50}$) for bendiocarb based on the DT_{50} determined in the hydrolysis study (A90220) for each pH:

pH	5	7	9
DT_{50}	46.5 days	48.1 hours	43.8 minutes
DT_{50} (h)	1116.0	48.1	0.73
k (h^{-1})	0.00062	0.01441	0.94952
1/k (h)	1610.05	69.39	1.05
log (1/k)	3.2068	1.8413	0.0225

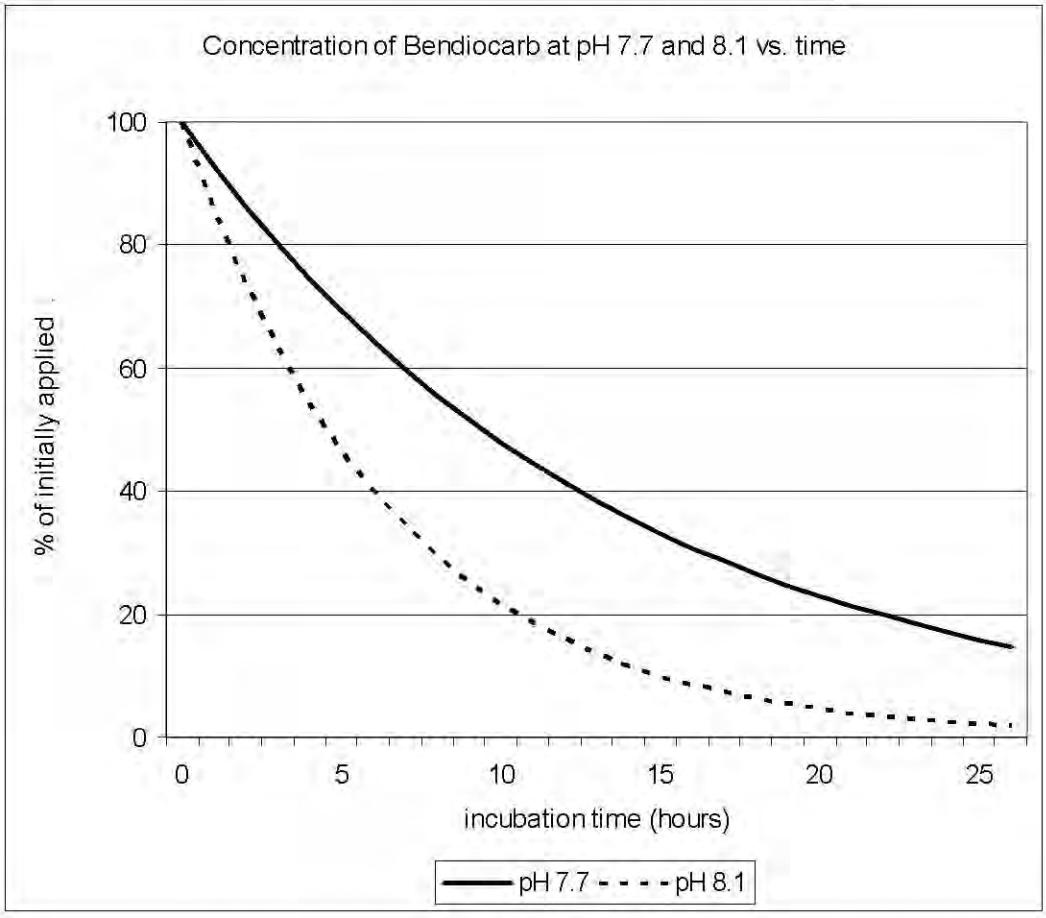
The linear regression of log (1/K) versus pH is presented in the graph below:
log (1/k) = -0.7995021 x pH + 7.27846 (r^2 = -0.99664)



Using this equation, the hydrolysis rate constant of bendiocarb corresponding to the initial and final pH (7.7 and 8.1, respectively) measured in the study on toxicity of bendiocarb to bacteria (MO-05-010465) was extrapolated. Based on the extrapolated rate constant, the concentration of bendiocarb remaining in the stirred solution at time (t) was calculated using the following equation: C(t) = C(0) x EXP(-k x t). The results are presented (as % of initially applied) in the following table and in the graph below:

	pH 7.7	pH 8.1
Time (h)	C(t) = C0*exp-kt	C(t) = C0*exp-kt

0	100	100
1	92,90	85,79
2	86,31	73,60
3	80,18	63,14
4	74,49	54,17
5	69,20	46,47
6	64,29	39,87
7	59,73	34,20
8	55,49	29,34
9	51,55	25,17
10	47,89	21,59
11	44,49	18,53
12	41,33	15,89
13	38,40	13,63
14	35,67	11,70
15	33,14	10,03
16	**30,79**	**8,61**
17	28,60	7,39
18	26,57	6,34
19	24.69	5.44



Considering the worst case situation of pH 8.1, where hydrolysis of bendiocarb should be the most important, the concentration of bendiocarb likely to have been present at the start of the 3-hour test (after 16 hours of stirring)

represents 8.3 % of the initially applied concentration. Therefore, the re-calculated EC_{50} corresponds to 70.135 mg bendiocarb/L (845 mg bendiocarb/L x 8.3 %).

7.4.2 Bioconcentration

			Official use only
		1. REFERENCE	
1.1	**Reference**	[redacted] (1988) Determination of the Accumulation and Elimination of [^{14}C]-Bendiocarb in Bluegill Sunfish (*Lepomis macrochirus*) [redacted] Document A90219 7.4.2/01 31 October 1988 Unpublished	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Bayer CropScience AG	
1.2.2	Companies with letter of access	n.a.	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	US EPA Guideline 72-6	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3. MATERIALS AND METHODS	
3.1	**Test material**	^{14}C-ring labelled bendiocarb and unlabelled bendiocarb	
3.1.1	Lot/Batch number	Unlabelled bendiocarb: CR 20859/2 Radiolabelled bendiocarb: CFQ4944	
3.1.2	Specification	As given in Section 2 for unlabelled bendiocarb	
3.1.3	Purity	Unlabelled bendiocarb: 97.2 % Radiolabelled bendiocarb: 98.5 % (w/w) by HPLC	
3.1.4	Further relevant properties	-	X
3.1.5	Radiolabelling	[phenyl-U-^{14}-C]-bendiocarb : 20.65 mg. specific activity : 107 uCi/mg	
3.1.6	Method of analysis	HPLC	
3.2	**Reference substance**	No	
3.2.1	Method of analysis for reference substance	n.a.	

3.3	**Testing/estimation procedure**		
3.3.1	Test system / performance	Bluegill sunfish were exposed to [^{14}C]-labelled bendiocarb for 10 days using a dynamic test system. The study was undertaken in three separate phases: (a) Stabilisation of the test system (b) Exposure of the fish for a period of 10 days (c) A depuration period when the fish were transferred and maintained in clean flowing water for a period of 3 days. Radiochemical analysis of the water was carried out at predetermined intervals during the exposure period and the water was also analysed for the test compound, using a high performance liquid chromatographic (HPLC) technique. During the test, fish in the exposure vessels and the control vessels were sacrificed and the total [^{14}C] residues were analysed in viscera (alimentary tract excluding heart), edible (flesh) and non-edible (carcass) fractions, at intervals during the exposure and depuration periods. Control fish were maintained in an identical test system to the exposed fish. Clean, temperature-controlled freshwater was passed through a similar peristaltic pump system of the same type used to deliver the test substance to the exposure fish tank.	
3.3.2	Estimation of bioconcentration	See 4.2	
		4. RESULTS	
4.1	**Experimental data**		
4.1.1	Mortality/ behaviour	Based on the initial 90 fish exposed, total mortalities in both the nominal 0.05 mg/1 [^{14}C]-bendiocarb concentration and the freshwater and solvent controls were less than 3 % (mean value) during the exposure period. No adverse effects were observed in the fish during the 14 day exposure to [^{14}C]-bendiocarb.	
4.1.2	Lipid content	No data	
4.1.3	Concentrations of test material during test	During the exposure period, the mean measured concentration of bendiocarb in the test vessel, as determined by liquid scintillation counting, was 0.0516 + 0.0123 mg/1. The mean measured concentration from HPLC analysis was 0.0402 + 0.011 mg/1 which indicated that little, if any, hydrolysis had occurred	
4.1.4	Bioconcentration factor (BCF)	Mean bioconcentration factors (BCF) for the edible, non-edible and viscera portions of the bluegills were 1.2x, 2.3x and 55.8x respectively. The overall (whole body) BCF was 6.0x.	
4.1.5	Uptake and depuration rate constants	During the 3 day depuration period the levels of radioactivity in the whole fish decreased rapidly with approximately 88 % elimination within one day and at day three, 97 % of the [^{14}C]-bendiocarb had been eliminated.	
4.1.6	Depuration time	3 days	
4.1.7	Metabolites	Because of the negligible bioconcentration in the edible tissue in particular, no metabolite identification was possible	

4.1.8	Other observations	-	
4.2	**Estimation of bioconcentration**	The above values are in good agreement with the predicted times to steady state (9 hrs) and BCF values 3.3x, based on a log P value of 1.44 and water solubility (0.28 g/l at pH 7) .	X
		5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Bluegill sunfish (*Lepomis macrochirus*) were exposed in a dynamic test system to a nominal concentration of 0.05 mg/1 of [^{14}C]-bendiocarb for a period of 10 days followed by a 3 day period of depuration in freshwater. During the exposure period, the mean measured concentration of bendiocarb in the test vessel, as determined by liquid scintillation counting, was 0.0516 + 0.0123 mg/1. The mean measured concentration from HPLC analysis was 0.0402 + 0.011 mg/1 which indicated that little, if any, hydrolysis had occurred. Amounts of radioactivity in the 1 hr, 6 hr and 1, 2, 3, 4, 7 and 10 days fish tissues (edible, non-edible and viscera) were determined during the exposure period by analysis of 5 fish at each time point and at 1 hr, 6 hr, 12 hr-and 1 and 3 days during the depuration period. Levels of radioactivity had reached a plateau after 6 hours and exposure was terminated after 10 days.	
5.2	**Results and discussion**	Mean bioconcentration factors (BCF) for the edible, non-edible and viscera portions of the bluegills were 1.2x, 2.3x and 55.8x respectively. The overall (whole body) BCF was 6.0x. Rapid elimination of [^{14}C]-bendiocarb residues occurred during depuration. Following the transfer of fish from the exposure vessel to freshwater, more than 89 % of the radioactivity was eliminated from the whole fish in one day and >97 % in three days. Because of the negligible bioconcentration in the edible tissue in particular, no metabolite identification was possible.	
5.3	**Conclusion**		
5.3.1	Reliability	1	
5.3.2	Deficiencies	No	

	EVALUATION BY COMPETENT AUTHORITIES
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	03/01/07
Materials and methods	Applicant's version is acceptable withe following comments: **3.1.4** Other physico-chemical data such as water solubility (0.28 g/l at pH 7) should be supplied, or reference made as where information can be found in this document.
Conclusion	Applicant's version is acceptable with the following comment: **4.2** Reported Log Pow value is 1.7 not 1.44. This means that the calculated value of BCF_{FISH} is 5.56 rather than 3.3. This gives very close agreement to the study's findings.
Reliability	1
Acceptability	Acceptable
Remarks	All endpoints transcribed from study correctly, apart from those mentioned in comment 4.2.
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

7.4.3 Effects on aquatic organisms, further studies

7.4.3.1 Prolonged toxicity to an appropriate species of fish

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ] Scientifically unjustified [ ]**	
Limited exposure [ ]	**Other justification [✓]**	
Detailed justification:	Based on the Technical Guidance Document, this study is not required as it does not add information as needed in the risk assessment.	X
Undertaking of intended data submission [ ]		

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	29/11/2006
Comments	The UK CA notes that the TGD lists a prolonged fish study as an additional study that is not usually required, as it does not add information as needed in the risk assessment, and that existing test guidelines are not sufficient. The UK CA agrees with the Applicant that for bendiocarb a prolonged fish study would not provide any additional information required for use in the aquatic risk assessment. This is because an acceptable chronic fish study (toxicity of bendiocarb to rainbow trout (*Salmo gairdneri*) embryos and larvae over 78 days) is available.
Conclusion	The UK CA agrees that no prolonged fish study is required.
Acceptability	Acceptable
Remarks	
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

7.4.3.2 Effects on reproduction and growth rate on an appropriate species of fish

			Official use only
		1. REFERENCE	
1.1	**Reference**	[redacted] (1989b) The Toxicity of Bendiocarb Technical to Rainbow Trout (*Salmo gairdneri*) Embryos and Larvae [redacted] Document A90214 7.4.3.2/01 16 May 1989 Unpublished	X
1.2	**Data protection**	Yes	
1.2.1	Data owner	Bayer CropScience AG	
1.2.2	Companies with letter of access	n.a.	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Springborn Life Sciences in-house protocol	X
2.2	**GLP**	Yes	
2.3	**Deviations**	No	X
		3. MATERIALS AND METHODS	
3.1	**Test material**	Bendiocarb	
3.1.1	Lot/Batch number	Lot#M36J	
3.1.2	Specification	As given in Section 2	
3.1.3	Purity	100 %	
3.1.4	Composition of product	n.a.	
3.1.5	Further relevant properties	-	
3.1.6	Method of analysis	Gas chromatography with electron capture detection	X
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	n.a.	
3.3	**Reference substance**	No	
3.3.1	Method of analysis for reference substance	n.a.	
3.4	**Testing procedure**		
3.4.1	Dilution water	See Table A7.4.3.2-1	

3.4.2	Test organisms	See Table A7.4.3.2-2	X
3.4.3	Handling of embryos and larvae (OECD 210/212)	The eggs and sperm were received at approximately 4°C. To fertilize the eggs one half of the sperm was placed in a stainless steel bowl, the eggs were added, and the bowl was swirled to mix the contents thoroughly. The remaining sperm was added to the bowl and again mixed by swirling. A small amount of control water (approximately 4°C) was then added to the sperm-egg mixture in the bowl, and the bowl was swirled again to ensure even distribution of the sperm over the eggs. The bowl containing the sperm and eggs was left undisturbed for approximately two minutes (long enough to complete the fertilization process) and the excess sperm rinsed from the eggs with control water. The usual 45-minute water hardening period was extended over the next 2 hours as the temperature of the embryos was raised to 7°C. Upon completion of the water hardening, fifty embryos were impartially distributed to each of 28 embryo incubation cups, two of which were then suspended in each duplicate test aquarium per exposure concentration and control. During the period of egg distribution, the temperature of the eggs was gradually raised to 10°C which was the temperature of the exposure solutions. During the initial 24 hours of the study the temperature of the exposure solutions was raised to 12°C. Dead embryos were counted daily until hatching was complete. During the early stages of development rainbow trout eggs are particularly susceptible to physical disturbances, therefore dead embryos were not removed until test day 19. Hatching was deemed complete on test day 29 when no more than 5 unhatched viable embryos remained in any embryo incubation cup. To initiate the larval exposure, the surviving larvae from each of the two embryo incubation cups within each aquarium were combined and 40 larvae were impartially selected and placed into their respective aquaria. When required, larvae from replicate A and B aquaria were combined to provide 40 organisms per aquarium. Larvae were fed live brine shrimp *(Artemia salina)* nauplii three times daily on weekdays and twice daily on weekends and holidays when the larvae reached swim-up stage and began actively feeding (approximately 17 days post-hatch). Aquaria were brushed and siphoned when necessary to remove excess food and faecal matter.	
3.4.4	Test system	See Table A7.4.3.2-3	
3.4.5	Test conditions	See Table A7.4.3.2-4	X
3.4.6	Duration of the test	78 days (30 days post swim up exposure)	
3.4.7	Test parameter	Survival of embryos at hatch and survival and growth of larvae after 30 days post swim up exposure.	

3.4.8	Sampling	The controls and all treatment levels (A and B replicates) were sampled on test day 20, and weekly thereafter (including the day hatching was determined complete) until test termination. The results of these analyses were used to establish that the nominal concentrations were representative of the actual concentration of bendiocarb present in the exposure aquaria. Each test solution sample was collected from the midpoint of the aquarium with a volumetric pipette. All samples (100 ml volume) were extracted at SLS and shipped frozen to the company for analyses.	X
3.4.9	Monitoring of TS concentration	See 3.4.8	
3.4.10	Statistics	One-way, single classification analyses of variance (ANOVAs) were conducted on each measured or calculated toxic endpoint; Dunnet's procedure	
		4. RESULTS	
4.1	**Range finding test**	Not performed, but test concentrations were selected based on the results of preliminary static and flow through acute exposures of juvenile rainbow trout to bendiocarb which were previously conducted.	
4.1.1	Concentration	n.a.	
4.1.2	Number/ percentage of animals showing adverse effects	n.a.	
4.1.3	Nature of adverse effects	n.a.	
4.2	**Results test substance**		
4.2.1	Initial concentrations of test substance	0, 0.047, 0.094, 0.19, 0.38 and 0.75 mg/L.	X
4.2.2	Actual concentrations of test substance	The highest dose rates were measured at 0.17 and 0.50 mg/L	X
4.2.3	Effect data	The most sensitive indicator of the toxicity of bendiocarb to the rainbow trout was larval growth expressed as length. At test termination, the mean total length of larvae exposed to the two highest mean measured concentrations of bendiocarb tested (0.50 and 0.17 mg/L) were significantly reduced as compared to the length of the control larvae. The mean total length of larvae in the 0.50 and 0.17 mg/L concentrations were 31 and 33 mm, respectively, as compared to 34 mm for the control larvae.	
4.2.4	Concentration / response curve	See Figure 2, page 29 in the original report	
4.2.5	Other effects	-	X

4.3	**Results of controls**		
4.3.1	Number/percentage of animals showing adverse effects	Survival at hatch and larvae survival were 97.5 % and 99 % respectively for water control and 92.5 % and 98 % for solvent control. No adverse effects were noted.	
4.3.2	Nature of adverse effects	n.a.	
4.4	**Test with reference substance**	Not performed	
4.4.1	Concentrations	n.a.	
4.4.2	Results	n.a.	
		5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Rainbow trout *(Salmo gairdneri)* embryos and larvae were continuously exposed for 78 days (30 post swim up exposure) to nominal bendiocarb concentrations ranging from 0.047 to 0.75 mg/L Observations were made on survival of embryos at hatch and on survival and growth of larvae after 30 days post swim up exposure.	
5.2	**Results and discussion**	The most sensitive indicator of the toxicity of bendiocarb to the rainbow trout was larval growth expressed as length. The mean percentage survival of organisms at hatch was unaffected by all concentrations of bendiocarb tested. Survival of rainbow trout larvae in the highest mean measured test concentration (0.50 mg/L) at test termination was 78 % which was significantly lower than the survival (98 %) of control (pooled dilution water and solvent control) organisms. At test termination, the mean total length of larvae exposed to the two highest mean measured concentrations of bendiocarb tested (0.50 and 0.17 mg/L) were significantly reduced as compared to the length of the control larvae. The mean total length of larvae in the 0.50 and 0.17 mg/L concentrations were 31 and 33 mm, respectively, as compared to 34 mm for the control larvae. The mean wet weight of larvae exposed to all treatment levels of bendiocarb tested was unaffected during the 78 day exposure. Effects of bendiocarb exposure were not observed at the three lowest concentration tested. Based on these data, the maximum acceptable toxicant concentration (MATC) of bendiocarb for rainbow trout embryos and larvae was extrapolated to be >0.07 mg/L and <0.17 mg/L (geometric mean MATC = 0.11 mg/L).	X
5.2.1	NOEC	0.07 mg/L	
5.2.2	LOEC	-	X
5.3	**Conclusion**		X
5.3.1	Reliability	2	
5.3.2	Deficiencies	No	

Table A7.4.3.2-1 Dilution water

Criteria	Details
Source	Well water which was pumped into an epoxy-coated concrete reservoir where it was supplemented with Town of Wareham untreated well water and aerated.
Alkalinity	19-24 mg/L, as $CaCO_3$
Hardness	19-30 mg/L as $CaCO_3$ (mean 28)
pH	6.8-7.5
Oxygen content	9.7 – 10.3 mg/L
Conductance	80 – 120 µmhos/cm
Holding water different from dilution water	No

Table A7.4.3.2-2 Test organisms

Criteria	Details
Species/strain	Unfertilized rainbow trout eggs and sperm were individually packaged and shipped under refrigeration from Mount Lassen Trout Farm, Red Bluffs, California.
Source	See above
Wild caught	No
Age/size	-
Kind of food	Larvae were fed live brine shrimp *(Artemia salina)* nauplii three times daily on weekdays and twice daily on weekends and holidays when the larvae reached swim-up stage and began actively feeding (approximately 17 days post-hatch).
Amount of food	See above
Feeding frequency	See above
Post-hatch transfer time	-
Time to first feeding	17 days
Feeding of animals during test	Yes
Treatment for disease within two weeks preceding test	No

Table A7.4.3.2-3 Test system

Criteria	Details
Test type	Flow-through
Renewal of test solution	8.7 volume replacements every 24 h
Volume of test vessels	39 x 20 x 25 cm with a constant test water volume of 11 L
Volume/animal	ca. 0.275 L
Number of animals/vessel	40
Number of vessels/ concentration	2
Test performed in closed vessels due to significant volatility of TS	No

Table A7.4.3.2-4 Test conditions

Criteria	Details
Test temperature	12 ± 1 °C
Dissolved oxygen	Not reported
pH	6.8 – 7.4
Adjustment of pH	No
Aeration of dilution water	Yes
Intensity of irradiation	20 – 100 footcandles
Photoperiod	16 h light and 8 h darkness

Table A7.4.3.2-5 Validity Criteria for Fish Tests According to OECD Guidelines 210/212

	Fulfilled	Not fulfilled
Concentration of dissolved oxygen >60 % saturation throughout the test	Not known	
Difference of water temperature <1.5 % between test chambers or successive days at any time during test; temperature within range for specific test species	X	
Overall survival of fertilised eggs in controls (and solvent controls) ≥ value, specified for the specific test species	X	
Test substance concentrations maintained within ± 20 % of mean measured values	X	
No effect on survival nor any other adverse effect found in solvent control	X	
Further criteria for poorly soluble test substances	**n.a.**	

Section A7 Annex Point IIIA XIII.2.2	**Ecotoxicological Profile Including Environmental Fate and Behaviour** A7.4.3.2 Effects on reproduction and growth rate on an appropriate species of fish

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	20/03/2007
	1.1 The reference given includes the phrase 'Bendiocarb Technical' whilst the study report title omits the word 'Technical'. The UK CA considers the study report to be more correct, as the bendiocarb used in this study has been reported as 100 % pure. **2.1** The study guideline is described as 'Springborn Life Sciences (SLS) in-house protocol' in this study summary, whilst the study report indicates that the guideline reference is 72-4, which the UK CA believes refers to US EPA FIFRA method 72-4. The applicant has also addressed the validity of this study relative to OECD guideline 210. The UK CA considers that the OECD guideline 210 is the preferred guideline for this type of study and has compared the information submitted against this standard. **2.3** The UK CA has noted the following differences when assessing this 1985 study (final report in 1989) against the current OECD guideline 210 (1992): - some information e.g. brood temperature, biomass loading rate, characteristics of the well water has not been recorded. However, the UK CA does not consider this of concern as control samples show appropriate development of the rainbow trout embryos and larvae - some of the methodology differs e.g. dead embryos were not removed until day 19 to minimise disturbance to the development of the rainbow trout eggs, and analysis was not done on all samples, just the concentrations affected. However, the UK CA considers the reasons for these differences acceptable.
Materials and methods	The Applicant's version is acceptable, noting the following: **3.1.6** The method of analysis is inadequately described. 100 ml samples were taken from the test aquaria and extracted with methylene chloride. The organic extracts were taken to near dryness and then made up in hexane and stored refrigerated, before being frozen and being transported to the analytical laboratory. The hexane extracts were applied to silica columns and eluted with 1+9 v/v ethyl acetate+ hexane (for NC 7312) and 1+3 v/v ethyl acetate+hexane (for bendiocarb). The separate fractions were derivatised with 1-fluoro-2,4-dinitrobenzene before analysis by GC-ECD. **3.4.2** Table A7.4.3.2-2 indicates that the time to first feeding is 17 days, whilst the study report indicates that it is 17 days post-hatch. As hatching was deemed completed on day 29, the UK CA has calculated that first feeding started around day 46 of the study. **3.4.5** Table A7.4.3.2-4 states the test temperature to be 12 ± 1 °C, and the the mean measured values range from 11 - 12 °C. However, there appears to have been some variability in the temperature during this study, as it has been noted in the study report that the temperature reached 14 °C on day 30 for that day. The UK CA does not consider this would significantly affect the results of this study as rainbow trout can be kept within the temperature range of 12 ± 2 °C

	according to OECD guideline 211. This table also states the pH range was 6.8 - 7.4, but in the study report the pH range was given as 6.8 - 7.5. In addition, a light intensity of 20 - 100 footcandles is given, which is correct but illumination was only provided after the larvae had completed development to the swim up stage. **3.4.8** Analyses were limited to those for samples taken from the two highest nominal concentrations of 0.38 mg l^{-1} and 0.75 mg l^{-1} from day 20 onwards. The analyses showed that the mean measured concentrations of bendiocarb were 48 % and 67 % of the nominal concentrations for the 0.38 mg l^{-1} and 0.75 mg l^{-1} samples respectively. A correction factor was determined from the difference observed between measured and nominal concentrations and this was applied to the lower test concentrations. The UK CA considers that the corrected values for the lower concentrations are an appropriate basis upon which to determine a NOEC / LOEC value. The analyses also showed that the amount of bendiocarb occurring as NC 7312 in the two highest nominal concentrations did not exceed 0.04 mg l^{-1}.
Results and discussion	The Applicant's version is acceptable, noting the following: **4.2.1** The concentrations given are nominal concentrations. **4.2.2** When corrected, according to the analytical data obtained, the mean measured test concentrations of bendiocarb obtained for the nominal values of 0.19 mg l^{-1}, 0.38 mg l^{-1} and 0.75 mg l^{-1} were 0.07 mg l^{-1}, 0.17 mg l^{-1} and 0.50 mg l^{-1} respectively. The UK CA notes that analysis was also carried out for NC 7312 in the samples taken, and that the levels present were very low (0.04 mg l^{-1} or lower). **4.2.5** In addition to larvae mean body length, additional endpoints were presented by the Applicant in the study summary – organism survival at hatching, larvae survival and mean wet weight of organisms at the end of the study. The UK CA agrees with the Applicant that these additional endpoints are less sensitive endpoints than the larvae mean body length that has been used to determine the NOEC/LOEC for this study.
Conclusion	The Applicant's version is acceptable, noting the following: **5.2** The UK CA agrees that rainbow trout larvae growth, expressed as length, is the most sensitive endpoint with significant effects being seen at the highest two test concentrations (nominal values of 0.38 and 0.75 mg l^{-1}). Some minor discrepancies on other endpoints were also noted, but these do not affect the NOEC of the study: i) the mean percentage survival of embryos at hatch was stated to be unaffected by all concentrations of bendiocarb tested, but the duplicate measurements of 17 % and 91 % (mean 54 %) for the highest test in Table 2 of the study report seem to suggest a significant difference compared to the average values for the solvent and water controls of 92.5 % and 97.5 % respectively. ii) the larvae survival was stated to be significantly affected at the highest test concentration only (Table 2 of the study report), although data later in the study report (page 200) suggest a significant effect is also seen at the second highest test concentration, making this endpoint as sensitive as (but not more sensitive

	than) the length of larvae. **5.2.2** The UK CA has noted that a LOEC can be determined of 0.17 mg l^{-1} based on mean measured concentrations. **5.3** The validity criteria for the study are not mentioned in the text of the study summary, although Table A7.4.3.4-5 has been included, where comparison with the criteria of OECD guideline 210 are given The UK CA notes that: - the criteria of dissolve oxygen > 60 % saturation has been met as the DO measurements range from 9.7 – 10.3 mg l^{-1}, - the difference in water temperature of < 1.5 % is unlikely to have been met when the temperature went up to 14 °C (on one day), but the UK CA does not consider this an issue, - the test substance concentration was not maintained at ± 20 % of the nominal values, but as measured values were used to define the endpoints this is not of concern to the UK CA.
Reliability	2
Acceptability	Acceptable
Remarks	All endpoints and data presented in the summary have been checked against the original study and are correct.
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

7.4.3.3 Bioaccumulation in an aquatic organism

7.4.3.3.1 Bioaccumulation in an appropriate species of fish

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ] Scientifically unjustified [✓]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	According to the technical guidance document in support of directive 98/8/EC, this test is required when there is the risk for secondary poisoning. As explained in the Environmental Risk Assessment, the first step in an assessment of secondary poisoning risk is to consider whether a chemical has the potential to bioaccumulate. The potential for bioaccumulation can be estimated from the values of the n-octanol/ water partition coefficient, log Kow. It is accepted that values of log Kow greater than or equal to 3 indicate that the substance may bioaccumulate (TGD, 2003). Since, bendiocarb has a log Kow of 1.72 the compound displays little potential for bioaccumulation. Furthermore, based on the study conducted under Point 7.4.2 showing a BCF of 6 with rapid elimination, a bioaccumulation test in an appropriate species of fish should not be required.	
Undertaking of intended data submission [ ]		

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	25 June 2007
Comments	Applicants justification for non-submission is accepted by the UK CA.
Conclusion	The UK CA agrees that no further tests are required to address this endpoint.
Acceptability	Acceptable
Remarks	
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

7.4.3.3.2 Bioaccumulation in an appropriate invertebrate species

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [✓]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	As there is no anticipated release to brackish or marine water, this study should not be required.	
Undertaking of intended data submission [ ]		

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	25 June 2007
Comments	Applicants justification for non-submission is accepted by the UK CA because of the additional points given under A7.4.3.3.1. Bioaccumulation in an appropriate species of fish.
Conclusion	The UK CA agrees that no further tests are required to address this endpoint.
Acceptability	Acceptable
Remarks	
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

7.4.3.4 Effects on reproduction and growth rate with an appropriate invertebrate species

			Official use only
		1. REFERENCE	
1.1	**Reference**	Smith, G.J. *et al* (1990) Flow-Through Chronic Toxicity of Bendiocarb to *Daphnia magna* [redacted] Document A90226 7.4.3.4/01 26 April 1990 Unpublished	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Bayer CropScience AG	
1.2.2	Companies with letter of access	n.a.	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	US EPA Guideline 72-4	X
2.2	**GLP**	Yes	
2.3	**Deviations**	No	X
		3. MATERIALS AND METHODS	
3.1	**Test material**	^{14}C-radiolabelled bendiocarb	X
3.1.1	Lot/Batch number	CFQ 4944 338 MBq	
3.1.2	Specification	Specific Activity 107 µiCi/mg	
3.1.3	Purity	95.15 % by HPLC	
3.1.4	Composition of product	n.a.	
3.1.5	Further relevant properties	-	
3.1.6	Method of analysis	The total ^{14}C radioactivity in the exposure solutions was measured by Liquid Scintillation Counting (LSC). Bendiocarb was qualitatively analyzed by TLC. Approximately 25 percent of the samples were analyzed by HPLC in order to confirm quantities of ^{14}C-bendiocarb as determined by TLC. For the sample collected on day 6, a reverse-phase system was utilized.	X
3.2	**Preparation of TS solution for poorly voluble or volatile test substances**	n.a.	
3.3	**Reference substance**	No	

3.3.1	Method of analysis for reference substance	n.a.	
3.4	**Testing procedure**		
3.4.1	Dilution water	See Table A7.4.3.4-1	X
3.4.2	Test organisms	See Table A7.4.3.4-2	
3.4.3	Handling of offspring	The young were counted and discarded either after they were pipetted from the test chamber or retained by a screen (only when clean beakers were incorporated in the system and at test termination after the adults were first transferred to a 100 ml holding beaker).	
3.4.4	Test system	See Table A7.4.3.4-3	
3.4.5	Test conditions	See Table A7.4.3.4-4	X
3.4.6	Duration of the test	21 days	
3.4.7	Test parameter	Survival, reproduction and growth of the freshwater invertebrate cladoceran *Daphnia magna*.	
3.4.8	Examination / Sampling	Water samples were collected on days 0, 1, 2, 6, 9, 13, 16, and 21 and the total ^{14}C activity was measured by Liquid Scintillation Counting (LSC).	X
3.4.9	Monitoring of TS concentration	See 3.4.8	
3.4.10	Statistics	The quantal data (survival) were analyzed using Fisher's Exact Test (a 2 x 2 contingency table). The nonquantal data (reproduction and total length) were analyzed using a one-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test.	
		4. RESULTS	
4.1	**Range finding test**	Test concentrations were based on a 96-hour static-renewal range-finding toxicity test.	
4.1.1	Concentrations	12.5, 25, 50, 100 and 200 µg/L	
4.1.2	Number / Percentage of animals showing adverse effects	In the 96-hour static-renewal range-finding test, there was 0 to 100 percent survival of the test organisms in the test material exposure concentrations and 100 percent survival in the controls. The dose response mortality pattern observed in the 12.5 to 200 µg/L nominal bendiocarb test concentrations at termination of the static-renewal range-finding test (96 hours) was used to establish the test material concentrations for the 21-day chronic test. Reproduction and growth were not reported.	
4.1.3	Nature of adverse effects	See 4.1.2	
4.2	**Results test substance**		
4.2.1	Initial concentrations of test substance	0.625, 1.25, 2.5, 5.0 and 10.0 µg/L.	X

4.2.2	Actual concentrations of test substance	0.74, 1.47, 2.71, 5.20 and 10.05 µg/L	X
4.2.3	Effect data	*Daphnia* survival was not significantly reduced (P <0.05) in any of the test material concentrations when compared to the acetone/reverse osmosis-well water controls as analyzed by Fisher's Exact Test. The 21-day LC_{50} value (based on the measured total radioactivity) was >10.05 µg bendiocarb equivalents/L. *Daphnia* reproduction (defined as total number of young per reproducing adult female) was significantly affected at a concentration of 2.71 µg/L when compared with that in the solvent control, but not at concentrations of 1.47 µg/L and below. The-no-observed-effects-concentration (NOEC) was 1.47 µg/L. The-lowest-observed-effects concentration (LOEC) was 2.71 µg/L. Growth of the organisms (as determined by measurement of body length) was not significantly affected at a concentration of 10.05 µg/L. The NOEC (growth) was 10.05 µg/L and the LOEC >10.05 µg/L. The maximum acceptable toxicant concentration (MATC) was >1.47 µg/L and <2.71 µg/L.	X
4.2.4	Concentration / response curve	Survival and growth were not affected. Reproduction was reduced at the 3 highest dose rates (full details in Table 6, p 34 of the original report).	X
4.2.5	Other effects	-	
4.3	**Results of controls**	No adverse effects	
4.4	**Test with reference substance**	Not performed	
4.4.1	Concentrations	n.a.	
4.4.2	Results	n.a.	
		5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The effects of bendiocarb on the survival, reproduction and growth of the freshwater invertebrate cladoceran *Daphnia magna* were determined in a 21-day flow-through study. The ^{14}C-bendiocarb test material in acetone was delivered continuously for 21 days via a 0.5 litre solenoid-activated proportional flow-through diluter system to duplicate test chambers containing 10 test organisms each. The diluter system also delivered the dilution control water. Water samples were collected on days 0, 1, 2, 6, 9, 13, 16, and 21 and the total ^{14}C activity was measured by Liquid Scintillation Counting (LSC). Characterization of ^{14}C-bendiocarb and the NC-7312 degradation product was determined by Thin-Layer Chromatography (TLC) and also by High Pressure Liquid Chromatography (HPLC).	

		Daphnia test organisms were obtained from a healthy parthenogenically reproducing stock. Survival of the test organisms was noted each day and reproduction (number of young) was noted at two to three day intervals and at the end of the experiment. On the final day of the test (day 21), length measurements (top of head to base of the spine) were made on all surviving adult daphnids.	
5.2	**Results and discussion**	The mean measured concentrations of the exposure solutions ranged from 100.5 to 118.1 % of nominal bendiocarb concentrations (0.625, 1.25, 2.5, 5.0 and 10.0 µg/L) as determined by LSC. The controls always had measured ^{14}C levels at or below background levels (<0.38 µg/L). Both TLC and HPLC analysis confirmed bendiocarb as the principle component of the exposure solutions. *Daphnia* survival was not significantly reduced (P <0.05) in any of the test material concentrations when compared to the acetone/reverse osmosis-well water controls as analyzed by Fisher's Exact Test. The 21-day LC_{50} value (based on the measured total radioactivity) was >10.05 µg bendiocarb equivalents/L. *Daphnia* reproduction (defined as total number of young per reproducing adult female) was significantly affected at a concentration of 2.71 µg/L when compared with that in the solvent control, but not at concentrations of 1.47 µg/L and below. The-no-observed-effects-concentration (NOEC) was 1.47 µg/L. The-lowest-observed-effects concentration (LOEC) was 2.71 µg/L. Growth of the organisms (as determined by measurement of body length) was not significantly affected at a concentration of 10.05 µg/L. The NOEC (growth) was 10.05 µg/L and the LOEC >10.05 µg/L. The maximum acceptable toxicant concentration (MATC) was >1.47 µg/L and <2.71 µg/L.	X
5.2.1	NOEC	1.47 µg/L (reproduction); 10.05 µg/L (growth)	
5.2.2	LOEC	2.71 µg/L (reproduction); >10.05 µg/L (growth)	
5.2.3	EC_{50} (EC_x)	21-day LC_{50} value (based on the measured total radioactivity) was >10.05 µg bendiocarb equivalents/L.	
5.3	**Conclusion**		X
5.3.1	Reliability	1	X
5.3.2	Deficiencies	No	

Table A7.4.3.4-1 Dilution water

Criteria	Details
Source	Blend of reverse osmosis-well water
Alkalinity	128-156 mg/L as $CaCO_3$
Hardness	148-188 mg/L as $CaCO_3$
pH	7.6 – 8.2
Ca / Mg ratio	-
Na / K ratio	-
Oxygen content	6.3-9.0 mg/L
Conductance	296-365 µmhos/cm
TOC	-
Holding water different from dilution water	No

Section A7 Annex Point IIIA XIII.3.4	**Ecotoxicological Profile Including Environmental Fate and Behaviour** A7.4.3.4 Effects on reproduction and growth rate with an appropriate invertebrate species

Table A7.4.3.4-2 Test organisms

Criteria	Details
Species / Clone	*Daphnia magna*
Source	U.S. Environmental Protection Agency (Environmental Research Laboratory, Duluth MN)
Age	<24 hours at test initiation
Breeding method	*Daphnia* were cultured in an environmental chamber under controlled conditions (temperature: 20 ± 2°C; photoperiod: 16 hours light – 8 hours dark; light intensity: 323 – 1076 lux). *Daphnia* were cultured in 1-L glass beakers (10 *Daphnia* per beaker) containing 800 ml of a blend of reverse osmosis-well water.
Kind of food	2.3 x 108 cells/litre *Selenastrum capricornutum* and 4.0 to 5.0 ml of a yeast/trout food/Cerophyl® suspension three times each week when the culture water was changed.
Amount of food	See above
Feeding frequency	See above
Pretreatment	Twenty-four hours before the start of the test, young were removed from the beakers to ensure that only daphnids less than 24 hours old would be used to start the test. Young daphnids (<24 hours old at test initiation) used as test animals in the chronic toxicity test were from 19-day old cultures. There was 90 percent survival of culture animals and an average of 11.0 young produced per female per reproductive day during the week before the toxicity test.
Feeding of animals during test	In the 21-day flow-through test, each test chamber received three times daily an aliquot of the green alga, *Selenastrum capricornutum*, at a concentration of 1.44 x 10^8 cells/L for days 0-5 of the study, 1.73 x 10^8 cells/1, for days 6-9 of the study and 2.30 x 10^8 cells/1, for days 10-21 of the study. A yeast-trout food-Cerophyl (YTC) suspension was also added once each day at a rate of 0.6 ml for days 0-5 of the study, once each day at a rate of 0.8 ml for days 6-9 of the study and twice each day at a rate of 0.8 ml for days 10-21 of the study.

Table A7.4.3.4-3 Test system

Criteria	Details
Test type	Flow-through
Renewal of test solution	5.75 volume replacements every 24 h
Volume of test vessels	1 litre containing 800 ml solution
Volume/animal	80 ml
Number of animals/vessel	10
Number of vessels/ concentration	2
Test performed in closed vessels due to significant volatility of TS	n.a.

Table A7.4.3.4-4 Test conditions

Criteria	Details
Test temperature	20 °C ± 2°C
Dissolved oxygen	6.3-9.0 mg/L
pH	7.6-8.2
Adjustment of pH	No
Aeration of dilution water	Yes
Intensity of irradiation	400 to 900 lux
Photoperiod	16 h light and 8 h darkness

Table A7.4.3.4-5 Validity Criteria for Invertebrate Reproduction Test According to OECD Guideline 211

	Fulfilled	Not fulfilled
Mortality of parent animals <20 % at test termination	**X**	
Mean number of live offspring produced per parent animal surviving at test termination ≥60	**X**	
Criteria for poorly soluble test substances	**n.a.**	

Section A7 Annex Point IIIA XIII.3.4	**Ecotoxicological Profile Including Environmental Fate and Behaviour** A7.4.3.4 Effects on reproduction and growth rate with an appropriate invertebrate species

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	20/03/2007
	2.1 Although the study was carried out to US EPA FIFRA Subdivision Guideline E 72-4, it is noted that the Applicant has compared the study against the validity criteria in OECD 211. Therefore, the UK CA has considered this study against the requirements within the OECD guideline 211 (*Daphnia magna* Reproduction Test), which is consistent with the Applicant's approach. **2.3** A number of minor amendments relative to the study plan were reported in the study report submitted by the Applicant. The UK CA is satisfied that none of them significantly affects the study. In addition, the UK CA has compared this 1990 study with the OECD guideline 211 (1998) and noted a number of points related to the test criteria and data interpretation. ***Test data:*** - no details are given in the study of whether there was a pre-acclimatisation period of 3 weeks or whether the blend of reverse osmosis-well water was the culture medium and no acclimatisation was necessary, - the carbon content of the feedstock was not recorded so it is unclear whether the feed met the 0.1 – 0.2 mg C/*Daphnia*/day required by OECD guideline 211, - the light intensity used was 400 – 900 lux, which is < 15- 20 µE $m^{-2}s^{-1}$ as stated in OECD guideline 211, - no details are given on when the first brood was produced as required by OECD guideline 211, - analytical measurements were not recorded on the controls, lowest and highest test concentrations (as required in OECD guideline 211 where the concentration was expected to be maintained at 100 ± 20 % of the nominal values), although they were carried out on the control and highest concentration regularly throughout the study. The UK CA considers these differences compared to the OECD guideline do not significantly affect the results as the validity criteria given in OECD guideline 211 have been met (see Section 5.3). ***Data Interpretation:*** - all juveniles (live and dead) were counted and included in the number of offspring, whilst OECD guideline 211 indicates that juveniles that have died within the study should be excluded. The UK CA has used the raw data provided by the Applicant to correct the total young observed to exclude young that were recorded as dead during the study (see Section 4.2.3); - the total no. of offspring per female have been expressed relative to the number of surviving females and not the initial number of females, which is required when mortality has been observed in the study. The UK CA has corrected for this (see Section 4.2.3).
Materials and methods	The Applicant's version is acceptable, noting the following:

	3.1 Bendiocarb was ^{14}C-radioabelled uniformly within the ring. **3.1.6** Analysis of the test material is inadequately described, further data is provided below: Total ^{14}C-radioactivity was determined by LSC (which encompasses both bendiocarb and its hydrolytic metabolite NC 7312). TLC was used to quantitatively, and not qualitatively, analyse for bendiocarb and NC 7312, although no details have been given as to how the identity of NC 7312 was confirmed. Details are also missing for the analysis of pyrogallol (mentioned in the study plan) but it appears to be absent from its expected position on a TLC gel. Samples from day 6 and 16 were extracted with methylene chloride, evaporated to near dryness and made up in acetonitrile before HPLC with UV (210nm) or radiochemical detection. **3.4.1** Table A7.4.3.4-1 contains some minor errors. According to the study report - the alkalinity should be 124-156 mgl^{-1} and not 128-156 mgl^{-1} as $CaCO_3$, - the hardness should be 148-192 mgl^{-1} and not 148-188 mgl^{-1} as $CaCO_3$, and - the conductivity should be 296-366 µmhos cm^{-1} and not 296-365 µmhos cm^{-1} The source of the test water is a blend of well water that has been via reverse osmosis with well water, to give water of a suitable hardness for the study. **3.4.5** The raw data in the study report shows that the light intensity range was 323 – 1076 lux. Therefore, Table A7.4.3.4-4 should have recorded the light intensity as 323 – 1076 lux and not 400 – 900 lux as quoted. **3.4.8** In addition to the sampling for LSC described here, samples were also taken on Days 0, 1, 2, 6, 9, 13, 16 and 21 for analysis by TLC and on days 6 & 16 for extraction and analysis by confirmatory HPLC.
Results and discussion	The Applicant's version is acceptable, noting the following: **4.2.1** The initial concentrations stated by the applicant are nominal values. **4.2.2** The actual concentrations determined are mean measured values based on ^{14}C measurements by Liquid Scintillation Counting. These ranged from 100.5 % to 118.1 % of the nominal values. Whilst these values confirm that the addition of bendiocarb into the test vessels has been successful, the concentrations given do not represent bendiocarb alone, but a mixture of bendiocarb and any of its metabolite NC 7312 from hydrolysis. The UK CA has discussed this further under their comment to section 5.2. **4.2.3** No effect data has been presented in this study summary. The UK CA considers that the following data, extracted from the raw data in the study report, provides useful information that should be included in this study summary:

Mean Measured Concentration (µg Bendiocarb equivalents per litre)	No. Daphnids in test at Day 0	% Survival at Day 21	No Daphnids alive at Day 21	Total Length at Day 21 (mm)	Mean Total No. Surviving Young per Reproducing Female
< 0.38 (Control)	20	100	20	4.24@	93.0
< 0.38 (Acetone Control)	20	95	19	4.23	86.1 (91.2*#)
0.74	20	90	18	4.22	84.6 (93.9*)
1.47	20	100	20	4.26	84.9
2.71	20	90	18	4.18	63.2 (71.7*#)
5.20	20	95	19	4.13@	56.0 (58.2#)
10.05	20	100	20	4.13	54.3 (56.2#)

Notes:
A value of < 0.38 equates to below two times the background level
The mean total number of surviving young per reproducing adult should be determined by dividing the total number of surviving young by the total number of initial adults (according to OECD guideline 211).
* These values are determined in the study report by dividing the total number of young by the number of surviving (not initial) adults, although for these concentrations the adult survival was < 100 %.
#These values are based on the total number of young observed, (including some dead young), although young that die before the end of the study should be excluded from the calculation (according to OECD guideline 211).
@ these values have been truncated rather than rounded up, and hence each value should be 0.01mm more than stated above.

The UK CA has corrected the total mean values for one/both of these errors in the table above (the original values remain in brackets), and the Applicant has confirmed that this does not affect the outcome of the study.

4.2.4 No concentration response curve was plotted within the study report, but the responses observed against concentration were clearly tabulated, so the UK CA does not consider this omission to be of any concern.

5.2 The UK CA agrees with the Applicant that both TLC and HPLC analysis confirmed bendiocarb as the principle component of the exposure solutions. However, no indication has been given in this study summary of the amount of bendiocarb or its metabolite NC 7312 present, although this information is in the study report. The UK CA considers the following information from the study report should be included in this study summary:

Day	Method	% Bendiocarb	% NC-7312
0	TLC	76.4	23.7
1	TLC	66.9	33.2
2	TLC	63.7	36.3
6	TLC	98.4	1.7
6	HPLC	99.0	0.2
9	TLC	68.5	31.5
13	TLC	68.7	31.4
16	TLC	81.4	18.7
16	HPLC	62.1	27.1
21	TLC	92.9, 75.0	7.2, 25.1
Average		**77.5**	**21.5**

Notes:
- fresh dosing solutions were used on day 6 and day 15
- duplicate samples were taken from the same exposure beaker on Day 21

The results given by the Applicant are based on ^{14}C determined by LSC and hence the concentrations encompass both bendiocarb and NC 7312. The UK CA considers that this is not a worst-case for bendiocarb, as NC 7312 is believed to have a much lower toxicity to *Daphnia magna* based on available acute toxicity data. The UK CA believes that the NOEC value of 1.47 µg l^{-1} should be adjusted to correct for the presence of NC 7312 – the Applicant has agreed and suggested using a correction factor of 60 % to ensure a conservative approach. Hence a NOEC for bendiocarb of 0.882 µg l^{-1} can be taken forward for use in risk assessment. Likewise, the LOEC for bendiocarb would be reduced to 1.626 µg l^{-1}.

Section A7 **Annex Point IIIA XIII.3.4**	**Ecotoxicological Profile Including Environmental Fate and Behaviour** A7.4.3.4 Effects on reproduction and growth rate with an appropriate invertebrate species

Conclusion	**5.3** The validity criteria for the study are not mentioned in the text of the study summary, although Table A7.4.3.4-5 has been included, which confirms that this study is acceptable according to the criteria of OECD guideline 211. **5.3.1** The reliability of this study has been reduced to 2, as the study and interpretation of the raw data is not fully consistent with the current standard, OECD guideline 211. In addition, the analytical methodology for NC 7312 and pyrogallol have not been adequately described and validated.
Reliability	2
Acceptability	Acceptable
Remarks	All endpoints and data presented in the summary have been checked against the original study report and are correct.

	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A7 **Annex Point IIIA XIII.3.4**	**Ecotoxicological Profile Including Environmental Fate and Behaviour** A7.4.3.5 Effects on any other specific, non-target organisms A7.4.3.5.1 Effects on sediment dwelling organisms

7.4.3.5 Effects on any other specific, non-target organisms (flora and fauna) believed to be at risk

7.4.3.5.1 Effects on sediment dwelling organisms

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [✓]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	As demonstrated in a water/sediment study there is a rapid degradation of bendiocarb (DT_{50}: 9 d) and a slow transfer of bendiocarb to the sediment. Consequently, this study should not be required.	X
Undertaking of intended data submission [ ]		

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	01/02/2007
Comments	The UKCA agrees that bendiocarb rapidly degrades with a DT_{50} of about 9 days in a water-sediment study, and notes that bendiocarb has a low log Kow of 1.7 (at 25 °C and pH 6.9). This suggests that the amount of bendiocarb that would reach sediment is likely to be very low, and that no risk assessment for bendiocarb in sediment should be required. Hence, the UK CA agrees with the Applicant's conclusion that no test on the effects of bendiocarb on sediment-dwelling organisms is necessary. This is consistent with the TGD for Risk Assessment, which indicates that if the log Kow < 3 then the requirement for sediment effects assessment is not triggered.
Conclusion	The UK CA agrees, in line with the TGD for Risk Assessment, that no test on the effects of bendiocarb on sediment-dwelling organisms is required.
Acceptability	Acceptable
Remarks	
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

7.4.3.5.2 Aquatic plant toxicity

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [✓]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	Bendiocarb is not an herbicide and was registered as an insecticide for crop protection and was not phytotoxic. Therefore, a study on aquatic plant toxicity should not be required.	X
Undertaking of intended data submission [ ]		

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	30/11/2006
Comments	The UK CA does not accept the Applicant's argument that because bendiocarb is not an herbicide, and not phytotoxic when used as an insecticide in crop protection, an aquatic plant toxicity test is automatically not required. However, the UK CA considers it unnecessary to request an aquatic plant toxicity test in order to do a preliminary risk assessment for the aquatic compartment, but does note that the lack of a study means that there is less aquatic toxicity data available upon which to base the risk assessment. If the aquatic PEC/PNEC ratio is unacceptable, an aquatic plant toxicity test is one option that could help in the refinement of the PEC/PNEC ratio.
Conclusion	The UK CA does not require an aquatic plant toxicity test to progress with the initial aquatic risk assessment.
Acceptability	Acceptable
Remarks	
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

7.5 Effects on terrestrial organisms

7.5.1 Terrestrial toxicity, initial tests

7.5.1.1 Inhibition to microbiological activity

			Official use only
		1. REFERENCE	
1.1	**Reference**	Warner, P.A. (1978a) The Effect of Bendiocarb on Soil Micro-organisms – Carbon Metabolism; 1- Starch [redacted] Document A90189 7.5.1.1/01 June 1978 Unpublished	X
1.2	**Data protection**	Yes	
1.2.1	Data owner	Bayer CropScience AG	
1.2.2	Companies with letter of access	n.a.	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No, but the study was conducted in line with good scientific practice.	X
2.2	**GLP**	No, the study was conducted prior to the introduction of GLP as a standard requirement.	
2.3	**Deviations**	No	
		3. MATERIALS AND METHODS	
3.1	**Test material**	Bendiocarb	
3.1.1	Lot/Batch number	Not specified	
3.1.2	Specification	As given in Section 2	
3.1.3	Purity	> 99 %	
3.1.4	Composition of product	Not applicable	
3.1.5	Further relevant properties	-	
3.1.6	Method of analysis	Not measured	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Not applicable	
3.3	**Reference substance**	No	

3.3.1	Method of analysis for reference substance	Not applicable	
3.4	**Testing procedure**		X
3.4.1	Culture medium	Not applicable	
3.4.2	Inoculum / test organisms	Soil microbial population (not identified)	
3.4.3	Test system	Unlabelled bendiocarb was added to 200 g samples of a sieved (2 mm aperture) sandy loam soil (see Table A7.5.1.1-1) to give a concentration of 5 and 50 mg/kg. Bendiocarb was added as a solution in ethanol (0.5 ml total volume). Aliquots (2 g) of ^{14}C-starch (10 µCi) were added to soils to give a 1 % w/w spiked soil. Control soils consisted of: a) aerobic soil + ^{14}C-starch only b) sterile soil + ^{14}C-starch and c) aerobic soil only.	
3.4.4	Test conditions	Sterilisation of the soil was carried out by autoclaving at 1 kg/cm² for 15 minutes. Duplicates were provided for each of five experiments giving a total of 10 soil samples. The moisture content of the soil was adjusted to 45 % field capacity and the soils incubated at 20°C ± 1°C. The sealed soil containers were flushed daily for 30 minutes with air. Evolved $^{14}CO_2$ was trapped in a mixture of ethanolamine/2-methoxy-ethanol 1:3. The $^{14}CO_2$ collected in the traps was quantified by taking samples of absorbent mixture every 2-3 days and radiocounting (LKB Wallac 81000 liquid scintillation counter).	
3.4.5	Duration of the test	36 days	
3.4.6	Test parameter	Carbon degradation (based on $^{14}CO_2$ evolution)	X
3.4.7	Analytical parameter	Not applicable	
3.4.8	Sampling	Days 4, 8, 12, 16, 19, 22, 25, 29, 32 and 36.	X
3.4.9	Monitoring of TS concentration	No	X
3.4.10	Controls	a) aerobic soil + ^{14}C-starch only b) sterile soil + ^{14}C-starch c) aerobic soil only	
3.4.11	Statistics	-	
		4. RESULTS	
4.1	**Preliminary test**		
4.1.1	Concentration	None	
4.1.2	Effect data	Not applicable	
4.2	**Results test substance**		
4.2.1	Initial concentrations of test substance	5 and 50 mg/kg.	

4.2.2	Actual concentrations of test substance	Not measured	X
4.2.3	Growth curves	-	
4.2.4	Cell concentration data	-	X
4.2.5	Concentration / response curve	-	X
4.2.6	Effect data	The amount of $^{14}CO_2$ evolved in 30 days from soils containing 5 and 50 mg/kg bendiocarb and ^{14}C-starch was similar to that evolved from ^{14}C-starch-treated soil alone. The results indicate that bendiocarb apparently stimulates the degradation of starch over the first 8 days. However, in all three soils the amount of $^{14}CO_2$ evolved over most time intervals was similar with a plateau reached between 15 and 30 days when little $^{14}CO_2$ was detected. The total $^{14}CO_2$ evolved over 30 days was 30-35 % of the original radioactivity applied to the soils.	X
4.2.7	Other observed effects	-	
4.3	**Results of controls**	The control soils consisting of sterile soil containing ^{14}C-starch and aerobic soil only both yielded very low levels of $^{14}CO_2$ after 30 days. This indicated that the evolution of $^{14}CO_2$ results from the degradation of ^{14}C-starch by aerobic micro-organisms.	
4.4	**Test with reference substance**		
4.4.1	Concentrations	None	
4.4.2	Results	Not applicable	
		5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The effect of bendiocarb on starch metabolism in a sandy loam soil has been investigated. Unlabelled bendiocarb was added to 200 g samples of a sieved (2 mm aperture) sandy loam soil (see Table A7.5.1.1-1) to give a concentration of 5 and 50 mg/kg. Aliquots (2 g) of ^{14}C-starch (10 µCi) were added to soils to give a 1 % w/w spiked soil. Control soils consisted of: a) aerobic soil + ^{14}C-starch only b) sterile soil + ^{14}C-starch and c) aerobic soil only. The moisture content of the soil was adjusted to 45 % field capacity and the soils were incubated at 20°C ± 1°C for 35 days with daily flushing and trapping of evolved $^{14}CO_2$. The levels of $^{14}CO_2$ evolved were quantified every 2-3 days and the study ended when the level of $^{14}CO_2$ evolution in treated and control soils was comparable.	X

5.2	**Results and discussion**	The amount of $^{14}CO_2$ evolved in 30 days from soils containing 5 and 50 mg/kg bendiocarb and ^{14}C-starch was similar to that evolved from ^{14}C-starch-treated soil alone. The total $^{14}CO_2$ evolved over 30 days was 30-35 % of the original radioactivity applied to the soils. The control soils consisting of sterile soil containing ^{14}C-starch and aerobic soil only both yielded very low levels of $^{14}CO_2$ after 30 days. This indicated that the evolution of $^{14}CO_2$ results from the degradation of ^{14}C-starch by aerobic micro organisms. These results indicate that the application of bendiocarb at 5 and 50 mg/kg to sandy loam soil has no detectable effect on those micro organisms responsible for starch metabolism.	X
5.3	**Conclusion**		X
5.3.1	Reliability	2	X
5.3.2	Deficiencies	None	X

Table A7.5.1.1-1 Soil Characteristics

Texture	Sand (%)	Silt (%)	Clay (%)	Organic carbon (%)	Moisture content (% field capacity)
Sandy Loam (Shelford – UK)	60.22	15.89	19.08	4.0	45

Section A7 **Annex Point IIA VII.7.4**	**Ecotoxicological Profile Including Environmental Fate and Behaviour** A7.5 Effects on terrestrial organisms A7.5.1 Terrestrial toxicity, initial tests A7.5.1.1 Inhibition to microbiological activity

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	4/3/2008
	1.1 The reference does not mention Schering Agrichemicals Ltd, to whom the study report A90189 belongs. Reference should include Schering, but acknowledge Bayer now own the data through merger/buyout. **2.1** The study was done in 1978 and not to any standard guideline. The UK CA has evaluated this study in its own right, and considered it in the context of current OECD guideline 217 (Soil Microorganism: Carbon transformation Test) to determine the significance of any differences.
Materials and methods	The Applicant's version is acceptable, noting that the structure of the study summary deviates slightly from that in the TNG for Dossier Preparation, and the following points: **3.4** The test method differs significantly from that given in the current OECD guideline 217. Deviations are given under the UK CA comments for Section 5.3.2. **3.4.6** The test parameter used to measure carbon transformation (not degradation as stated in the study summary) was $^{14}CO_2$. **3.4.8** Samples were aerated daily to release CO_2 and analysis, by scintillation counting, was carried out of the combined $^{14}CO_2$ released over each 2-3 days. Hence, data were not recorded for the first few individual days of the study. **3.4.9** Analysis is not a requirement of the existing OECD guideline 217. However, the lack of analysis of the test substance bendiocarb, when bendiocarb is known to hydrolyse, means it is unclear how much test substance remained in the soil during the 36 days of the test.
Results and discussion	The Applicant's version is acceptable, noting the following: **4.2.2** These are the nominal concentrations of bendiocarb spiked on to the soil (in an ethanolic solution). According to the Applicant (Doc III-A section A10) the concentrations are based on dry weight of soil, but this is not documented in the study report. **4.2.4** No data were given in the study report on the amount of biomass of microorganims present in the test soil. **4.2.5** The UK CA notes that a Conentration/Response Curve, of $^{14}CO_2$ evolution (as % of applied radioactivity) against time, is available on page 5 of the study report. **4.2.6** The Applicant has not presented any of the available data in this study summary. The UK considers the following table should be included:

Day of Study	Mean % applied radioactivity released as $^{14}CO_2$				
	50 ppm bendiocarb + starch	5 ppm bendiocarb + starch	Aerobic control + starch	Sterile control + starch	Aerobic without starch
4	23.1	21.5	13.1	0.06	0.059
8	25.1	24.85	20.5	0.074	0.067
12	26.8	28.9	26.8	0.11	0.076
16	27.9	29.05	28.4	0.11	0.087
19	27.0*	29.9	30.05	0.41	0.11
22	29.05	30.85	30.2*	0.42	0.12
25	30.05	31.5	35.5*	0.50	0.125
29	31.15	32.05	36.6*	0.50	0.125
32	27.4*	32.7	37.8*	0.51	0.125
36	27.5*	33.5	39.2*	0.52	0.13

- single analysis only, other analyses are duplicates.

5.1 The methodology used differs significantly from that in the current OECD guideline 217, and the UK CA considers the differences could affect the reliability of the result and its applicability for use in the terrestrial risk assessment of bendiocarb.

5.2 The UK CA considers the results to be subjective as no statistical analysis done

Conclusion

The Applicant's version is acceptable, noting the following:

5.3 No validity criteria were available for the original study. However, the UK CA notes that the validity criteria given in OECD guideline 217 include one that states $^{14}CO_2$ release between replicate control samples should be < 15 %. This is not the case for all the analyses done on the control replicates in this study.

5.3.1 The reliability has been reduced from 2 to 3 as

- the number of differences compared to the current OECD guideline 217 (see below)
- the variability in the data, and
- the subjective, rather than statistical, interpretation of the data are of sufficient concern that the UK CA cannot accept this study as giving a reliable NOEC for use in reducing the assessment factor in the determination of a terrestrial PNEC.

5.3.2 The UK has noted that the main differences between the study and current OECD guideline 217 are:
- the source and history of the soil used was not identified, and whilst the sand

	content was acceptable, the organic content was 4 % compared to 0.5-1.5 % required by OECD guideline 217 to ensure carbon starvation - the biomass of microorganism presents was not quantitated in any way so it is unclear whether it is > 1 % of the total soil organic carbon - the bendiocarb was dosed on to the soil in ethanol (organic solvents are not considered acceptable under OECD guideline 217 because of their effect on soil microflora), and there was no indication of how the soil was mixed - the carbon source added was ^{14}C-starch and not ^{14}C glucose, as required by the OECD guideline - only 2 and not 5 test concentrations were used, with duplicate rather than triplicate analyses being performed on them. On a number of days only a single measurement was made for a specific sample due to contamination issues that have not been adequately explained. - there is no indication as to whether the study was done in the dark - variability between control replicates exceeded the < 15 % validity criteria on all occasions - no statistical procedures were used to determine the results
Reliability	3
Acceptability	Unacceptable
Remarks	The UK CA considers that this study suggests that nominal concentrations in the region of 50 mg/kg of bendiocarb are unlikely to significantly adversely affect soil microorganisms. However, - the number of differences compared to the current OECD guideline 217, - the variability in the data, and - the subjective, rather than statistical, interpretation of the data are of sufficient concern that the UK CA cannot accept this study as giving a reliable NOEC for use in the determination of a terrestrial PNEC. However, it should be noted that the Applicant has addressed the potential for a data gap in Appendix 1 of Doc IIA using a series of non-key data to support that bendiocarb is not of significant toxicity to soil microorganisms. All endpoints and data presented in the summary have been checked against the original study and are correct
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A7 **Annex Point IIIA XIII.3.2**	**Ecotoxicological Profile Including Environmental Fate and Behaviour** A7.5 Effects on terrestrial organisms A7.5.1 Terrestrial toxicity, initial tests A7.5.1.2 Acute toxicity test to earthworms or other soil non-target organisms

7.5.1.2 Acute toxicity test to earthworms or other soil non-target organisms

			Official use only
		1. REFERENCE	
1.1	**Reference**	Lechelt-Kunze, C. (2005) Bendiocarb (tech): Acute Toxicity to Earthworms (*Eisenia fetida*) tested in Artificial Soil [redacted] Document MO-05-010333 7.5.1.2/01 5 July 2005 Unpublished.	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Bayer CropScience AG	
1.2.2	Companies with letter of access	n.a.	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	OECD 207.	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3. MATERIALS AND METHODS	
3.1	**Test material**	Bendiocarb	
3.1.1	Lot/Batch number	B930101	
3.1.2	Specification	As given in Section 2.	
3.1.3	Purity	97.62 %	
3.1.4	Composition of product	Not applicable	
3.1.5	Further relevant properties	-	
3.1.6	Method of analysis	Not analysed	
3.2	**Reference substance**		
3.2.1	Method of analysis for reference substance	Not analysed	
3.3	**Testing procedure**		
3.3.1	Preparation of the test substance	See Table A7.5.1.2-2	X
3.3.2	Application of the test substance	See Table A7.5.1.2-2	

Section A7 Annex Point IIIA XIII.3.2	**Ecotoxicological Profile Including Environmental Fate and Behaviour** A7.5 Effects on terrestrial organisms A7.5.1 Terrestrial toxicity, initial tests A7.5.1.2 Acute toxicity test to earthworms or other soil non-target organisms

3.3.3	Test organisms	See Table A7.5.1.2-1	
3.3.4	Test system	See Table A7.5.1.2-2	
3.3.5	Test conditions	See Table A7.5.1.2-3	X
3.3.6	Test duration	14 days	
3.3.7	Test parameter	Mortality and growth	X
3.3.8	Examination	Days 7 and 14	
3.3.9	Monitoring of TS concentration	Not applicable	
3.3.10	Statistics	Probit analysis (Finney)	X
		4. RESULTS	
4.1	**Filter paper test**	Not applicable	
4.2	**Soil test**		
4.2.1	Initial concentrations of test substance	0.1, 0.18, 0.32, 0.56, 1.0, 3.2, 10, 100, 178, 316, 562 and 1000 mg a.s./kg dry weight soil	X
4.2.2	Effect data (mortality)	See Table A7.5.1.2-4	
4.2.3	Concentration / effect curve	See Figure A7.5.1.2-1	
4.2.4	Other effects	Earthworms became cramped at 0.56 mg a.s./kg dry weight soil	X
4.3	**Results of controls**	See Table A7.5.1.2-4	
4.4	**Test with reference substance**	Chloroacetamide A.R.	
4.4.1	Concentrations	5.6, 10, 18, 24 and 32 mg/kg dry weight soil	
4.4.2	Results	LC_{50} (14 days) 9.3 mg/kg dry weight soil	
		5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Adult *Eisenia fetida andrei* (4 x 10 animals per concentration, the overall average weight was 0.33 g) were exposed for 14 days in an artificial soil to the nominal concentrations of 0.1, 0.18, 0.32, 0.56, 1.0, 3.2, 10, 100, 178, 316, 562 and 1000 mg bendiocarb technical (97.62 %)/ kg dry weight soil. The artificial soil comprised 10 % peat.	
5.2	**Results and discussion**	Earthworms became cramped at a concentration 0.56 mg a.s./kg dry weight soil. The LC_{50} (14 days) was 163 mg a.s./kg dry weight soil (95 % confidence limits not determined due to mathematical reasons). Related to weight alterations and symptoms, the no-observed-effect-concentration (NOEC) was 0.32 mg a.s./kg dry weight soil, the lowest-observed-effect-concentration (LOEC) was 0.56 mg a.s./kg dry weight soil.	

5.2.1	LC_0	0.32 mg a.s./kg dry weight soil	
5.2.2	LC_{50}	163 mg a.s./kg dry weight soil	
5.2.3	LC_{100}	562 mg a.s./kg dry weight soil	
5.3	**Conclusion**		X
5.3.1	Other conclusions	-	
5.3.2	Reliability	1	X
5.3.3	Deficiencies	No	

Table A7.5.1.2-1 Test organisms

Criteria	Details
Species / strain	*Eisenia fetida andrei*
Source	In-house
Culturing techniques	-
Age / weight	> 2 months; average weight 0.33 g
Pretreatment	None

Section A7 **Annex Point IIIA XIII.3.2**	**Ecotoxicological Profile Including Environmental Fate and Behaviour** A7.5 Effects on terrestrial organisms A7.5.1 Terrestrial toxicity, initial tests A7.5.1.2 Acute toxicity test to earthworms or other soil non-target organisms

Table A7.5.1.2-2 Test system

Criteria	Details
Artificial soil	Sphagnum peat: 10 % Kaolinite clay: 20 % Quartz sand: 70 % Calcium carbonate: 0.4 %
Test mixture	To obtain the nominal test concentrations of 1000, 562, 316, 178 and 100 mg test item/kg dry weight soil, nominal amounts of 500, 281, 158, 89 and 50 mg test item were weighed into a petri dish respectively and quartz sand (washed and calcined) was added up to a final weight of 5 g for each of the four replicates. To obtain the test concentrations of 10, 3.2, 1.0, 0.56, 0.32, 0.18 and 0.1 mg test item/kg dry weight soil, nominal amounts of 5 g, 1.6 g, 500 mg, 280 mg, 160 mg, 90 mg and 50 mg stock mixture were weighed in a same procedure as the other concentrations. The stock mixture was prepared by weighing 42.5 mg of the test item into a petri dish and adding quartz sand (washed and calcined) up to a final weight of 42.5909 g (stock mixture). Test item and quartz sand were transferred to a mortar and thoroughly mixed with a pestle. Each application mixture (5 g) was transferred separately to artificial soil (500 g dry weight) and mixed thoroughly using a laboratory mixer. To the control replicates 5 g of quartz sand (without test item) was mixed into the soil. Finally 50 ml of deionized water were added and mixed into each replicate to achieve between 40 % and 60 % of the maximum water holding capacity (WHC_{max}).
Size, volume and material of test container	1.5 L preserving jars with glass lids
Amount of artificial soil (kg)/container	0.5
Nominal levels of test concentrations	100, 178, 316, 562 and 1000 mg/kg soil
Number of replicates/concentration	Four
Number of earthworms/test concentration	40
Number of earthworms/container	10
Light source	Artificial
Test performed in closed vessels due to significant volatility of test substrate	No

Section A7 **Annex Point IIIA XIII.3.2**	**Ecotoxicological Profile Including Environmental Fate and Behaviour** A7.5 Effects on terrestrial organisms A7.5.1 Terrestrial toxicity, initial tests A7.5.1.2 Acute toxicity test to earthworms or other soil non-target organisms

Table A7.5.1.2-3 Test conditions

Criteria	Details
Test temperature	20 ± 2°C
Moisture content	40 – 60 %
pH	5.97 – 6.08
Adjustment of pH	No
Light intensity / photoperiod	400 – 800 lux (constant)
Relevant degradation products	Not applicable

Table A7.5.1.2-4 Effects of Bendiocarb (tech.) on *Eisenia fetida* after 14 days exposure

Test concentrations (mg test item/kg dry weight soil) [a]	Mortality (%)	
	After 7 days	After 14 days
Control	0	0
0.1	0	0
0.18	0	0
0.32	0	0
0.56*	0	0
1.0*	0	0
3.2*	0	0
10*	0	5 ± 10
100*	0	5 ± 6
178*	13 ± 5	43 ± 13
316*	25 ± 13	93 ± 5
562*	58 ± 21	100
1000	100	100

Mortality (%) was calculated from the means of 4 replicates each containing ten earthworms.
(a) test concentrations are nominal concentrations
* earthworms became cramped

Section A7 Annex Point IIIA XIII.3.2	Ecotoxicological Profile Including Environmental Fate and Behaviour A7.5 Effects on terrestrial organisms A7.5.1 Terrestrial toxicity, initial tests A7.5.1.2 Acute toxicity test to earthworms or other soil non-target organisms

Table A7.5.1.2-5 Effect data

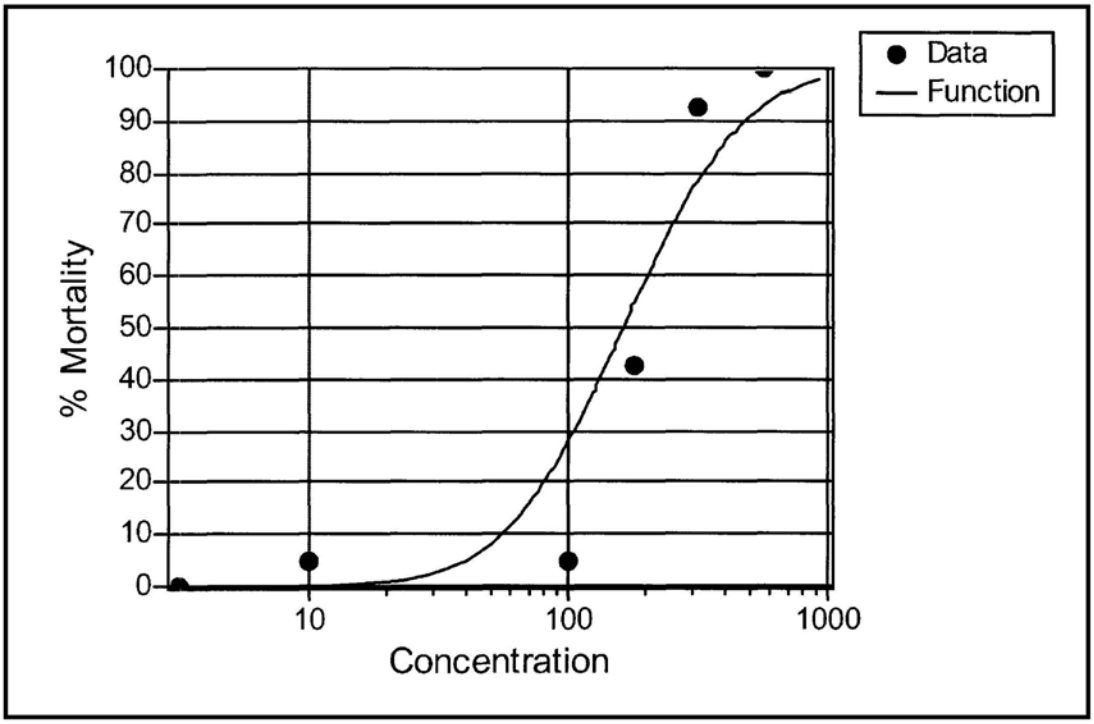
	14 d [mg a.s./kg dry weight soil][1]
LC_0	0.32
LC_{50}	163
LC_{100}	562

1 based on nominal concentrations

Table A7.5.1.2-6 Validity Criteria for Acute Earthworm Test According to OECD Guideline 207

	Fulfilled	Not fulfilled
Mortality of control animals <10 %	X	

Figure A7.5.1.2-1 Dose-Effect-Curve to *Eisenia fetida* after 14 days exposure



Section A7 **Annex Point IIIA XIII.3.2**	**Ecotoxicological Profile Including Environmental Fate and Behaviour** A7.5 Effects on terrestrial organisms A7.5.1 Terrestrial toxicity, initial tests A7.5.1.2 Acute toxicity test to earthworms or other soil non-target organisms

	EVALUATION BY COMPETENT AUTHORITIES
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	20/03/2007
Materials and methods	The Applicant's version is acceptable, noting the following: **3.3.1**. Table A7.5.1.2-2 suggests that the only test concentrations used were 100, 178, 316, 562 and 1000 mg kg^{-1} soil. Additional lower concentrations (i.e. 0.1, 0.18, 0.32, 0.56, 1.0, 3.2 and 10 mg kg^{-1} soil) were also included in this study, as indicated in Section 4.2.1 amd Table 7.5.1.2-4. **3.3.5** In Table A7.5.1.2-3 the moisture content of the soil was stated to be 40 - 60 %, when that required by the OECD guideline 207 is a water content of about 35 % (of the dry weight) and those values given in the study report range from 24.9 – 25.7 % . In addition, the UK CA considers that the calculated values for the % of WHCmax ranging from 52.4 – 54.7 are wrong, and should range from 39.3 – 40.1 %. However, the UK CA does not consider that a slightly lower water content will significantly affect the outcome of the study **3.3.7** The test parameter should indicate that growth was a sublethal effect and monitored by body weight. In addition other non-lethal symptoms were observed i.e. cramping. **3.3.10** According to the study report the LC_{50} and 95 % confidence limits were calculated by probit analysis. However, for both bendiocarb and the reference substance, it has also been stated that the confidence limits could not be determined due to mathematical reasons. The Applicant has subsequently recalculated the data to provide a revised 14 d LC_{50} of 188 mg a.s. kg^{-1} and 95 % confidence limits of 169 – 209 mg a.s. kg^{-1}.
Results and discussions	The Applicant's version is acceptable, noting the following: **4.2.1** The concentrations given are initial nominal concentrations. The UK CA considers that, as bendiocarb degrades, these concentrations will not be maintained throughout the test. As a result, time-weighted average (TWA) concentrations were requested from the Applicant. The Applicant considered that TWA concentrations were not appropriate and provided a justification based on measured concentrations not being required by OECD 207 and a request that nominal concentrations could be compared with initial PECsoil rather than refined PECsoil. The UK CA accepted this point and will base its risk assessment on a comparison of nominal PNEC v initial PECsoil. **4.2.4** In addition to the symptom data (earthworms becoming cramped at 0.56 mg kg^{-1} and above) the study also monitored growth (as weight) at Day 0 and 14. A reduction in growth was observed at nominal concentrations of 0.56 mg kg^{-1} and above. **5.2** The results given are based on nominal concentrations, although bendiocarb

Section A7 **Annex Point IIIA XIII.3.2**	**Ecotoxicological Profile Including Environmental Fate and Behaviour** A7.5 Effects on terrestrial organisms A7.5.1 Terrestrial toxicity, initial tests A7.5.1.2 Acute toxicity test to earthworms or other soil non-target organisms

	is known to degrade. As a result, the terrestrial risk assessment will be based on a comparison of nominal PNEC v initial PECsoil.
Conclusion	The Applicant's version is acceptable, noting the following: **5.3** The validity criteria in OECD guideline 207 are not mentioned in this study summary, although the study report does confirm that the study is valid. **5.3.2** The reliability has been reduced from 1 to 2, as the lack of measured or time-weighted data mean that the results given do not accurately reflect the toxicity of bendiocarb alone.
Reliability	2
Acceptability	Acceptable
Remarks	All endpoints and data presented in the summary have been checked against the original study and are correct

	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A7 Annex Point	**Ecotoxicological Profile Including Environmental Fate and Behaviour** A7.5 Effects on terrestrial organisms A7.5.1 Terrestrial toxicity, initial tests A7.5.1.3 Acute toxicity to plants

7.5.1.3 Acute toxicity to plants

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [✓]	**Other justification [ ]**	
Detailed justification:	Based on the use pattern of the biocidal product, no exposure to plants is anticipated when it is properly used by trained pest control operators. Furthermore, bendiocarb was registered as an insecticide for crop protection and was not phytotoxic. Therefore an acute toxicity study to plants is not required.	X
Undertaking of intended data submission [ ]		
EVALUATION BY COMPETENT AUTHORITIES		

	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	01/02/2007
Evaluation of applicant's justification	The UK CA does not agree with the Applicant that because bendiocarb is not phytotoxic, when used as an insecticide in crop protection, a plant toxicity test is automatically not required. However, the UK CA does accept the Applicant's argument that based on this type of product and use pattern, including use by professional operators only, no significant direct exposure to plants is likely. However, the exposure of soil to bendiocarb is expected, so plants could be affected indirectly via the soil. The UK CA considers it unnecessary to request an acute plant toxicity test in order to do a preliminary risk assessment for the terrestrial compartment, but does note that the lack of a study means that there is less terrestrial toxicity data available upon which to base the risk assessment. If the terrestrial PEC/PNEC ratio is unacceptable, a terrestrial plant toxicity test is one option that could help in the refinement of the PEC/PNEC ratio.
Conclusion	The UKCA does not require an acute terrestrial plant toxicity test to progress with the initial terrestrial risk assessment.
Remarks	

	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A7 **Annex Point IIIA XIII.3.2**	**Ecotoxicological Profile Including Environmental Fate and Behaviour** A7.5.2 Terrestrial tests, long-term tests A7.5.2.1Reproduction study with other soil non-target macro-organisms

7.5.2 Terrestrial tests, long-term tests

7.5.2.1Reproduction study with other soil non-target macro-organisms

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [✓]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	The risk assessment for the terrestrial compartment, based on the results from the acute toxicity test on earthworm and the realistic environmental exposure indicates no concern for the terrestrial compartment. Therefore, reproduction study should not be required.	
Undertaking of intended data submission [ ]		

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	01/12/2006
Evaluation of applicant's justification	The UK CA agrees with the Applicant that if the terrestrial risk assessment for bendiocarb, based on the acute toxicity test for earthworm, shows no concern for the terrestrial compartment then this study will not be required.
Conclusion	The UK CA does not require this reproduction test on soil macroorganisms to progress with the initial terrestrial risk assessment.
Remarks	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

7.5.2.2 Long-term test with terrestrial plants

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [✓]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	As there is no continual exposure of plants, this study should not be required.	
Undertaking of intended data submission [ ]		

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	01/02/2007
Comments	The UK CA accepts the Applicant's argument that based on this type of product and use pattern, including use by professional operators only, continual direct exposure to plants is unlikely. As the proposed application of bendiocarb is for a single annual use, and bendiocarb rapidly degrades, chronic exposure of the soil (and hence chronic indirect exposure to plants) is unlikely. The lack of continued exposure of the soil to bendiocarb suggests that a long-term terrestrial plant test is not required.
Conclusion	The UK CA does not require a long-term plant test to progress with the initial terrestrial risk assessment.
Acceptability	Acceptable
Remarks	
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

7.5.3 Effects on birds

7.5.3.1.1 Acute oral toxicity

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [✓]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	As the product is not used as a bait or granule or powder, this study should not be required.	
Undertaking of intended data submission [ ]		

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	04/12/2006
Evaluation of applicant's justification	The UK CA accepts the Applicant's justification that as the product is not a bait, granule or powder then this study is not required for this product type (PT 18, insecticide) and use pattern.
Conclusion	Applicant's justification is acceptable.
Remarks	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

7.5.3.1.2 Short-term toxicity

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [✓]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	As the product is not used as a bait, granule or powder, this study should not be required.	
Undertaking of intended data submission [ ]		

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	04/12/2006
Evaluation of applicant's justification	The UK CA accepts the Applicant's justification that as the product is not a bait, granule or powder then this study is not required for this product type (PT 18, insecticide) and use pattern.
Conclusion	Applicant's justification is acceptable.
Remarks	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

7.5.3.1.3 Effects on reproduction

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [✓]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	As the product is not used as a bait, granule or powder, this study should not be required.	
Undertaking of intended data submission [ ]		

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	04/12/2006
Evaluation of applicant's justification	The UK CA accepts the Applicant's justification that as the product is not a bait, granule or powder then this study is not required for this product type (PT 18, insecticide) and use pattern.
Conclusion	Applicant's justification is acceptable.
Remarks	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A7 Annex Point IIIA XIII.3.1	**Ecotoxicological Profile Including Environmental Fate and Behaviour** A7.5.4 Effects on honeybees A7.5.4.1 Acute toxicity to honeybees and other beneficial arthropods

7.5.4 Effects on honeybees

7.5.4.1 Acute toxicity to honeybees and other beneficial arthropods

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [✓]	**Other justification [ ]**	
Detailed justification:	Bendiocarb is toxic to bees (on the label, Ficam W is to be used to control wasps and wild bees). However, the treated places are not visited by bees (ground, around buildings) and no application is carried out on plants and flower beds. Therefore, no exposure to bees is anticipated. With regard to the contamination of bees by treated wasps nests, no exposure is anticipated because Pest Control Operators are taking the treated nets back and according to the label: "Action should be taken to prevent foraging bees gaining access to the treated bees nests preferably by removing the combs or blocking nest entrance". Therefore, exposure (if any) to honey bees and other beneficial arthropods would in any case be extremely localised to the immediate vicinity of the treated areas. Consequently, on population level, no significant risk to honey bees and other beneficial arthropods is anticipated. Thus, studies on bees and other beneficial arthropods should not be required.	
Undertaking of intended data submission [ ]		

EVALUATION BY COMPETENT AUTHORITIES

EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	03/03/2008
Evaluation of applicant's justification	The UK CA agrees with the Applicant that bendiocarb is likely to be toxic to bees. As a result of this, it is used in products to control wild bees. The Applicant has noted that exposure of bees by contamination from treated wasp nets should not occur, as the use of bendiocarb is controlled by limiting its use to professional operators and adequate instructions. The UK CA accepts that contamination of bees will be limited, and localised, if its use is limited to professional operators and appropriate risk mitigation measures are followed. Hence there should be no significant risk to honey bees at the population level.
Conclusion	Acceptable
Remarks	The UK CA notes that, in order to protect the honey bees, the use of bendiocarb must be limited to professional operators and appropriate risk mitigation measures must be followed. This is discussed further as part of the risk assessment in Doc II-B, Doc II-C and Doc I.

Section A7 **Annex Point IIIA XIII.3.1**	**Ecotoxicological Profile Including Environmental Fate and Behaviour** A7.5.4 Effects on honeybees A7.5.4.1 Acute toxicity to honeybees and other beneficial arthropods

	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

7.5.5 Bioconcentration, terrestrial

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [✓]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	As explained in the Environmental Risk Assessment, the first step in an assessment of secondary poisoning risk is to consider whether a chemical has the potential to bioaccumulate. The potential for bioaccumulation can be estimated from the values of the n-octanol/ water partition coefficient, log Kow. It is accepted that values of log Kow greater than or equal to 3 indicate that the substance may bioaccumulate (TGD, 2003). Since, bendiocarb has a log Kow of 1.72 the compound displays little potential for bioaccumulation. Therefore, a bioconcentration, terrestrial study should not be required.	
Undertaking of intended data submission [ ]		

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	25/06/07
Evaluation of applicant's justification	Applicant's justification is acceptable.
Conclusion	Applicant's justification is acceptable.
Remarks	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

7.5.5.1 Bioconcentration, further studies

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [✓]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	As explained in the Environmental Risk Assessment, the first step in an assessment of secondary poisoning risk is to consider whether a chemical has the potential to bioaccumulate. The potential for bioaccumulation can be estimated from the values of the n-octanol/ water partition coefficient, log Kow. It is accepted that values of log Kow greater than or equal to 3 indicate that the substance may bioaccumulate (TGD, 2003). Since, bendiocarb has a log Kow of 1.72 the compound displays little potential for bioaccumulation. Therefore, further studies should not be required.	
Undertaking of intended data submission [ ]		

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	26/06/07
Evaluation of applicant's justification	Applicant's justification is acceptable.
Conclusion	Applicant's justification is acceptable.
Remarks	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

7.5.6 Effects on other terrestrial non-target organisms

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [✓]	**Other justification [ ]**	
Detailed justification:	The risk assessment for the terrestrial compartment, based on the results from the acute toxicity test on earthworm and the realistic environmental exposure indicates no concern for the terrestrial compartment. Therefore, long-term and field studies should not be required.	
Undertaking of intended data submission [ ]		

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	01/02/07
Evaluation of applicant's justification	The UK CA agrees that if the terrestrial risk assessment for bendiocarb, based on the acute toxicity test for earthworm, shows no concern for the terrestrial compartment then these studies will not be needed. Applicant's justification is acceptable.
Conclusion	Applicant's justification is acceptable.
Remarks	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A7 **Annex Point IIIA XIII 3.4**	**Ecotoxicological Profile Including Environmental Fate and Behaviour** A7.5.7 Effects on mammals A7.5.7.1 Direct and/or indirect exposure to mammals – further tests A7.5.7.1.1 Acute oral toxicity

7.5.7 Effects on mammals

7.5.7.1Direct and/or indirect exposure to mammals – further tests

7.5.7.1.1 Acute oral toxicity

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [✓]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	Sufficient laboratory studies are available on a range of mammals. This study should therefore not be needed.	
Undertaking of intended data submission [ ]		

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	24/01/2007
Evaluation of applicant's justification	The UK CA agrees that further studies on mammals should not be required, as the use pattern for bendiocarb (i.e. as surface spray rather than granule bait) removes risk for mammals. Applicant's justification is acceptable.
Conclusion	Applicant's justification is acceptable.
Remarks	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A7 **Annex Point IIIA XIII 3.4**	**Ecotoxicological Profile Including Environmental Fate and Behaviour** A7.5.7 Effects on mammals A7.5.7.1 Direct and/or indirect exposure to mammals – further tests A7.5.7.1.2 Short-term toxicity

7.5.7.1.2 Short-term toxicity

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [✓]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	Sufficient laboratory studies are available on a range of mammals. This study should therefore not be needed.	
Undertaking of intended data submission [ ]		

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	24/01/2007
Evaluation of applicant's justification	The UK CA agrees that further studies on mammals should not be required, as the use pattern for bendiocarb (i.e. as surface spray rather than granule bait) removes risk for mammals. Applicant's justification is acceptable.
Conclusion	Applicant's justification is acceptable.
Remarks	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	